PRP8 inteins of *Penicillium*: structure, evolution and expression in *E. coli*

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Inteins are selfish genetic elements that excise themselves from the host protein during post translational processing, and re-ligate the host protein with a peptide bond. In addition to this splicing activity, most reported inteins also contain an endonuclease domain that is important in intein propagation, called homing. Recently fungal inteins have been identified within the *prp8* gene of the basidiomycete *Cryptococcus neoformans*. The *prp8* gene encodes a highly conserved mRNA splicing protein found as part of the spliceosome. Meanwhile allelic PRP8 inteins have been identified in several species of yeast and filamentous ascomycetes.

In this study, selected members of the ascomycetous genus *Penicillium* were investigated for the presence of inteins inside the PRP8 protein. We identified PRP8 inteins in *P. expansum*, *P. claviforme* and *P. chrysogenum*, but not in *P. thomii.* The Penicillium PRP8 inteins are inserted in the same position as the inteins found in the PRP8 protein of the basidiomycete *C. neoformans* and the ascomycetes *Aspergillus nidulans* and *Aspergillus fumigatus,* respectively. Phylogenetic analyses revealed that the small Penicillium PRP8 inteins are closely related to the endonuclease containing inteins of *A. fumigatus* and *A. nidulans*. In contrast to the *Aspergillus* PRP8 inteins, the *Penicillium* PRP8 inteins do not contain an endonuclease domain. We demonstrated that the *Penicillium* PRP8 inteins undergo autocatalytical protein splicing when heterologously expressed in a model host protein in *E. coli*.

XIp-2

Evolution of mating-type (MAT) genes of discomycete fungi

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Mating-type (MAT) genes are important as 'master-loci' regulating sexual reproduction in filamentous fungal species. MAT genes have been cloned from certain classes of ascomycete fungi and it has been shown for heterothallic (obligate outbreeding) species that isolates of opposing mating type contain highly dissimilar stretches of DNA known as 'idiomorphs', which contain from one to three open reading frames (ORF's). There is low homology between species, but idiomorphs can be divided into two MAT families, depending on the presence of alpha encoding domain or HMG encoding protein motifs. At present, work is underway to characterise the mating-type regions of plant pathogenic *Tapesia* species. Sequence analysis has confirmed the presence of an alpha encoding domain and HMG encoding protein motif within the MAT-1 idiomorph, and a single HMG encoding protein motif within the MAT-2 idiomorph. However a previously reported putative metallothionein like gene within the MAT-1 idiomorph was not detected. Resulting sequence data has been used to develop a species-specific, mating-type molecular diagnostic test for *Tapesia* species, which will be used to determine species identity and mating type of field isolates.

Extrolites are of paramount importance for classification and evolution in filamentous fungi

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Extrolites (outwards directed chemical differentiation products in fungi of functional or ecological importance) have been neglected in fungal classification and cladification, yet they are the most clear-cut diagnostic taxonomic features known. Extrolites include secondary metabolites, accumulated organic acids, extracellular enzymes, iron chelators, hydrophobins, adhesins, chaperones etc. and the profiles of such extrolites are highly species specific. We submit that these extrolites are of paramount importance in taxonomy and phylogeny at the species level. The use of especially secondary metabolites in *Aspergillus* and *Penicillium* taxonomy has shown that these alone can be used to discover species, yet is is adviseable to use both morphological, physiological and chemical differentiation in a more complete classification. Such taxonomies are backed up by features based on DNA sequencing, but the latter data are rather poor for establishing classifications and only indirect indicators of phylogeny (molecular clock estimations). It is expected that even though DNA sequence based biosystematics is in vogue now, real molecular biosystematics based on extrolites and morphology will be the taxonomy of the future. However once extrolite/morphology/physiology classifications are in place, sequencing of the gene clusters coding for these ecotype features can be done, giving valuable indications of gene, organism and species evolution. Such real classifications can also be used in more effective annotations of full genome sequenced isolates.Examples from the genus *Aspergillus* will be given, with emphasis on the full genome sequenced species.

XIp-4

The alkaloid pathway and evolution of chemical races in *Claviceps purpurea*

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Ergot alkaloids are secondary metabolites produced by the ascomycete *Claviceps purpurea*. Due to structural homologies to neurotransmitters they can act e.g. at the serotonin- or dopamine-receptors and are used in a variety of clinical conditions.

We isolated and cloned genes organized in a cluster (68.5 kb) that could mediate pathway specific steps of the alkaloid biosynthesis [1]. All cluster genes are co-regulated and are only activated under alkaloid producing conditions [2]. The cluster comprises the gene *dma*W which encodes the key enzyme dimethylallyltryptophansynthase (DMATS) that is responsible for the first alkaloid pathway-specific step, four nonribosomal peptide synthetases (NRPS) named *lps*A1, A2, B and C, several reductases and dehydrogenases (*eas*A, D, E, G), a predicted methyltransferase (*eas*F) and other putative oxygenases and hydroxylases (*eas*H1, H2) not characterized so far.

Targeted inactivation of one NRPS gene (*lps*B) led to an ergopeptine-nonproducing mutant which – unlike the parent producer strain – accumulated D-lysergic acid (D-LA). *Lps*B was shown to encode the monomodular NRPS LPS2 responsible for the activation of D-LA [3].

Knock-out experiments with the gene *clo*A (P450-monooxygenase) led to an ergopeptine-nonproducing mutant which instead accumulated agroclavine and elymoclavine. Biochemical characterization of CLOA showed that it catalyzes the step of conversion of clavines to D-LA. Therefore it acts as a critical enzyme in the alkaloid pathway bridging the biosynthesis of two different families of alkaloids [4].

The knock-out of the putative catalase-encoding alkaloid cluster gene *eas*C led to an alkaloid-nonproducing mutant in which the transcripts of other genes in the cluster are down-regulated. We attempted heterologous expression of *eas*C in *Pichia pastoris* to decide whether there is a regulatory or a structural role for this enzyme. First results of these experiments will be discussed.

Another aim is to compare different strains of *C. purpurea* with respect to their potential to produce different types of alkaloids (chemical races). Comparison of the cluster sequences of strain P1 (ergotamine producer) with that of strain ECC93 (ergocristine producer) showed high conservation of most cluster genes, but significant variation in the NRPS modules, strongly suggesting that evolution of chemical races is confined to evolution of NRPS module specificity [2].

[1] Tudzynski P *et al.*, Mol Gen Genet (1999) 261: 133-41
[2] Haarmann T *et al.*, Phytochemistry (2005) 66: 1312-20
[3] Correia T *et al.*, Chem Biol (2003) 10: 1281-92
[4] Haarmann T *et al.*, ChemBioChem (2006) in press

Molecular genetics and evolutionary aspects of the alkaloid pathway in *Claviceps* spec.

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Claviceps purpurea is an ubiquitous phytopathogenic ascomycete which infects nearly 600 species of grasses. It replaces the host ovary during the infection and produces ergot alkaloids in the resting structures the so called sclerotia. These secondary metabolites are cyclol structured indol derivatives which show structural homology to some neurotransmitters. This leads to the treatment of ergot alkaloids in a variety of clinical conditions.

By means of chromosome walking combined with cDNA-screening a 68.5 kb gene-cluster could be identified [1]. Expression studies delineated that all the cluster genes are co-regulated and only activated under alkaloid producing conditions. Molecular biological and biochemical analysis of several of these genes revealed their function in the alkaloid biosynthesis and is carried on for further genes [2,3].

We are also interested in interspecies evolution of the alkaloid biosynthetic pathway. Because the production of alkaloids is not limited to *C. purpurea* we work on a comparison of the cluster sequences of three *Claviceps* species which produce different classes of ergot alkaloids as endproducts. A cooperation with the group of C. Schardl (USA) provided 20 kb of the “left portion” of the cluster sequence in *C. fusiformis* and further sequencing of the “right portion” using TAIL-PCR is in progress. Analysis showed that the cluster sequence of *C. fusiformis* includes two genes which are not needed for the production of Clavines, the endproducts of the pathway in this species. To test if these genes are still functional, we started a complementation approach using corresponding knock-out mutants of *C. purpurea*. First results indicate that the genes are expressed but not functional at least not in the *C. purpurea* background. To determine these assumptions we complement *C. fusiformis* with *cp*P450-1 of *C. purpurea*, which encodes for a monooxygenase that mediates the further metabolisation of clavines. Successful complementation will be checked by *Northern* and HPLC analysis and should hopefully show a modified alkaloid pattern. For *C. paspali* which produces lysergic amids as endproducts construction of a genomic library, genebank screening and further analysis are on the way.

[1] Tudzynski *et al.*: Evidence for an ergot alkaloid gene cluster in *Claviceps purpurea*. Mol Gen Genet; 261:133-141 (1999).
[2] Haarmann *et al.*: Identification of the cytochrome P450 monooxygenase that bridges the clavine and ergoline alkaloid pathway. Chem Biochem; (in press).
[3] Correia *et al.*: Molecular cloning and analysis of the ergopeptine assembly system in the ergot fungus *Claviceps purpurea*. Chem Biol; 10:1281-1292 (2003).

XIp-6

Molecular analysis and evolution of gibberellin biosynthetic gene clusters in fungi

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Gibberellins (GAs) are diterpenoid plant hormones that are also produced as secondary metabolites by the ascomycete *Fusarium fujikuroi* (teleomorph: *Gibberella fujikuroi*) and some other fungi and by some bacteria. In the last years, the GA biosynthetic genes of higher plants and *F. fujikuroi* (gene cluster) have been cloned and functionally characterized. Due to fundamental differences in the GA-biosyntheses at the chemical (pathway), biochemical (enzymes) and genetic levels, the hypothesis of a horizontal gene transfer from the plants to the fungus can now be excluded. To elucidate the origin of GA- or similar gene clusters in fungi, we investigate the GA gene cluster in a second, not closely related GA-producing fungus, *Sphaceloma manihoticola*. This fungus is a cassava pathogen causing superelongation of the internodes similar to *F. fujikuroi* on rice. In contrast to *F. fujikuroi*, the fungus produces GA_4 and GA_9 as final products which are intermediates of the GA pathway in *F. fujikuroi*. No traces of GA_1, GA_7 and GA_3 were found suggesting that the 13-hydroxylase (*P450-3*) and the GA_4 desaturase (*des*), catalyzing the last two steps in *F. fujikuroi,* are missing or at least inactive in *S. manihoticola*.

The screening of a genomic library and subsequent chromosome walking revealed a GA biosynthesis cluster similar to that in *F. fujikuroi*. As in *Fusarium*, the two monooxygenase genes (*P450-1* and *P450-4*) as well as the *ggs2* and *cps/ks* homologous genes share bidirectional promoters. The order and direction of transcription of the putative GA genes differ in some cases from that in the *Fusarium* cluster. As already expected, homologues of *P450-3* and *des*, localized at the right and left borders in the *Fusarium* gene cluster are missing in *S. manihoticola*.

To confirm, that the *cps/ks*-like gene catalyzes the same reactions as in *F. fujikuroi*, we complemented a *Fusarium cps/ks* mutant with the *Sphaceloma* homologue. Northern blot analysis revealed a very weak expression level of the *Sphaceloma* gene in the *Fusarium* background, probably due to different regulation mechanisms. To overcome this problem, we fused the strong *Fusarium* promoter with the *Sphaceloma cps/ks* gene.

A BLAST search for *cps/ks*-and *ggs*-like genes, which are physically linked sharing the same promoter, displayed homologous complexes in the published genomes of *Magnaporthe grisea* and *Phoma betae*. These findings lead to the suggestion of a fungi-specific evolution of diterpenoid gene clusters.

Excision of the fumonisin gene cluster in *Fusarium verticillioides* strains isolated from banana

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Fumonisins are polyketide-derived mycotoxins produced mainly by *Fusarium* species in the *Gibberella fujikuroi* complex, especially *F. verticillioides* isolated from maize. A cluster of 15 putative fumonisin biosynthetic genes, the Fumonisin Gene Cluster (FGC), has been described in *F. verticillioides*. However, several strains of *Fusarium verticillioides* isolated from banana were described as non fumonisin producers and attempts to amplify by PCR or reveal by southern blot genes of the FGC were negatives. Here we report preliminary results indicating that a sequence of 44 kb including the main part of the FGC has been excised from one strain (MUCL 31965) of *F. verticillioides* isolated from banana. The excision hypothesis is supported by the fact that the 3' end of the last gene of the FGC (*FUM19*) is still present.

XIp-8

Crawler, a novel transposable element of *Tc1/mariner* superfamily in *Aspergillus oryzae* transposes under stress conditions

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A number of transposable elements (including both of class I and class II transposons) in *Aspergilli* have been found in the past decade. Among them, class II transposons, *Vader* and *Ant1* in *A.* niger, were proved to have transposition activity, because they were isolated when the resident *niaD* gene was used as a transposon trap. We have recently identified a novel *Tc1/mariner*-type transposable element, named *Crawler*, in an *A. oryzae* industrial strain OSI1013 as an insertion in the coding region of the *niaD* gene. *Crawler* is approx. 1.3 kb in length with terminal inverted repeats of 22 bp and has an open reading frame encoding a putative transposase of 357 amino acid residues. The transposase shows overall sequence similarity to that encoded by *impala* of *Fusarium oxysporum* (31% identity). A dinucleotide (TA) direct repeat sequence flanking the element exists, which is a typical target site duplication upon insertion of transposons belonging to *Tc1/mariner* superfamily. The element is present in multiple copies (>16) in the strain OSI1013, but is found as a single defective copy with RIP-like mutations in the strain RIB40 that was used for genome analysis. Expression of the transposase-encoding gene (*aotA*) was found in standard growth medium, and was stimulated slightly by stress treatments, such as heat shock and $CuSO_4$. When conidiospores of OSI1013 were screened for mutations by chlorate resistance under stress conditions, we could isolate successfully a number of mutant strains harboring insertion of *Crawler* in the *crnA* gene or in the *niaD* gene. In contrast, insertion event was seldom detected in the mutants obtained without stress. In addition, judged from a result of selection of revertants capable of assimilating nitrate from a resultant *crnA* mutant, excision events of *Crawler* occurred at a frequency of 10^{-4} - 10^{-5} by treatment of heat shock or $CuSO_4$. All excision sites of the revertants sequenced so far contained the same footprint of 5 nucleotides, 5'-CTTTA-3'. To the best of our knowledge, this is the first observation that the resident transposable element is able to transpose under stress conditions in *Aspergillus* species.

Analysis of *Botrytis cinerea* populations in the Eger and Tokaj wine regions - a multiloci approach

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Botryotinia fuckeliana (anamorph: *Botrytis cinerea*) is a phytopathogenic fungus that causes grey mould decease on a wide range of plants in temperate regions worldwide. *B. cinerea* has been shown to have several variable genetic and physiological traits, such as transposa and vacuma and it has developed resistance against most of the fungicides used to control it.

Our aim was to evaluate the genetic diversity of *B. cinerea* in the Eger and Tokaj wine regions. Sixty samples were analysed. Sequence analysis of minisatellite (MSB1), intergenic spacer (IGS1) and translation elongation factor 1 (*tef1*) showed polymorphism in all the tree DNA fragments, although the difference was not high in the case of the IGS1 and the *tef1* sequences. In general, sequence analysis revealed a high degree of genetic diversity, with no widespread of clonal lineages. The analysis of MSB1 sequences indicated tree bigger groups. Parsimony phylogenetic analysis of MSB1, *tef1* and IGS1 sequences indicates only one closely related group (clone) of *B. cinerea*. We could not find any geographical preference of the different genotypes groups. The combination of alleles suggests the presence of sexual reproduction in the area, while the disperse distribution of the genotype indicates high migration rate.

XIp-10

Isolation, molecular characterization and location of telomeric sequences of basidiomycete *Pleurotus ostreatus* var. *florida*

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The white rot fungus *Pleurotus ostreatus* is an edible basidiomycete of increasing biotechnological interest due to its ability to degrade both wood and chemicals related to lignin degradation products. Telomeres are specialized structures at the end of all eukaryotic chromosomes. Ensure chromosome stability and protect the ends from degradation and from fusing with other chromosomes. Telomeres sequences are extraordinary highly conserved in evolution. The loss of telomeric repeats triggers replicative senescence in cells. For identification of restriction telomeric fragments in a previously described linkage map of *Pleurotus ostreatus* var*. florida* (Larraya *et al.*, 2000), dikaryotic and eighty monokaryotic genomic DNAs were digested with diferents restriction enzymes (*Bam*HI, *Bgl*II, *Hind*III, *Eco*RI, *Pst*I, *Sal*I, *Xba*I and *Xho*I) electrophoresed and transferred to nylon membranes. Numerous polymorphic bands were observed when membranes were hibridized with human telomericd probe $(TTAGGG)_{132}$ (heterologous probe). Telomeric restriction fragments were genetically mapped to a previously described linkage map of *Pleurotus ostreatus* var.*florida*, using RFLPs identified by a human telomeric probe (tandemly repeating TTAGGG hexanucleotide). Segregation of each telomeric restriction fragment was recorded as the presence vs. absence of a hibridizing band. Segregation data for seventy three telomeric restriction fragments was used as an input table to be analysed as described by Ritter *et al.*, (1990) and by Ritter and Salamini (1996) by using the MAPRF program software. Eighteen out of twenty two telomeres were identified. Telomere and telomere-associated (TA) DNA sequences of the basidiomycete *Pleurotus ostreatus* were isolated by using a modified version of single-specific-primer polymerase chain reaction (SSP-PCR) technique (Sohapal *et al.*, 2000). Telomeres of Pleurotus ostreatus contain at least twenty five copies of non-coding tandemly repeated sequence (TTAGGG).

Aft1 an active transposon in *Aspergillus fumigatus*

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Through bioinformatic analysis a *Mariner* type transposon – *Aft1* with high homology to the *Tan1* transposon of *A. niger* was identified. This transposon is present in high numbers (>20 full length copies) in AF293 and encodes a transposase which contains all the functional motifs of an active transposon. Southern blot analysis and PCR revealed this transposon to be present in all six *A. fumigatus* strains tested with considerable variation in the distribution of *Aft1* between strains. PCR over four *Aft1* transposon insertion sites identified by bioinformatic analysis revealed these insertions are only present in AF293 suggesting this element has been active in the past. Activity of the *Aft1* element was tested by cloning the element into a zeocin resistance cassette (between the promoter and Zeocin resistance gene) and screening for zeocin resistant colonies. PCR of the zeocin cassette region of these resistant colonies revealed excision of the transposon and in most cases a 2 b.p footprint. Several colonies showed partial excision of the transposon with 200-300 b.p fragments remaining. The potential use of this transposon in tagging experiments is currently being examined.

XIp-12

Differentiation of zygomycetous strains on the basis of high affinity iron permease (*FTR1*) sequences

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Zygomycetous fungi have been reported as agents of frequently fatal opportunistic mycoses in man and animals. However, most of the infections caused by these fungi have been identified in clinical microbiological laboratories only as zygomycosis or mucormycosis without species or at least genus determination. The majority of the known DNA based identification methods do not resolve the closely related species, development of new techniques for strain identification and typing is therefore beneficial for both the clinical and the biotechnological fields.

Iron transporters, such as high affinity iron permease (*FTR1*) have been suggested to function as virulence factors. The high affinity iron permease gene contains both highly conservative and variable regions applicable for phylogenetic comparisons. The aim of this study was a comparative analysis of this gene of different zygomycetous strains.

Conserved regions of *Candida albicans* and *Rhizopus oryzae FTR1* genes have been analysed to design degenerate primers for polymerase chain reaction. These were used to amplify the homologous regions of 25 strains from 5 different genera (*Rhizopus*, *Mucor*, *Backusella*, *Rhizomucor* and *Syncephalastrum*). DNA and the deduced protein sequences were aligned and phylogenetic analyses were carried out. In this tree, the strains of distantly related *Mucor* species formed a monophyletic group. *Backusella* was also located in this cluster. Interestingly, the *R. microsporus* isolate seems to be distant from the other *Rhizopus* isolates and form a sister group with *Mucor* strains. The *R. oryzae* isolates formed a group completely different with a significant distance from the *R. microsporus* isolate. With the degenerated primers used in this study, *Rhizomucor* and *Syncephalastrum* can be distinguished from other species, because their amplicon sizes are higher due to the presence of introns.

Alignment of the amplified *FTR1* sequences revealed small regions characteristic to the studied species. These regions proved to be suitable to design oligonucleotid primers for species-specific amplifications. Significant differences in the *FTR1* sequences of the *R. microsporus* and *R. oryzae* strain make possible to differentiate these closely related species. The examined strains can be differentiated on the basis of PCR-RFLP, after the *Alu*I digestion of the amplified regions *R. oryzae* isolates can be divided into 3 further groups.

This research was supported in part by grants from the Hungarian Scientific Research Fund (OTKA T37471, F46658, D48537) and GVOP-3.1.1.-2004-05-0471.

Conflicting phylogenetic position of *Schizosaccharomyces pombe*

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The phylogenetic position of the fission yeast *Schizosaccharomyces pombe* in the fungal Tree of Life is still controversial. Three alternative phylogenetic positions have been proposed in the literature namely 1. a position basal to the Hemiascomycetes and Euascomycetes, 2. a position as a sister group to the Euascomycetes with the Hemiascomycetes as a basal branch or 3. a sister group to the Hemiascomycetes with Euascomycetes as a basal branch. Here we compared 91 clusters of orthologous proteins containing a single orthologue that are shared by 19 eukaryote genomes. The major part of these 91 orthologues supports a phylogenetic position of *S. pombe* as a basal lineage among the Ascomycetes, thus supporting the second proposition. Interestingly, part of the orthologous proteins supported a fourth, not yet described alternative, in which *S. pombe* is basal to both Basidiomycetes and Ascomycetes. All both phylogenetic trees are well supported. We believe that both reflect correctly the phylogenetic history of the species concerned. This apparent paradox may point to a heterogeneous nuclear genome of the fungi. Importantly, this needs to be taken in consideration for a correct understanding of the Tree of Life.

XIp-14

DNA sequence analysis and metabolic profiles demonstrate existence of two marine *Dendryphiella* species that do not belong to the genus *Scolecobasidium*

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The two species of marine *Dendryphiella* - *D. arenaria* and *D. salina* are usually identified based on differences in their conidial morphology. The assumed high taxonomic value of this parameter led to the recent inclusion of *Dendryphiella* in the genus *Scolecobasidium*. However, due to the significant dissimilarity in the physioecological and other morphological characters, there is still disagreement on the placement of these species within *Scolecobasidium*. We were interested, therefore, in determining the position of the marine *Dendryphiella* species with respect to the modern molecular phylogeny of ascomycetes. Furthermore, we determined to what extent the species can be differentiated on the basis of their metabolic profiles and analysis of their gene sequences. The *Dendryphiella* strains were isolated from various substrates collected along coastal areas in subtropical and temperate waters. Genomic DNA was extracted from strains of both *D. arenaria* and *D. salina* and from several *Scolecobasidium* species. Analysis of partial *rpb*2 sequences was used to find the next closest taxonomic relatives. The infragenic structure of marine *Dendryphiella* was detected by analysis of the variable ITS1 and 2 of the rDNA repeat and the introns of *tef*1 gene. Production of enzymes using cultural methods and API ZYM assay, as well as BIOLOG Phenotype Microarrays were used to assess the ability of *Dendryphiella* strains to utilize different substrata. The *Dendryphiella* species from different geographical locations exhibited similar enzyme profiles, but differed significantly in their carbon utilization profiles. Sequence analysis of ITS1 and 2 and *tef*1 genes showed that the marine *Dendryphiella* strains form two sister clades, which correspond to *D. arenaria* and *D. salina*. Both species belong to the *Pleosporaceae* family, with *Pleospora herbarum* (*Stemphyllium botryosum*) as the next very close taxonomic relative. All *Scolecobasidium* species sequenced form a distinct genetically isolated phylogenetic group outside of the class *Loculoascomycetes*. Though the exact phylogenetic position of *Scolecobasidium* remains unclear, we have shown that the resemblance of conidial morphology of *D. arenaria* and *D. salina* to the species of *Scolecobasidium* is not a result of a close genetic relationship.

Mitochondrial plasmids in *Trichoderma* strains associated with green mould disease of commercially grown mushrooms

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Trichoderma species are common fungi found in many cultivated and natural soils, and used as biological control agents of fungus-associated plant diseases. However, green mould epidemics on commercially grown mushrooms caused by*Trichoderma* species spread in the last two decades both in Europe and North America.

Double-stranded DNA plasmids were found in the mitochondria of numerous filamentous fungi. The plasmid carrying fungi generally do not show any symptoms, but special phenotypes are associated with their presence in certain cases. Little is known about the extrachromosomal genetic elements of *Trichoderma* species. Although plasmid ladders derived from circular mitochondrial plasmids have already been reported in some *Trichoderma* isolates, the complete sequence of only a single 2.6 kb plasmid detected in *T. harzianum* strain T95 is known. In the present study we investigated the presence of extrachromosomal dsDNA molecules in *Trichoderma* isolates derived from Hungarian *Agaricus* compost and *Pleurotus* substrate samples as well as from the CBS culture collection.

In addition to a large DNA band, two smaller bands, 1.7 and 5.0 kb in size, and two bands in the very-high-molecular-weight region (>23 kb) were observed in the undigested total DNA preparation of *T. aggressivum* f. *aggressivum* strain CBS 450.95. These fragments were resistant to S1 nuclease and RNase treatment, indicating their double-stranded DNA nature. Hybridization experiments carried out using the 5.0 kb fragment as probe revealed that these fragments exhibit sequence homology with each other. The DNA samples derived from the isolated mitochondria of the strain also contained the fragments, indicating the mitochondrial localization of the plasmid molecules. Similar sized bands exhibiting sequence homology with the labeled 5.0 kb fragment were observed in the undigested total DNA preparations of strains *T. aggressivum* f. *aggressivum* strain CBS 100527, *T. aggressivum* f. sp. *europaeum* strain B1, and *Trichoderma* sp. DAOM 175924 C15 as well. It was pointed out in the case of *Neurospora* and *Fusarium* that mitochondrial plasmids seem to be highly mobile and their horizontal transfer occurs frequently in nature. A similar mechanism could have caused the widespread appearance of these plasmids in these green mould associated *Trichoderma* strains. Further studies are in progress to clarify whether these plasmids have any effect on the virulence of the harboring strains.

This work was supported by the Hungarian Government with a grant OM-00083/2004.

XIp-16

Characterization of *Trichoderma* species from Caspian sea area using RAPD and nucleotide sequence of ITS-rDNA region

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Trichoderma is one of the most studied genera of fungi as a biocontrol agent for many plant pathogenic fungi or target organism for genetic engineering. In this research the *Trichoderma* species of south regions of the Caspian Sea that there is no complete record of their distribution and variation were studied. Five hundred and thirty isolates belonging to sect. *Trichoderma* and sect. *Longibrachiatum* were isolated from different parts of this area mainly from soil and a few from wood and were identified by molecular and morphological characteristics. Two molecular methods including RAPD analysis and sequencing of two genes (tef and internal transcribed spacer region of rRNA) were used for identification of isolates that were not distinguishable easily by morphological characters. In RAPD analysis six primers were used to differentiate morphologically selected isolates which two of them were more suitable and confirmed the differences among the isolates. The internal transcribed spacer region (ITS1, ITS2 and the 5.8S gene) of 22 Caspian Sea isolates and Translation Elongation Factor of 18 of them were sequenced. Using these morphological and molecular characteristics different species were identified which are as follow:
Section *Longibrachiatum* comprised *T. citrinoviride*, *T. reesei, T. longibrachiatum, T. ghanense* , *T. parceramosum*, *T. saturnisporium* and *Hypocrea orientalis* and section *Trichoderma* comprised *T. harzianum*, *T. atroviride*, *T. asprellum*, *T. erinaceum*, *T. koningii* and *Trichoderma sp*.1, *Trichoderma sp*.2, *Trichoderma sp*. 3
Results from analysis the sequences of *tef* gene confirmed the results obtained from analyzing ITS region sequences. In this study some of the isolates of *T. koningii* species aggregate showed considerable differences in molecular and morphological characters and these isolates can be new species that were regarded as *Trichoderma sp*.

ITS sequence analysis for taxonomic study of *Xylaria* species

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Xylaria is a large and relatively well-known fungus group in the family Xylariaceae and it is represented in most countries in the world. These fungi occur on wood, leaves, seeds, dung, and soil or in a few cases are associated with insects. Many *Xylaria* species have been reported as endophytes living inside healthy plant tissue without apparent damage to the host. Recently, endophytes have been widely investigated because of their ability to produce either new or interesting secondary metabolites, some of which have proven to be bioactive. The taxonomic studies of *Xylaria* have been based on morphological and cultural characteristics but their high morphological variation among species is the major problem. Furthermore, the identification of endophytic fungi is often difficult since they are very infrequently to form their teleomorph stage in culture. Therefore, the molecular taxonomic study based on ribosomal nucleotide sequences, which presented in this study, was undertaken to identify *Xylaria* species and this molecular data was then used to align with endophytic fungus sequences available in GenBank database. Nucleotide sequences of ITS1, 5.8S, and ITS2 rDNA of 22 species from 98 teleomophic *Xylaria* isolates collected from different locations were amplified and analyzed. The comparison of *Xylaria* nucleotide sequences to endophytic fungal sequences revealed that most endophytic fungus sequences closed to sequences of *X. juruensis* SUT140, *X. multiplex* ST2298, *X. pesidii* SUT125, and *Xylaria* sp. SUT127 examined respectively. The alignment of *Xylaria* sequences exhibited the greatest variation in the ITS1 region whereas 5.8S sequence gave approximately 99% similarity for all isolates. The phylogenetic tree of *Xylaria* species was constructed and exhibited clearly separation of each species. The relationship between *Xylaria* species and endophytic fungi was also included. These molecular results were proven to be valuable for the taxonomic investigation of *Xylaria* species with their high morphological variation. In addition, a database of this molecular data would be useful for the designation of species specific primers and/or probes to detect endophytic fungi, which are difficult to identify.

XIp-18

Phylogenetic analysis of *Aspergillus* section *Terrei*

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Aspergillus section *Terrei* historically includes isolates with strongly columnar, cinnamon to orange-brown biseriate conidial heads (1). Although *Aspergillus terreus* was the only species belonging to this section until recently, molecular studies have since indicated that this section should be expanded to include a number of other species (2). *Aspergillus terreus* is an opportunistic human pathogen, and economically important as the main producer of lovastatin, a cholesterol lowering drug. In this study, our aim was to examine the genetic variability of *A. terreus* and closely related species using molecular and analytical techniques. Lovastatin was produced by seven isolates belonging to the species *A. terreus* as proved by HPLC analysis. RAPD analyses were carried out using 25 different random primers. Neighbor-joining analysis of RAPD data resulted in clustering of the *A. terreus* isolates into distinct groups. The ITS region of *A. terreus* and related isolates was also sequenced. Phylogenetic analysis of sequence data let us classify the isolates into different clades which mostly correspond to the species *A. terreus*, *A. flavipes*, *A. niveus*, *A. carneus* and *A. janus*/*A. janus var. brevis*. *A. allahabadii*, *A. terreus var. aureus* and *A. niveus var. indicus* belonged to the *A. niveus* clade, while an *Aspergillus* isolate previously classified as *A. niveus* was most closely related to *A. flavipes* isolates. *A. anthodesmis* formed a distinct branch on the tree. Although it was previously suggested based on 28 S rDNA sequence data that *Aspergillus* section *Terrei* should include *A. carneus* and *A. niveus* isolates, phylogenetic analysis of ITS sequences indicate that *A. flavipes* isolates are as closely related to *A. terreus* as *A. carneus* isolates. Our data suggest that sections *Terrei* and *Flavipedes* should be merged. However, further loci should be analyzed to draw more definite conclusions.

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Characterization of *Fusarium solani* isolates from Ethiopia using Amplified Fragment Length Polymorphism (AFLP) and DNA sequence information

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Fusarium solani is a fungal pathogen that causes diseases on at least 87 genera and many hundreds of species of plants. This fungus represents one of the two *Fusarium* species most commonly isolated from agricultural soils and plant tissues in Ethiopia. We studied 43 Ethiopian *F. solani* isolates using Amplified Fragment Length Polymorphism (AFLP) and nucleotide sequences of the Translation Elongation Factor 1a (TEF-1a) and b-tubulin genes. Phylogenetic analyses of the TEF-1a sequence data aggregated all the Ethiopian isolates in a strongly supported group. This group correlated with Clade 3 of the *F. solani-Nectria haematococca* species complex that has been characterized previously. Within this clade, the Ethiopian *F. solani* isolates separated into six well-supported lineages, corresponding with the six lineages that emerged from the AFLP analyses. AFLPs further separated the six lineages into two clusters. The presence of three b-tubulin nucleotide sites that were fixed differently between the two clusters also supported the separation of these clusters. However, the genetic differentiation between the two clusters was small. Taken together, these data suggest that the examined Ethiopian *F. solani* isolates represent two different entities that are either in the process of separating into two different species or groups that have recently converged into a single species.

XIp-20

Genetic characteristics of *Gibberella fujikuroi* mating population A (Fusarium verticillioides) from corn in the Philippines

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Gibberella fujikuroi (Fusarium verticillioides) is one of the most important fungal pathogens of corn worldwide. The pathogen produces mycotoxin fumonisins that are potentially harmful to human and animal health. Forty isolates of *G. fujikuroi* from Laguna, Philippines were characterized by mating type and mating population. The identity of the isolates was confirmed by sexual crosses with standard tester strains. Thirty-four (85%) were mating type A^+ and six (15%) were A^-. Twenty isolates were further characterized by fertility and vegetative compatibility group (VCG) in the laboratory and tested for their stalk rot aggressiveness under field conditions across two environments using the toothpick inoculation method. In two greenhouse trials, inhibition of seedling emergence, seedling height, fresh and dry weight were also determined. Analysis of fertility revealed 50% of the population were female fertile. Significant (P = 0.05) differences in aggressiveness toward corn of some isolates were observed for both experimental locations while vegetative compatibility grouping by pairing nit mutants identified 19 vegetative compatibility groups for this population with a genotype diversity of 0.95. All isolates were pathogenic to corn seedlings and mature plants compared to non-inoculated control. The predominance of *G. fujikuroi* mating population A suggests that Philippine corn is contaminated with fumonisins.

Significance of variability in *F. verticillioides* and *F. proliferatum*

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F. verticillioides and F. proliferatum are widespread pathogens of important crops, especially maize. Both species are toxigenic and are considered the main source of fumonisins in food and feed products, responsible of serious chronic and acute diseases in human and animals. Phylogenetic analyses using DNA sequence data have provided useful information in relation to the toxin profiles, to the development of rapid, specific and accurate diagnostic methods, and to the genetic variability, population structure and host preferences of the critical species necessary to understand their distribution and dynamics, and therefore, to their control. Additionally, it is important to determinate the ability to produce fumonisins and evaluate differences of biosynthesis at an intraspecific level.

The aim of this work was to analyse and compare the intraspecific variability of the main fumonising-producing species *F. verticillioides* and *F. proliferatum*. A sample of *F. verticillioides* and *F. proliferatum* isolated from a naturally infected field of maize were analysed and their haplotypes were determined on the basis of IGS and EF-1α gene sequences, as well as determination of mating type (MAT-1 or MAT-2 alleles). These strains were compared with strains of the same species representing different geographical origins and hosts by a phylogenetic analysis by PAUP using the partial genomic sequences of the EF-1α gene. Additionally, we determined the ability to synthesize fumonisins of the *F. verticillioides* and *F. proliferatum* haplotypes by analyzing the expression of *fum1*, critical step in the fumonisin biosynthetic pathway, by real time RT-PCR. The results obtained in this work indicated higher variability of *F. proliferatum* than *F. verticillioides* both at local and at global scale. The higher variability of *F. proliferatum* might partially be explained by a possible higher incidence of sexual reproduction. Host preferences seemed to be more restricted in the case of *F. verticillioides* than in *F. proliferatum*, although a certain adaptation to host might be occurring in *F. proliferatum.* The analysis of the ability to produce fumonisin confirmed the absence of a positive relationship with prevalence of the strains on infected maize.

This work was supported by the Spanish MCyT (AGL 2004-07549-C05-05) and M.J. by a MCyT fellowship.

XIp-22

Applying of RAPD-PCR for determination of genetic variability of *Bipolaris sorokiniana* originated from spring barley

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Bipolaris sorokiniana (*Cochliobolus sativus*) is one of the most dangerous pathogens of spring barley and infects all parts of plant in all stages. It is a serious problem, especially in organic systems, were a pesticide applying is prohibited. Infection by *B. sorokiniana* can be reduced by development of resistance in spring barley. This development requires knowledge of the structure of the pathogen population. Such information is lacking, so research on breeding practices connected with resistance by spring barley of this important pathogen is badly needed. The purpose of our study was to determine intraspecies genetic variability among *B. sorokiniana* strains that originated from different regions of Poland, various farming systems, different spring barley cultivars and plant parts.

The research materials were over 160 isolates of *B. sorokiniana* taken in 2002-2005 from roots, stem bases and leaves with disease symptoms and from harvested grain. Pure colonies of *B. sorokiniana* obtained at PDA medium were the basis for monosporic culture preparation. The mycelium, obtained at liquid PDA, was filtered, washed with TE buffer, and total DNA was extracted according to a modification of the method of Doyle and Doyle (1990). DNA samples were diluted in TE buffer to give a concentration of 20 ng μl^{-1} taken for further studies.

Genetic-variability analysis of *B. sorokiniana* isolates was carried out by RAPD-PCR (Random Amplified Polymorphic DNA). Out of the 120 primers (Operon Technologies Inc.) tested, 8 showed evidence of polymorphism between isolates and were used for studies with all isolates.

RAPD-PCR reactions were performed in 12.5-μl volumes containing PCR Core Kit and 5 picomoles of primer and 100 ng of DNA. Amplification was performed in a thermocycler, according to the following program: 94^{o}C for 3 min followed by 40 cycles of 94^{o}C for 1 min, 37^{o}C for 1 min, 72^{o}C for 2 min, and a final extension at 72^{o}C for 5 min.

Analysis of dendrogram revealed high genetic variability among the isolates of *Bipolaris sorokiniana* obtained from spring barley. It is difficult to state directly the cause of differences among isolates, because in a measure almost all factors as farming system, barley cultivar and also plant part effected on variability. No direct effect of year of isolation or isolate originated from which the pathogen was isolated was apparent.

Comparison of *Candida* species on the basis of their phospholipase D sequences

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Phospholipase D (PLD) of Candida albicans is suggested to be an important regulator of dimorphic transition, which plays a role in fungal virulence (1). Analysis of *PLD* gene sequences containing conserved regions separated with more or less variable sections can be a useful tool for phylogenetic investigations as well as for strain identification. The aim of the present work was to test the value of *Candida PLD* sequences from this aspect.

Thirteen different *Candida* isolates, representing species (*C. albicans*, *C. glabrata*, *C. tropicalis*, *C. dubliniensis*, *C. parapsilosis*, *C. krusei*, *C. guillermondii*, *C. lusitaniae*, *C. norvegica*, *C. norvegense*, *C. zeylanoides*, *C. inconspicua* and *C. lypolitica*) concerned as opportunistic pathogens causing diseases in immunocompromised patients. were involved in the study. A degenerated primer pair was constructed on the basis of known *C. albicans* and *C. glabrata PLD* sequences (accession numbers: AB010810 and XM448908, respectively) for PCR reactions. The amplified *PLD* fragments contain parts of two conserved regions interspaced with a variable sequence and have various lengths between 497 and 617 bps. Sequences of both *C. albicans* and *C. glabrata* amplified in this study was identical with sequences obtained from databases. Using the alignments of the DNA and the putative amino acid sequences, phylogenetic analyses were carried out. Our results are in good agreement with previous phylogenetic studies such as the analysis of the ITS regions or the 26S rRNA genes (2). The *C. inconspicua*, which has not been included in previous studies, showed the closest homology to *C. norvegense*. Alignment of the amplified *PLD* sequences revealed small regions characteristic to the studied species. These regions seem to be suitable to design oligonucleotid primers for species-specific amplifications.

This research was supported in part by grants from the Hungarian Scientific Research Fund (OTKA T37471, F46658, D48537) and GVOP-3.1.1.-2004-05-0471.

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XIp-24

Identification of *Colletotrichum* species isolated from Iran based on morphological and molecular characteristics

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Colletotrichum is one of the most important genera of plant pathogenic fungi worldwide. It causes economically important diseases in different crops and ornamentals which are known as anthracnose. *Colletotrichum* has also been used as a model system for studying infection processes, resistance mechanisms and host-plant interactions, and a biological control agent against weeds.

In this study *Colletotrichum* species were isolated from different crops and ornamental plants, specially from potato fields, of two Iran provinces (Hamedan and Lorestan) during the years 2004 and 2005. The isolates were identified based on morphological criteria (conidial and appressorial shape and size, and presence or absence of setae and sclerotia) and the sequences of internal transcribed spacer regions (ITS1, ITS2 and 5.8S gene) of rRNA gene. Based on these characteristics nine species including *C. acutatun*, *C. boninense*, *C. coccodes*, *C. dematium*, *C. destructivum*, *C. gloeosporioides*, *C. musae, C. trichellum* and *C. truncatum* were identified and three of them were new for the mycoflor of Iran. In addition, the genetic diversity of *C. coccodes*, the causal agent of potato black dot, was studied because this species has recently become a very important pathogen in potato producing regions of Iran. No genetic diversity were seen among *C. coccodes* isolates according to the sequences of ITS region .

ITS analysis of DNA from *Trametes* species

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Isolates of the genus *Trametes* belong to the best analyzed white-rot fungi with regards of their lignolytic activities. However, the relationships in this genus on the molecular level is not resolved. A large portion of ITS sequences placed under the name *Trametes* in the NCBI database are indeed misidentifications. Likewise, ITS sequences deposited under other fungal names by phylogenetic analysis appear to come from *Trametes* isolates. Amongst the ITS sequences from different *Trametes* isolate, there is also no clear-cut identification of different species. Here we present ITS sequence data for species clarification on the molecular level.

Financial support of the laboratory by the Deutsche Bundesstiftung Umwelt is gratefully acknowledged. This work is supported by the BMBF in the network project “Forst-Holz-Wertschöpfungskette Buche/Küstentanne” of the Kompetenznetzwerk für Nachhaltige Holznutzung.

XIp-26

Species diversity of *Fusarium* from chinese barley

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Infection of small grain cereals with Fusarium species not only leads to yield losses but also to the contamination of the produce with mycotoxins that have various detrimental effects on consumers. This concern has led to the enforcement by the EU of maximum levels for the mycotoxins deoxynivalenol (DON) and zearalenone in cereals and additional legislation for other mycotoxins produced by Fusarium (and other fungi) is expected. In barley (and malt and beer) the situation is even more strict, since most brewing companies have a zero tolerance for DON.

As China is an increasing stakeholder in the barley/malt/beer chain, both as producer as well as consumer, we monitored the presence of Fusarium species during the growing season of 2005. We performed hierarchical sampling of barley heads in nine provinces along the Yangtze River, covering the most important barley growing areas of the country. In total, we collected 1894 isolates from infected barley heads collectively called the "Fusarium Barley Bank from China". Monospore cultures were generated of all isolates, and they were prepared for long term storage. From all isolates highly purified DNA was extracted and we are currentlycharacterizing the “Fusarium Barley Bank” by PCR methods. We used multiplex PCR using species-specific primers previously designed to monitor Fusarium populations in The Netherlands (1). Preliminary analysis show that the F. graminearum complex is by far the most dominant species identified, accounting for over 80% of all identified isolates, with minor occurrences of F. proliferatum and F. culmorum. This is largely in agreement with previous reports that identified F. graminearum lineage 6, also known as F. asiaticum in wheat fields in Zhejiang province (2). Clear differences were found between the different provinces in China. For instance, F. graminearum was found as the only species in Yunnan, Anhui, Fujian and Shanghai provinces. A substantial number of isolates could not be identified with the primers used, suggesting that populations in China may be more complex that in Europe. Future analyses will focus on the chemotype of the isolates, differentiation of species belonging to the Fusarium graminearum complex and genetic analyses of populations using SSR markers.

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Transposon elements and mycelial compatibility groups of *Botrytis cinerea* in the Eger and Tokaj wine regions

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The development of grey mould on the grape berries is among the most striking viticultural phenomena with complex preconditions and implications. The ascomycete *Botryotinia fuckeliana* (anamorph: *Botrytis cinerea*) is the causal agent of both the destructive grey mould (pourriture grise) as well as the noble rot (pourriture noble), an infection that results in wines with a special quality and most pleasant bouquet. In Hungary, the Tokaj wine district has a reputation for the production of these great sweet pourriture noble wines, called „aszú". On the other hand, in the nearby Eger wine district it is the pourriture grise that usually causes serious losses.

B. cinerea has been shown to possess two types of transposons: class I (called Boty) and class II (called Flipper). Studies on French and Chilean isolates revealed three types of isolates: (1) having both transposable elements (2) having no transposable elements, and (3) containing transposable element Boty alone. Isolates containing only the Flipper transposon element showed the highest ratio both in the Eger an in the Tokaj wine district. This genotype is extremely rare or absent in France and Chile.

Characterization of groups of vegetatively (somatically) compatible individuals provides a powerful approach to subdividing a species into discrete populations in filamentous ascomycetous fungi. Mycelial incompatibility test was used to define mycelial compatibility groups (MCGs) of *B. cinerea*. Classifying all strains into distinct groups was difficult, and both dark interaction line and mycelial-free space were observed in interactions. The existance of multiply MCGs indicates that hyphal fusions are not common in the *B. cinerea* populations cinerea in the Eger and Tokaj wine regions.