BRITISH MYCOLOGICAL SOCIETY
Annual Scientific Meeting 2005

EXPLOITATION OF FUNGI

University of Manchester: September 5-8 2005

Abstracts

Invited and Offered Papers

SESSION 1 – COMPARATIVE AND FUNCTIONAL FUNGAL GENOMICS

Invited Papers

Scott E Baker, Jon K Magnuson, Ellen A Panisko, Christopher Wend, Ziyu Dai, Linda Lasure
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Genome and proteome analysis of industrial fungi

The enzymatic and metabolic diversity of fungi make fungal bioprocesses important in the development of fuels and chemicals derived from biomass. For example, citric acid production on an industrial scale by the filamentous ascomycete fungus, *Aspergillus niger* represents one of the most efficient, highest yielding bioprocesses in practice and is a model for future filamentous fungal fermentations that will become a key part of US Department of Energy's (DOE) vision of the biorefinery, where multiple products, such as, organic acids and ethanol, are produced from renewable biomass. The US DOE has funded genome sequencing projects for *Aspergillus niger, Trichoderma reesei* and *Phanerochaete chrysosporium*. As common members of the microbial communities found in soils these fungi play a significant role in the global carbon cycle with a wide array of hydrolytic and oxidative enzymes involved in the breakdown of plant lignocellulose. The Pacific Northwest National Laboratory Fungal Biotechnology Team has made use of the genomic sequence and predicted protein databases to better understand export of various biomass degrading enzymes, molecular mechanisms critical to fermentation process development, and mechanisms involved in the control of fungal morphology. Our current project, funded through the DOE Office of Biomass Programs, is aimed at developing enabling technology around fungal bioprocesses that will be used in the biorefinery using high throughput, global methodologies. We are currently collaborating with the DOE Joint Genome Institute on an *A. niger* genome project, and have used *T. reesei* as a testbed for proteomic method development. The progress of the *A. niger* genome project and current results from our *T. reesei* proteomic studies will be presented.

Ralph A Dean
Center for Integrated Fungal Research, Dept. Plant Pathology, North Carolina State University, USA (radean2@unity.ncsu.edu)

Fungal pathogen genomes: A new perspective on pathogenesis

In a few short years we have emerged from darkness to the promised land. The fungal biology community through emmence cooperation and effort can now begin to reap knowledge from a growing list of fungal genomes, including *Magnaporthe grisea* and several other pathogenic species. However, even with the wealth of new information - access to the raw components of the pathogen's offensive arsenal - how far have we come to understand the molecular basis of disease? I will discuss some of the novel discoveries in *M. grisea* that have only come to light as a result of having access to genome sequences, such as novel classes of secreted proteins, surface receptors and large suites of enzymes involved in secondary metabolism that may play a role in the disease process. I will also highlight some of the limitations of the current state of knowledge, such as inadequate gene predictions, lack of sensible annotation of the majority of predicted genes and little knowledge of gene function. To address the latter I will discuss recent results from functional analyses including transcription profiling and gene knockout experiments. I will close with thoughts on strategies and other resources needed to fully appreciate host-pathogen interactions.

Ben Bower[1], Cherry Lin[1], Nigel Dunn-Coleman[1], Giuseppe Macino[2], Carlo Cogoni[2]
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The use of RNAi to suppress gene function in industrial fungi

RNAi mediated gene silencing has been shown to be a powerful tool in understanding gene function in a number of eukaryotes. With the sequencing of a number of fungal genomes including the industrially important fungi *Aspergillus niger* and *Trichoderma reesei,* we have investigated the potential use of RNAi to study gene function in these fungi. We have demonstrated RNAi in both of these fungi and have used RNAi to manipulate the expression on a number of genes. The results of this work will be presented.

James E Galagan
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Insights into eukaryotic biology through genome comparisons of three Aspergilli

We will describe the results of a comparative analysis of 3 related but highly divergent species of Aspergillus comprising a model organism (*A. nidulans*), a human pathogen (*A. fumigatus*), and an industrial agent (*A. oryzae*). We have quantified the rate of lineage-specific rearrangements, and demonstrate that rates of large and small scale eukaryotic genome evolution are not correlated in these species. Our analysis also sheds new light on sexual reproduction in these Aspergilli. Both *A. fumigatus* and *A. oryzae* are thought to produce only asexually, and the lack of sex has precluded genetics. However, our analysis led to an experimentally validated model of mating-type locus evolution that suggests the potential for sexual reproduction in these two species.

We identified numerous sequences actively conserved across the wide evolutionary distance separating the Aspergilli. Using a computational approach, we have identified several sequences with likely biological significance. These include two novel TPP riboswitches, and a conserved PUF binding motif. Finally, we analyzed conserved regulatory elements called upstream open reading frames (uORFs). We generated genomic and experimental evidence suggesting that uORFs play a larger role in regulating eukaryotic gene expression that has been previously suspected.

Offered Papers

S Muthumeenakshi[1], CW Rogers[1], S Sreenivasaprasad[1], MP Challen[1], JM Whipps[1], JR Green[2]
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Molecular basis of sclerotial mycoparasitism of *Coniothyrium minitans*

Coniothyrium minitans is a well-known natural sclerotial mycoparasite of the important plant pathogen *Sclerotinia sclerotiorum*. Despite the fact that this is a well studied interaction and *C. minitans* is exploited commercially in some parts of the world, very little is understood of the process of sclerotial colonisation at the molecular level. In this work, we are using a range of gene identification and characterisation approaches to unravel the mechanisms of mycoparasitism.

Suppression subtractive hybridisation was carried out between cDNA from *C. minitans* grown in culture and *C. minitans* colonising sclerotia of *S. sclerotiorum*. A subtracted library of 682 clones containing cDNA fragments of putative upregulated genes was established and 192 clones were sequenced. 82 unique sequences were identified and assigned putative functions following bioinformatic analysis. Dot blot and virtual Northern analyses revealed upregulation of nearly 70 genes from *C. minitans* during sclerotial colonisation. Exploiting PCR based methods and a genomic library macroarray, we have cloned pKAC, *pmk*1, *cmg*1 and *chi*1 genes shown to be involved ion signalling and colonisation in parasitic interactions. Insertional mutagenesis carried out in *C. minitans* using REMI and T-DNA tagging approaches resulted in eight pathogenicity mutants. Molecular characterisation and analysis of these pathogenicity mutants enabled the identification of several novel putative pathogenicity genes.

Gene silencing or disruption and complementation technologies are being tested to enable the functional analysis of *C. minitans* genes that have been identified and to investigate their role in sclerotial parasitism.

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Proteomic analysis of *Peronospora viciae*-pea leaf interactions

Peas comprise the largest planted area of field vegetables in the UK and downy mildew, caused by the oomycete pathogen *Peronospora viciae*, is the most common foliar disease of this crop with up to 55% losses in yield observed where plant resistance is ineffective. This presentation reports data from experiments using two-dimensional difference gel electrophoresis and mass spectrometry (MS) to compare the protein profile of the pathogen on the leaf surface to that of the pathogen within the leaf during infection. Proteins were isolated from dormant spores, germinating spores and pea leaves infected by *P. viciae*. Those proteins whose abundance was observed to either increase or decrease at the different developmental stages were selected for identification by MS analysis. Details of methodologies and preliminary results will be presented. The data are interpreted in relation to the proteins integral to infection and signalling mechanisms in this plant-pathogen interaction.

John Thomas[1], Ana Costa[1,2], Andy Bailey[2], Gary Foster[2], Mike Challen [1] & Peter Mills[1]
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Identification and characterisation of *Agaricus bisporus* genes differentially expressed during *Verticillium fungicola* infection

The mycoparasite *Verticillium fungicola* causes 'dry bubble' disease in the mushroom *Agaricus bisporus*. Work is in progress to identify mushroom genes expressed during pathogen infection. An SSH strategy was used to recover ca 100 unique differentially expressed sequences; 26 down-regulated genes, 74 up-regulated genes. Bioinformatic analysis suggests that the response genes identified encompass a range of biological functions; stress, signalling, protein synthesis, cell wall structure and function.

Specific full-length genes are being recovered from a cDNA library constructed from lesions of *A. bisporus* infected with *V. fungicola*, and will enable silencing approaches to further investigate the role of the identified genes in disease. Functional analysis of *A. bisporus* response genes will be determined through disease infection trials with silenced lines. A high-throughput method of functional analysis (RNAi) is being developed using *A. bisporus* model genes. RNAi hairpin constructs were transformed into *A. bisporus* using *Agrobacterium tumefaciens* and selection for hygromycin resistance. Screening transformants by PCR confirmed presence of T-DNA in

transformants; transcription of the hairpin units was confirmed using RT-PCR. Quantitative PCR is being used to analyse target gene transcripts of RNAi transformants.

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Comparative analysis of the genomes of two *Aspergillus fumigatus* clinical isolates and *Neosartorya fischeri*.
The genomes of two *A. fumigatus* clinical isolates (Af293 and CEA10) and the type strain of *N. fischeri* have been sequenced. *Neosartorya fischeri* was selected to aid the annotation effort as it is the most closely related known sexual species to *A. fumigatus*. As might be expected, the two *A. fumigatus* genomes are of a similar size (~29 Mb) with a similar number of genes (~10,000) and they share extensive blocks of synteny between themselves and *N. fischeri*. However, perhaps unexpectedly, there are unique genes in each genome that are not present in the other two genomes. For instance, there are 211 genes in Af293 that are not present in CEA10 and 235 unique genes in CEA10. Analysis of these genes has shown that a large proportion (>40%) code for proteins with no similarity matches to proteins in any other organism and there is a trend for them to be located towards the telomeres. Interestingly, many of these unique genes are clustered together between syntenic blocks and some of these clusters are presumed to be required for the production of secondary metabolites. It will also be of interest to look at those genes that are only present in these three genomes and that are not present in other *Aspergillus* genomes.

Dan Eastwood, Kerry Burton
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Using RNAi to understand the causes of quality-loss in the fruitbody of the mushroom *Agaricus bisporus*
Agaricus bisporus, the white cultivated mushroom, is one of the world's most important industrial fungi with an annual production value of £3bn. Recent advances have also highlighted the potential use of *A. bisporus* in the heterologous production of novel proteins. Breeding and cultivation technologies have been employed to improve mushroom crop characteristics, including colonisation rate, disease resistance and yield. However, control of post-harvest quality and, therefore, shelf-life has proved elusive. Mushroom quality is defined by a uniform colour, texture and maturity, which is lost as harvested mushrooms continue to mature to achieve their function of spore production and release.
Experiments were designed to understand the biology of the harvested mushroom by developing procedures to down-regulate target genes through RNA interference (RNAi). Twenty genes with increased transcription in the harvested mushroom were identified by differential screening. Profiling experiments have identified where and when these genes are expressed and allowed the selection of target genes for down-regulation by RNAi. Gene-specific RNAi cassettes were created and transferred to *A. bisporus* via *Agrobacterium tumefaciens*-mediated transformation. Stable transformants were taken through to crop trails. Results will be presented of the expression levels of the targeted genes and the effects on mushroom post-harvest quality characteristics.

SESSION 2 – BIOACTIVE MOLECULES

Invited Papers

Russell Cox, Thomas Simpson, Colin Lazarus, Andy Bailey, Song Zhongshu, Frank Glod, Kirstin Eley, Deirdre Hurley, Ying Zhang, Kate Harley, Tom Nicholson, Lewis Bingle
Schools of Chemistry and Biological Sciences, University of Bristol, Bristol, UK (r.j.cox@bris.ac.uk)
Biosynthesis of pyridones and tetramic acids in fungi - linking polyketides and non-ribosomal peptides
Filamentous fungi produce a very wide array of secondary metabolites - and individual species contain gene clusters encoding the biosynthetic proteins for the synthesis of dozens of individual compounds. Often the compounds themselves show useful or harmful biological activity. However, it is very difficult to relate specific compounds with specific gene clusters in any given organism - and for organisms where genome sequences are not available this problem is magnified. We have devised a strategy for the rapid cloning of genes associated with the biosynthesis of specific secondary metabolites by using very selective degenerate PCR primers for the production of selective gene probes, combined with the use of cDNA libraries prepared at timepoints corresponding to maximal secondary metabolite production. In this way we have rapidly obtained the genes encoding the core synthases involved in fusarin, squalestatin and tenellin production in *Fusarium venenatum, Phoma* sp. and *Beauveria bassiana* respectively.

Timm Anke
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Fungal metabolites as lead structures for agriculture
When grown in submerged culture in artificial media, about thirty to forty percent of all ascomycetes and basidiomycetes produce metabolites inhibitory to bacteria and/or fungi. In addition, many phytopathogens produce phytotoxic compounds. The structures of these compounds are often very elaborate and differ markedly

depending on their origin from ascomycetes or basidiomycetes. While most of the antimicrobial "secondary metabolites" are thought to have a function for their producers e. g. in securing a habitat or substrate, very few have become useful therapeutics in human medicine. While for medical uses natural products can be produced by fermentation, this is with few exceptions (e.g. spinosad) not feasible in agriculture because of economic restraints. Here, the field is dominated by compounds derived by cost efficient chemical synthesis. The natural compound can serve as lead for the synthesis of simpler and more effective agrochemicals or can provide new insights into potentially useful targets. Up to now the only fungal metabolites having served as lead compounds for the development of successful agricultural fungicides are the strobilurins and oudemansins. These basidiomycete antibiotics are examples for the structural adaptations required to obtain an ecologically safe and effective commercial fungicide.

Barry Scott
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Symbiosis expressed secondary metabolite genes from a mutualistic fungal endophyte confer bioprotection to the symbiotum
Epichloë endophytes are biotrophic fungi that systemically colonise the intercellular spaces of vegetative and reproductive tissues of grasses of the subfamily Pooideae to form mutualistic symbiotic associations (symbiota). The production of secondary metabolites by these endophytic fungi confers a number of bioprotective benefits to the symbiotum. Using Epichloë festucae as our experimental system we have recently cloned genes for the synthesis of two classes of these bioprotective molecules; peramine, a pyrrolopyrazine that is the product of a non-ribosomal peptide synthetase (NRPS), and lolitrem B, an indole diterpene. The gene for peramine synthetase, *perA*, was cloned by PCR and shown to encode a NRPS comprised of two modules. Deletion and complementation analysis confirmed *perA* is a NRPS for peramine biosynthesis. A cluster of genes for the biosynthesis of lolitrems was isolated by a combination of PCR and chromosome walking using primers designed to orthologues and candidate endophyte ESTs. The lolitrem (*ltm*) gene cluster is a complex genetic locus of three mini-clusters interspersed with large blocks of retrotransposon DNA sequences. Deletion and complementation analysis confirmed that two of these genes are required for lolitrem biosynthesis. All 10 *ltm* genes are highly expressed in planta and not detectable in axenic culture.

Geoffrey Turner
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What can genomics tell us about secondary metabolism in Aspergillus?
As a genetic model system, *Aspergillus nidulans* has been studied intensively with respect to penicillin and sterigmatocystin biosynthesis, two secondary metabolic pathways encoded by clustered genes. Such studies have been essential to the understanding of the biochemistry of these pathways, and the regulation of expression of the genes involved. However, the identification, isolation and characterisation of these large gene clusters required considerable effort.
The recent completion of the genome sequences of *A. nidulans* and *A. fumigatus* provides an opportunity to examine the total secondary metabolic capacity of these fungi, and also to gain insights into the evolution of such pathways by comparative genomics. Most secondary metabolic gene clusters can be recognised by the presence of key genes, such as those encoding polyketide synthases (PKS) and non-ribosomal peptide synthetases (NRPS), each of which has characteristic domain structures. While pre-genomic approaches started with known metabolic products followed by strategies to find the gene, we are now faced with a large collection of putative secondary metabolic gene clusters for which we have no known metabolic product.
Some of the current approaches to identifying the gene products of the unknown clusters will be discussed.

SESSION 3 – PROTEIN FOLDING AND SECRETION IN FUNGI

Invited Papers
Reinhard Fischer
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Microtubules, motors and polarized growth in *Aspergillus nidulans*
We identified 11 kinesins in the genome of *A. nidulans* and took three unknown kinesins KinA, KipA and KipB for detailed studies. The conventional kinesin motor, KinA delivers cell wall components and corresponding enzymes enclosed in secretion vesicles to a vesicle supply center, called the Spitzenkorper.
We found that microtubules merge at the center of growing tips and discovered that the kinesin motor KipA, related to Tea2 of *Schizosaccharomyces pombe*, is required for their temporal anchorage. In a ΔkipA hyphae loose directionality and grow in curves. GFP-KipA localizes to the microtubule plus end and jams behind them, suggesting that KipA is a moving motor. Using KipA as a microtubule plus end marker we determined the

location of MTOCs in *A. nidulans* compartments close to nuclei, in the cytoplasm and at septa. For full activity of the MTOCs a novel MTOC associated protein, ApsB, is required.
The third kinesin-like motor protein studied here, is KipB a member of the Kip3 family of microtubule depolymerases. *kip*B mutation caused a delay in anaphase and an increase of bent mitotic spindles, a decrease in depolymerization of cytoplasmic microtubules during mitosis and a failure of spindle positioning in the cell.

Martin Schroeder
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Nitrogen sensing by an intracellular stress signalling pathway
Protein folding is the rate- and yield limiting step for protein expression. Secretory proteins fold into their native conformation in the endoplasmic reticulum (ER). Disruption of protein folding in the ER by expression of mutant or overexpression of wild-type proteins activates a stress response known as the Unfolded Protein Response (UPR). In yeast, the kinase endoribonuclease Ire1p transduces the unfolded protein signal across the ER membrane. Ire1p cleaves both exon intron junctions in the mRNA encoding the basic leucine zipper (bZIP) transcription factor Hac1p. tRNA ligase (Rlg1p) joins the H*AC1* exons. Hac1p, together with the bZIP protein Gcn4p, activates transcription of genes encoding ER resident molecular chaperones which relieve the unfolded protein stress. The UPR also represses nitrogen starvation responses of diploid budding yeast, i.e. pseudohyphal growth and meiosis. In meiosis, Hac1p represses transcription of the early meiotic genes (EMGs). Repression of EMGs by Hac1p required the catalytic activity of the Rpd3p-Sin3p histone deacetylase complex (HDAC) and recruitment of the HDAC to EMG promoters. Severe nitrogen starvation blocked H*AC1* mRNA splicing in exponentially growing cells. I propose that through monitoring the influx of nascent, unfolded polypeptide chains into the ER the UPR monitors environmental nitrogen levels.

Colin J Stirling
Faculty of Life Sciences, University of Manchester
Co-ordination of Molecular Chaperones: many hands make light work!
Hsp70s are a ubiquitous family of molecular chaperones involved in many vital cellular processes including protein folding, quality control, organelle biogenesis and stress response. Central to their chaperone function is their ability to reversibly bind unfolded polypeptide chains. This activity requires an ATP-dependent conformational change that is regulated by specific co-chaperone partners. Eukaryotic cells encode numerous distinct Hsp70s of which several have been shown to functionally overlap. It had been assumed that such overlap reflected simple functional redundancy but our recent data suggests that Hsp70s interact with one another in order to co-ordinate their different activities**.**

David B Archer, Sally E Barnes, Thomas Guillemette
School of Biology, University Park, Nottingham, NG7 2RD, UK (david.archer@nottingham.ac.uk)
Protein secretion and associated stresses in *Aspergillus*
Aspergillus spp. are filamentous fungi that secrete enzymes to support their saprophytic and, particularly in the case of *A. fumigatus,* pathogenic lifestyles. Many *Aspergillus* enzymes are prepared commercially for a wide range of applications. The recent availability of genome sequences for *A. fumigatus*, *A. nidulans* and *A. oryzae,* together with the anticipated genome sequences for *A. niger, A. terreus* and *A. flavus* has provided an unprecedented resource for exploring the molecular basis of the lifestyles of these Aspergilli. In relation to the secretion of proteins, it is now possible to predict the complement of enzymes used by the aspergilli to degrade polymeric material in the environment and, in conjunction with analyses of gene expression, to explore the regulation of expression of those genes in response to particular environmental cues. It is also possible to predict the protein components that are involved in the process of secretion and to compare that with the mammalian and yeast systems that have been described. Many genes will also be exploited commercially to provide new enzymic activities and, in most cases, those selected genes will be over-expressed to provide acceptable yields of enzyme. That approach puts a strain on the secretory system and can lead to induction of the unfolded protein response (UPR). The UPR is also induced when *Aspergillus* spp. are used as cell factories to produce heterologous enzymes. We have used the *A. niger* genome sequence, made available to us by DSM, and Affymetrix gene chips to explore the UPR, endoplasmic reticulum-associated protein degradation (ERAD) and repression under secretion stress (RESS).

Offered Papers

Bleddyn Hughes[1], Carol A Munro[1], Steve Bates[1], Donna M MacCallum[1], Abdelmadjid Atrih[2], Gwyneth Bertram[1], Suzanne Hamilton[1], Michael AJ Ferguson[2], Alistair JP Brown[1], Frank C Odds[1], Neil AR Gow[1]

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Glycosylation of cell wall proteins of *Candida albicans* and its importance for cell wall integrity and virulence.

The cell wall of the fungal pathogen *Candida albicans* is enriched with glycoproteins which are involved in adhesion and virulence. Therefore we are studying the importance of genes encoding mannosyltransferases involved in the glycosylation process in relation to fungal pathogenesis. Protein glycosylation of yeasts is characterised by high levels of mannosylation in both *N*- and *O*-linked glycans. Additionally, N-linked glycans may carry extensive, hypermannosylated outer-chain structures which are unique to fungi. Deletion of the *C. albicans* homologue of *och1*, which in *Saccharomyces cerevisiae* encodes a mannosyltransferase required for addition of the first *a*-1,6 mannose residue of the outer chain, resulted in a highly compromised strain which forms clumps of cells in liquid culture and dimpled colonies on solid media. The *och1*Δ null mutant lacks outer-chain *N*-glycans and is essentially avirulent in a murine model of pathogenesis. *O*-glycans in *Candida* can be between 1 and 5 mannose residues in length, with two enzymes (Mnt1 and Mnt2) responsible for the addition of second, third and fifth mannose residues. A double knockout mutant *mnt*1Δ*mnt*2Δ is able to produce *O*-glycans of up to three mannose residues at low efficiency, indicating the presence of further mannosyltransferases involved in *O*-glycosylation. The double mutant is attenuated in virulence, underlining the importance of *O*-glycosylation in pathogenicity.

M Gabriela Roca[1], Alan E Wheals[2], Nick D Read[1]

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Conidial anastomosis tubes in filamentous fungi

In filamentous fungi a colony can arise from a single spore but it has been long appreciated that conidia and conidial germlings in close vicinity to each other commonly undergo fusion to produce an interconnected network of germlings. These germlings are connected by specialized hyphae called conidial anastomosis tubes (CATs). CATs have been described in 73 species of filamentous fungi covering 21 genera, and develop in nature and in culture. They have been shown to be morphologically and physiologically distinct from germ tubes in Colletotrichum and Neurospora, and under separate genetic control in Neurospora. CATs are short, thin, usually unbranched and arise from conidia or germ tubes. Their formation is conidium density dependent, and CATs grow towards each other. MAP kinase mutants of Neurospora are blocked in CAT induction. Nuclei pass through fused CATs and are potential agents of gene exchange between individuals of the same and different species. CAT fusion may also serve to improve the chances of colony establishment. In this presentation the defining characteristics of CATs are described, recent insights that have been gained into their cell biology are reviewed, possible roles of CATs are suggested, and key questions which need to be addressed about their biology are defined.

Roberto Parra, Dave Aldred, Naresh Magan

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A novel immobilized design for the production of the heterologous protein lysozyme by a genetically engineered *Aspergillus niger* strain

A novel immobilization design for increasing the final concentration of the heterologous protein lysozyme by a genetically engineered fungus *Aspergillus niger* B1 was developed. A central composition design was used to investigate different immobilized polymer types (alginate and pectate), polymer concentration (2 and 4% (w/v)), inoculum support ratios (1:2 and 1:4) and gel-inducing agent concentration ($CaCl_2$, 2 and 3.5% (w/v). Studies of the kinetics of production showed that after 10 days optimum lysozyme was produced. Lysozyme production was significantly affected by polymer type, polymer concentration and inoculum support ratio. Overall, immobilization in Ca-pectate resulted in higher lysozyme production compared to that in Ca-alginate. Similar effects were observed when the polymer concentration was reduced. Regardless of polymer type and concentration, increasing the fungal inoculum level increased lysozyme production. A significantly higher lysozyme yield was achieved with Ca-pectate in comparison to Ca-alginate (approx. 20-23 and 0.5-2 mg l_{-1} respectively). The maximum lysozyme yield achieved was about 23 mg^{-1} by immobilization in Ca-pectate 2% (w/v) with 33% (v/v) mycelium and 3.5% (w/v) gel-inducing agent ($CaCl_2$). Response surface methodology was used to investigate the effect of pH and water activity. The best medium pH was 4.5-5.0, and bead water activity was 0.94 for optimum lysozyme yield, regardless of polymer type.

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Translational regulation in the unfolded protein response of *Saccharomyces cerevisiae*

The unfolded protein response (UPR) is initiated in the lumen of the endoplasmic reticulum of eukaryotes by the accumulation of unfolded proteins. We have used the reducing agent dithiothrietol (DTT) to initiate the UPR in *Saccharomyces cerevisiae* and have then fractionated mRNA into polysomic and non-polysomic fractions. Those mRNA fractions were used to probe Affymetrix gene chips in order to assess the impact that the UPR has on translational control, rather than the well-described transcriptional control mediated by the transcription factor HAC1. We have shown that 26 transcripts exhibited a two-fold or greater shift from the non-polysomic to polysomic fractions upon exposure to DTT. The affected genes include HAC1 as well as the UPR targets ERO1, DER1 and SEC66. These findings were validated by independent means.

Mikko Arvas[1], Jari Rautio[1], Bart Smit[1], Tiina Pakula[1], Karin Lanthaler[2], Geoff Robson[2], Markku Saloheimo[1], Merja Penttilä[1]
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System-wide responses to heterologous protein production in the fungus *Trichoderma reesei*

Trichoderma reesei is an industrial protein production host famous for its exceptional protein secretion capability. Protein production can cause stress to cells by compromised protein folding or transport in the secretory pathway. *T. reesei* is known to respond to secretion stress by unfolded protein response (UPR) and repression through secretion stress (RESS). We have studied the effects of heterologous protein production in bioreactor cultivations on the transcriptome and proteome of *T. reesei*. As an example of secreted and UPR causing protein, a transformant producing human tissue plasminogen activator (tPA), was studied with semi-genomic transcription profiling methods, cDNA subtraction libraries and cDNA-AFLP and 2D proteomics. In addition effect of chemical dithiothreitol (DTT) and a transformant over-expressing IREI protein (UPR pathway sensor protein) were analysed. Data from various secretion stress transcription profiling experiments in *S. cerevisiae* were combined from literature and compared to results from *T. reesei*. The transcriptional responses of *T. reesei* and *S. cerevisiae* show clear overlap, especially with UPR related genes involved in protein translocation, folding and glycosylation in the ER, but also some interesting differences.

As an example of a protein causing no UPR, a transformant producing *Melanocarpus albomyces* laccase was studied with oligonucleotide microarrays. Moderate downregulation of secreted proteins was detected.

HOWARD EGGINS OFFERED PAPERS SESSION

Graham D Wright, Jochen Arlt, Wilson CK Poon, Nick D Read
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Manipulating fungi with light

Optical tweezers were first described in the 1980's and have been implemented in a wide variety research across many disciplines; however, the attention received from fungal biologists has been limited. In an attempt to correct the balance we have evaluated what can be achieved using optical tweezers to micromanipulate filamentous fungi. Utilising the properties of laser light, tweezers permit the non-invasive trapping and movement of cells and organelles. We have built a simple, safe and user-friendly optical tweezer system that can be mounted on a commercial microscope and is, importantly, easy to use for biologists whom lack optics experience. The tweezers can be used synchronously with DIC, phase contrast and fluorescence microscopy techniques to enable enhanced visualization and manipulation of living cells and organelles. The position and orientation of whole cells with respect to one another can be controlled and a variety of organelles can be manipulated within cells. Inert beads can be used as tools for the measurement of forces generated by fungal cells, and for the localized delivery of chemicals to cells. We are presently using the tweezers as powerful experimental tools to address a range of novel mycological questions.

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Screening for anti-rice blast compounds

Rice is Thailand's most economically important crop. However, agricultural yield losses of rice continue to be a problem, particularly those caused by phytopathogenic fungi such as *Magnaporthe grisea.* Currently, the only form of control of the disease is to use synthetic fungicides. This not only triggers resistance of the target pests but also causes chemical accumulation in environments. Therefore, the use of biological control agents is seen as an attractive alternative. Thailand is rich in biological diversity including fungi. At the BIOTEC Culture Collection, over 13,000 strains of fungi have been deposited which could yield many new and useful metabolites. A rapid and easy assay for screening anti-blast agents was established employing a fluorescent dye to indicate viability of spores in a 96-well plate format. The initial results from our preliminary screening of 700 samples showed that 0.7-1 % of the crude extracts possess anti-fungal properties. Two of the potential fungal strains exhibiting growth inhibition activity of blast are *Aschersonia tamurai* and *Trichoderma viride* isolated from infected insects (Hermoptera) and soil, respectively. The soil fungus is of particular interest because it was previously reported to synthesise peptide antibiotics. The active compounds will be further characterised using bioassay guided techniques.

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Identification and characterisation of fungal communities involved in polyester polyurethane degradation

Microbes are known to cause deterioration of polyester polyurethane (PU) within the environment, but little is known of the organisms responsible. To remedy this, PU coupons were buried for five months in two types of soil, one high in carbon/pH 6.9 (soil 1), the other lower in carbon, at pH 5.5 (soil 2). Fungal communities were then recovered from the surface of the buried PU and compared to the native soil fungal communities using culture-based and molecular methods. The percentage of polyurethane degrading fungi recovered from both soils, and from the plastic buried in soil 2 was not significantly different (37-45% of the total population); however, significantly more PU-degrading fungi were recovered from the plastic buried in soil 1 (58%). Hence, growth on plastic enriched for PU degrading fungi under high carbon/neutral pH conditions, whereas no such enrichment was observed under acidic/low carbon conditions. Community species composition was analysed using denaturing gradient gel electrophoresis (DGGE) profiling. Lower diversity in the plastic-bound communities (21 bands in their DGGE profiles compared with ~60 in the soil profiles) and radical differences in species composition (less than 30% of bands were common to both the soil and plastic profiles) indicated that growth on plastic selected for specific, minor members of the native soil communities. A *Fusarium* sp., an *Alternaria* sp. and *Geomyces pannorum* were polyurethane degraders frequently isolated from the plastic surface. In conclusion, growth on plastic was influenced by soil conditions and resulted in reduced fungal diversity compared to native soil communities.

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Competition of heterozygous deletant *Saccharomyces cerevisiae* in a grape juice environment

The existence of a complete set of 6000 single-gene deletant strains of the yeast *Saccharomyces cerevisiae* represents an important tool for qualitative and quantitative analysis of gene function. The presence of unique barcodes and a selective marker in each deletion cassette allows parallel analysis of strains using microarray hybridisation to quantify strain abundance and thus a means of assessing the contribution of individual genes to fitness under a range of environmental conditions. Using a pool of diploid heterozygous deletants, it was possible to identify strains which were either haploinsufficient or haploproficient (i.e. increased fitness when one rather than both gene copies were present) under different fermentation conditions.

'Competition' experiments were conducted, in two fermentor systems, using red and white juices and in the presence and absence of competitor organisms. Microarray data were also validated in growth curve experiments. Changes in fitness identified in grape juice were compared to responses in synthetic nutrient limited media. Whilst fruit juices are generally N-limited, there was incomplete overlap between the strains showing altered fitness in juice vs. N-limited media. Comparison of continuous and semi batch fermentations suggested that growth rate in exponential phase was not the only determinant of fitness.

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Calcium signalling in *Neurospora crassa* in response to mechanical perturbation

In the natural environment filamentous fungi respond to a range of contact stimuli (e.g. physical surfaces, obstacles, `microtopographical features, compression and shaking) which elicit various growth and developmental responses. We are investigating the role of Ca_{2+} signalling in response to mechanical perturbation using *Neurospora crassa* expressing codon-optimized aequorin to measure cytosolic free Ca_{2+} ($[Ca_{2+}]c$). We have found that mechanical perturbation (microinjection of growth medium) of fungal cultures generates reproducible transient increases in $[Ca_{2+}]c$ in vegetative hyphae (in 18 h-old cultures), germ tubes (3-6 h-old cultures) but not ungerminated conidia. The $[Ca_{2+}]c$ response to mechanical perturbation was shown to be dose dependent with respect to the force of mechanical perturbation. Adding the Ca_{2+} chelator BAPTA demonstrated that extracellular Ca_{2+} is required for the $[Ca_{2+}]c$ response. Inhibition of the $[Ca_{2+}]c$ response with Gd_{3+} has suggested the involvement of stretch-activated Ca_{2+} channels. We are presently investigating which Ca_{2+}-channels, -pumps and/or antiporters are involved in generating the $[Ca_{2+}]c$ response to mechanical perturbation. For this purpose, we are conducting a more detailed pharmacological analysis of the $[Ca_{2+}]c$ response, and are measuring $[Ca_{2+}]c$ in different Ca_{2+}-transporter deletion mutants which we are transforming with the aequorin gene.

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An ultrastructural study of the marine oomycete endoparasites

The ultrastructural morphology of two marine oomycete endoparasites (*Olpidiopsis* sp. and *Haliphthoros* sp.) was examined. *Olpidiopsis* is a parasite of the economically important red seaweed, *Porphyra*, and *Halipthoros* is a parasite of marine crustaceans. Both of these species appear to be located near the base of the molecular phylogenetic tree, branching before both the main Saprolegnian and Peronosporalean clades. The zoospore of *Olpidiopsis* sp. possessed electron-dense body near the flagellar base similar to the structure reported as K-body in *Olpidiopsis saprolegniae*. The "perinuclear mitochondria" were found in the immature zoosporangium of *Olpidiopsis* sp. This feature has reported in another marine parasite of red algae, *Petersenia palmaria*. These results suggest that *Olpidiopsis* sp. possibly be an intermediate organism between *O. saprolegniae* and *P. palmariae*. Furthermore, although the flagellar transitional region has known as the comparatively conservative structure in the higher taxonomical groups, two types have been reported in the class Oomycetes. The double helix has been reported at the flagellar transitional region in the major lineages (Saprolegniales and Peronosporales), whereas in *O. saprolegniae* only a single helix is reported. In this study, the double helix was found in the zoospore of *Haliphthoros*. These morphological features may provide valuable clues as to the likely evolutionary development and phylogenetic origins of the economically important oomycete fungi. It also suggests that there may be more diversity amongst these simple holocarpic endoparasites than is apparent from their overlala mrophology.

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Chromium toxicity in *Saccharomyces cerevisiae* arises from oxidative damage to proteins

Oxidative damage in fungi and other organisms occurs during exposure to the toxic metal chromium, but it is not certain whether such oxidation accounts for the toxicity of Cr. Here, a *sod1Δ* mutant of *S. cerevisiae* (defective for the Cu,Zn-superoxide dismutase) was hyper-sensitive to Cr(VI) toxicity under aerobic conditions, but this phenotype was suppressed under anaerobic conditions. Cells expressing a Sod1p variant (Sod1H46C) showed that the superoxide dismutase activity rather than the metal-binding function of Sod1p was required for Cr-resistance. To help identify the macromolecular target(s) of Cr-dependent oxidative damage, cells deficient for reduction of phospholipid hydroperoxides (*gpx3Δ*, and *gpx1Δgpx2Δgpx3Δ*) and for the repair of DNA oxidation (*ogg1Δ*, and *rad30Δogg1Δ*) were tested, but were found not to be Cr-sensitive. In contrast, *msraΔ* and *msrbΔ* mutants defective for peptide methionine sulfoxide reductase (MSR) activity were Cr-sensitive, and cells overexpressing these enzymes were Cr-resistant. Consistent with the inference that protein oxidation causes Cr toxicity, a ~20-fold increase in the cellular levels of protein carbonyls occurred during Cr exposure. Glycolytic enzymes and heat shock proteins were specifically targeted by Cr-dependent oxidative damage. This study establishes an oxidative mode of Cr toxicity in *S. cerevisiae*, which primarily involves oxidative damage to cellular proteins.

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Endocytosis: A filamentous fungal perspective

Endocytosis has been well characterized in budding yeast and animal cells, and to a lesser extent in plant cells. In contrast, much less is known about this process in filamentous fungi although it is likely to have important roles in membrane recycling, membrane degradation, and the uptake of signal molecules. This presentation will provide evidence from studies on *Neurospora crassa*, which support the occurrence of endocytosis in filamentous fungi.

To gain more insight into the endocytic process in *Neurospora* GFP fusions have been made of some endocytic proteins. They show a similar localization as their budding yeast homologues. For the clathrin light chain this means that it is most likely, as is the case in budding yeast, not involved in the early stages of endocytosis. This in contrast to its function and localization in mammalian cells. The Las17/WASP homologue in *Neurospora* shows a similar localization to motile pathches on the plasma membrane as it does in yeast.

To prove function complementation experiments in budding yeast have been performed to assess whether the phenotype can be rescued with the *Neurospora* homologue.

To prove endocytic internalization a *Neurospora* Maltose permease homologue was labelled with GFP, and further live-cell imaging studies of this and other proteins will be performed and presented on this paperr.

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Secondary metabolites of marine fungi

Terrestrial micro-organisms have traditionally been a valuable source of natural products for the development of pharmaceuticals and agrochemicals. However to avoid redundant discoveries, natural product chemists and biologists have turned to previously unexplored habitats, e.g. the marine environment, as a source of novel metabolites. Since fungi are likely to produce such metabolites in an environment where the metabolites play a role in interactions and do not disperse in the environment, fungi associated with other organisms should be a good source of novel metabolites. Additionally, novel metabolites are to be expected from fungi that have not been adequately studied, e.g. obligate marine fungi. However, to date most new compounds have been from marine-derived fungi that may be incidental to the habitat. The taxa of the fungi we isolated from sponges suggest that these were of terrestrial origin, whereas physiological results on fungi isolated from algae suggest adaptation to the marine habitat. Molecular methods have shown that the fungi recovered from marine algae using traditional methods of culture are not necessarily those primarily associated with the host. Consequently, conditions of isolation and culture should be optimized to obtain those strains that presumably represent obligate marine fungi and a source of novel metabolites.

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Pharming proteins in the mushroom *Agaricus bisporus*

Biologics are therapeutic proteins with a huge global market ($28bn in 2002 forecast to rise to $70bm in 2006). Current production, using fermentation technology, has high production costs. Alternative production systems are under active examination, largely based on crops but with the environmental risks associated with field crops, contained production systems are now viewed as essential, i.e. glasshouse based or mushrooms. Mushroom (*Agaricus bisporus*) culture offers a number of advantages for pharmaceutical protein production: high biomass yields (3,200 tonnes/ha/yr), containment, low costs, modular and scaleable production and rapid vegetative scale-up from transgenic cultures to crops (no seed bulking-up required). However, transformation technology for *Agaricus* is recent and not all the required molecular technologies are in place. A number of foreign genes have been expressed in mushrooms including alpha-galactosidase, human growth hormone, cyanovirin-N and endostatin, but at low yields. The model protein, Green Fluorescent Protein (GFP), has been produced at 2.4 µg/g f.wt, and improved protein yields are being investigated by exploitation of regulatory sequences from the mushroom (e.g. fruitbody-specific gene promoters and introns). Pharming constructs have been transformed into *A. bisporus* using *Agrobacterium*, and levels of GFP quantified in mycelium and mushroom fruitbodies.

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Nutrition – a major factor that influences the virulence of the entomogenous fungus *Metarhizium anisopliae*

Nutrition influenced virulence of the insect pathogenic fungus, *Metarhizium anisopliae*. Virulent conidia were produced on nutrient poor or osmotic stress media while less virulent conidia were produced on nutrient rich media. Several strain independent parameters were identified that could be used to monitor the virulence of *M. anisopliae*. Virulent conidia typically had high levels of spore bound Pr1, CN ratio below 5.2:1 and high

germination rates. Real Time-PCR revealed that virulent conidia from insects contained high levels of transcripts of *pr1*A and other pathogenicity-related genes. Virulent conidia from 1% yeast extract had higher levels of transcripts of these pathogenicity-related genes than the least virulent conidia from CN 35:1 medium (= SDA), however, the levels were significantly lower than those from insect-derived ("passaged") conidia.

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Cultivation independent tools for detection, identification, and monitoring of entomopathogenic fungi in the soil
Specific and sensitive detection tools are important when investigating ecological aspects of entomopathogenic fungi in the soil. In addition, such tools are crucial for identification and efficient monitoring of fungal biocontrol agents (BCA). Traditionally, techniques depend on cultivation of the organism and subsequent morphological, biochemical or genetic identification. Our goal is to develop specific and efficient tools that circumvent time consuming cultivation by applying PCR based techniques on bulk soil DNA extracts.
We have used two experimental systems to establish such tools. In the first system we have used species specific primers targeting ITS rDNA regions to investigate the so far unresolved overwintering strategy of *Pandora neoaphidis* (Zygomycota), a fungus with potential use in aphid biocontrol. The specific PCR technique allowed for detection of the fungus in soil samples obtained from natural habitats in fall and spring, suggesting an overwintering stage in soil.
In the second system we employ six microsatellite markers to identify and monitor *Beauveria brongniartii* (Mitosporic fungi) BCA strains commercially applied to control the soil dwelling larvae of the Maybeetle (*Melolontha melolontha*). Our results show that this technique allows monitoring of applied strains as well as indigenous populations of *B. brongniartii* in the same assay.

THE BERKELEY LECTURE

Simon Avery
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Stress and the single cell
Individual cells within clonal populations of unicellular fungi exhibit marked variabilities in their phenotypes. Such heterogeneity is typified by differing sensitivities to stress among isogenic cells. It is believed that this 'phenotypic heterogeneity' may have fitness advantages for cell populations during environmental perturbation, by increasing the survival chances of at least some cells to allow persistence of the organism. Heterogeneity may be driven by ordered genetic regulation or by stochastic processes. This presentation will outline our current understanding of the underlying bases of heterogeneity, together with its potential evolvability and implications for fungal populations, with emphasis on studies using the yeast *Saccharomyces cerevisiae.*

SESSION 4 – FUNGAL BIOREMEDIATION

Invited Papers
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Bioluminescence-based fungal biosensors
Biological assays are becoming more widely adopted in bioremediation projects. These assays have an advantage over conventional chemical analysis as they are able to determine the bioavailable fraction of pollutants. Bioluminescence-based biosensors are well suited to toxicity testing because light output is linked to metabolic activity and thus changes correspond to pollutant doses. Bioluminescent bacterial biosensors are widely used to assess the potential toxicity of pollutants, but the need for eukaryotic sensors, as well as the ecological importance of fungi, has resulted in the development of fungal biosensors. A bioluminescence-based yeast biosensor, *luc*-marked *Saccharomyces cerevisiae*, was developed and used for toxicity testing. The potential of naturally bioluminescent fungi as biosensors was explored using *Armillaria mellea, Mycena citricolor* and *Omphalotus olearius*. They grew as bioluminescent, globular mycelia in submerged cultures and the results suggested that fungal bioluminescence is linked to metabolic activity. *A. mellea* and *M. citricolor* were used to develop a novel, bioluminescence-based, filamentous fungal bioassay for toxicity testing. Bioluminescence showed characteristic dose-responses to pollutants, such as copper, in both standard solutions and environmental samples. The bioluminescent fungi can be used as biosensors in a way that extends and complements the bioluminescent bacterial biosensors available for ecotoxicity testing.

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White-rot fungi and xenobiotics

Land farming, biopiling and windrowing techniques are increasingly being used for the treatment of former industrial land contaminated with hydrocarbon compounds including petroleum and oil spillages. However a number of practical problems remain:

- A slow rate of mass transfer of the hydrophobic pollutants from the soil particles to the liquid phase to make contact with metabolically active organisms.
- A requirement for improved oxygen mass transfer - the enzymes involved in PAH metabolism have an absolute requirement for molecular oxygen.

Laboratory studies have shown that white rot fungi will degrade recalcitrant organo-pollutants more extensively than any other microbial group, using an oxidative enzyme system involving ligninolytic enzymes and redox mediators. Their oxidative attack could shift the binding equilibrium of xenobiotics with soil in the direction of release to the liquid phase, or polymerise them so that they are no longer bioavailable. However soil is not the natural habitat of white-rot fungi, and fungal growth and the ability to express the extracellular oxidative enzyme system are not synonymous, and the presence of readily available carbohydrates can be inhibitory to optimal expression of the ligninolytic system. This paper will review strategies for accelerating the rate and extent of bioremediation of hydrocarbon contaminated land using white-rot fungi.

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Fungal genomics and bioremediation

Genomics allows us to identity new gene products by the proteins they encode (proteomics) and by the enzymatic reactions they control (metabolomics). To date, the power of bioinformatics for deciphering of whole genomes has focused more on agricultural, industrial and medical genes than on those with environmental importance. Nevertheless, using homology searches, putative genes for novel cellulases, hemicelluloses, lipases, oxygenases, peroxidases, proteases and other enzymes with potential for use in solving environmental problems are readily found. To date, genome projects for *Phanerochaete chrysosporium* and *Trichoderma reesei* have focused on their application in biomass degradation; data mining will also provide new treasures for scientists interested in harnessing the unique capacity of fungi to secrete organic acids and degradative enzymes that can be exploited in bioremediation. Examples will be provided from the genomes of *Aspergillus fumigatus, A. nidulans, A. oryzae, P. chrysosporium, T. reesei* and other selected filamentous species that have been the focus of genome research. The revolution in fungal genomics provides an opportunity for both discovering new enzymes and for studying degradative processes at a new level of complexity.

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Metal and mineral transformations

Fungi play a crucial role in biogeochemical processes, and these are also relevant to bioremediation of metal-contaminated habitats and wastes. Main biochemical mechanisms of mineral dissolution are acidolysis, complexolysis, and redoxolysis. Solubilization of toxic metal minerals by mycorrhizal fungi was related to their metal tolerance, with the majority of species dissolving toxic metal minerals through acidification. When oxalic acid was excreted, however, dissolution shifted to complexolysis and subsequently resulted in the precipitation of metal oxalates. Fungi can also immobilize soluble metal species through sorption, accumulation, and precipitation. Fungi are efficient metal bioaccumulators with carboxylate and phosphate being the main ligands coordinating metals within the biomass. Fungi can also form a variety of secondary minerals (both amorphous and crystalline). Fungal ability to dissolve minerals increases soil nutrient availability (e.g. phosphate). However fungal dissolution of toxic metal phosphates may enhance toxic metal availability in the soil and should be considered in assessment of chemical remediation approaches. Fungal metal and mineral transformations are not only of potential for the development of bioremediation strategies for metal-contaminated soils and wastes, but also to leaching, biosorption and metal recovery/recycling approaches, as well as site revegetation and regeneration.

Offered Papers

Mariangela Girlanda, Rossana Segreto, Sergio E Favero-Longo, Consolata Siniscalco, Paola Bonfante, Silvia Perotto

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Rhizodegradation of organic pollutants: exploiting the interactions between roots and saprotrophic microfungi

In situ bioremediation of organic pollutant-contaminated soils based on the degradative capacities of saprotrophic fungi may be limited by reduced maintenance and activity of the fungal inoculum under natural conditions. Exploiting plant-microfungal interactions in the rhizosphere may overcome such a limitation and thus provide a tool to tackle recalcitrant organic chemicals in the environment. We have analysed microfungal community structure in an aged industrial soil contaminated by a mixture of pollutants, and we have characterized the ability of selected isolates to degrade nonylphenol, a widespread, recalcitrant endocrine-disrupting compound, with estrogenic effects on fish and the ability to stimulate the growth of human breast cancer cells. In vitro and greenhouse experiments (sterile sand substrate amended with 2000 ppm nonylphenol and nonsterile soil from the industrial estate) were carried out using a consortium of six microfungal species, tested alone or in association with *Populus nigra* or *Pisum sativum*. The fungal inoculum was able to grow in the presence of the pollutant, with significantly higher growth stimulation and nonylphenol degradation (50-85%) when in association with poplar plants. These findings indicate the potential of rhizosphere activities for bioremediation of nonylphenol-contaminated soils, although the nature and effects of the degradation products need to be addressed.

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The production pattern of carbohydrases during the decomposition of *Magnolia lilifera* leaves

Endophytic fungi live in healthy plants and can adapt to a change in the mode of life to be saprobes when leaves decay. In a study of endophytes and the succession of saprobes on senescent leaves of *Magnolia liliifera*, we found some species of endophytes do behave as saprobes. To verify the pattern of degrading enzyme production from dominant saprobes during the leaf decay, enzyme activity was assayed. Fresh senescent leaves of *M. liliifera* were treated as the test group and divided into groups, and incubated in trays covered with gauze. The trays were placed outdoors and sprayed with sterile water every day. The leaves were collected over a period of 88 days and assays for cellulase, xylanase and mannanase were carried out using sterilised leaves as the control. A succession in enzyme production starting with xylanase, followed by mannanase and finally cellulose was observed. We concluded that some endophytes within leaves of *M. liliifera* can become saprobes and produce degrading enzymes during leaf decay.

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Colonization and invasion of porous matrices by filamentous fungi

The activities of fungi in the transformation and cycling of both organic and inorganic compounds are of profound global significance. However their activity in rock and mineral environments, and on related derived matrices such as concrete and mortar, is only just beginning to be realized. Fungi are capable of proliferating in these low nutrient situations and, once established, can penetrate the pore system of the substrate. During growth in the subsurface their excretion of potentially corrosive metabolites, such as protons and organic acids, is thought to play a role in the biodeterioration of the material. Our current research is investigating the colonization and invasion of the concrete surface and subsurface. In particular, we are studying the composition of the subsurface fungal community and attempting to model fungal growth in response to topographical features using micro-engineered structures. By combining light and confocal microscopy with statistical analyses we have investigated the contour following (thigmotropic) responses of fungi to different chemical and physical environments. We have discovered significant spatial variations in thigmotropic responses even within single fungal colonies which may help our understanding of the invasion of the concrete pore network.

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Ligninolytic cork colonising fungi consortium and its ability to degrade PCP

Pentacloroanisol (PCP) was considered a priority toxic substance by the EC commission and the FDA. It can lead to unpredictable physiologic responses, causing abnormal chromosomal aberrations and carcinoma formation in humans, yet, mostly because of its use in pesticides and wood preservatives is dispersed in all ecosystems. PCP natural attenuation through microbial degradation involves some classes of depolymerising enzymes which are also involved in lignin degradation. The high surface-to-cell ratio of filamentous fungi, together with the natural overlap between fungi ability to biodegradability and bioremediation makes fungi better degraders under certain niches, like contaminated soils. Ligninolytic fungi species among the cork colonising fungi consortium are particularly adapted to degrade cork main constituents – lignin/suberin, tolerate high concentrations of the toxic and may produce an a common PCP fungal derivative, poly-chlorinated-anisol (PCA) . Their ability to degrade the structure of cork cell walls was followed by microscopy and the preferentially degraded constituents were suggested through the analysis of the colonised tissues mechanic performance. The stimulation effect of key environmental parameters on the fungi consortium PCP degradation rate is being screened.

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Differential breakdown of mixtures of pesticides by *Trametes versicolor* and *Phanerochaete chrysosporium* by production of hydrolytic enzymes under different soil water potentials in soil microcosms

Microcosm studies of soil modified to 0.7 and 2.8 MPa water potential contaminated with a mixture of pesticides (simazine, trifluralin and dieldrin, 10 mg kg-1 soil) were inoculated with *T.versicolor* and *P.chrysosporium* grown on wood chips and temporal studies used to examine degradation rates at 15^{o}C for up to 12 weeks. The two test isolates successfully grew and produced extracellular enzymes in soil at both water potentials. Respiratory activity was enhanced in soil inoculated with the test isolates, and was generally higher in the presence of the pesticide mixture, which suggested increased mineralization. Cellulase and dehydrogenase was also higher in inoculated soil than in the control especially after 84 days incubation. Laccase was produced at very high levels, only when *T.versicolor* was present. Degradation of the three pesticides by *T.versicolor*, after 6 weeks, was enhanced by 46, 57 and 51% respectively for simazine, trifluralin and dieldrin over that in natural soil. *P.chrysosporium* also significantly enhanced degradation rates over controls, especially in the wetter treatment. There was a correlation between some enzyme production in soil, degradation rates and other indicator criteria. This study shows that even under quite dry conditions (twice the wilting point of plants) bioremediation and enzyme activity can occur effectively.

SESSION 5 – FUNGAL BIOCONTROL OF PESTS

Invited Papers

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Fungal control of subterranean pests

Traditional crops are under increasing threat from a number of subterranean pests that have proven particularly difficult to control. In Europe these include the larvae of Scarabs (e.g. *Melolontha melolontha, Amphimallon solstitialis, A. majale, Phyllopertha horticola, Hoplia philanthus),* a number of different larvae of Elateridae (e.g. *Agriotes lineatus, A. obscurus, Limonius* spp.) and Curculinidae (e.g. *Bothynoderes punctiventris, Otiorhynchus sulcatus), Daktulosphaira vitifoliae* (Phylloxeridae) and the new exotic pest *Diabrotica virgifera* (Chrysomelidae). It is not surprising that these pests are difficult to control because a major problem is their hidden niche and the inherent difficulties of penetrating their habitat with appropriate control agents. In most of the affected agricultural systems in Europe use of chemical insecticides is undesirable or impossible.

Currently the application of virulent, ecologically competent strains of insect-pathogenic fungi appears the best option. Fungal pathogens are endemic in pest populations and fulfil the key criteria of BCAs by effectiveness, autodissemination and their excellent persistence. This paper provides examples of the successful use of the entomopathogenic Hyphomycetes *Beauveria* and *Metarhizium* in subterranean pest control in European agriculture, forestry and horticulture. Preventive control approaches based on the subterranean pests listed above will be discussed.

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Development and use of mycoherbicides (using *Stagonospora* as a model)

Several weeds such as the bindweeds *Convolvulus arvensis* and *Calystegia sepium*, which are considered among the twelve economically most important weeds, are difficult to control with conventional physical and chemical methods. Biological control with fungal biocontrol agents (FBCA) offers a promising alternative. However, the employment of such FBCA's implies not only the identification of highly effective strains and the development of cheap formulation and production techniques, but also an extensive risk assessment including tracking of FBCA's and their toxic metabolites in the environment. *Stagonospora convolvuli* strain LA39 effectively controls field- and hedge bindweed in the field. For this strain formulation and application techniques were developed and currently markers for environmental tracking are designed. A major metabolite produced by LA39 is elsinochrome A (EA). To assess the risk of EA production by LA39, different methods, such as toxicity tests, stability tests and methods for environmental monitoring were developed. Our results revealed that EA is toxic to a wide array of organisms. However, EA could never be detected in LA39-treated bindweed or crop plants, and the EA content in the applied biocontrol product is far too low to result in a toxic environmental concentration. Furthermore EA is quickly degraded by sunlight. Taken together we conclude that LA39 is a promising candidate for the development of a successful biocontrol product against bindweed and that harmful side effects of EA production can be excluded.

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Trichoderma-fungal interactions

Trichoderma-based biofungicides are a reality in agriculture, with more than 50 formulations today available as registered products worldwide. Several strategies have been applied to identify the main genes and compounds involved in this complex cross-talk between the fungal antagonist and the microbial pathogen, as mediated by the plant. Proteome and genome analysis have greatly enhanced our ability to conduct holistic and genome-based functional studies. We have identified and determined the role of a variety of novel genes and gene-products, including ABC transporters, enzymes and other proteins that produce or act as novel elicitors of induced systemic resistance, proteins recognized by the plant as avirulence factors, as well as molecules that generally activate the antagonistic activity in *Trichoderma* spp. We have cloned mycoparasitism-related promoters and used them in combination with GFP and other markers to study the interaction *in vivo* and *in situ* between *Trichoderma* and the fungal pathogen or the plant. Finally, we have transgenically improved the ability of the antagonist to kill other microbes and to activate plant defence mechanisms.

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Fungal Parasites of Invertebrates: Multimodal Biocontrol Agents?

Nematophagous and entomopathogenic fungi (NEF) infect hosts (nematodes and insects) with similar structures (i.e. egg-shell and cuticle) or life strategies. NEF belong to a wide range of fungal taxa and are mostly facultative parasites. To date, research has covered their mode of action on their "cannonical" hosts (i.e. nematodes for nematophagous fungi). Their infection cycles share common strategies (i.e. adhesion to the host) or metabolites (i.e. proteases and chitinases for host penetration). Some species (i.e. *Lecanicillium lecanii*) can even be isolated from both infected nematodes and insects. Life histories and experiments indicate that many NEF live saprophytically and can infect hosts other that their own. Therefore, NEF display a multimodal behaviour that can widen their usage as biocontrol agents (broader host range). Recent studies on the endophytic behaviour of NEF point to new biocontrol activities (modulation of plant defences), or even new (more targeted) means of delivery in agroecosystems which may increase effectivity of the inoculum in adverse environments such as the rhizosphrere. This multimodal behaviour may also shed light on the very nature of biocontrol and may help to understand why a soil can be suppressive to –for instance- plant-parasitic nematodes.

Poster Abstracts

COMPARATIVE AND FUNCTIONAL FUNGAL GENOMICS

1.1

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Molecular analysis of mating types in the genus *Agaricus*

The genus *Agaricus* exhibits a diverse range of breeding life styles; homothallic, heterothallic and secondary homothallic. We are characterising mating-type genes from different *A. bisporus* varieties and other *Agaricus* species to study the role and function of these genes in the evolution of different breeding behaviours. In the 2 – spored, secondarily homothallic, cultivated *A. bisporus* var. *bisporus*, a unifactorial mating-type locus determines compatibility. Haploid nuclei of opposite mating-type are most often partitioned into two spores to yield self-fertile heterokaryons. In heterothallic, 4 – spored varieties, meiotic nuclei are individually compartmentalised and non-self-fertile homokaryons must mate to generate fertile heterokaryons. Other *Agaricus* species are homothallic (eg. *A. subfloccosus*) where all progeny are self-fertile, and appear genetically homogeneous.

We have cloned a pair of divergently transcribed homeobox genes from the *A. bisporus* mating-type locus. Allelic variants of different A specificities were characterised to determine whether a single pair of genes can generate the 14 different mating types identified in wild collections. Current work aims to characterise homeobox mating genes from *A. bitorquis* (heterothallic) and *A. subfloccosus* (homothallic), and to search for pheromone and receptor genes in the genus**.**

1.2

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Sexuality and asexuality in *Aspergillus* species

The genus *Aspergillus* comprises species that reproduce by asexual and/or sexual means, and includes species with both homothallic (sexually self fertile) and heterothallic (obligate outbreeding) breeding systems. Thus they provide good model systems to study the evolution of sexual breeding systems and the molecular genetic basis of asexuality. In addition, the aspergilli include species of importance in the biotechnology, food production and medical sectors. Thus there are possible applied benefits to understanding the nature of reproduction in these species. We are investigating reproduction in the supposed 'asexual' species *A. fumigatus* (an opportunistic pathogen) and *A. oryzae* (used in the Asian food and biotechnology industries). A series of complementary genomic and experimental approaches have been used to investigate possible reasons for asexuality in these species. Genome analysis of both *A. fumigatus* and *A. oryzae* revealed the presence of a set of genes known to be involved with sexual reproduction in heterothallic filamentous ascomycetes. Isolates used in the genome sequence projects contained either a MAT-2 high-mobility group gene or a MAT-1 alpha-domain gene in *A. fumigatus* and *A. oryzae* respectively. Further experimental work identified the presence of sexually compatible MAT-1 and MAT-2 isolates of *A. fumigatus* and *A. oryzae* in a preliminary screen of isolates. Evidence for recombination was also gained in population genetic studies of *A. fumigatus*. Finally, expression of certain key genes involved with sexual reproduction (mating-type, pheromone precursor and pheromone receptor genes) was demonstrated for both species. Taken as a whole, these data suggest that both *A. fumigatus* and *A. oryzae* have a recent evolutionary history of sexuality, and might retain the capacity to undergo sexual reproduction.

1.3

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Metabolic and genetic diversity of marine *Dendryphiella* species from different geographical locations

Two species of *Dendryphiella* are known to be of marine origin, *D. arenaria* and *D. salina*. These species are usually identified based on differences in their spore morphology and colony appearance. We were interested in determining to what extent the species can be differentiated on the basis of their metabolic and molecular profiles, but also to see if climatic and/or geographical zone of origin influence these parameters. The *Dendryphiella* strains were isolated from subtropical (Gulf of Mexico) and temperate (North Sea, Baltic Sea, Mediterranean Sea, English Channel) waters. BIOLOG Phenotype microarrays were used to assess their ability to utilize different substrates. Genomic DNA was extracted from representative strains and used for RAPD analysis, and for sequencing of ITS1 and 2 and partial tef1 gene in order to infer phylogenetic differences. The *Dendryphiella* strains from various climatic zones differed in their subtrate utilization profiles, which could be a result of adaptation to their respective habitats. Although RAPD analysis showed differences between the isolates, the similarity of their ITS1 and 2 and tef1 gene sequences indicates that probably our isolates belong to a single phylogenetic species.

1.4

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Identifcation of genes involved in *Aspergillus nidulans* hyphal development

Filamentous fungi, such as *Aspergillus niger* and *Aspergillus oryzae* have been used secrete large amounts of native proteins for many industries. More recently the production of heterologous proteins has been attempted with limited success, as production yields obtained were often low. The link between the hyphae and protein secretion, primarily from the hyphal tip has been noted and several studies to improve protein secretion capacities through the production of hyperbranching mutants have been carried out.

A limited number of *Aspergillus* mutants are known to affect hyphal morphology, growth and polarity, including *cotA*, and *hbr* mutants. With the availability of genome sequences for the 3 species of Aspergillus and microarray technology, the identification of genes involved in morphological development can be established by transcriptome analysis of hyperbranching mutants and using bioinformatics to identify *Aspergillus* homologues of known genes.

The temperature sensitive *A. nidulans* mutant *hbrB3* exhibits hyperseptation and shows a marked increase in hyphal branching at the restrictive temperature 40oC. RNA was isolated from the mutant strain *hbrB3* and a wild type strain, R153, grown under continuous culture conditions at both 30^{o}C and 40^{o}C, and reverse transcribed with *cy3* and *cy5*. Analysis of microarrays probed with fluorescently labelled cDNA from these strains will be used to identify additional genes and signalling pathways involved in the morphology of hyperbranching.

1.5

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Using web-robots to make a comprehensive comparative search for developmental gene sequences in the genomes of animals, plants and fungi.

The major kingdoms of eukaryotes probably separated from one another at a stage prior to the multicellular grade of organisation. So there is no logical reason to expect that these Kingdoms will share features that contribute to multicellular developmental biology. There are now sufficient filamentous fungal genomes in the public sequence databases to make direct sequence comparisons with animal and plant genomes a worthwhile exercise, although a comparative manual search of genomic resources would be extremely arduous. However, database querying is a good candidate for programming. Here we report a fully comprehensive data-mining exercise using specially-programmed Internet web-robots (that automate the tasks associated with the usual sequence comparisons), which has revealed the following homologies:

Kingdom	**Sequence homologies**
Fungi only	4
Fungi and plant	58
Fungi and animal	73
Animal and plant	34
Plant only	68
Animal only	231
All three kingdoms	85
Total	553

The poster will discuss the nature of the sequences in the categories shown in the Table. The observations contribute to the idea that general features of eukaryotes (like signalling and export pathways, uptake, transport, nuclear behaviour, etc.) are common to animals, plants and fungi, but the overall "management" processes on which development depends are distinct.

1.6

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Genetic control of hyphal morphology in *A. nidulans*

The molecular biology behind the development of *A. nidulans* hyphae has been studied for many years. The physiology of mycelia can be altered by varying the growth medium, however it has been shown that there is very strong genetic control of the process of mycelial development. BemA, the *A. nidulans* homologue of the *S. cerevisiae* protein Bem1p was identified through genome sequence comparison and we have shown that it plays a similar role to in yeast, where it acts as a scaffold protein involved in cell polarity establishment. The HbrB protein was identified in a screen for temperature sensitive mutants. It has no known domains and is only present

in some closely related fungal species. When grown at a restrictive temperature cells do not show much apical extension, but produce many lateral branches and exhibit hyperseptation. An *alcA-hbrB* strain was created previously and under promoter repressing conditions growth is stunted and the cells are very swollen. Recombinant PCR has been used to generate linear fragments for promoter replacement and fluorescent protein tagging of these proteins. These new strains will facilitate further characterization of the two proteins and indicate their role in the control of hyphal development in *A. nidulans*.

1.7

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Evidence of RIP (repeat-induced point mutation) in transposase sequences of *Aspergillus oryzae*

A DNA methyl-binding column was used to isolate genomic fragments enriched for DNA-methylation from *Aspergillus parasiticus*. One of the isolated sequences presented 67% identity at the protein level with the transposase from the transposable element Tan1 of *Aspergillus niger* var. awamori, and was found to be present in at least 20 copies in the *Aspergillus oryzae* database. Analysis of four copies showed evidence of C:G to T:A transitions in at least 98.2% of the mutations found over a 1032-1180 bp region spanning a large part of the transposase sequence. Using copy specific primers three sequences were amplified from a different strain of *A. oryzae* and a similar pattern of C:G to T:A transitions was found. These transitions are indicative of a lighter version of RIP, discovered in *Neurospora crassa*, where cytosine-methylation is believed to be involved. Using methylation-sensitive Southern blotting, no evidence of methylation was found in the transposase sequences in these two *A. oryzae* strains as well as one *A. parasiticus* and one *Aspergillus flavus* strain.

1.8

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Hybridisation array technology coupled with chemostat culture: gene expression studies in *Aspergillus nidulans*

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*.

1.9

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The role of genetic resource centres in the exploitation of fungi

The successful exploitation of any fungus, be it for research, educational or commercial utilisation, will at some stage, require the involvement of a Genetic Resources Centre [GRC]. GRC's provide an array of services including: preservation, maintenance and supply of organisms, facilities for confidential safe and patent deposit and taxonomic verification. Many GRC's operate to strict quality management standards.

For a culture that exhibits novel properties, for example, the production of bioactive molecules, it is imperative that the integrity of the fungus is maintained during preservation and storage. GRC's employ state-of-the-art preservation techniques to ensure that organisms are maintained in optimal condition. Cryopreservation and lyophilisation are commonly used, but novel methodologies such as alginate encapsulation can be developed for preservation recalcitrant fungi. A series of post-preservation biochemical and genomic tests can be applied to assess the success of preservation. A review of the current status of GRC's and how they support the exploitation of fungi through the application of preservation technologies and quality management systems will be discussed.

Reference: Ryan, M.J. & Smith, D. (2004). Fungal Genetic Resource Centres and the Genomic Challenge. Mycological Research 108: 1351-1362.

BIOACTIVE MOLECULES

2.1

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The occurrence and distribution of endophytic fungi isolated from marine and shoreline plants including an account of agroactive metabolite production

The occurrence and distribution of endophytic fungi of salt marsh and shoreline plants in Southern England was recorded in respect of: plant organs, plant species and habitat. Using high throughput screening technology methanol extracts of the mycelia of axenic cultures of endophytes grown on a variety of media were assessed for fungicidal, insecticidal and herbicidal activity. The results of screening 524 isolates showed a high degree of confirmed activity (~60%) with 133 (25%) expressing high specific or broad spectrum activity indicating endophytes from the selected habitats represented a promising group to investigate for lead structures for new agrochemicals. Activity ranged from fungicidal (53%) to insecticidal and herbicidal (both at 11%) together with mixed activities. The use of a plant based non-synthetic medium produced an interesting high level (47%) of activity in the mycelial extracts. The differing patterns of bioactivities expressed by the endophytes are discussed in the context of the biology of the host plant.

2.2

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Use of electronic nose technology for detection of pharmaceutically useful metabolites by correlation with volatile production patterns

We have examined the effect of different free and immobilised bioreactor systems on temporal volatile production patterns and related this to production of a cholesterol lowering drug, squalistatin S1. Using a Conducting polymer sensor array based electronic nose system it was possible to differentiate changes in volatile production patterns with time for 72 hrs. The changes in sensor parameters were related to the quantified amounts of S1 produced in the bioreactor system over 96 hrs. The sensor responses varied with nutritional conditions favouring secondary metabolite production. This study suggests that opportunities exist for applying volatile production patterns for rapid screening and identification of optimised nutritional and physiological parameters for optimising production systems. Studies are in progress to further examine the correlation between volatile production fingerprints and production of useful secondary metabolites.

2.3

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Use of an electronic nose for early detection and discrimination between dermatophytes

There has been interest in developing methods for the early diagnoses and discrimination between the three-four important dermatophytes isolated from skin and scalps of patients (*T.mentagrophytes*, *T.rubrum, T.verrucoum* and *T.violaceum*). There is knowledge to show that there are different volatile fingerprints produced by different species of fungi. We have used two different sensor array systems to examine the volatile fingerprints produced by these species in vitro to evaluate the potential for early discrimination and differentiation. This has shown that it is possible to differentiate between two of the four species within 72-96 hrs. The sensitivity of detection was examined for *T.mentagrophytes* and it was found possible to differentiate between log4, log6 and log8 inoculum levels within 96 hrs. This approach is being optimised for examination of patient samples.

2.4

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Characterization of bioactive compounds produced by the Zingiberaceous endophytic *Chaetomium globosum* CMUZE0132:

Endophytic and saprobic isolates from representative species of Zingiberaceae were screened for the production of antifungal metabolites. The endophytic *Chaetomium* strain CMUZE0132, cultured in F1 medium, showed the highest inhibitory activity. This strain was identified as *C. globosum* based on morphology and the ITS rDNA sequence analysis at 98% similarity. Fractions from culture extracts obtained from HP 20 resin and silica gel columns were bioassayed against *Candida albicans, P. avellaneum, Pyricularia oryzae* and *Phytophtora* sp. The highest levels of inhibition were found in one from fraction against the filamentous fungi, but not against *C.*

albicans. The Rf value for this fraction on TLC was 0.38. This compound was shown to be a hydrophobic peptide with a molecular weight of 659.29. Candidate formula is $C_{25}H_{44}O_{15}N_6$. The possible structures are L-threonine, N-[N-[N-[N-(N-L-threonyl-L-seryl)-L-seryl]-L-α-glutamyl]-L-isoleucyl]-(9CI) or L-Lysine, N2-[N2-[N-(N-acetylmuramoyl)-L-seryl]-D-α-glutaminyl]-(9CI). Further confirmation is required.

2.5

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Origin and roles of fungal flavour volatiles.

The unique flavour of *Agaricus bisporus* is due to the release of a set of eight-carbon volatile compounds, the biosynthetic pathway of which has not been elucidated. Beside their influence on crop quality, these 8-carbon volatile compounds play an important part in developmental regulation i.e. triggering the switch from vegetative to reproductive growth in mushrooms, and are also used by insects to detect the presence of fungi.

8-carbon volatiles are derived from the oxygenation and the cleavage of the polyunsaturated fatty acid linoleic acid. This reaction has similarities to the plant system, but also major differences. Examination of the enzymic mechanisms and the fatty acid chemistry suggested that the enzyme involved in the oxygenation step could be a lipoxygenase (as found in plants) or a heme-dioxygenase, similar to the recently isolated linoleate diol synthase from the fungus *Gaeumannomyces graminis*.

In order to characterise the biochemical pathway leading to eight-carbon volatile production, we investigated fatty acid and lipids distribution in *Agaricus bisporus*, as well as hydroperoxide and volatile compounds levels. In parallel, we searched for candidate genes susceptible to encode the enzyme responsible for this novel oxidation route in fungi.

The combination of analytical methods, such as GC-MS, with molecular approaches based on degenerate PCR, RT-PCR and library screening provided us with a broad range of results. Novel lipid metabolism genes have been identified and their respective functions have been established through heterologous expression and functional assays. This together with the relationship between fatty acids and volatile compounds enabled a better understanding of mushroom volatiles biosynthesis and lipid metabolism.

2.6

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Characterization of secondary metabolites produced by *Penicillium* species colonizing cork slabs using ESI-MS

During cork manufacturing the humid cork slabs are extensively colonised by fungi, where a dynamic succession of distinct species occurs, mostly controlled by water activity evolution. Taxonomic studies indicated the dominance of *Chrysonilia sitophila* but the number of species of *Penicillium* genera (e.g. *P. brevicompactum, P. chrysogenum, P. glandicola and P. olsonii)* also isolated, pointed out to its relevance in cork and as such should be investigated in more detail. Fungi implications were only explored regarding the presence of metabolites known to impart sensorial defects in wine, but a more complete survey remains to be investigated. These compounds, which may own a good (xanthones with cytostatic activity) or harmful (mycotoxins) biological effect, are synthesised due to the critical interaction between fungi and substrate break-down products. Both facts justify a rather extensive survey of cork occurring fungi secondary metabolites. The production profile, at different growth periods, during fungi colonisation of cork semi-solid media is on-going. Metabolites characterisation was done by mass spectrometry using electrospray ionization, eventually further structural characterization will be performed by MS/MS assays. . Several factors affecting fungi producing profile are also being analysed, namely water activity, and may well result in revision of cork stoppers productive scheme.

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PROTEIN FOLDING AND SECRETION IN FUNGI

3.1

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PLAY, an abundant ascospore cell wall protein in *Talaromyces macrosporus*.

Ascospores formed by the fungus *Talaromyces macrosporus* belong to the most resilient eucaryotic structures observed to date. The ascospores can survive prolonged heat treatments at 85 ºC and as such survives pasteurisation treatments with ease. The spores can also survive high pressure treatments (800 MPa) which are used as a novel non-thermal preservation method for food products. Moreover, these treatments activate the dormant ascospores to germinate, which is characterised by trehalose breakdown, glucose release and a sudden ejection of the inner cell through the outer, very thick, ascospore cell wall. Heat activation was accompanied by changes in the ascospore cell wall structure as judged by an increase of the permeability for fluorescent probes, X-ray diffraction patterns and electron paramagnetic resonance studies on cell wall fractions. Activation in buffer was very low at 65 ºC, but strongly increased at 70 ºC or higher. These temperatures were also associated with the release of large amounts of a small protein from the cell (wall). This protein was estimated as being 5% or more of the total amount of protein of these cells. We have obtained the sequence of a small protein (called PLAY), which contains one short intron. The genome of *Talaromyces macrosporus* contains one copy of this gene and its expression is strictly related to the formation of fruit bodies (that contain ascospores).

3.2

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Biochemical and molecular studies of the cytoplasmic α1,2-manosidase from *Candida albicans*

We have been demonstrated the presence of three α-mannosidases in *C. albicans*, one located in the membrane fraction (E-M) and two other soluble enzymes E-I and E-II*. In this work we demonstrate that the three enzymes are α-1,2 mannosidases of the glycosyl hydrolase (GH) family 47. Molecular studies showed the presence of only one gene that encode for α1,2-mannosidase, suggesting that the other proteins are derived from the same gene product. Assays of proteolysis demonstrated that the α-mannosidase E-II is generated *in vitro* during the process of cell rupture. Studies of intracellular localization of these enzymes using protoplasts showed that the enzyme E-I is in the cytoplasmic compartment and is generated by proteolysis of the ER-α1,2-mannosidase (E-M). The proteolytic processing is carried out in the Golgi and involves the participation of Kex2. The mechanism of exit of E-I from Golgi to the cytoplasm is not yet known. The soluble enzyme participates in the trimming of free oligosaccharide of the cytoplasmic compartment, suggesting a different role to the described for members of the GH family 47. This is the first report of cytoplasmic α1,2-mannosidases in lower eukaryotes.

*Mora-Montes et al., (2004) Glycobiology 14: 593-598.

3.3

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Polychlorinated phenols induced stress on cork colonising moulds

The manufacturing process of cork stoppers includes a maturing stage after boiling the cork slabs. During this period, the slabs are completely covered with moulds, which find in cork an environment of temperature, water activity and substrate availability favourable for their development. Those moulds can methylate polychlorinated phenols (PCPs), as a detoxification process, and lead to the appearance of polychloroanisoles (PCAs), which are associated with cork taint defect in bottled wines, and are responsible for loses in the cork industry. PCP´s were frequently used as biocide, fungicides, mainly as wood preservatives being a common soil and water pollutant.

The goal of this work is the study of the mechanisms involved on the PCP´s degradation by the moulds that colonize cork slabs. Several species have been screened for their ability to grow in presence of the PCP´s and/or to metabolise these compounds. Growth inhibition was assessed in the presence of different concentrations of PCPs, mainly 2,4,6-trichlorophenol and pentachlorophenol, in solid and liquid cultures. Moreover, the production of some enzymes (peroxidases and laccases) was studied using molecular and biochemical approaches.

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3.4

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The role of cell surface mannans in *Candida albicans*

The outer layer of the cell wall of *Candida albicans* is heavily enriched in glycosylated proteins that play critical roles in cell adherence, and act as major antigens and in the immunoregulation of the host. A null mutant in the Golgi manganese transporter gene *PMR1* was viable in vivo, had greatly reduced N- and phosphomannan and

was attenuated in virulence. Therefore protein mannosylation is required for pathogenesis. The *C. albicans* O-linked mannan consists of a tetrasaccharide in which Mnt1p and Mnt2p participate as partially functionally redundant enzymes in the assembly of the terminal two alpha-1,2-mannose residues. Deletion of either *MNT1*, *MNT2* or both *MNT1* and *MNT2* resulted in strains with reduced adherence to epithelia and attenuation of virulence. This suggests that O-mannan functions as a ligand in interactions with host surfaces. Mutants with deletions in the *MNN4* gene are almost devoid in phosphomannan, which has been implicated in recognition of C. albicans by macrophages, but were phagocytosed normally. Deletion of *OCH1* resulted in elimination of the outer N-mannan chains, induction of the cell wall salvage pathway and loss of virulence. Analysis of glycosylation mutants demonstrates that the carbohydrate epitopes of mannoproteins play key roles in pathogenesis of *C. albicans*.

3.5

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The *Aspergillus niger* MADS-box transcription factor RlmA is required for cell wall reinforcement in response to cell wall stress.

In *Aspergillus niger*, the genes coding for glutamine:fructose-6-phosphate amidotransferase (*gfaA*) and α-1,3-glucan synthase (*agsA*) are induced in response to cell wall stress. *In silico* analysis of the promoter region of the two genes revealed the presence of putative DNA binding sites for transcription factors involved in stress responses, including sites identical to the *Saccharomyces cerevisiae* Rlm1p and Msn2p/Msn4p transcription factors. Promoter analysis indicated that the induction of the *agsA* gene in response to cell wall stress is fully dependent on a putative Rlm1p binding site in its promoter region. Database searches revealed the presence of *S. cerevisiae* Rlm1p homologues in most filamentous fungi examined, including A. niger. Deletion of the RLM1 homologue, named *rlmA* in *A. niger*, completely eliminated the induction of *agsA* and resulted in a two-fold reduced induction of *gfaA* during Calcofluor White-induced cell wall stress. In addition, the deletion strain was more sensitive towards cell wall stress agents. Our results indicate that *A. niger* responds to cell wall stress by transcriptional activation of cell wall reinforcing genes including *agsA* and *gfaA* through an Rlm1p-like transcription factor. We propose that such a cell wall salvage mechanism is wide-spread in filamentous fungi.

3.6

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Imaging the last stages of protein secretion in the filmentous fungus *Trichoderma reesei*

Available data suggest that the basic principles of secretion obtained from studies on *S. cerevisiae* and animal cells also apply to filamentous fungi. Although considerable research has been performed on the secretion process in filamentous fungi, little is known about their secretory machinery. The transport of proteins between the cellular compartments occurs in membrane-bounded vesicles that bud from the donor and fuse with the acceptor compartment. Membrane bound proteins called SNAREs (Soluble N-ethylaleimide-sensitive factor Attachment protein REceptors) play a key role in membrane fusion. SNAREs have been found to be involved in most membrane fusion events in the cell. This study describes the cloning and characterization of a putative exocytotic t-SNARE (*sso1*) from *Trichoderma reesei*. We cloned *the T. reesei* homologue of *Neurospora crassa nsyn2*. This protein was labelled with the Cerulean fluorescent protein mCer and the fusion protein expressed in *T. reesei*. Transformants expressing the fusion protein exhibit a clear labelling of the plasma membrane. The fusion protein is uniformly distributed along the hyphae with no tip-focused gradient. A v-SNARE SNCI was labelled with mRFP to analyze its interaction with the SSOI protein using fluorescence resonance energy transfer (FRET) imaging.

FUNGAL BIOREMEDIATION

4.1

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Effect of CaCO3 on morphology and essential metal accumulation by *Morchella* sp. colony

The ascomycete Morel mushroom (*Morchella* sp) exhibits high ecotypes variability, which are found in a wide range of habitats that differ in their chemical, physical, biological and nutritional properties. As such, morels have adopted several different morphological pathways to endure diverse and changing environments: fruit bodies, sclerotia, conidia and mycelial mat. Although morels have been found in several places around the world in slightly acidic soil, in Israel they are found in lime-soil with high pH levels. In this work we have studied the

effect of lime (CaCO3) on *Morchella* colonial growth and morphology in pure culture. It was found that mycelial linear growth increased, and sclerotia appeared earlier upon addition of CaCO3 to the agar medium. Calcium carbonate increased the pH of the medium, but caused a decrease in electrical conductivity. Increasing medium pH adjusted by other buffering agents such as Tris-HCl or K-Phosphate buffer showed a similar effect on mycelial growth rate, but not on sclerotia production. Further study showed lower accumulation of essential metal ions in the biomass of CaCO3 enriched treatments. It is suggested that the decrease in metal-ions availability has a role in the CaCO3 impact on the fungal colonial morphology.

4.2

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Fungal phenolic degradation and catalytic polymerization of the products by metal compounds

Fungal degradation of phenol (and related aromatic compounds) has been well documented. However, the interactions between phenolic degradation products and metals have received little attention. Fungi are able to mobilize metals and metalloids from minerals by several mechanisms, including organic acid and/or proton excretion. Our work proposes an additional mechanism where the fungal metabolism of aromatic compounds could indirectly result in mineral transformations by abiotic reactions with resulting degradation products. We have isolated fungi which have the ability to degrade phenol at high concentrations and which could also dissolve metal oxides during phenol degradation. It was found that catechol, one of the important intermediates of phenol degradation, was polymerized by calcium oxide and cuprite. We can conclude that interactions between organic and inorganic substances are complex and influenced by microbial activity, and these may have important effects on pollutant breakdown and transformation.

4.3

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The importance of soil and saprotrophic fungi in radionuclide sorption within organic soil systems

The potential of soil micro-organisms to enhance the retention of 137Cs and 85Sr in organic systems was assessed in a series of experiments. To determine the true potential of litter and soil microorganisms to accumulate 137Cs and 85Sr in situ, we used a biologically active, mineral-free, organic material, prepared from washed leaf litter, to minimise interference from clay minerals. Biological uptake and release was differentiated from abiotic processes by comparing the sorption of radionuclides in sterilised organic material with sterile material inoculated with soil extracts or single fungal strains. Our results show conclusively that living components of soil systems are of primary importance in the uptake of radionuclides in organic material. The presence of soil microorganisms significantly enhanced the retention of Cs in organic systems but sorption of 85Sr was not significantly influenced. A non-linear temperature response indicative of biological activity was observed for the retention of radionuclides in biotic systems, temperature did not significantly affect uptake in abiotic systems. Experiments with single strains of soil and saprotrophic fungi indicate that fungi may account for part of the strong, irreversible binding observed in biotic systems.

4.4

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The molecular analysis and geochemical influence on lithic fungal community diversity

Fungi are ubiquitous and associated with an extensive variety of rocks and minerals occurring over a wide range of geographical zones. In this research, the contrasting elemental profile of adjacent layers from a sandstone outcrop was examined to find out whether differences in mineralogical composition influenced fungal diversity. Culture-independent molecular approaches were used in combination with geochemical analyses, community-level physiological profiling and scanning electron microscopy to assess the effects of mineralogical variation on community composition. A DNA-based community fingerprinting approach (ARISA: automated ribosomal intergenic spacer analysis) combined with construction of fungal rRNA gene clone libraries was used to determine community structure and to identify some of the constituent fungal populations. Molecular data was combined with X-ray diffraction and X-ray fluorescence in combination with multivariate statistics to identify those chemical properties that notably influenced fungal populations. It was found that the sandstone supported a varied fungal diversity and community ribotype profiles differed between mineralogically-distinct sandstones in close proximity to each other. Canonical correspondence analysis (CCA) illustrated relationships between certain fungal ribotypes and particular chemical elements with Na and Ca having a considerable impact on community structure signifying that fungal diversity was affected by the specific elemental composition of

sandstone. We can conclude that these molecular and statistical methods provide a powerful tool for resolving rock-inhabiting fungal communities, and their relationship to the mineral nature of the rock substrate.

4.5

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Strontium accumulation and transformation by fungi

The surfaces of many nuclear facilities may be contaminated with different radionuclides of which strontium-90 may be an important component. Sr-90 is the most important strontium radioisotope in the environment and has a relatively long half-life of around 30 years. Decontamination of contaminated surfaces is clearly an important objective of the decommissioning portfolio. Strontium is chemically similar to calcium, and this similarity to calcium allows strontium to be localised to the same sites within the human body, e.g. bones and teeth. A novel approach to decontamination is the application of microbial systems and products and it is now recognised that microorganisms have considerable potential for bioremediation of a variety of contaminants, and some processes are in commercial operation. Fungi have received less attention than bacteria despite these organisms being major biotic components of soil and highly important in organic and inorganic biogeochemistry, including toxic metal cycling. This work has examined the interactions of strontium with selected fungal species chosen for their established properties relating to soil and mineral transformations. It was found that growth responses were variable with inhibitory effects being low and only occurring under specific conditions. Oxalic-acid producing species were able to precipitate secondary oxalate minerals external to the mycelium.

4.6

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Transformation of organic phosphorus during decomposition of spruce needle litter

By using the 31P NMR spectroscopy we studied P transformation during decomposition of spruce litter. Needle litter, collected from OL horizon under native mountain stand of *Picea abies* [L.] Karst. and sterilized by gamma radiation, was inoculated by individual strains and mixtures of autochtonous saprotrophic fungi. After long-term cultivation in the dark at laboratory temperature, HA were isolated and measured their 31P NMR spectra. Spectra of these HA were compared with spectra of alkaline extract of fresh spruce needles. Our results confirmed that the 31P NMR spectroscopy represent an excellent instrumental method for studying transformations of soil organic matter during decomposition of plant residues. This sensitive method is selective namely to the activity of fungi (spectral areas from -4 to -22 ppm and 15-20 ppm, respectively). Important information can be gained also from the phosphate monoester/diester ratio, DNA-P content (area near 0 ppm) and/or from the content of mineralized P (orthophosphates). Individual fungal strains showed significant differences in dominant forms of phosphorus they transformed.

4.7

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White-rot fungal laccases as oxygen reduction catalysts: Building a biofuel cell

Laccases are blue copper oxidases which occur widely in nature and catalyse the oxidation of aromatic compounds by molecular oxygen. The physiological functions of these extracellular glycoproteins are still under intense investigation although they are implicated in the synthesis and/or degradation of the biopolymer lignin, wound response mechanisms and pathogenic virulence in certain fungi. The laccases isolated from white-rot fungi such as *Trametes versicolor* and *Coriolus hirsutus* reduce oxygen at high potentials. Technological interest in these enzymes arises from their relevance in lignin degradation, 'green' remediation procedures and as cathode catalysts for fuel cells.

Laccases have broad specificity enabling them to catalyse the one electron oxidation of a range of substrates with concomitant four electron reduction of atmospheric oxygen to water. Central to catalytic function are four divalent copper ions characterised according to their spectroscopic properties. These comprise the type 1 (T1) EPR active, mononuclear site, the EPR active type 2 (T2) site and the EPR silent, binuclear site. It has long been known however that these enzymes are inhibited by anions, particularly fluoride and the ubiquitous chloride ion[1]. Early studies employing EPR spectroscopy indicated that F- binds at the T2 site but otherwise details of halide inhibition have remained obscure[2].

The Oxford Biofuel Cell Concept employs laccases as cathodic catalysts. The electrochemical technique, protein film voltammetry offers a finely-tuned approach to the study of enzymatic inhibition. These studies have allowed quantitative insight into the suppression of catalytic activity and more specifically, the kinetics and energetics of anion inhibition in fungal laccases.

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4.8

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Isolation and growth of pyrene-degrading fungi

Polycyclic aromatic hydrocarbons (PAHs) are common pollutants found in soil and groundwater as a result of human activities such as the combustion of fossil fuels, the incineration of wastes and the accidental spilling of oils. Fungi are able to metabolize a wide variety of PAHs, converting them either completely to carbon dioxide or to various metabolites. PAH-contaminated soil samples were collected from a variety of sites. To isolate pyrene-degrading fungi, serial dilution methods, both by direct and selective enrichment inoculations, were used. A solid PBBM (phosphate-buffered basal medium) supplied with pyrene as a sole carbon source was used as a selective medium for the isolation and cultivation of pyrene degrading organisms. After two weeks incubation, over 70 fast-growing fungal strains were selected. According to their morphologies, ten different isolates were chosen and grown in liquid PBBM and the pyrene degradation rate was analyzed by HPLC. Several isolates were able to degrade >90% of the supplied pyrene after two weeks incubation.

4.9

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Responses of ectomycorrhizal fungi to mercury

Mercury is a potent toxic substance that is concentrated through the food chain. The combined effects of atmospheric deposition of mercury and leachable, sandy soil accentuate metal bioavailability in areas like the New Jersey Pine Barrens. Ectomycorrhizae (ECM) are important regulators of nutrient and metal uptake by woody plants, and dominant tree species of the Pine Barrens are ectomycorrhizal . This laboratory study was designed to investigate the effects off mercury on ECM radial expansion. Five Pine Barrens ECM were incubated on nutrient-amended agar plates with initial concentrations of mercury ranging from 0-100uM for up to three months. Biweekly measurements of colony radii were taken. *Amanita muscaria* was the only isolate consistently able to survive concentrations of mercury at or above 50uM. At 25uM, expansion was least inhibited in *Laccaria laccata* (20%). Growth inhibition of *L. laccata, Piloderma bicolor* and *Pisolithus tinctorius* was not proportional to mercury concentration at 0-40uM. *P. tinctorius*, often used in forest restoration, was sensitive to mercury at 5uM initial concentration. These results suggest variable levels of sensitivity to mercury among ECM and multiple mechanisms of mercury-ECM interactions. How these responses affect tree fitness in mercury-contaminated soils is currently being investigated.

4.10

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Role of mycorrhizas in toxic metal mineral transformations

Plant roots and their symbiotic and free-living microbial populations alter the physico-chemical characteristics of the rhizosphere making it different from bulk soil and affecting toxic metal speciation. The mechanisms by which fungi and plants obtain phosphate are of particular interest since solubilization of inorganic phosphates can result in release of associated metals. Zinc phosphate was of least toxicity and the most easily solubilized by axenic ericoid and ectomycorrhizal fungi among tested insoluble cadmium-, copper-, lead- and zinc-containing minerals. Solubilization of toxic metal minerals was related to fungal metal tolerance. Zinc phosphate solubilization by *Paxillus involutus/Pinus sylvestris* ectomycorrhizal associations and protection of host plants against toxic metal mobilized from the mineral also depended on the phosphorus status of the growth matrix. Zinc, copper and lead mobilized from minerals were oxygen-coordinated within the fungal/ectomycorrhizal biomass. "Heterotrophic leaching" of toxic metal(s) from insoluble minerals comprised acidification and ligand-promoted dissolution mediated by different organic acid anions: if oxalic acid was produced, precipitation of metal oxalates resulted.

4.11

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Toxic metal speciation in fungi

Fungi can be highly efficient biogeochemical agents and accumulators of soluble and particulate forms of metals. This project aims to understand the physico-chemical mechanisms involved in toxic metal transformations and accumulation by fungi and mycorrhizal associations. Because of the amorphous state or poor crystallinity of metal complexes within biomass and relatively low metal concentrations, determining metal speciation in biological systems remains a challenging problem in the case of fungal and mycorrhizal biomass. Synchrotron-based element-specific X-ray absorption spectroscopy (XAS) is an ideal technique for studying element complexation in environmental samples varying from biological to mineralogical in nature. We exposed fungi and ectomycorrhizas to a variety of copper-, zinc- and lead-containing minerals. XAS-studies revealed that oxygen ligands (phosphate, carboxylate) played a major role in metal coordination within the fungal and ectomycorrhizal biomass during accumulation of mobilized toxic metals. Coordination of toxic metals within biomass depended on the fungal species, initial mineral composition, nutrients (especially the nitrogen source) and the physiological state/age of the fungal mycelium.

4.12

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The presentation of white rot fungi to contaminated soils using green waste composts: A practical approach

The use of lignin-degrading white rot fungi for the remediation of petroleum contaminated land has been studied intensively; however, their incorporation into soil remains a major challenge. This study describes first steps towards a practical and non expensive approach for the introduction of white-rot fungi into a hydrocarbon-contaminated site via composted green waste.

Compost and wood-chips were transferred into perforated carrying bags (800 perforations per 1m2) to ensure a sufficient aeration which is needed by white rot fungi in order to oxidise organic hydrocarbons. To identify the optimum combinations of white rot fungi for bioremediation purposes each bag was inoculated with a nutrient solution containing defined numbers of spores or mycelia of a range of white rot fungi (*Phanerochaete chrysosporium* and a mix of *Phanerochaete chrysosporium Pleurotus ostreatus, Coriolus versicolor* and *Stropharia rugosoannulata*). Control bags were treated with both nutrient solution and water.

In a small scale *ex situ* treatment the compost was mixed with petroleum contaminated soil (TPH 52.4 g/kg) (1 : 6 ratio of compost: soil) and placed into windrows which were turned weekly to ensure a high oxygen supply to all micro organisms. In this study results concerning the impact of the treated compost in terms of bioremediation are presented.

FUNGAL BIOCONTROL OF PESTS

5.1

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Production of the biocontrol agent *Phlebiopsis gigantea* in controlled environmental and nutritional media

Heterobasidion annosum, a tree pathogen, is ubiquitous in the environment and causes financial losses for the forestry industry. It has been found that the saprophyte, *Phlebiopsis gigantea*, can reduce losses from *H. annosum*, by out-competing for woody resources. The natural population of spores of *P. gigantea* can be augmented by the application of a spore suspension, to the stumps, at the time of tree-felling. Environmental studies have been carried out to assess the fitness of different isolates of the antagonist relative the pathogen. Competitiveness was affected by environmental factors, especially water availability. These indicate that the antagonist is not able to suppress the pathogen under all conditions.

Studies have been carried out to examine potential for liquid or solid substrate fermentation systems for optimising production of *P.gigantea*. Liquid culture studies were variable regardless of available nutrients and ecophysiological stresses imposed. However, temporal studies on solid substrate based on *Pinus sylvestris* sawdust gave >Log10 ^{7} viable oidia g^{-1} in the best moisture content treatments. Scale up, preservation studies and analyses of the endogenous reserves of the best treatments are in progress to identify specific quality characteristics.

5.2

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Chitinase production and phosphate solubilization from potential biological control agents *Trichoderma viride* and *Azomonas* sp.

From 2002-2004, six species of *Trichoderma* and one species of *Azomonas* were isolated from rhizosphere soil samples of 20 Vetiver zizanicides ecotypes and 11 V. nemoralis ecotypes in Prachuab Khiri Khan Province, Thailand. *Trichoderma viride* showed highly effected antagonistic activity in tests against some plant pathogenic fungi, such as *Phytophthora palmivora, P. parasitica, Pythium* sp. and *Rhizoctonia solani*. Furthermore, *T. viride* produced moderate amounts of chitinase and organic acids for phosphate solubilization. Azomonas sp. produced large amounts of organic acids to solubilize phosphate and calcium to increase soil fertility and enhance plant production.

5.3

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Fungal control of aphids in lettuce and Brassica crops

The pest aphids infesting the foliage of lettuce and Brassica crops (*Nasonovia ribisnigri, Myzus persicae*, *Brevicoryne brassicae*) are increasingly difficult to control in the UK. This is because the range of effective insecticides has decreased as a result of the withdrawal of products following the UK/EU pesticides reviews and an increase of insecticide resistance in field populations of *M. persicae* and *N. ribisnigri*. Although new active ingredients have become available, they do not provide a complete solution to the problem. As part of a project to develop an IPM strategy for aphid control on these crops, four fungal biopesticides were evaluated.

In laboratory bioassays, all four fungal biopesticides caused mortality in the three aphid species. Differences in susceptibility were observed between aphid species and between treatments. *Myzus persicae* was the most susceptible aphid species. The most virulent biopesticide examined was a product based on *Beauveria bassiana*. Treatment with this product resulted consistently in fungal-induced mortality, regardless of aphid species, and it was selected for further evaluation in a field experiment. A reduction in the development of *B. brassicae* populations was observed in the field, but the other two aphid species were not controlled. This may be due to difficulties in contacting these species using foliar sprays and to adverse effects of the weather on fungal performance.

5.4

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Control of *Fusarium moniliforme* (bakanae disease) by using endophytic fungi in rice

Fifty endophytic fungi isolated from leaf blades, leaf sheaths and roots of apparently healthy rice plants were tested *in vitro* for the efficacy of being antagonists to *Fusarium moniliforme*, a causal agent of bakanae disease by dual culture method. *Acremonium* sp. 0119, *Aspergillus* sp. 0035, *Coelomycetes* 1 0117, *Coelomycetes* 1 0071, *Talaromyces* sp. 0003, *Nodulosporium* sp. 0019, *Nodulosporium* sp. 0021, *Nodulosporium* sp. 0020 and *Eupenicillium* sp. 0007 were the highest percentages of inhibition group (55.45-58.76%). Five endophytic fungi were selected to determine the efficacy of controlling the bakanae disease in vivo. It was found that *Acremonium* sp. 0119, *Coelomycetes* 1 0117, *Aspergillus* sp. 0036, *Neosartorya* sp. 0026 and *Nodulosporium* sp. 0020 were increasing seed germination of infected seed (78.75, 71.75, 80.50, 77.50 and 81.75%, respectively) when compared to infected seed germination which untreated with endophytic fungi (54.25%). Fresh weight and dry weight of seedling were also increasing after treated seeds with the endophytic fungi before planting.

5.5

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Attributes of specialists and generalists entomogenous fungi for insect pest control

There is considerable interest in the development of entomogenous fungi for the control of arthropod pests of economic and medical importance because they offer an environmentally friendly alternative to chemical pesticides. Fungi are usually host specific, relatively inexpensive to produce, and can be used with other biocontrol agents (predators and parasitoids) in integrated pest management (IPM) programmes. There are over 700 species of entomogenous fungi, some of which are being developed for pest control while others have already been commercialised.

Entomogenous fungi can be broadly divided into two categories, the specialists and the generalists. Specialist fungi are restricted to specific arthropod species while generalists infect arthropods from several different

families or orders. Most specialist species belong to the order Entomophthorales (Zygomycotina) while most generalists belong to the class Hyphomycete (Mitosporic Fungi formerly Deuteromycotina).
This poster briefly outlines the salient attributes of specialist and generalist insect pathogenic fungi. Particular attention is drawn to the infection processes of Hyphomycete and Entomopthoralean fungi.

5.6

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Essential oil of *Chenopodium ambrosioides* as botanical fungitoxicant against toxigenic strain of *Asperpgillus flavus*

Essential oil extracted from leaves of Chenopodium *ambrosioides* Linn. (Chenopodiaceae) was tested against the toxigenic strain of test fungus - *Aspergillus flavus* Link. The oil completely inhibited the mycelial growth at 100 ppm. The oil exhibited broad fungitoxic spectrum against *A. niger, A. fumigatus, Botryodiploidea theobromae, Fusarium oxysporum, Sclerotium* sp. *Macrophomina phaseolina, Cladosporium cladosporoides, Helminthosporium oryzae* and *Pythium debaryanum* at 100 ppm. The oil showed significant efficacy in inhibiting the aflatoxin B1 production by the toxigenic strain of *A. flavus*. *Chenopodium* oil also exhibited potent antioxidant activity when tested by ABTS method. GLC of *Chenopodium* oil showed three major and four minor compounds. All these observations suggest the possible exploitation of the *Chenopodium* oil as botanical fungitoxicant in ecofriendly control of post harvest biodeterioration of food commodities from toxigenic storage fungi.
Keeping view in the side effects of synthetic pesticides and global importance of some plant products (neem products) in plant protection , the *Chenopodium* oil may be recommended as an ideal botanical fungitoxicant .

5.7

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Analysis of expressed sequence tags from the mycoparasite *Pythium oligandrum*

Employing biological control strategies to suppress root-infecting oomycetes using bacterial, fungal or oomycete antagonists, is an area of increasing research interest. This work is driven in part by concerns over the use of chemicals in the environment and the drive to look for alternative disease control strategies.
The oomycetous biological control agent *P. oligandrum* is an effective antagonist of various plant pathogens and has also been shown to promote plant growth and induce systemic acquired resistance in tomatoes. The aim of the current project is to identify and characterise effector molecules from *P. oligandrum* that are involved in the mycoparasitic interaction with *Phytophthora infestans*. As an initial attempt to identify candidate genes, a pilot EST library consisting of 1000 sequences has been created from mycelia. This will also assist in comparative genomics between other oomycete species.
Analysis of this library has identified several sequences that are predicted to be secreted, that are possibly involved in the mycoparsitic interaction, including a lipase, protease, protease inhibitor, beta-glucosidae and several others. Several sequences have also been found that could potentially be involved in the interaction with the plant host.

5.8

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Noteworthy *Talaromyces* with a special emphasis on *T. flavus* as a biological control agent

Soil samples were collected from thirty-two locations in Thailand during March 2002 to February 2005. The soil plate, dilution plate , alcohol and heat treatment methods and Gochenaur's glucose ammonium nitrate agar were used to isolate *Talaromyces* spp. Identification was based on macro-and microscopic characters when cultured on different media and after observation under stereo, light and scanning electron microscopes. Camera lucida drawings was made. A total of 290 isolates of *Talaromyces* were recorded , comprising 9 species and 3 varieties including *Talaromyces austrocalifornicus, T. bacillisporus, T. flavus var. flavus, T. flavus var. macrospermus, T. helicus* var. *helicus, T. luteus, T. rotundus, T. stipitatus, T. trachyspermus* and *T. wortmanii*. Among 170 strains of *T. flavus*, 20 strains were used for antagonistic tests to 9 plant pathogenic fungi in vitro and in the green house. The antagonistic tests indicated that most strains of *T. flavus* could effectively control *Phytophthora palmivora in vitro.* All strains of *T. flavus* produced moderate control of *Phytophthora parasitica, Fusarium oxysporum, F. semitectum, Colletotrichum capsici,* and *C. gloeosporioides*, but did not control *Lasiodiplodia theobromae, Rhizoctonia oryzae* and *Sclerotium rolfsii in vitro*. Antagonistic tests of 20 strains of T. *flavus* with 9 plant pathogenic fungi in the green house are ongoing.

5.9

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"*In vitro*" antagonism towards *Plasmopara viticola* from an endophyte *Acremonium* sp. in grapevine

The association of an *Acremonium* sp., strain A20 with grapevine leaves has been investigated, utilizing a recently optimised technique for endophyte visualization in dicotyledon plants. The fungus has been shown to occur in veins of symptomless leaves, at a latent stage and in a silent equilibrium with its host, where it finds nutrients and protection. When an abiotic or biotic stress affected the grapevine, then the endophyte was activated, moving from veins to the parenchimatic tissues. In case of an injury, *Acremonium* sp. reached and invaded the offended tissue with degradation of cells, followed by sporification. In the presence of *P. viticola*, the hyphomycete moved to the injured cell, colonizing all phytopathogen structures, like mycelium, sporangiophore branches and microsporangia, or anteridia, oogonia and oospores, then sporified itself. The phenological and sanitary conditions of the grapevines played a role in determining the activation of *Acremonium* sp. and its protective action towards biotic stresses. On the same time, the antibiotic activity of cultural filtrates, crude extracts, as well as acremines A-D obtained from A20 cultures have been tested towards agamic structures of the pathogen. Germinability of microsporangia decreased in all tests, compared to the respective controls. The recently characterized acremines A-D are metabolites which possess a cyclohexene or aromatic part substituted or cyclized with a hemiterpene unit. Assuming a break-point of 60% inhibition, only acremine C exceeded this value at concentrations >0.5 mM.

5.10

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Biological control of bindweed with *Stagonospora convolvuli* LA39: Do fungal metabolites pose a risk to the environment or enter the food chain?

The development of fungal biocontrol agents (FBCAs) as alternatives to chemical pesticides is of increasing public interest. A prerequisite for the safe use of FBCAs is a thorough assessments of risks associated with their application especially with respect to the production of toxic fungal metabolites. However, tools to assess the toxicity of these metabolites and to monitor them in the environment are often not available. We have established several methods to assess the risk of fungal metabolites using the bindweed biocontrol strain *Stagonospora convolvuli* LA39 and one of its major metabolites elsinochrome A (EA) as a model. Potential risks were evaluated following a three-point strategy: First, the toxicity of the metabolite against a wide variety of organism was tested. Second, the possibility of the metabolite to enter the food chain was evaluated by investigating if the metabolite is present in the applied product or whether it is produced by the FBCA in the environment. Third, the environmental stability of the metabolite was examined. Following this strategy we could show that although EA displays a broad-spectrum toxicity, its levels in the applied product are far too low to cause toxic effects to any organisms. Moreover we could never detect EA in treated bindweed leaves, not to mention in crop plants. We conclude that EA produced by the fungal biocontrol agent *S. convolvuli* LA39 does not enter the food chain or pose a risk to the environment**.**

5.11

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Infection mechanisms in oomycete pathogens of nematodes

Zoosporic oomycete pathogens of nematodes have developed a number of ingenious infection strategies. Some produce zoospores that are chemotactically attracted to their hosts and encyst around natural orifices on the host surface. The hyphal germ tubes then rupture the animal cuticle or invade via these natural openings. However, in the majority of species, the zoospore phase simply disperses the spores from the immediate vicinity of the host or is suppressed entirely. The encysted zoospores or aplanospores in these species germinate to produce bud like outgrowths coated with adhesive that are passively picked up by passing hosts. The final group are exemplified by the genus Haptoglossa. The zoospores or aplanospores germinate to produce specialised infection cells, known as gun cells. These show great morphological diversity and some species produce more than one form of such cell. Most infection (gun) cells have an inverted tube in which there is a needle-like structure, locked in position by an elaborate series of investing 'cones'. Upon contact with a passing host the tube everts and the needle ruptures the host cuticle allowing the everting 'tube' to enter the host and act as a conduit for the spore cytoplasm. Within a few milliseconds and infective sporidium is injected in the unsuspecting victim. Some of the likely phylogenetic relationships between these pathogens will also be briefly discussed.

5.12

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***Sphaerellopsis filum*, a hyperparasite for biological control of carnation rust**

The rust fungus on carnation,*Uromyces duanthi*, was collected from mountainous areas in Chiang Mai and Chiang Rai Provinces of Thailand in December 2003. Severe rust pustules with unidentified black spots were found on carnation leaves and stems. The specimens were examined under a stereo microscope. Numerous black pycnidia were observed on uredinia of the rust fungus. A fine needle was used to transfer pycnidia from the specimens to a mount on a slide and the material was examined with a light microscope. Morphological characters revealed that the Coelomycete, *Sphaerellopsis filum*, was the carnation rust hyperparasite. This mycorparasite was cultivated on potato dextrose agar (PDA) supplemented with 2.5 g Bacto peptone, carnation leaf agar, and V-8 juice agar and incubated at 28° C. The fungus sporulated well on V-8 juice agar. Antogonistic activity tests are being conducted. The specimens are being maintained for further investigation in the National Herbarium at Department of Agriculture, Ministry of Agriculture and Co-orperatives.

GENERAL TOPICS

G.1

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Molecular taxonomic studies of selected members of the Xylariaceae

Representative species of a number of genera belonging to the family Xylariaceae (Ascomycotina) were collected from different regions of Thailand. These were identified using traditional morphological characters of both teleomorph and anamorph stages (when available) and together with selected temperate and non-Thai species were subjected to molecular examination. Nucleotide sequences of ITS1, 5.8S and ITS rDNA were analysed. In general the ITS1 region exhibited the greatest variation among the species studied whereas 5.8S and ITS2 regions were more conserved. The molecular data indicated that *Xylaria* is a monophyletic group which is separated from the genera *Biscogniauxia, Camillea, Daldinia* and *Hypoxylon*. The data also supported a clear distinction between *Astrocystis* and *Rosellinia* which contrasts with some modern authors views. Furthermore it was found that closely related species groups or genera appeared as separate entities but retained a close similarity. In most cases the molecular data supported the traditional taxonomic groupings.

G.2

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The dual role of peroxisomes in *Magnaporthe grisea* pathogenicity

Magnaporthe grisea, the causative agent of rice blast disease, serves as a good model to study fungal pathogenesis owing to its established genetic and molecular manipulation systems as well as the availability of its genome sequence. During a screen of random T-DNA insertional mutants, TMP6-2 was identified as a nonpathogenic isolate. Flanking sequence tag data revealed that the T-DNA insertion in TMP6-2 occurred in *pex6*, a gene essential for peroxisome biogenesis. Peroxisomes are ubiquitous organelles with key metabolic functions such as fatty acid metabolism. Further studies conducted in a *pex6*Δ strain confirmed defects in peroxisome-related functions such as inability to utilize complex fatty acids as sole carbon source and mislocalization of GFP tagged with a peroxisomal targeting signal. Detailed characterization of *pex6*Δ indicated that acetyl-CoA generated by peroxisomal activity in appressoria is essential for melanin biosynthesis. Deposition of a melanin layer underneath the appressorial cell wall enables the appressorium to maintain a high turgor pressure needed to penetrate the host epidermis. Thin-section EM revealed that pex6Δ appressoria lacked melanin completely and are unable to penetrate host surfaces. Phenotypic defects such as aberrant appressoria and the inability to plug septal pores under hypotonic conditions could be attributed directly to the loss of peroxisome-derived Woronin bodies in the *pex6*Δ strain.

G.3

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Leaf litter fungi from Central, Eastern and Western Thailand

Organic debris, including fallen and submerged leaves and twigs, was collected from five locations including two National Parks (Kaeng Krachan and Erawan), one wildlife Santuary (Khao Khieo), Muang District of

Kanchanaburi, Kasetsart University Campus, and a garden in Bangkok from April to June 2005. The moist chamber method and dilution plates were employed. Microfungi were examined on leaf litter under a stereo microscope after a few days to one month of incubation in a moist chamber. A fine needle was used to transfer spores and fruiting bodies to make permanent slides and to transfer spores to agar media in a pretri-dishes. A total of 33 genera of microfungi were reported from teak, mango, eucalyptus, bamboo, orchid, and unidentified leaf litter. Included among these were 22 Hyphomycetes, 6 Coelomycetes, and 5 Ascomycetes: *Alternaria, Arthrobotrys, Arthrinium, Aspergillus, Beltrania, Chaetomium, Chaetomella, Cladosporium, Ciliochorella, Colletotrichum, Curvularia, Dictyoarthrinium, Drechslera, Fusarium, Glomerella, Helicosporium, Idriella, Lasiodiplodia, Macrophoma, Memnoniella, Myrothecium, Nectria, Nigrospora, Opioceras, Penicillium, Pestalotiopsis, Periconia, Phoma, Stachybotrys, Trichoderma, Volutella, Wiesneriomyces, Xylaria* and *Zygosporium*. Tests for antagonistic activity using five selected species of leaf litter fungi against 9 plant pathogenic fungi in vitro are currently underway.

G.4

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A novel role for reactive oxygen species in regulating a fungal-plant mutualistic interaction

While much is known about the signals and mechanisms that lead to pathogenic interactions between plants and fungi comparatively little is known about mutualistic fungal symbiotic interactions. We describe here a new role for reactive oxygen species (ROS) in regulating the mutualistic interaction between a clavicipitaceous fungal endophyte, *Epichloë festucae*, and its grass host, *Lolium perenne*. In wild-type associations *E. festucae* grow systemically in the intercellular space as infrequently branched hyphae parallel to the leaf axis. In a plasmid insertional mutagenesis screen to identify important fungal symbiotic genes a mutant was isolated that altered the interaction from mutualistic to antagonistic. This mutant has a single-copy plasmid insertion in the coding region of a NADPH oxidase, *noxA*. Plants infected with the *noxA* mutant become severely stunted and in most cases eventually die. The fungal biomass is dramatically increased and the hyphae become hyper-branched and swollen. Complementation and deletion analysis confirmed that the mutant phenotype is caused by disruption of *noxA*. Deletion of a second NADPH oxidase, *noxB*, had no effect on the *E. festucae*-perennial ryegrass symbiosis. ROS accumulation was detected cytochemically near cell walls of fungal hyphae in wild-type but not in *noxA* mutant associations. These results demonstrate that fungal ROS production is critical in maintaining a mutualistic plant interaction.

G.5

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Unravelling the mechanism driving cell-to-cell heterogeneity in cadmium resistance of *S. cerevisiae*

Single cells within a population of genetically uniform micro-organisms exhibit variability in their phenotypes. It has been proposed that such mechanisms provide an evolutionary advantage, increasing the survival chances of some cells when faced with potentially lethal stresses and allowing persistence of the organism under conditions which do not favour growth. We have observed marked cell-to-cell variability in the reduced-glutathione (GSH) contents of *S. cerevisiae*, and this variability was correlated with heterogeneity in the resistances of individual cells to cadmium. A mutant defective for Gsh1p exhibited uniform Cd sensitivity. Cd and GSH are linked in a pathway of Cd detoxification, via vacuolar accumulation of bis(glutathionato)cadmium conjugates. Deletion of YCF1 or PEP5 (also required in this pathway) also abolished the heterogeneity in Cd resistance of *S. cerevisiae*. The transcription factors responsible for stress-responsive induction of GSH1 made varying contributions to Gsh1p/Ycf1p-dependent heterogeneity. In order to gain insight to the mechanism underlying cell-to-cell variability in this Cd detoxification pathway, experiments have been initiated to identify a process that is the source of the heterogeneity. Our results to date suggest that oscillatory transcriptional regulation of a single gene is the heterogeneity generator. The results reveal a driver of heterogeneity within *S. cerevisiae* cultures, which could impact on the fitness of populations when faced with a range of xenobiotics.

G.6

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Proteomic analysis of the differential responses to biotrophic and necrotrophic pathogens in pea

Peas comprise the largest planted area of field vegetables in the UK and downy mildew, caused by the oomycete pathogen *Peronospora viciae*, is the most common foliar disease of this crop with up to 55% losses in yield observed where plant resistance is ineffective. Control of downy mildew is achieved through the use of resistant pea cultivars and treatment with fungicides. Resistance to these fungicides is now a problem, so optimal application is required to prolong their effectiveness in controlling downy mildew outbreaks. This DEFRA-funded work in

conjunction with CSL uses proteomics and mass spectrometry to identify protein biomarkers that have increased or decreased abundance during infection of pea by *P. viciae*. The specificity of these proteins to the downy mildew infection is being assessed by examining proteins differentially regulated during infection of pea by other pathogens including the biotroph *Erysiphe pisi* and necrotroph *Botrytis cinerea*. Antibodies against the identified biomarkers will be used in biosensor devices for early disease diagnosis, allowing fungicides to be applied more efficiently and effectively. Fundamental information on proteins key to the infection process may lead to novel control strategies for downy mildew diseases, and data on specific proteins will be presented**.**

G.7

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Yeast-cell individuality during oxidative stress: Interplay of copper transport and Cu, Zn-superoxide dismutase activities

Cell-to-cell variability in the resistance of *S. cerevisiae* to the pro-oxidant menadione is enhanced in mutants (*mac1*, *ctr1*, *ccs1*, *sod1*) defective for copper trafficking and sequestration. This enhanced variability yields higher %survival in mutant populations versus wild type at high menadione doses, supporting the hypothesis that phenotypic heterogeneity can confer benefits on cell populations during adversity. Overexpression of SOD1 (encoding Cu, Zn superoxide dismutase) in the *sod1*Δ mutant restored homogeneous menadione resistance. In contrast, SOD1 overexpression in *mac1*Δ, *ccs1*Δ and particularly *ctr1*Δ strains only gave further increases in heterogeneity. This result did not rule out the possibility that the heterogeneity phenotypes of these mutants are due to altered Cu delivery to, and activity of, Sod1p – SOD1 overexpression in these strains may increase the amount of inactive Cu-deficient apo-Sod1p. To address this, media were supplemented with $Cu(NO_3)_2$, which partly corrected the increased heterogeneity of the mutants (especially *ctr1*Δ). However, exogenous Cu had negligible effect in SOD1-overexpressing mutant strains. Copper had no effect on wild-type heterogeneity. Thus, Cu transport from Ctr1p to Sod1p maintains relatively homogeneous resistance to pro-oxidants. Disruption of this 'buffering' promotes heterogeneity, which can have benefits under severe stress. Further work is required to elucidate the more-complex effects of SOD1 overexpression and Cu supplementation on these heterogeneity phenotypes.

G.8

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Do estimates of airborne spore concentrations differ depending on the sampling volume used?

The numbers and types of fungal spores present in air vary considerably, in response to biotic and abiotic factors. Such factors include the proximity of substrata suitable for fungal growth and the prevailing weather conditions. In addition to this natural variation, the choice of sampling apparatus, sampling volume, exposure medium etc. can influence spore counts. The aim of this work was to determine if the calculated viable airborne spore concentration differs depending on the sampling volume used. Preliminary results had suggested that high-volume sampling might compromise the integrity of the bioaerosol, thereby leading to under-estimation of spore concentrations. In order to ascertain this, five sampling volumes ranging from 60-180 litres were compared. Petri dishes containing Dicloran-Rose Bengal Chlorotetracycline agar (n=5) were exposed to outdoor air at University College Dublin, in a Surface Air System (SAS™) Super 180 air sampler on the 15th February 2005 in three replicate experiments. Using a mixed linear model in SAS® and ANOVA estimates, it was shown that there were no significant differences ($p<0.05$) in the total spore counts for the volumes of air sampled. It was therefore concluded that estimated spore concentrations were not dependent on the sampling volume, at least in the range investigated.

G.9

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Molecular characterisation of MVX disease dsRNA elements from *Agaricus bisporus*

Mushroom Virus X (MVX) disease affects the cultivated button mushroom, *Agaricus bisporus* and comprises a range of symptoms that reduce crop yield and quality. Twenty-six dsRNA elements are associated with MVX that range in size (640 bp - 20.2 kbp) and do not all appear together. Three dsRNAs (16.2, 9.4 and 2.4 kbp) routinely occur in asymptomatic mushrooms.

Sequence analysis of specific MVX elements is being progressed as part of the effort to characterize the complex of viruses. In a 14.4 kbp dsRNA element (MVX14.4), putative RNA-dependant RNA polymerase (RdRp) and helicase functional domains were identified. Highest similarities for MVX14.4 were observed with unencapsidated dsRNAs (the Endornaviruses); e.g. *Vicia faba* RdRp (BlastX score 209; E value 8e-52), *Lagenaria siceraria* RdRp (BlastX score 207; E value 5e-51). Phylogenetic analysis of RdRp sequences, indicates that the

MVX14.4 dsRNA is more closely related to the Endornaviruses and ssRNA plant viruses than to other dsRNA mycoviruses.
As part of a long-term strategy to develop MVX resistance in mushrooms, post-transcriptional gene silencing mechanisms are being progressed. The RdRp and helicase sequences are being introduced into MVX-infected and MVX-free mushrooms to determine whether RNAi can mediate changes in dsRNA expression and transmission.

G.10

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The effects of monoterpenes on soil fungi in coniferous forest ecosystems

Monoterpenes are volatile compounds found in many higher plants and particularly conifers. They are thought to be involved in plant defence and are found in high concentrations not only in living needles but also in the litter layer of soils.
In coniferous forests, fungi dominate the microbial community. In these nutrient-poor ecosystems, fungi are the drivers of carbon and nutrient cycling processes. Although monoterpenes are present in high concentrations in the litter layer and despite their proven antifungal properties, very little is known about their effects on the fungi that occur in forest soils and litters. If monoterpenes have differing effects on different species or functional groups among the soil fungi, this could alter fungal community structure and potentially alter rates of carbon and nutrient cycling in forest ecosystems.
A field study has been started to determine the rate of loss of monoterpenes from litters of *Picea sitchensis* and *Picea abies* as they decompose. I am also investigating the impacts of monoterpenes on a range of pure cultures of fungal species typical of coniferous forests. I then intend to study the effects on colonization of needle litters by saprotrophic fungi, and colonisation of conifer seedlings by ectomycorrhizas.

G.11

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Coordinated regulation of chitin synthesis in *Candida albicans*

β-1,3 glucan and chitin represent the major structural elements of the fungal cell wall. Chitin represents a small and variable fraction of the total cell composition but is essential for growth, viability and morphogenesis. Understanding the regulation of chitin synthesis is therefore critical for analysis of cellular growth and morphology. Since chitin is not found in mammals but is essential for fungal growth and viability, chitin also represents a potential target for antifungal drugs. In the fungal pathogen *Candida albicans*, chitin is synthesised by the products of four CHS genes. We are exploring the coordinated regulation of chitin synthesis in terms of the growth, differentiation and responses of *C. albicans* to environmental stresses. The individual roles of the four enzymes have been investigated by systematic gene disruption and by transcriptional analysis using promoter exchange experiments and a promoter-*lacZ* fusion strategy. Three signalling pathways have been identified that differentially regulate *CHS* gene expression. The anti-fungal drug Caspofungin has also been shown to activate CHS gene expression, to increase chitin synthase activity and to increase chitin content. The morphology of chitin fibrils synthesised by each of the four enzymes has been shown to be different and may have distinct roles at specific cellular locations. Work is currently underway to determine the precise spatial distribution of each of the four enzymes to determine how they act in the concerted synthesis of chitin at different cellular locations.

G.12

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SsRNA virus disease of *Pleurotus ostratus*

A mycovirus, named oyster mushroom spherical virus (OMSV), was isolated from cultivated oyster mushrooms with a severe epidemic of oyster mushroom Die-back disease. OMSV was a 27-nm spherical virus encapsidating a single-stranded RNA (ssRNA) of 5.784 kb with a coat protein of approximately 28.5 kDa. The nucleotide sequence of the virus revealed that its genomic RNA was positive strand, containing 5784 bases with seven open reading frames (ORFs).
To examine a correlation between the presence of OMSV and the disease, we eliminated OMSV from diseased isolates by adenosine 3',5'-cyclic monophosphate(cAMP) treatment. The curing of the virus converted abnormal phenotypes into normal phenotypes involving normal mycelial growth, the formation of normal fruiting bodies, and increased yield. This suggests that OMSV could be a causative agent for the disease symptoms.

G.13

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Trichogyne fusion and nuclear behavior during sexual reproduction in *Neurospora crassa*

Neurospora crassa is a heterothallic filamentous fungus which requires the fusion of nuclei of opposite mating types (mat A and mat a) to complete sexual reproduction. The initial stages of sexual reproduction involve a specialized 'female' receptive hypha (the trichogyne) growing out from the ascogonium of the protoperithecium, and fusing with a 'male' cell (a conidium). The conidium releases a peptide pheromone which causes the trichogyne to grow towards it. We have developed techniques to analyse the trichogyne-conidium interaction using live-cell imaging at high spatial resolution with confocal microscopy. Questions we are addressing include: (1) what are the mechanics of trichogyne homing towards, and fusion with macroconidia, microconidia and arthroconidia? (2) How do the opposite mating type nuclei behave before and after fusion, and do they interact in the trichogyne?

G.14

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A revision of Tubeufiaceae based on morphological and molecular data (LSU rDNA)

The family Tubeufiaceae is circumscribed for fungi in the Pleosporales that possesses superficial, white, pallid to bright, or ascomata which may darken at maturity. The family currently includes 21 genera with varied taxonomic histories, resulting from disparate opinions regarding several different morphological characters. In this study, 28S rDNA from 20 species combined with previous morphological studies are used to elucidate the phylogeny of members within the Tubeufiaceae. All new sequences were aligned with additional sequences in Pleosporales obtained from GenBank, while *Dyrithiopsis lakefuxiznensis* and *Xylaria acuta* were used as outgroups. The data set (50 taxa) was analyzed using maximum parsimony, maximum likelihood and the bayesian method. Tubeufiaceae forms a polyphyletic group while *Acanthostigma*, *Boerlagiomyces* and *Letendraea* do not belong to Tubeufiaceae. In this study, a revision of the family Tubeufiaceae is provided based on results obtained from morphological characters and phylogenetic analyses.

G.15

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The morphological study of coprophilous ascomycetes from dung in Thailand

Coprophilous ascomycetes were isolated from thirteen dung samples of wildlife and domestic animals collected from eighteen locations in Thailand. Various isolation methods, such as the moist chamber technique, soil plate, alcohol and heat treatment were used. Identifications were based on morphological characters such as colony growth pattern on artificial media. Microscopic features were examined under stereo-, light and scanning electron microscopes and camera lucida drawings was employed. Nineteen genera of coprophilous Ascomycetes were found comprising twenty-six species including ten Plectomycetes: *Emericella rugulosa, Emericella variecolor, Eupenicillium parvum, Eurotium amsterodami, Hamigera avellanea, Neosartorya fischeri, Neosartorya fumigata, Talaromycetes bacillisporus, Talaromyces flavus* and *Talaromyces rotundus*, four Discomycetes: *Ascobolus* sp., *Ascodesmis macrospora, Ascodesmis sphaerospora* and *Saccobolus glaber* and twelve Pyrenomycetes: *Cercophora* sp., *Chaetomium crispatum , Chaetomium cupreum, Chaetomium globosum, Delitschia* sp., *Echinopodospora* sp., *Gelasinospora brevispora, Podosordaria* sp., *Podospora curvicolla, Sordaria fimicola, Sporormiella minima* and *Thielavia terricola*. Antagonistic activity tests using of *Sordaria fimicola* and *Ascodesmis macrospora* with 7 plant pathogenic fungi *in vitro* are currently underway.

G.16

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Evolution of mating-type (MAT) genes of *Tapesia*, and their use as molecular diagnostics.

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 opposite mating type contain highly dissimilar

stretches of DNA know 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 domain or HMG protein motifs. At present, work is underway to characterise the mating-type regions of plant pathogenic *Tapesia* species. The resulting sequence data is being used in the development of species-specific, mating-type molecular diagnostic markers that could be used on field isolates. Sequence analysis will also provide insights into the evolution of sexuality in Ascomycete fungi by indicating whether Discomycete fungi has an idiomorph organisation similar to Pyrenomycete or Loculoascomycete classes, or if there is a novel gene arrangement.

G.17

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Isolation of two rumen anaerobic fungi *Piromyces minutus* and *Piromyces rhizinflata* from sheep in Iran

Two types of *Piromyces* anaerobic fungi (P1 and P2) were isolated from sheep rumen using anaerobic liquid medium (medium C) in serum bottle. They were then purified by the use of a roll bottle technique. Identification of the isolates was based on morphological characteristics of the fungal zoospores and their thallus. Both isolates had monocentric thalli and uniflagellate globosely zoospores (5-10 µm). The sporangial developments of P1 were endogenous and had oval or spherical (30-90 µm) shapes while the P2 isolates had endogenous or exogenous sporangium development with a long (30 µm), pyriform, oval, or spherical (100-160 µm) shapes. The morphological characteristics of thalli, sporangia, and zoospores of the two isolates indicated that they could be two different species of *Piromyces* and most probably are "*Piromyces minutus*" (P1) and "*Piromyces rhizinflata*" (P2).

G.18

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Study on endophytic strains of genus *Acremonium* in symptomless grapevines

The endophytism of *Acremonium* sp. in asymptomatic grapevines, previously ascertained in cultivars Regina and Catarratto breed in pots, was ascertained also in cv. Insolia, in field. During the phenologycal cycle of the host, by isolation tests done each month in 2003, the colonies of the hyphomycete were obtained from all the sampled organs: buds, leaves, stalks, shoots and grape-seeds. The endophytic isolation frequency, even if varied depending upon organs and seasons, reached the maximum value as far leaves as concerned (19,2% during winter); restricted percentage values were obtained as far as buds are concerned (7,4 and 9,2 during spring and summer, respectively) and as far as shoots are concerned (not more then 1,8%), while as far as stalks and grape-seeds are concerned the hyphomycete was occasional found (2,6 and 0,3% during summer and autumn). The strains of genus *Acremonium*, coming from different organs always resulted not vegetatively compatibile; the genetic variability has been studied through DNA random amplification (PCR). The results define the ecological role of endophytic strains of genus Acremonium, in symptomless grapevines. They actually indicate that host-colonization appears as organ-specific which is the typical feature of most endophytic micro-organisms in healthy plants.

G.19

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Potential use of microbial volatile production patterns for discrimination between soil treatments

There have been increasing concerns about irreversible loss of soil quality in Mediterranean areas. An early and rapid detection of changes in soil microbial activity and volatile production patterns may be key to protect soil from permanent degradation.

An electronic nose (e-nose) consisting of an array of 14 conducting polymer sensors was employed for analysis of three different soil types: an Arable soil rich in organic matter; a Mediterranean soil collected in the Algarve, South Portugal; and volcanic ash (NASA Jonhson Space Center). The potential of e-nose for discriminating between these soil types based on microbial volatile production patterns was tested. Moreover, its ability to detect temporal changes in soil microbial activity following the incorporation of glucose and cereal straw was also evaluated.

Principal Component Analysis (PCA) of data showed a clear discrimination between these soil types and different soil treatments, accounting for 90% of the variance within the data set. This approach could therefore be used to detect early changes in microbial activity and volatile patterns, in a rapid and reproducible way. It suggests that volatile fingerprints could be employed as a monitoring tool for changes in soil quality and to supplement other methods for assessing soil microbial status.

G.20

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***Trichophyton rubrum*: initial events in the infection process.**

This project centres around a study of factors involved in cell wall biosynthesis, adhesion and initial infection events in *Trichopyton rubrum*.

Dermatophytes are cosmopolitan superficial pathogens of animals and humans, found throughout the world. They invade keratinous tissues such as skin, hair and nails causing the conditions commonly known as athlete's foot and ringworm. Dermatophytosis caused by *Trichophyton rubrum* is the most prevalent fungal infection worldwide, and this pathogen has been shortlisted for sequencing by the Fungal Genome Initiative.

Despite the ubiquity of dermatophytes, there is scant knowledge of their basic biology or molecular biology. This project examines aspects of the lifecycle of *T. rubrum* that may be important in the initiation of infection. The fungal cell wall is critical in this process, as cellular integrity must be maintained throughout infection. The wall is a complex mesh of glucans, chitin and glycoproteins mediating host perception, adhesion and subsequent invasion. Detecting fungal adhesion to surfaces has enabled investigation of the effectiveness of plant-derived compounds at disrupting the adhesion process.

We have used biological and molecular techniques to study the *T. rubrum* infection process, examining germinating *T. rubrum* conidia using current chemistries and novel molecules to target specific enzymes in the cell wall biosynthetic pathway, disrupting cell wall synthesis.

We have sequenced two *T. rubrum* chitin synthase genes as well as elucidating partial sequence of genes encoding catalytic and regulatory subunits of the glucan synthase complex. DNA-mediated transformation for *T. rubrum* is under development; meanwhile, we are exploiting heterologous complementation in yeast.

G.21

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Water availability and metabolomic profiles of *Epicoccum nigrum* and *Sarophorum palmicola* grown in solid substrate fermentation systems.

There has been interest in using environmental screening procedures for enhancing the metabolomic production profiles by fungi which are of pharmaceutical interest. This study examined two ecologically distinct species, *Epicoccum nigrum* and *Sarophorum palmicola*, in solid substrate fermentation systems over a range of water availability conditions (water activity, aW). Total secondary metabolite profiles were obtained by HPLC + diode array detection after 14 days incubation on cereal-based substrates in relation to four aw treatments. Temporal studies (24 d, *E. nigrum* and 18 d, *S. palmicola*) showed that metabolite production profiles varied markedly between the two fungi. For *E. nigrum*, metabolite production generally increased with reduction in aw, and was optimal in the range 0.99 – 0.97. In contrast to this, for *S. palmicola*, metabolite production was restricted to the highest aW level used, 0.998, and declined to zero at 0.99 aw. Statistical analysis revealed that time, aw and their interactions were significant in all cases ($p<0.001$). The potential for using ecophysiological stresses for enhancing natural product discovery is discussed.

G.22

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Medium optimization for the production of the secondary metabolite squalestatin S1 by a *Phoma* sp. combining orthogonal design and response surface methodology.

In the present work, a combined statistical approach of orthogonal design (L27(313), response surface techniques and polynomial regression were applied to optimize the composition and concentration of a liquid fermentation medium for the production of squalestatin S1 by a fungus (a *Phoma* species). Optimal conditions for maximal titres and productivity were determined based on thirteen parameters at three different levels. Initially, a screening design methodology was used to evaluate the process variables which were relevant to S1 titre and the response surfaces applied to find optimal regions for production. The sources of carbon and concentration, and their interactions with oily precursors were statistically significant factors. The combined orthogonal design and response surface methodology predicted optimal conditions for of 273 mg l-1 of squalestatin S1. Confirmatory experiments of the optimal medium composition produced titres of 434 mg l-1 in a five day fermentation at 25oC. This represented a 60% improvement in the maximum titre predicted, and a two-fold higher productivity when compared with reported S1 yields of various fungal species. This combined statistical approach enables rapid identification and integration of key medium parameters for optimising secondary metabolite production and could be very useful in pharmaceutical screening programmes.

G.23

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Evolutionary fitness in itraconazole susceptible and resistant *Aspergillus fumigatus* clinical isolates

Introduction: *Aspergillus fumigatus* is the most common aetiological agent of aspergillosis. Invasive aspergillosis is a major cause of death in leukaemic and organ transplant patients. The increasing rates of recovery of antimicrobial resistant micro-organisms in hospital and community settings are of growing concern. The evolutionary changes of drug resistance and its fitness penalty have been extensively studied in bacteria, viruses and yeasts. In many organisms, mutations that confer resistance to drugs confer a biological fitness cost that is expressed as decreased growth rate, survival or virulence. Loss of fitness causes resistant organisms to become disadvantaged relative to susceptible strains in the absence of drug pressure.

Methods: Selected itraconazole susceptible and resistant clinical isolates of *A. fumigatus* were studied. Various measures of growth rate (colony radial growth rate and specific growth rate), germination time and conidial yields have been determined on various media. Mutations in the 14 alpha lanosterol demethylase gene, cyp51A, were determined.

Results: There was a correlation between resistance and specific growth rate ($p = 0.03$). Generally, resistant isolates grow more slowly than susceptible isolates. Three resistance profiles were observed in these clinical isolates. One isolate was resistant to itraconazole only, two isolates were resistant to itraconazole and ravuconazole, and two isolates were resistant to itraconazole, voriconazole, ravuconazole, and posaconazole. Sequencing the cyp51A gene of these isolates revealed three different amino acid substitutions at three different codons.

Conclusions: It is hoped that this and additional molecular studies will highlight mutations that are the potential cause(s) of resistance in the various isolates and that correlations can be made between different types of mutation (e.g. single nucleotide polymorphisms in the demethylase gene versus overexpression of efflux pumps) and measures of evolutionary fitness.

G.24

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Regulation of hyphal growth and morphogenesis by RhoGTPases in filamentous homobasidiomycetes

Filamentous fungi grow by polar extension of hyphae. This growth pattern changes at differentiation, at mating, and at pathogenic or symbiotic interactions. The small Rho GTPases Cdc42, Rac and Rho play an important role in regulating cell polarity and in linking the extracellular and intracellular signals to reorganization of actin cytoskeleton. Recently, the small GTPase Cdc42 of the ectomycorrhizal homobasidiomycete *Suillus bovinus* was cloned and its function investigated. The replacement of polar growth of vegetative hyphae by isotropic growth with actin and SbCdc42 redistribution suggested that SbCdc42 plays a central role in *S. bovinus* hyphal morphogenesis at ectomycorrhiza formation. These observations could not be experimentally verified since the transformation system of the fungus is only under development. However, the ectopic expression of constitutively active Sccdc42 allele in Schizophyllum commune closely related to S. bovinus was shown to induce branch emergence at exceptional sites and isotropic growth of the hypha next to the septum indicating that ScCdc42 regulates the branch site selection and subsequent hyphal development. In transformants with the constitutively active Sccdc42 allele the tip growth of apical cells in the leading hyphae was normal suggesting involvement of another RhoGTPase in the regulation of apical polarity in leading hyphae.

G.25

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Intracellular laccases activity of the vegetative mycelium of different *Pleurotus* species

Laccases, manganese-peroxidases and lignin peroxidases belong to the enzymatic complex that degrade lignin and are classified as phenol-oxidases. Laccases are secreted in multiple isoforms, depending mainly on the fungal species and environmental conditions. These enzymes have been found in white-rot fungi such as *Pleurotus* species, which are often cultivated on ligninocellulosic substrates (e.g. maize, barley or wheat straw).

In this research, the constitutive intracellular laccases activity of the vegetative mycelium of ten strains of *Pleurotus* was determined in vitro and by zymograms, using different substrates. Differences in the in vitro activities were observed between all the strains, however, zymogram patterns were only similar for strains within same species, independently of any of the three substrates (2,6-dimethoxyphenol, □-anisidine or □-tolidine) used. The differences observed in the number and positions of the isoforms in the gel suggest that laccase zymograms can be used to differentiate species of this organism.

G.26

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Seasonal Variation in Ectomycorrhizal Fungi upon Beech - Fagus sylvatica : a comparison between summer and winter species composition using molecular analysis

Ectomycorrhizae form a significant part of the wood-wide web paradigm; however the diversity and spatial heterogeneity of ectomycorrhizal species forming these symbiotic communities has not been systematically characterised. Little research has investigated the relationships of ectomycorrhizal communities and the age of host trees - this relationship can be viewed as the succession of ectomycorrhizae upon a host root. This matter is further complicated by temporal changes in the community structure over an unknown timespan – possibly of the order of years. Recent advances in molecular methodology have facilitated the identification of mycorrhizae from the associative tissue, which cannot be characterised to a species level based on gross anatomical results alone. The aims of this project were to investigate the diversity of ectomycorrhizae in summer months; repeat an earlier investigation between ectomycorrhizal diversity and host age using the summer sampling results and to identify the ectomycorrhizal morphotypes of interest in both winter (previously collected) and summer sampling using ITS sequence data. Ectomycorrhizal communities were sampled and ITS sequences were generated from a single woodland. Ordination techniques demonstrated that there is a significant association of specific morphotypes with adult trees (as opposed to seedling or sapling). A comparison in the ectomycorrhizal community composition shows that a level of seasonal variation may occur within woodland with a different composition of species forming the community in summer and winter.

G.27

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Phospholipases of *Aspergillus fumigatus*

Aspergillus fumigatus is an opportunistic pathogen responsible for around 90% of all cases of disseminated *Aspergillus* infections in immunocompromised patients. Infections normally arise through inhalation of spores which penetrate the alveoli and come into contact with surfactant, composed of around 80% phospholipid. Extracellular phospholipase activity has previously been demonstrated. In the presence of phosphatidylcholine, the colony radial growth rate of *A.fumigatus* increased two-fold by decreasing branching frequency without affecting the overall specific growth rate. Phosphorylcholine, a degradation product of the action of phospholipase C was shown to also significantly increase the colony radial growth rate whereas the breakdown products of other phospholipases did not. Real time PCR revealed a greater level of expression in the presence compared to the absence of phospholipid in the media. The stimulation of surface growth in response to PLC mediated phosphatidylcholine may have implications for colonisation and invasion of lung tissue.