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POSTERS

Analysis of sterol regulation in the *Saccharomycotina.*

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Many fungal pathogens grow on superficial and in internal sites in infected hosts, regions that have considerably different oxygen levels. A better understanding of the influence of hypoxia (low oxygen) on virulence and pathogenesis may lead to improved treatments against systemic fungal infections. Hypoxia induces filamentation of *Candida albicans*, which is associated with virulence. However other pathogenic *Candida* species do not grow as filaments, and presumably respond to hypoxia in different ways. In many fungal species (including the *Saccharomyces* and *Candida* clades) the transcription factor Upc2 plays a major role in regulating expression of the sterol pathway, particularly in low oxygen conditions. In other fungi, such as fission yeast *Schizosaccharomyces pombe* and the human pathogens *Aspergillus fumigatus* and *Cryptococcus neoformans*, expression of sterol genes is regulated by SREBP (Sterol Regulatory Element Binding Protein). SREPB is an ancient regulator that is conserved in mammals (including humans). The genome of *Yarrowia lipolytica*, an out-group of Saccharomyces and *Candida* clade species, contains homologues of both Upc2 and SREBP. By carrying out gene deletions in this species, we have shown that the appearance of Upc2 coincides with the acquisition of sterol pathway regulation. Our results also suggest that the remnants of the SREBP proteins regulate morphology and filamentous growth.

Efg1 regulates morphology and biofilm formation in *Candida parapsilosis.*

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The incidence of infection by *Candida parapsilosis* has risen substantially in recent years, possibly because it is easily transmitted to patients from contaminated external sources such as medical devices, catheters, or the hands of health care workers. One of the known virulence factors of *C. parapsilosis* is its ability to form biofilms on indwelling medical devices. In the related species *Candida albicans*, the transcription factor EFG1 is a major regulator of biofilm development and hyphal growth (Ramage et al., 2002). Efg1 is also an important regulator of white-to-opaque switching (Zordan et al., 2007) The role of EFG1 in *Candida parapsilosis* has not yet been studied in detail. Here we show that in contrast to *C. albicans* the Efg1 ortholog in *Candida parapsilosis* is a major regulator of a morphological switch at the colony level, from wrinkled to smooth phenotypes. The rate of switching is greatly increased in an efg1 knockout. The phenotypes of the two colony types are significantly different, suggesting that that there are differences in the cell walls. Smooth cells with an *efg1* deletion are more sensitive to congo red, caspofungin and calcofluor white. In addition, deleting efg1 reduces biofilm formation in in vitro models, particularly for smooth cells. Biofilm reduction is not as significant in in vivo models. Analysis of ChIP-seq and RNA-seq data shows that Efg1 binds to the promoters of several transcription factors and regulates expression of cell wall genes. Results suggest an important role of Efg1 in cell wall regulation and biofilm formation and an ancient role in morphological transition.

Investigating the biological activities of a bacterial metabolite in two model organisms.

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Naturally occurring antifungal compounds are abundant and very diverse and are mostly considered to be produced to regulate the growth of competing organisms in environments such as the rhizosphere of plants. However, in recent years the concept of antibiotics as signalling molecules has emerged and receives rising attention. In this study we are investigating the effects of the secondary metabolite 2,4-diacetylphloroglucinol (DAPG), which is produced by a few *Pseudomonas* spp. frequently associated to the rhizosphere. This metabolite exhibits a broad spectrum of antimicrobial activity but little is known about its cellular targets or possible fungal resistance mechanisms. We are using two model organisms, *Saccharomyces cerevisiae* and *Neurospora crassa*, to address these questions. DAPG treatment impairs cell growth in both organisms and specifically causes loss of membrane potential in mitochondria suggesting that electron transport is a target. A screen of the yeast deletion library revealed that alterations of several different processes, such as protein biosynthesis and DNA repair, can confer resistance. We also found that in both *S. cervisiae* and *N. crassa*, DAPG induces a transient cytoplasmic Ca2+ signal. The relevance of this signal is part of our current investigations but it may indicate a possible role of DAPG as a signal. The outcomes of this study could facilitate understanding the mode of action of antifungals/antibiotics and their role in inter-and intra-species communication but also help exploitation of this metabolite for agri-biotech and other applications.

Trichosporon mucoides Fungaemia in a Patient with Solid Organ Tumor.

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Introduction. We report a case of *Trichosporon mucoides* central line associated fungaemia in a 20 year old female with disseminated Ewing's sarcoma. *Trichosporon* species are fungi that commonly inhabit the soil and are known to be present in the normal flora of the skin and gastrointestinal tract of humans. *Trichosporon* spp. more commonly cause superficial infections such as white piedra. However, recently there has been an upsurge of invasive Trichosporinosis in patients with haematological malignancies and recent transplants. Although Candida is by far the most common cause of disseminated yeast infections in humans, *Trichosporon* is making itself known as a less common but still important causative organism in these cases.

Aim. To report a case of central line related fungaemia caused by *Trichosporon* mucoides in a patient with solid organ tumor and its management.

Method: Case report with a review of the literature.

Case Report: Our patient is a 20-year old female with newly diagnosed disseminated Ewing's sarcoma. She presented with ongoing pyrexias and raised inflammatory markers following treatment with broad-spectrum antibiotics for presumed urinary tract infection. Central line related fungaemia was diagnosed following two separate blood cultures obtained four days apart via her PICC line with a good growth of *Trichosporon mucoides*. The line was removed and the patient was treated with liposomal amphotericin B.

Conclusion: This is our first reported case of *Trichosporon mucoides* line related fungemia highlighting an unusual presentation in solid organ tumor. Early detection and appropriate treatment is essential for a good patient outcome.

Epidemiology and Antifungal Susceptibility of *Aspergillus fumigatus* in an Irish Cystic Fibrosis Patient Cohort

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Aspergillus fumigatus is an opportunistic pathogen known to cause a spectrum of diseases including Allergic Bronchopulmonary Aspergillosis (ABPA) and life-threatening angioinvasive pulmonary disease. *A. fumigatus* is the most common agent causing fungal infections in Chronic Lung Disease such as Cystic Fibrosis (CF), most often presenting as ABPA, whereas *A. fumigatus* commonly presents as Invasive Aspergillosis (IA) in immunocompromised individuals. There are a number of antifungal agents available for the treatment of *A. fumigatus* infections but triazole antifungal resistant *A. fumigatus* strains in CF have been reported [1]. In this study the epidemiology of *A. fumigatus* in an Irish patient population consisting of a CF patient population pre and/or post itraconazole treatment in a major CF Centre (Hospital 1) and a non-CF patient population from a major Teaching Hospital (Hospital 2) was investigated and the anti-fungal susceptibility of all isolates collected was determined.

A. fumigatus isolates from colonized adult CF patients (n=19) and from non-CF patients were collected (n=37). All isolates from the study were confirmed as *A. fumigatus* by PCR and sequencing of the ITS region. Isolates were genotyped using the Short Tandem Repeat assay for *A. fumigatus* (STR*Af* assay) [2]. Minimum Inhibitory Concentrations (MICs) of all *A. fumigatus* isolates to nine anti-fungal drugs were tested using the Sensititre Plate system (TREK Diagnostic Systems, Magellan Biosciences)

Three distinct *A. fumigatus* colonization patterns were observed in the CF cohort, (1) persistent colonization over time with the same genotype (>2 consecutive samples with indistinguishable genotypes), (2) non-persistent colonization with distinguishable genotypes over time and (3) patients sharing an indistinguishable genotype suggesting the possibility of a common source of acquisition. No shared genotypes between the two hospitals were found. These colonization patterns were observed in both CF and non-CF patients. No antifungal drug resistance was observed from any study isolate, even for isolates collected following exposure to itraconazole for 6 weeks. Twelve of 56 *A. fumigatus* isolates had MICs of 2µg/ml for amphotericin B and further investigation is required here.

No *A. fumigatus* genotype was linked with any one underlying disease or colonisation pattern. This suggests the impact of *A. fumigatus* on the patient may be a host trait. Some patients shared indistinguishable genotypes suggesting a common source. No triazole antifungal resistant strains of *A. fumigatus* were detected during this study. Twelve strains had higher than expected amphotericin B MICs which warrants further investigation.

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2. de Valk HA, Meis JF, Curfs IM, Muehlethaler K, Mouton JW, Klaassen CH. Use of a novel panel of nine short tandem repeats for exact and high-resolution fingerprinting of *Aspergillus fumigatus* isolates. J Clin Microbiol. 2005 ; 43:4112-4120.

Investigating potential links between gene composition and protein thermostability in filamentous fungi.

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In Nature, a variety of pathogenic and saprophytic microorganisms play a vital role in the deconstruction and decomposition of plants and plant-derived residues and wastes. While this microbial ecosystem comprises both fungi and bacteria that co-exist and compete in this natural composting process, the filamentous fungi occupy a pivotal role as key players in the biological conversion

of plants and plant-derived wastes. During the different phases of natural biodegradation, the temperature profile of the composting biomass changes, with the most efficient decomposition taking place during the thermophilic phase. The temperatures in the thermophilic phase generally range from 40-60°C, but can reach up to 70°C. Understanding the phenomenon of plant decomposition and the role of different microorganisms in this process is as central to developing new strategies to combat microbial spoilage of food crops. However, it is also essential for the development of new 'green' and 'white' biotechnology approaches to harness the potential of non-food crops and plant-derived wastes for bioenergy and commodity products.

We have investigated the enzyme machinery and enzyme systems produced by thermophilic, saprophytic fungi. Our work has shown that some of these fungi produce multiple cellulases, hemicellulases and other enzymes that are essential for conversion of plant cell wall biopolymers to simple building blocks. Many of the enzymes are thermostable and act as very effective and efficient biocatalysts. More recently, this work has involved investigating structural and biochemical factors that may enhance the stability of specific biomass-degrading enzymes in comparison with their mesophilic counterparts. Our findings suggest that, although individual covalent and non-covalent interactions may influence enhanced protein stability, no one structural feature is singularly responsible for thermostablity, which also supports reports from other researchers in the field. In this report, we discuss some of the findings to-date and explore whether or not potential clues exist at a genetic level for specific thermostable biomass-degrading enzymes from specific thermophilic fungi, in comparison with the same enzymes from selected mesophilic fungi, and less thermostable enzymes from then same thermophilic fungi.

POSTERS

Analysis of hypoxic regulation in *Candida glabrata* and related species.

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Pathogenic *Candida* species colonize hypoxic (low oxygen concentration) environments such as deep in tissue, or in periodontal spaces. We used microarrays to determine the transcriptional response of *Candida glabrata* to low oxygen levels, and to evaluate the role of specific transcription factors. We showed that lowering sterol levels acts as a signal for reduced oxygen, by comparing the transcriptional response of cells treated with ketoconazole (which targets azoles) to those grown in low oxygen conditions. We also determined the transcription profile of *C. glabrata* cells that are deleted for the transcription factor RXL1 (ROX1-like), in hypoxia and normoxia. *C. glabrata* RXL1 is a paralogue but not an orthologue of S. cerevisiae ROX1, a major regulator of the hypoxic response in yeast. C. glabrata has lost the ROX1 orthologue, and *S. cerevisiae* has lost RXL1. We show that in *C. glabrata* RXL1 acts as a repressor of expression of hypoxic genes. The hypoxic response in *C. glabrata* is therefore conserved with other species such as S. cerevisiae but the regulation is different.

We also investigated the regulation of the hypoxic response *in Naumovozyma castellii,* a close relative of *C. glabrata* and *S. cerevisiae*, which contains both ROX1 and RXL1 orthologues. *N. castelli* has a very unusual hypoxic response, with no increased gene expression and very little reduced gene expression. However, we found that *N. castelli* has a similar response to *C. glabrata* to treatment with cobalt chloride ($CoCl_2$), a commonly used hypoxia-mimicking agent. We used microarrays and RNA-seq to show that $CoCl_2$ induces expression of sterol and mitochondrial genes in both *N. castellii* and *C. glabrata*. ROX1 is required for the $CoCl_2$ induction of heme synthesis, sterol synthesis and mitochondrial function. Our results suggest that Rox1 has an ancestral role in regulating the hypoxic response and that Rxl1 has taken over this role in the human fungal pathogen *C. glabrata*.

Biomass to biofuel: Towards the bioengineering of *Saccharomyces* species for cellulose degradation.

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As the world enters the post-fossil fuel era, there is a need to exploit environmentally sustainable energy sources. One such potential energy source is the use of biofuels derived from lignocellulose-based biomass. Cellulose, the main component of lignocellulose, is made up of repeating units of the disaccharide cellobiose, which is comprised of two glucose molecules linked by a Î"-1,4 glycosidic bond. The most characterized microorganism known to ferment simple sugars to ethanol are yeasts belonging to the *Saccharomyces stricto sensu* group, however *Saccharomyces* species do not possess the cellulases required to degrade cellulose. However, the filamentous fungi *Trichoderma reesei* encodes all three major types of cellulases, namely endoglucanases, cellobiohydrolases also and Î"-glucosidases.

The aim of this project was to combine the fermentative capacity of *Saccharomyces* species with the cellulolytic ability of T. reesei to simultaneously saccharify and ferment a cellulose substrate into ethanol. Three cellulolytic genes of *T. reesei* were cloned separately into the bakers yeast *Saccharomyces cerevisiae* and also into a proprietary stress tolerant strain of the industrial yeast S. pastorianus. Each cellulase encoding gene was expressed under the control of a *S. cerevisiae* constitutive promoter. Functional cellulase activity was observed in transformed yeast. The resultant cellulase producing S. cerevisiae and S. pastorianus strains were separately co-cultured with phosphoric acid swollen cellulose (PASC) to produce ethanol. Experiments are being conducted to optimize the activity levels of recombinant cellulases, fermentation conditions and strain selection to increase ethanol production.

The quantification and optimisation of recombinant beta-glucosidase in various *Sacchraomyces* species.

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As our energy requirements and oil prices rise, the need for a more sustainable and environmental friendly fuel source has become a necessity. Biomass has been identified as a possible replacement due to its abundant and renewable nature, with the waste portion namely lignocellulose being of great interest. Lignocellulose is composed of three main components, lignin, hemicellulose and cellulose with the latter two being our main interest. To optimise alcohol production, the use industrial yeast strains, which are conditioned to producing and tolerating high levels of alcohol, is a logical choice, although currently these yeast are unable to naturally ferment both cellulose and hemicellulose.

The overall aim of this project is to reconstitute the lignocellulosic

degrading machinery from cellulose degrading fungal species within industrial strains, allowing for the effective hydrolysis of lignocellulosic biomass into fermentable sugars and subsequent alcohol production.

Building on previous work, the expression of the lignocellulosic degrading gene beta-glucosidase, which is responsible for the final stage in cellulose degradation has been quantified in various *Saccharomyces* species. Environmental conditions were varied to optimise production and secretion of the enzyme, resulting in increased activity. Using these new strains growth on cellobiose was examined.

Roles of telomere-associated (*TLO*) genes in the pathogenesis of *C. albicans* and *C. dubliniensis.*

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Candida albicans is widely regarded as the most pathogenic yeast species. Surprisingly the very closely related species *Candida dubliniensis* is far less pathogenic. Comparative genomic analysis of the two species revealed that the genomes are very similar however there is a significant disparity in the copy number of a family of telomere-associated (*TLO*) genes, which encode putative transcriptional regulators. The *C. albicans* genome contains 14 *TLO* genes whereas the far less pathogenic *C. dubliniensis* only encodes two. We hypothesise that this discrepancy in *TLO* copy number may contribute to the differential virulence of these two highly related species.

We have previously shown that the homozygous deletion of both *TLO1* and *TLO2* in *C. dubliniensis* (*tloDD*) significantly reduces its ability to form true hyphae. Subsequent work has also shown that the *tloDD* mutant grows poorly in the presence of alternative carbon sources, such as galactose and succinate, and is more sensitive to oxidative stress induced by H2O2 or menadione. Expression of *CdTLO1* and *CdTLO2* from a doxycycline-inducible promoter in *C. dubliniensis tloDD* restored levels of true hypha formation to 80% and 40% of wild-type respectively. Similarly, expression of selected *C. albicans TLO* genes in the *tloDD* mutant also restored true hypha formation suggesting that the *TLO* families play similar roles in the two species. DNA microarray studies have shown that *C. dubliniensis tloDD* displays altered expression of a number of genes encoding filamentation regulators (*EFG1*, *UME6*), hyphal cell wall proteins (*HWP1,RBT5*), oxidative stress response genes (*SOD1-6*) and genes essential to galactose metabolism (*GAL1,7, 10*).

The presence of a conserved putative Med2-binding domain (associated with RNApol II mediator complex) in the CaTlo and CdTlo proteins suggest that they are a family of transcriptional regulators and we are currently investigating the relationship between the Tlo and Med families.

Whole Genome Sequence Analysis of ten *Candida dubliniensis* isolates.

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Aim: Previous multilocus sequence typing (MLST) analysis has shown that the population structure of *Candida dubliniensis* is significantly less divergent than that of its closest relative, *Candida albicans*. Furthermore, *C. dubliniensis* has undergone loss in some gene families such as the agglutinin-like sequence (*ALS*) and telomere-associated (*TLO*) families, and pseudogenization of genes proposed to play a role in pathogenesis. The present study undertakes a detailed analysis of genomic content and variation of ten *C. dubliniensis* isolates that represent each of three previously identified MLST clades.

Methods: Ten isolates were sequenced using the Solexa/Illumina technology, and reads were aligned against the reference CD36 sequence using SHORE software v5, allowing determination of normalized coverage scores for all *C. dubliniensis* open reading frames (ORFs) and identification of single nucleotide polymorphisms (SNPs).

Results: Homozygous and heterozygous SNPs were quantified for each of the ten isolates. The majority of SNPs were homozygous in *C. dubliniensis*, in contrast to *C. albicans*, and has been observed previously using MLST. Phylogenetic analysis using homozygous and heterozygous SNP data also identified a population structure that has previously been shown using MLST, consisting of three closely related clades. Normalized coverage scores were obtained for each ORF across the ten isolates. The majority of ORFs that exhibited significant variation or undercoverage amongst the ten isolates encoded transposable elements or hypothetical proteins. In some cases these undercoverage scores may be the result of poor alignment to, or ORF duplication in the reference CD36 sequence. The CaALS DNA sequences were used in separate Basic Local Alignment Search Tool (BLAST) analyses against de novo assemblies of seven isolates, and identified seven *CaALS* homologues in each. No homologues of *CaALS3* or *CaALS5* were identified in any of the assemblies, as previously observed in CD36. The *CaTLO* DNA sequences were used in BLAST searches against the same de novo assemblies and identified two homologues, *CdTLO1* and *CdTLO2*. Interestingly, the genomic region identified upon BLAST search of *CdTLO2* in isolate CD06037 did not align with the reference *CdTLO2* sequence. Further global genomic alignment data and the low coverage score (0.012) for this ORF further suggested that *CdTLO2* is absent in CD06037. Alignment data also identified the movement of *CdTLO2* from chromosome R to chromosome 3 in isolate Wu284.

Conclusions: Our data correlate with previous work that indicates a preference for homozygous SNPs, and a low level of intraspecies divergence in *C. dubliniensis*. No homologues of *CaALS3* or *CaALS5* genes were found, and only the *CdTLO1* or *CdTLO2* genes were identified in the *C. dubliniensis* isolates, suggesting that these gene families are truly reduced in *C. dubliniensis*.

Generation and analysis of putative Tor1 hyperactive mutants of *Candida albicans*.

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Candida albicans is a commensal inhabitant of the alimentary canal capable of opportunistic infections in a debilitated or compromised host. Responsible for a wide range of superficial to life threatening infections, *Candida* spp. are the fourth most common cause of nosocomial bloodstream infections. The ability to adapt to various nutritive environments presented by the host is essential to its survival as commensal or pathogen. Thus, understanding how cell growth is controlled in response to environmental signals remains an active field of research.

Target of rapamycin (TOR), a conserved protein kinase, is highly conserved from mammalian to yeast cells. In eukaryotes, TOR is responsible in regulating metabolism and the cell cycle. In *Saccharomyces cerevisiae*, TOR is stimulated by amino acids and an active TOR kinase promotes ribosome biogenesis and glycolysis, as well as repressing genes involved in nitrogen scavenging. Current literature presents several lines of evidence indicating that in *Candida* spp., TOR may play a role in the transition of yeast to hyphae.

The aim of this project is to determine if the TOR1 fungal kinase is a central regulator of virulence. It is hypothesised that an active kinase represses virulence and filament formation. Subsequently, we aim to determine whether strategies to activate this kinase could be valuable in controlling infection. Preliminary work has involved generating a TOR hyperactive mutant using an overexpression strategy with the Enolase 1 promoter (ENOp).

ENOp was placed upstream of VAM6, a protein reported to control TOR complex 1 in S. cerevisiae, in order to promote TOR hyperactivity. Further to this, ENOp was also placed upstream of TOR1 itself and the recently characterized G-protein RHB1.

We will primarily focus on determining the phenotypic differences between mutant and WT strains. Additionally, we will examine whether nutrient depletion or the addition of the drug rapamycin can restore filamentation. Changes in gene expression and the host-cell interactions will be examined.

Chromatin remodelling during stationary phase in *Saccharomyces cerevisiae.*

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Eukaryotic genomes are organised as the DNA:protein complex chromatin. The fundamental subunit of chromatin is the nucleosome which comprises 147 base pairs of DNA wrapped around an octamer of histone proteins. This structure is generally repressive to any process, such as transcription, which requires access to the DNA. However, chromatin can undergo changes in its structure which can alter its function.

Histones are the substrate for numerous post-translational modification (PTMs) including acetylation and methylation. Of particular interest is the modification of histone H2B by the addition of ubiquitin (ub), a 76 amino acid protein. In the budding yeast Saccharomyces cerevisiae histone H2B is ubiquitylated at lysine residue 123 in the C-terminal tail. H2B ubiquitylation plays a role in transcription elongation and regulates the downstream methylation of histone H3 at lysine residues 4 (H3K4) and 79 (H3K79).

Recent work has shown that yeast stationary phase (SP) cultures comprise two distinct populations of cells with divergent developmental fates. SP cultures compromise older mother cells that undergo apoptosis and necrosis, and younger daughter cells which become quiescent. The daughter cells retain the ability to re-enter the cell cycles upon nutrient replenishment.

Little is known about the fate of chromatin during SP except that chromosomes become condensed. This event accompanies a general shutdown of transcription. In this project the two SP populations have been separated and the chromatin in each population has been examined. The results have shown that H2B ubiquitylation is lost upon entry into SP, whereas other histone PTMs associated with active transcription are retained during SP. We have also shown that H3K79 di-methylation is specifically depleted in the quiescent population. We are currently investigating the significance of this chromatin remodelling during SP.

Investigating gene repression by the Tup1p-Ssn6p complex in *Saccharomyces cerevisiae.*

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Transcriptional repression is an important part of gene regulation. In the budding yeast *Saccharomyces cerevisiae* the Tup1p-Ssn6p corepressor complex is recruited to gene promoters to repress transcription in response to nutrient depletion, DNA damage and numerous other signals. The current model for Tup1-Ssn6-mediated repression dictates that Tup1p promotes repression, while Ssn6p acts as an adaptor between Tup1 and the target gene. The aim of this project is to (i) elucidate the contribution of the Tup1p and Ssn6p subunits of the complex to gene repression and (ii), determine if Tup1p and Ssn6p can regulate gene repression independently from each other. The results will help elucidate the precise mechanism of action of gene repression by the evolutionary conserved Tup1p-Ssn6p corepressor complex.

Fungal SMC bioconversion: A potential greener technology.

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The Irish mushroom industry produces over 400,000 tons of spent mushroom compost (SMC) annually. Currently, this by-product is employed as a soil conditioner; however, the application for SMC is considered very limited due to associated environmental and health impacts. The relatively high carbohydrate content of SMC makes it a suitable alternative feedstock in the biofuel and biorefinery sectors. Biochemical analysis of SMC indicates its potential use as an inexpensive nutrient source for microbial enzyme production. Ligno-cellulolytic enzymes are produced by different fungi. Many of the xylanase- and cellulase-rich commercial enzyme preparations are derived from fungal sources. In this study, we compare the proximate, carbohydrate and metal ion compositions of SMC from different sources and SMC from one of the sources at different annual time points. We also compare the conversion of SMC using the commercial enzyme preparations and an in-house preparation from a thermophilic fungal source. The study shows that the fungal enzymes can be used to generate hydrolysates rich in fermentable sugars for downstream bioenergy production. This process avoids the use of harsh chemical reagents and high operating temperatures which would be beneficial for large scale industrial development and commercialisation.

