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Mini-Review

The first fifty microarray studies in filamentous fungi

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Microarray studies have examined global gene expression in over 20 species of filamentous fungi encompassing a wide variety of research areas. The majority have addressed aspects of metabolism or pathogenicity. Metabolic studies have revealed important differences in the transcriptional regulation of genes for primary metabolic pathways between filamentous fungi and yeast. Transcriptional profiles for genes involved in secondary metabolism have also been established. Genes required for the biosynthesis of both useful and detrimental secondary metabolites have been identified. Due to the economic, ecological and medical implications, it is not surprising that many studies have used microarray analysis to examine gene expression in pathogenic filamentous fungi. Genes involved in various stages of pathogenicity have been identified, including those thought to be important for adaptation to the host environment. While most of the studies have simulated pathogenic conditions *in vitro*, a small number have also reported fungal gene expression within their plant hosts. This review summarizes the first 50 microarray studies in filamentous fungi and highlights areas for future investigation.

Introduction

Microarray analysis allows the identification of differentially regulated genes from multiple distinct samples differing in environmental condition, developmental stage or genetic background. The first fungal microarray studies were reported in the budding yeast *Saccharomyces cerevisiae* 10 years ago (DeRisi *et al.*, 1997; Lashkari *et al.*, 1997). Since then microarrays have been fabricated for more than 20 species of filamentous fungi. The majority have been constructed using PCR products amplified from expressed sequence tag (EST) libraries. More recently, arrays including all predicted ORFs have enabled more comprehensive genome-wide expression studies. This review describes the earliest 50 microarray studies retrieved by a search of the Web of Science database using the query 'microarray* AND fungus'. The first 50 microarray studies in filamentous fungi have covered a broad spectrum of research areas, including metabolism, development, pathogenesis, symbiosis and industrial applications (Table 1).

Metabolism

Microarray studies of primary metabolism have revealed striking differences between filamentous fungi and budding yeast. Bonaccorsi *et al.* (2006) examined the effects of altering oxygen availability on cultures of the obligatory aerobe *Trichoderma reesei*. The majority of genes for the glycolytic pathway and TCA cycle were strongly repressed under oxygen-free conditions. Such a reduced expression of glycolytic genes would be insufficient to maintain anaerobic metabolism for prolonged periods without oxygen. In contrast, *S. cerevisiae* has been previously shown to up-regulate glycolytic genes in oxygen-free conditions, allowing metabolism to continue anaerobically (Kwast *et al.*, 2002). Several studies have examined the effects of glucose starvation on primary metabolic genes (Chambergo *et al.*, 2002; Maeda *et al.*, 2004; Xie *et al.*, 2004). In *S. cerevisiae* strong glucose repression of TCA cycle genes means that glucose is preferentially metabolized anaerobically, and all TCA cycle genes are strongly up-regulated during glucose starvation (DeRisi *et al.*, 1997). In *T. reesei* several TCA cycle genes were only partially up-regulated while others were unaffected by glucose starvation (Chambergo *et al.*, 2002). In *Neurospora crassa* and *Aspergillus oryzae* some TCA cycle genes were even down-regulated by glucose starvation (Maeda *et al.*, 2004; Xie *et al.*, 2004). It is clear that the same degree of glucose repression does not operate in filamentous fungi and that aerobic respiration has a greater role in glucose metabolism by filamentous fungi than by *S. cerevisiae.*

Glucose starvation experiments have also revealed differences in ethanol utilization among filamentous fungi. Similar to the case in *S. cerevisiae*, the alcohol dehydrogenase responsible for ethanol assimilation in *T. reesei* was found to be constitutively expressed before and during glucose starvation (DeRisi *et al.*, 1997; Chambergo *et al.*, 2002). This is in contrast to *A. oryzae* and *N. crassa*, where it was strongly induced during glucose starvation (Maeda *et al.*, 2004; Xie *et al.*, 2004). Glucose repression of alcohol dehydrogenase is well characterized in *Aspergillus nidulans* and is mediated through the transcription factor CreA (Flipphi *et al.*, 2002). CreA is also known to repress many other metabolic genes under glucose-rich conditions,

Table 1. The first fifty microarray studies in filamentous fungi

Organism	Results* and validation†	Genes/ESTs represented and format‡	Reference
	Metabolism		
Aspergillus nidulans	Identified terrequinone A biosynthetic gene cluster (N)	9541§ (Nim)	Bok *et al.* (2006)
Trichoderma reesei	Identified oxygen regulated genes (N, RT)	2000 (cDNA)	Bonaccorsi *et al.* (2006)
Aspergillus parasiticus‖	Identified aflatoxin biosynthesis genes (RT)	5002 (cDNA)	Price *et al.* (2006)
Phanerochaete chrysosporium	Obtained profiles of P450 monooxygenase genes (RT)	190 (oligo)	Doddapaneni & Yadav (2005)
Aspergillus parasiticus‖	Identified aflatoxin biosynthesis genes (PM)	753 (cDNA)	Price *et al.* (2005)
Aspergillus oryzae	Obtained profiles of metabolic and industrially important genes (N)	2070 (cDNA)	Maeda *et al.* (2004)
Fusarium verticillioides	Identified fumonisin biosynthesis genes (RT)	716 (cDNA)	Pirttilä *et al.* (2004)
Aspergillus nidulans	Evaluated subtraction library enriched with polysaccharide metabolism genes (N)	728 (cDNA)	Ray *et al.* (2004)
Aspergillus nidulans	Evaluated array by examining known metabolic genes (PN)	3752 (cDNA)	Sims *et al.* (2004)
Neurospora crassa	Identified glucose-regulated genes (N)	1343 (cDNA)	Xie *et al.* (2004)
Trametes gallica¶	Identified nitrogen-regulated genes (NV)	2596 (cDNA)	Sun *et al.* (2004)
Neurospora crassa	Identified nutrient-regulated genes (NV)	4700 (cDNA)	Aign & Hoheisel (2003)
Aspergillus parasiticus‖	Identified aflatoxin biosynthesis genes (NV)	753 (cDNA)	O'Brian *et al.* (2003)
Trichoderma reesei	Identified glucose-regulated genes (N)	1151 (cDNA)	Chambergo *et al.* (2002)
	Development		
Sordaria macrospora#	Identified fruiting body development genes (N)	~1000 (cDNA)	Pöggeler *et al.* (2006)
Neurospora crassa	Identified conidial germination genes (PN)	3366 (oligo)	Kasuga *et al.* (2005)
Neurospora crassa	Identified MAP-kinase-regulated genes (N)	1343 (cDNA)	Li *et al.* (2005)
Sordaria macrospora#	Identified fruiting body development genes (N)	~1000 (cDNA)	Nowrousian *et al.* (2005)
Metarhizium anisopliae	Identified ageing genes (RT)	1730 (cDNA)	Wang *et al.* (2005b)
Neurospora crassa	Identified clock-controlled genes (N)	1343 (cDNA)	Correa *et al.* (2003)
Neurospora crassa	Identified clock-controlled genes (N)	~1000 (cDNA)	Nowrousian *et al.* (2003)
Neurospora crassa	Identified light-induced genes (N)	1343 (cDNA)	Lewis *et al.* (2002)
	Pathogenesis		
Aspergillus fumigatus	Identified voriconazole adaptation genes (RT)	9516§ (cDNA)	Ferreira *et al.* (2006)
Fusarium graminearum	Evaluated array by analysing fungal samples *in vitro* and *in planta* (NV)	~14 000§ (Affy)	Güldener *et al.* (2006)
Uromyces fabae	Identified parasitic growth genes (PN)	512 (cDNA)	Jakupović *et al.* (2006)
Gibberella zeae	Evaluated subtraction library enriched with sexual reproduction genes (N)	291 (cDNA)	Lee *et al.* (2006)
Fusarium oxysporum f. sp. *vasinfectum***	Identified pathogenic genes (RT)	6000 (cDNA)	McFadden *et al.* (2006)
Gibberella zeae	Identified perithecium development genes (RT)	1550 (cDNA)	Qi *et al.* (2006)
Blumeria graminis	Identified development and pathogenic genes (RT)	2027 (cDNA)	Both *et al.* (2005a)
Blumeria graminis	Obtained profiles of metabolic genes during infection (RT)	2027 (cDNA)	Both *et al.* (2005b)
Fusarium oxysporum f. sp. *lycopersici*	Identified nitrogen-responsive genes (N)	1000 (cDNA)	Divon *et al.* (2005)
Metarhizium anisopliae	Identified host-specific pathogenic genes (RT)	837 (cDNA)	Freimoser *et al.* (2005)
Fusarium species††	Evaluated array designed to identify *Fusarium* species (N/A)	57 (DNA)	Nicolaisen *et al.* (2005)
Aspergillus fumigatus	Identified temperature-regulated genes (NV)	9516§ (cDNA)	Nierman *et al.* (2005)
Metarhizium anisopliae	Identified pathogenic and saprophytic adaptation genes (SL)	1730 (cDNA)	Wang *et al.* (2005a)
Metarhizium anisopliae	Identified host-specific pathogenic genes (NV)	1730 (cDNA)	Wang & Leger (2005)
Cryphonectria parasitica	Identified specific hypovirus-responsive genes (RT)	2200 (cDNA)	Allen & Nuss (2004a)
Cryphonectria parasitica	Identified mitochondrial hypovirulence-regulated genes (RT)	2200 (cDNA)	Allen & Nuss (2004b)

Table 1. cont.

Organism	Results* and validation†	Genes/ESTs represented and format‡	Reference
Cryphonectria parasitica	Identified G-protein regulated genes associated with hypovirus infection (RT)	2200 (cDNA)	Dawe *et al.* (2004)
Cryphonectria parasitica	Identified hypovirus-responsive genes (RT)	2200 (cDNA)	Allen *et al.* (2003)
Magnaporthe grisea	Identified appressoria development genes (N)	3500 (cDNA)	Takano *et al.* (2003)
	Symbiosis		
Neotyphodium species/Epichloë festucae‡‡	Evaluated arrays by comparing expression in different endophytes (NV)	3806/5038 (cDNA)	Felitti *et al.* (2006)
Paxillus involutus§§	Identified rapidly evolving genes (N/A)	2161 (cDNA)	Le Quéré *et al.* (2006)
Paxillus involutus§§	Identified mycorrhizal development genes (RT)	2161 (cDNA)	Le Quéré *et al.* (2005)
Paxillus involutus§§	Identified tissue-specific symbiosis-regulated genes (NV)	2161 (cDNA)	Wright *et al.* (2005)
Paxillus involutus§§	Identified host-specific symbiosis-regulated genes (N)	2161 (cDNA)	Johansson *et al.* (2004)
Paxillus involutus§§	Obtained profiles of symbiosis-regulated genes (NV)	2161 (cDNA)	Le Quéré *et al.* (2004)
	Industrial applications		
Aspergillus niger	Evaluated subtraction library enriched with dithiothreitol stress genes (SL)	Whole genome‖ ‖ (Affy)	MacKenzie *et al.* (2005)
Aspergillus nidulans	Identified unfolded protein response genes (NV)	5579 (cDNA)	Sims *et al.* (2005)
Trichoderma reesei	Obtained profiles of biomass-degrading genes (N)	5131 (oligo)	Foreman *et al.* (2003)

*Further details can be found in the text.

†Microarray studies are often validated by comparing expression data for a small number of genes (5–10) with results obtained from another analytical method. In general, the results obtained from the microarray studies in this review correlated well with Northern or real-time quantitative PCR data. The method used for validation is given in parentheses: N, Northern analysis; PN, compared to previously published Northern; PM, compared to previously published microarray; RT, real-time quantitative PCR; SL, compared to genes isolated from subtraction library; N/A, not applicable; NV, no validation reported.

‡Affy, Affymetrix GeneChips; DNA, short oligonucleotides based on genomic DNA; cDNA, PCR amplicons on coated glass slides; Nim, Nimblegen arrays; oligo, long oligonucleotides.

§Microarray based on whole genome.

‖*Aspergillus flavus* cDNA microarry hybridized with *Aspergillus parasiticus* probes.

¶*Phanerochaete chrysosporium* cDNA microarry hybridized with *Trametes gallica* probes.

#*Neurospora crassa* cDNA microarry hybridized with *Sordaria macrospora* probes.

**Microarray represents ESTs from both *Fusarium oxysporum* f. sp. *vasinfectum* and its host *Gossypium hirsutum*.

††Microarray represents a variety of *Fusarium* species that infect cereal grain.

‡‡Two microarrays were used, one representing 3806 *Neotyphodium coenophialum* and *Neotyphodium lolii* genes and the other representing 4195 *Neotyphodium* and 920 *Epichloë festucae* genes.

§§Microarray represents ESTs from both *Paxillus involutus* and its ectomycorrhizal partner *Betula pendula*.

‖ ‖Further information is available to academic groups and non-profit organizations (hans.roubos@dsm.com).

playing a major role in carbon catabolite repression (Flipphi *et al.*, 2003). Sims *et al.* (2004) evaluated an *A. nidulans* microarray by examining the expression of previously characterized metabolic genes. CreA-mediated glucose repression was observed in cells which were shifted from ethanol to glucose. Aign & Hoheisel (2003) also explored the effects of carbon source, comparing cultures grown on minimal or acetate medium to complete medium. The majority of differentially regulated genes were up-regulated on minimal or acetate medium, with one-third representing central metabolic genes.

In contrast to *S. cerevisiae* and other yeasts, filamentous fungi produce a vast array of secondary metabolites. Secondary metabolite genes are often clustered together in the genome and regulated by a common transcription factor. The nuclear protein LaeA is a global regulator of secondary metabolite clusters in *A. nidulans*. Bok *et al.* (2006) compared expression in an *laeA* deletion (Δ*laeA*) mutant and a wild-type strain to identify clusters controlled by LaeA. Genome mining identified one particular cluster in which all five genes were down-regulated in the Δ*laeA* strain. Analysis of the secondary metabolite produced by the cluster revealed the antitumour compound terrequinone A, a metabolite not previously described in *A. nidulans*.

Aflatoxins are highly carcinogenic secondary metabolites which contaminate a wide variety of crops, including maize.

AflR regulates transcription of genes required for the biosynthesis of aflatoxin in *Aspergillus parasiticus* (Yu *et al.*, 1996). Price *et al.* (2006) compared expression of an *aflR* deletion mutant (Δ*aflR*) with its parental wild-type. Microarray analysis revealed 23 genes up-regulated in the wild-type. These included three genes outside the aflatoxin pathway cluster which were previously not associated with aflatoxin biosynthesis. Earlier studies investigated the influence of fungal development (O'Brian *et al.*, 2003) and culture conditions (Price *et al.*, 2005) on aflatoxin production. Price *et al.* (2005) compared expression in culture conditions conducive and not conducive to aflatoxin production. Cluster analysis was used to identify genes with similar expression profiles to known aflatoxin biosynthesis genes. A similar strategy was employed to identify genes associated with fumonisin biosynthesis in the plant pathogen *Fusarium verticillioides* (Pirttilä *et al.*, 2004). Nineteen genes had similar expression profiles to known fumonisin genes. Six of these were homologous to genes with characterized functions including the high-affinity (Zrt1p) and low-affinity (Msc2p) zinc transporters in *S. cerevisiae.*

Development

Filamentous fungi provide excellent models to study development in multicellular eukaryotic organisms. Kasuga *et al.* (2005) used microarrays to investigate the genetic mechanisms important for conidial germination in *N. crassa.* Expression was compared at distinct morphological events such as isotropic swelling, germ tube emergence and the formation of a mycelial mat. A total of 1287 genes were differentially regulated and the majority grouped into seven clusters with similar expression patterns. Transcriptional profiles were consistent with previously published data and corresponded with known biochemical processes associated with conidial germination. The results were also compared to previous microarray studies detailing spore germination in the dimorphic fungus *Ustilago maydis* (Zahiri *et al.*, 2005) and the amoeba *Dictyostelium discoideum* (Xu *et al.*, 2004). Of the 1287 genes differentially regulated in *N. crassa*, 25 were orthologous to those regulated in *U. maydis* and 16 orthologous to those regulated in *D. discoideum.* Kasuga *et al.* (2005) concluded that the transcriptional similarities between these diverse organisms suggest that mechanisms of spore germination could be evolutionarily conserved.

Nowrousian *et al.* (2005) examined the complex developmental process of fruiting body formation in *Sordaria macrospora.* Expression profiles of three developmental mutants (*pro1*, *pro11* and *pro22*) unable to produce mature perithecia were compared to a wild-type strain. Genes encoding proteins associated with cell wall biogenesis and fungal development were significantly down-regulated in the mutant strains. Sexual development in *S. macrospora* was further examined by Pöggeler *et al.* (2006), who identified genes regulated by the mating type gene *smta-1.* Since *smta-1* is also unable to produce mature perithecia it is not surprising that 10 of the genes identified were also differentially expressed in the three *pro* mutants (Nowrousian *et al.*, 2005; Pöggeler *et al.*, 2006). The *N. crassa* MAP kinase MAK-2 and its target PP-1 are also required for sexual development (Li *et al.*, 2005). Li *et al.* (2005) compared expression in a wild-type to Δ*mak-2* and Δ*pp-1* deletion mutants. Interestingly, two of the genes up-regulated in both Δ*mak-2* and Δ*pp-1* are rhythmically expressed circadian-clock-controlled genes (Bell-Pedersen *et al.*, 1996).

Circadian-clock-controlled genes have been characterized in cyanobacteria, fungi, plants and animals (Young & Kay, 2001). Sleep patterns, hormone cycles and body temperature in humans and photosynthesis in plants rely on the rhythmic expression of clock-controlled genes (Takahashi & Zatz, 1982). Temperature and light keep them in synchrony with the environment. Circadian rhythms are important for development in *N. crassa* (Sargent *et al.*, 1966). Conidiation peaks at the transition from dark to light or from cold to warm, a time known as subjective dawn. Several studies have used microarrays to identify novel clock-controlled genes in *N. crassa* (Correa *et al.*, 2003; Nowrousian *et al.*, 2003). Expression was compared at various time points throughout the circadian cycle and clustering used to identify rhythmically expressed genes with similar expression patterns. The genes identified coded for proteins with diverse functions, including protein synthesis, metabolism and development (Correa *et al.*, 2003; Nowrousian *et al.*, 2003).

The products of *frq*, *wc-1* and *wc-2* form an auto-regulatory negative feedback cycle known as the FRQ/WCC oscillator that is required for circadian regulation in *N. crassa* (Nowrousian *et al.*, 2003). Nowrousian *et al.* (2003) used microarray analysis to demonstrate that the FRQ/WCC oscillator is responsive to temperature. Of the 27 clock-controlled genes identified in the study, 14 were also temperature regulated. However, temperature regulation of all 14 genes was lost in an *frq* knockout mutant (Δ*frq*). This result suggests that the FRQ/WCC oscillator mediates temperature-induced synchrony of *N. crassa* clock-controlled genes. In addition to its role as a circadian regulator, WC-1 regulates all known blue-light-responsive clock-independent genes (Lewis *et al.*, 2002). Lewis *et al.* (2002) used microarrays to identify genes that are induced by light and genes that are induced by overexpression of WC-1. Since only four of the 22 light-induced genes identified are also induced by WC-1 overexpression it was concluded that elevated levels of WC-1 alone are not sufficient to activate all light-responsive genes.

Pathogenesis

Microarray studies have addressed fungal pathogens of both plants and animals. Many plant-pathogenic fungi rely on the formation of appressoria (swollen hyphae required for anchorage and penetration) to infect their hosts. Turgor pressure generated by the appressorium combined with

enzyme degradation allows these fungi to penetrate the plant cuticle. Takano *et al.* (2003) aimed to identify genes important for appressorium formation in *Magnaporthe grisea* using cultures grown on appressoria-inducing GelBond medium. Sixty-seven genes were up-regulated in developing appressoria compared to vegetative mycelia. Interestingly, transcripts for 19 of these genes were present at higher levels in dormant conidia compared to vegetative mycelia. These included a homologue of a characterized virulence gene from *Collectotrichum gloeosporioides* (*cap20*) (Hwang *et al.*, 1995) and Δ^{24}-sterol C-methyl transferase. Sterol biosynthesis has also been linked to pathogenicity in *C. gloeosporioides* (Kim *et al.*, 2002) and the insect pathogen *Metarhizium anisopliae* (Wang *et al.*, 2005a). The results suggest that a pool of pre-existing conidial mRNA may contain transcripts important for pathogenesis.

The *Blumeria graminis cap20* homologue was also significantly up-regulated in developing appressoria (Both *et al.*, 2005a). Gene expression was examined in this plant pathogen as part of a wider study of fungal development during infection of barley (Both *et al.*, 2005a, b). RNA was collected from eight stages of infection representing the complete asexual cycle of the fungus. Ten other potential pathogenicity genes were identified whose expression correlated with the *cap20* homologue. Five were homologous to previously characterized virulence genes and five were homologous to genes with previously proposed pathogenicity roles. Microarray analysis of fungal genes expressed *in planta* has also revealed pathogenic genes in the rust fungus *Uromyces fabae* (Jakupovic *et al.*, 2006) and the cotton pathogen *Fusarium oxysporum* f. sp. *vasinfectum* (McFadden *et al.*, 2006). McFadden *et al.* (2006) identified a putative oxidoreductase gene, with homologues in both pathogenic and non-pathogenic fungi, which was expressed 500-fold higher *in planta* compared to vegetative mycelia. Furthermore, *in planta* expression of the gene in pathogenic isolates was greater than that in nonpathogenic isolates. Expression was also positively correlated with vascular browning, a characteristic symptom of *Fusarium* wilt infection (McFadden *et al.*, 2006).

Cryphonectria parasitica is the causative agent of chestnut blight and infects chestnut trees worldwide. Hypoviruses have been shown to reduce virulence in *C. parasitica* and are important for its biological control (Allen *et al.*, 2003). Allen *et al.* (2003) compared fungal expression in a strain of *C. parasitica* infected with the hypovirus CHV1-EP713 to a virus-free parent. A group of 295 differentially regulated genes belonged to a variety of functional groups including metabolism, development and cell wall growth. A homologue of *M. grisea* Mst12, a transcription factor regulating infectious hyphal growth, was amongst those down-regulated in the virus-infected strain. A major connection between viral hypovirulence and G-protein-mediated signal transduction was established when expression of two mutant strains (Δ*cpg-1* and Δ*cpgb-1*) missing Gα and Gβ G-protein subunits was examined (Dawe *et al.*, 2004). Almost half (45 %) of the genes previously shown to be regulated by hypovirus infection were also differentially expressed in at least one of the G-protein mutants compared to the parental wild-type.

Microarray analysis has also been used to investigate infection in animal pathogens. Perhaps the best-studied animal pathogens are dimorphic, not filamentous, and so are not described here (recent microarray studies in *Candida albicans* include Hromatka *et al.*, 2005; Sandovsky-Losica *et al.*, 2006; Lepak *et al.*, 2006). The human pathogen *Aspergillus fumigatus* must adapt to its host temperature for successful infection. Nierman *et al.* (2005) examined the thermotolerance of the fungus by comparing expression at temperatures representing pathogenic (37 °C) and non-pathogenic (30 °C and 48 °C) environments. Many genes encoding heat-shock proteins were differentially regulated, suggesting their importance for temperature adaptation. Voriconazole is used to treat patients infected with *A. fumigatus*. However, its use is often limited by drug resistance (Ferreira *et al.*, 2006). Ferreira *et al.* (2006) examined the ability of the fungus to adapt to this antifungal agent. Cluster analysis revealed one group of rapidly up-regulated genes containing an ABC multidrug transporter and a glutathione *S*-transferase. Both genes are thought to have roles in voriconazole detoxification and are potentially required for adaptation to the drug.

Adaptation by the insect pathogen *Metarhizium anisopliae* during infection of its host has also been addressed using microarray analysis. *M. anisopliae* must adapt to both the cuticle and haemolymph of its host (Wang *et al.*, 2005a). Microarray analysis revealed subsets of genes associated with its adaptation which included genes for cell wall reorganization and adaptors to osmotic stress (Wang *et al.*, 2005a). *M. anisopliae* was also observed to adapt specifically to different insect hosts (Freimoser *et al.*, 2005). One particular hydrophobin (a family of proteins which mediate fungal attachment to hydrophobic surfaces) was up-regulated on medium containing cockroach or beetle cuticle but unaffected on medium containing gypsy moth cuticle. The differential regulation of this and other hydrophobins (Freimoser *et al.*, 2005) further links cell wall changes to fungal adaptation.

Symbiosis

Ectomycorrhizae (ECM) are ecologically important symbiotic associations consisting of both plant and fungal tissue. While the fungus gains a source of photosynthetic sugars, the plant benefits from improved nutrient uptake. Several studies have investigated global expression during the development of ECM between *Betula pendula* (birch) and *Paxillus involutus*. Both fungal and plant genes specifically regulated in the ectomycorrhizal symbiosis have been identified.

Fungal genes specifically regulated in the ECM have a variety of putative functions. A predicted fungal hexose transporter

was up-regulated during the entire course of ECM development (Le Quéré *et al.*, 2005). This is significant because the fungal partner in the symbiotic relationship receives sugars from the plant (Söderström *et al.*, 1988). Le Quéré *et al.* (2005) speculated that the putative heoxose transporter is a candidate for fungal hexose assimilation in the ECM tissue. Homologues of proteins in the electron transport chain were also highly up-regulated during ECM development (Le Quéré *et al.*, 2005). However, the lowered expression of the TCA cycle enzyme malate dehydrogenase suggested that the potential increase in respiration was not connected to glucose metabolism. Fungal genes with putative functions in the secretory pathway were also differentially expressed in ECM tissue (Johansson *et al.*, 2004; Wright *et al.*, 2005). Interestingly, many were expressed more strongly in rhizomorphs, tubular hyphal aggregates that function in the transport of nutrients to the ECM root tissue, than in ECM root tissue itself (Wright *et al.*, 2005).

Consistent with the fungal partner, ECM-regulated plant genes also have a variety of putative functions. Many predicted metabolic genes, including several encoding TCA cycle and electron transport chain enzymes, were down-regulated in ECM tissue (Johansson *et al.*, 2004). Johansson *et al.* (2004) suggested that the reduced expression of these enzymes indicated a lower rate of aerobic respiration in ECM compared to free-living roots. Genes with putative roles in plant defence were also differentially expressed in ECM tissue (Johansson *et al.*, 2004; Le Quéré *et al.*, 2005). While many were up-regulated in early stages of ECM development they were not significantly regulated in later stages (Le Quéré *et al.*, 2005).

Recently microarrays have been fabricated for the grass fungal endophytes *Neotyphodium coenophialum*, *Neotyphodium lolii* and *Epichloë festucae* (Felitti *et al.*, 2006). Several proof-of-concept hybridizations were reported comparing different saprophytic growth conditions (Felitti *et al.*, 2006). The microarrays should allow further insight into the mutualistic associations between plants and fungi.

Industrial applications

Filamentous fungi are used extensively in industrial fermentations. Maeda *et al.* (2004) used microarray analysis to examine the expression of industrially important enzymes secreted by *A. oryzae*. A variety of solid-state culture conditions containing different waste biomass materials were compared. Growth on wheat bran induced expression of the richest set of industrially important hydrolytic enzymes and validated its current industrial use. In a similar study Foreman *et al.* (2003) examined the regulation of commercially important biomass-degrading enzymes in *T. reesei*. The majority of putative and characterized biomass-degrading enzymes represented on the array were up-regulated in both sophorose- and lactose-supplemented cultures (Foreman *et al.*, 2003).

Filamentous fungi are also used as hosts to produce commercially important heterologous proteins. Industrial-scale production of heterologous proteins often results in lower yields than those achieved for native proteins. A molecular understanding of the secretory system is important for improving efficiency of recombinant protein secretion. Sims *et al.* (2005) used microarray analysis to identify genes associated with recombinant protein production in *A. nidulans*. Expression was compared in a recombinant bovine-chymosin-producing strain and its wild-type parent. The study revealed a variety of secretion-related genes involved with the unfolded protein response (UPR). Manipulation of UPR genes in *Aspergillus* species has been shown to improve yields of heterologous proteins (Moralejo *et al.*, 2001; Conesa *et al.*, 2002). MacKenzie *et al.* (2005) aimed to identify further UPR genes in *Aspergillus niger* using the secretion blocker DTT, thought to mimic the effects of recombinant protein secretion. Subtractive hybridization and microarray analysis were used to identify genes regulated by DTT. While a number of stress-responsive genes were identified they did not appear to be related to the UPR, suggesting that DTT does not specifically induce the UPR.

Future prospects: the next fifty

Microarray analysis is still in its infancy and much potential remains for further study in all of the areas covered in this review. Of the studies investigating pathogenesis only a few have reported expression in plant hosts and none have reported expression in animal hosts. There is certainly much potential for further investigation of in-host expression, provided that complications of obtaining sufficient quantities of fungal RNA and interference from host RNA can be overcome. It would also be of great interest to compare genes induced during pathogenic interactions to those induced in symbiotic relationships. Such a comparison may distinguish genes associated with host adaptation from those related to pathogenicity. Of the 50 studies detailed in this report it is surprising that none have investigated the cell cycle. Microarray studies in both budding and fission yeast have identified many periodically expressed cell cycle genes not previously connected to the cell cycle (Spellman *et al.*, 1998; Bähler, 2005; Peng *et al.*, 2005). There is great potential for similar studies in filamentous fungi, particularly in *A. nidulans*, where many cell cycle mutants are available.

Comparing expression profiles of different species of filamentous fungi may provide insight into how this diverse group of fungi has evolved. Interestingly, microarrays have only been fabricated for three filamentous species of basidiomycetes (*Paxillus involutus*, *Phanerochaete chrysosporium* and *Uromyces fabae*) and none represent filamentous zygomycetes. The potential for comparative functional genomics within filamentous fungi would greatly benefit from more microarrays of filamentous members from these groups. Several studies have described successful cross-species hybridizations in closely related species of filamentous fungi. An Affymetrix GeneChip recently fabricated for

the plant pathogen *F. graminearum* has been shown to efficiently detect genes from four other closely related species of *Fusarium* (Güldener *et al.*, 2006). Cross-species analysis could dramatically increase the number of studies available for comparative functional genomics.

The recent completion of several fungal genome projects and subsequent fabrication of whole-genome microarrays has further fuelled the potential for global gene expression studies in filamentous fungi. The first 50 microarray studies in filamentous fungi were published over a four-year period. With the explosion of fungal microarray studies the next 50 will appear much faster.

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