Genes with increased transcript levels following harvest of the sporophore of *Agaricus bisporus* have multiple physiological roles

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We screened a cDNA library generated from harvested and stored sporophores of *Agaricus bisporus* and identified 19 genes with higher transcript levels than at the time of harvest. Five of these genes had no detectable mRNA levels prior to detachment from the mycelium. Sequence analysis of ten clones revealed significant similarities to known genes, these code for proteins involved in polymer breakdown and metabolism, cell wall synthesis, stress tolerance, cytochrome P450 activity and DNA binding. The diversity of functions of these genes suggests the changes in the sporophore after harvest involve several different physiological processes.

INTRODUCTION

Morphogenesis of the homobasidiomycete *Agaricus bisporus* involves the differentiation of a vegetative mycelium into a multicellular sporophore, or mushroom. The sporophore consists of the stipe (stalk) and the pileus (cap) which is covered with a 'skin' layer. The underside of the cap is differentiated into gill tissue that bears basidiospores. During development the stipe grows longitudinally and the cap expands, exposing the gills ready for spore dispersal. The resources required for sporophore and basidiospore production are supplied by the vegetative mycelium, which is in contact with the substrate, i.e. leaf litter in natural habitats or compost if cultivated. After sporulation the sporophore senesces and dies, presumably to avoid over-commitment of resources to the sporophore at the expense of the mycelium. The regulation of this final stage of development is not known. Characteristics of natural mushroom senescence include darkening tissue colour, loss of texture and cellular disruption and collapse (McGarry & Burton 1994, Umar & van Griensven 1997).

Cultivated *A. bisporus* sporophores are often harvested at an immature stage prior to sporulation. The harvest is both a wounding event and a massive disruption to the metabolism of the sporophore, since the sporophore is detached abruptly from its supply of nutrients and water. However, despite the damage, the sporophore continues to develop similarly to the non-harvested mushroom (Hammond 1979); the stipe grows longitudinally, the cap continues to expand, gill formation occurs and spores are produced (Braaksma *et al*. 1998). Prolonged storage results in tissue changes comparable with natural senescence observed in the uncut sporophore (Fig. 1); tissue discolouration, loss of texture, localised cellular collapse and cell emptying (Evered & Burton 1995, McGarry & Burton 1994, Umar & van Griensven 1997). However, other workers suggest the harvested sporophore does not senesce, but instead is subject to a 'post-harvest stress disorder' in response to water and nutritional limitation (Moore 1998, Umar & van Griensven 1997).

Detached sporophores continue to have high rates of respiration linked to a switch from nutrient import to the breakdown of storage compounds (Hammond & Nichols 1975). Mannitol, trehalose, glycogen and soluble protein levels decrease (Burton *et al*. 1997, Hammond & Nichols 1975) while levels of the components of the cell wall and of urea increase (Hammond 1979). Compositional changes in the detached *A. bisporus* sporophore are not uniform throughout the different tissues of the mushroom (Burton *et al*. 1997, Donker & Braaksma 1999, Hammond & Nichols 1975). There is additional evidence of the mass movement of resources from the stipe to the cap and gill tissues during development in the harvested sporophore (Donker & van As 1999, Hammond & Nichols 1975). These post-harvest metabolic changes are the result of existing enzymes and pathways that are able to operate reversibly, for example mannitol dehydrogenase (Morton, Dickenson & Hammond 1985), the *de novo* production of new proteins and the down-regulation of other proteins. The only described example of *de novo* protein synthesis occurring in the *A. bisporus* sporophore postharvest is that of serine proteinase (Burton *et al*. 1997) and is controlled at the level of transcription (Kingsnorth, Eastwood & Burton 2001). Serine proteinase levels also increase during natural senescence in *A. bisporus* (Burton *et al*. 1997). However, little additional information is available on the biochemistry and genetic regulation of the harvested mushroom.

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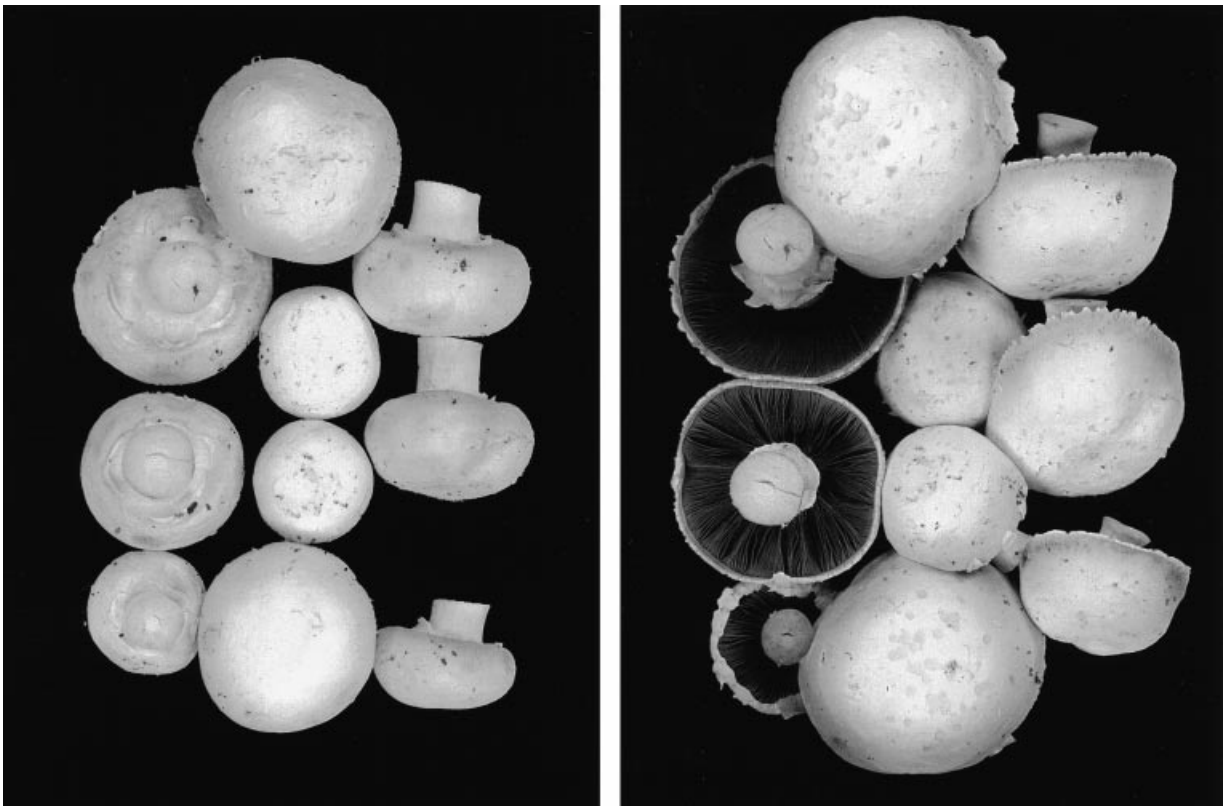

Fig. 1. Postharvest changes in the mushroom *Agaricus bisporus*, observed at harvest (day 0) and after three days storage (day 3) at 18 °C, and 90–95% relative humidity.

We are interested in the postharvest biology of the mushroom because its high commercial value can be lost after harvest due to quality loss. In this study we aim to gain an insight into the biochemistry and genetic regulation in sporophores postharvest, by identifying genes with higher transcript levels after harvest. cDNA derived from sporophores stored for 2 d was compared with cDNA from freshly harvested sporophores by differential screening. Genes with higher transcript levels 2 d after harvest were selected, sequenced and characterised.

MATERIALS AND METHODS

Strains

Agaricus bisporus strain U3 (Sylvan, UK) was used throughout the study. Mushrooms were grown according to commercial practice at the Horticulture Research International mushroom cropping unit. During cropping, mushroom sporophores are produced in synchronous weekly flushes; sporophores used experimentally were from the second flush only. Sporophores were harvested and either frozen immediately in liquid nitrogen (termed day 0), or stored for 2 d in a controlled environment, 18 °C and 90–95% relative humidity (day 2), before freezing in liquid nitrogen. Frozen samples were stored at −80 °C.

The *Escherichia coli* strain XL1-Blue (Stratagene) was used for plasmid maintenance and cloning. The pBluescriptSK (Stratagene) vector was used for routine cloning.

RNA isolation

RNA was isolated from fresh mushroom tissues and from tissues stored for 2 d following harvest. RNA isolation was carried out according to established protocols (Sambrook, Fritsch & Maniatis 1989). Absorbance measurements at 260 and 280 nm were used to assess RNA concentration and purity (Sambrook *et al.* 1989). RNA integrity was determined by formaldehyde agarose gel electrophoresis (Rosen & Villa-Kormaroff 1993). Poly(A)$^+$ RNA was isolated from the total RNA using affinity chromatography oligo d(T) cellulose mRNA purification kit (Amersham Pharmacia Biotech).

cDNA library construction

A cDNA library was constructed with the λ-ZAP Express™ cDNA synthesis kit (Stratagene) from 5 μg poly(A)$^+$ RNA extracted from day 2 sporophores. The primary library of approximately 10^5 clones was amplified according to Stratagene protocols.

Preparation of an ordered cDNA library

Phagemid clones (pBK-CMV) were excised from the λ-ZAP cDNA library and cloned as described in the manufacturer's instructions (Stratagene) and selected on Luria-Bertani (LB) agar medium in Petri plates containing 50 μg ml^{-1} kanamycin; each plate contained 0.8 mg X-gal and 0.8 mg IPTG. White colonies were isolated and inoculated into wells of a microtitre dish containing 200 μl of 'media 96' (tryptone 10 g l^{-1}, yeast

extract 5 g l^{-1}, NaCl 5 g l^{-1}, KH_2PO_4 6.3 g l^{-1}, sodium citrate 0.45 g l^{-1}, $MgSO_4$ 90 mg l^{-1}, $(NH_4)_2SO_4$ 0.9 g l^{-1}, glycerol 5% (v/v)) and 50 μg ml^{-1} kanamycin. The microtitre plates were incubated overnight at 37 °, frozen and stored at −80 °.

Differential screening

Differential screening was carried out according to established protocols (Sambrook *et al*. 1989). Bacteria from the ordered library were transferred onto duplicate nylon membranes (Roche Diagnostics) and prehybridised separately.

Differential probes were prepared with cDNA created from 5 μg poly(A)$^+$ RNA extracted from day 0 and day 2 sporophores. Synthesis of cDNA was achieved through reverse transcription of poly(A)$^+$ RNA with the Ready-To-Go T-primed cDNA synthesis kit (Amersham Pharmacia Biotech). The cDNA was purified with the QIAquick nucleotide removal kit (Qiagen) prior to being used as a template for random priming, incorporating [α-^{32}P]dCTP (*Redi*prime kit, Amersham Pharmacia Biotech). The duplicate membranes were hybridised with denatured cDNA probe from either day 0 or day 2 sporophores. Putative, differentially expressed cDNA clones were identified and the corresponding phagemid colonies re-screened with cDNA probes extracted from different day 0 and day 2 sporophores. The inserts of the phagemid clones which showed a greater transcript level at day 2 were used as probes in a Northern blot tertiary screen. Total RNA from day 0 and day 2 sporophores were separated with formaldehyde agarose gel electrophoresis prior to Northern blot analysis (Sambrook *et al*. 1989).

Agaricus bisporus hemicellulase (*hem*1) and 28S rRNA genes were used as controls. *hem*1 is constitutively expressed at a constant level under a range of conditions in *Aspergillus nidulans* (Bradshaw *et al*. 1993) and had been previously used as a loading control with *A. bisporus* (Yagűe *et al*. 1997). The 28S rRNA gene has also been used as a loading control for total RNA samples (de Groot *et al*. 1998).

Nucleic acid sequence determination analysis

DNA sequencing reactions were carried out using the ABI Prism™ BigDye terminator cycle sequencing kit (Perkin–Elmer). Electrophoresis and acquisition of sequence data were carried out by the DNA Sequencing Unit at the University of Durham.

Sequences were analysed with the DNAStar program (DNAStar, USA). Homology studies on both nucleic acid and amino acid sequences were carried out using the FASTA, BlastX and BlastP algorithms available on the European Bioinformatics Institute (EBI) (www.ebi.ac.uk) homepage and the OWL (www.biochem.ucl.ac.uk/bsm/dbbrowser/OWL/OWL) composite protein sequence database. Database searches were carried out using the default settings. In addition, the encoded proteins were also analysed using links available through the ExPASy (www.expasy.com) homepage. Each encoded protein was analysed for conserved domains, motifs and fingerprints; protein structural analyses; profile and pattern searches; protein localisation markers and transmembrane spanning regions.

Accession numbers

The sequences identified in this study were submitted to the EMBL database (accession nos AJ271690 to AJ271708).

RESULTS AND DISCUSSION

Isolation of differentially expressed cDNA

We screened 2992 arbitrarily selected clones from a cDNA library, constructed from the mRNA of *Agaricus bisporus* sporophores that had been stored for two days following harvest. We initially identified 154 clones that hybridised to cDNA derived from sporophores harvested and stored for two days (day 2), and not to cDNA from freshly harvested sporophores (day 0). A second round screening, using RNA extracts from different day 0 and day 2 sporophores, reduced the number of clones to 40. The tertiary screen (using the cDNAs as probes) reduced the number of clones with higher transcript levels two days after harvest to 38. These 38 clones were sequenced fully and compared. Clones with very similar sequences. i.e. over 98% sequence similarity were grouped together in gene families. From the 38 clones, 19 gene families were identified, and all families contained potential open reading frames.

Identification of genes with higher transcript levels

The nucleic acid and protein sequences of the 19 genes/gene families were compared with other known sequences from the databases. Whole sequences, motifs and structural characteristics were analysed. Ten of the 19 gene families had strong similarities to the sequences of known proteins; another two showed no overall similarity, but their derived protein sequences contained motifs with which functions have been associated (Table 1). The remaining seven had no similarities with proteins of known function. Five of the genes (SHS 1, 3, 7, 8 and 14) had no detectable expression at day 0 (Fig. 2). The remaining genes were transcribed at low levels prior to harvest and up-regulated greatly following detachment and 2 days' storage (Fig. 2).

Some of the genes with sequences similar to proteins with known biochemical functions could be considered according to probable cellular function, e.g. nitrogen metabolism and mobilisation, polysaccharide degradation, cell wall synthesis, stress tolerance, and cytochrome P450. The genes were identified in this study by comparing RNA from freshly harvested sporophores with RNA from harvested and stored sporophores, and are therefore referred to as **s**porophore **h**arvested and **s**tored (SHS) genes. This does not imply however that their increased transcription is due specifically to the harvest, as other processes are also occurring e.g. growth and development.

Nitrogen metabolism and mobilisation

Genes encoding two enzymes involved in nitrogen metabolism (leucine aminopeptidase and argininosuccinate lyase) had higher transcript levels following sporophore harvest and storage. Leucine aminopeptidase (SHS 1) catalyses the release

Table 1. Characteristics of cDNA clones from *Agaricus bisporus* sporophores which show elevated transcript levels after harvest and storage at 18 °C, 90–95% relative humidity.

Clone (EMBL No)	No of clones in library	Size of cDNA Insert (kb)	Size of mRNA (kb)	Putative Gene Product	BLASTP Search Results		
					Probability*	Similarity %	Similarity Ratio†
SHS 1 (AJ271690)	1	1.2	1.4	Leucine aminopeptidase EC 3.4.11.10	$1.9e^{-53}$	55	197/357
SHS 2 (AJ271691)	1	0.7	1.3	Argininosuccinate lyase EC 4.3.2.1	$1.9e^{-78}$	80	190/235
SHS 3 (AJ271692)	5	0.7	1.6	Glucuronyl hydrolase	$2.5e^{-17}$	49	173/353
SHS 4 (AJ271693)	1	0.4	1.6	β(1-6) Glucan synthase	$4.5e^{-05}$	42	107/251
SHS 5 (AJ271694)	1	0.5	1.1	Superoxide dismutase EC 1.15.1.1	$4.0e^{-53}$	84	111/131
SHS 6 (AJ271695)	1	0.4	0.8	Metallothionein	$2.1e^{-07}$	56	14/25
SHS 7 (AJ271707)	1	1.2	1.7	Cytochrome P450 I EC 1.14.14.1	$5.3e^{-67}$	69	173/250
SHS 8 (AJ271708)	1	1.3	1.7	Cytochrome P450 II EC 1.14.14.1	$2.4e^{-46}$	63	143/224
SHS 9 (AJ271696)	1	0.6	0.6	Cruciform DNA binding protein	$3.0e^{-25}$	76	74/97
SHS 10 (AJ271697)	2	0.7	1.0	Riboflavin aldehyde-forming enzyme	$6.9e^{-10}$	54	35/64
SHS 11 (AJ271698)	1	2.3	2.5	Contains leucine zipper motif	NA	NA	NA
SHS 12 (AJ271699)	1	0.5	1.1	Contains glycine-proline rich motif	NA	NA	NA
SHS 13 (AJ271700)	8	0.6	1.0	Contains glycine/serine-rich motif	NA	NA	NA
SHS 14 (AJ271702)	2	1.2	1.6	Unknown	NA	NA	NA
SHS 15 (AJ271701)	3	1.3	1.3	Unknown	NA	NA	NA
SHS 16 (AJ271703)	3	0.6	0.7	Transmembrane protein	NA	NA	NA
SHS 17 (AJ271704)	1	0.4	1.5	Unknown	NA	NA	NA
SHS 18 (AJ271705)	3	0.8	1.3	Unknown	NA	NA	NA
SHS 19 (AJ271706)	1	0.5	0.6	Unknown	NA	NA	NA

* The probability of the database match occurring randomly.

† Ratio of $\frac{\text{similar amino acids of } A.bisporus \text{ protein}}{\text{region compared of best fitting protein}}$.

of amino acids from the N-terminus of peptides. The soluble protein levels decline in the *Agaricus bisporus* sporophore after harvest while the level and activity of serine proteinase increases (Burton *et al*. 1997). However serine proteinase has a narrow substrate specificity and the final products are short chain peptides upon which leucine aminopeptidase may act (Burton *et al*. 1993). Argininosuccinate lyase (SHS 2) is an integral enzyme of the urea cycle. The level of urea increases 9 fold in the sporophore between day 0 and day 4 post-harvest storage (Hammond 1979). The urea cycle is probably linked to the citric acid cycle via argininosuccinate lyase so that amino acids released during proteolysis are de-aminated, nitrogen is accumulated as urea and carbon skeletons are decarboxylated, leading to the generation of high energy phosphate bonds.

Polysaccharide breakdown

Five clones identified in the tertiary screen have the same derived protein sequence which is similar (49%) to unsaturated glucuronyl hydrolase (SHS 3) identified from a *Bacillus* sp. (GenBank AP001514). The protein probably belongs to the family of glycosyl hydrolase enzymes (EC 3.2.1–3.2.3). These enzymes have conserved catalytic domains, yet utilise a wide range of substrates (Henrissat *et al*. 1995). Unsaturated glucuronyl hydrolase is an exoglycanase which releases glucuronyl monosaccharides from di-, tri- and tetrasaccharides formed during polysaccharide degradation by polysaccharide lyases (Hashimoto *et al*. 1999). The substrates of the previously described unsaturated glucuronyl hydrolase are glucuronic acid-containing degradation products of mucopolysaccharides, e.g. chondroitin and hyaluronate, which are often associated with the extracellular matrix (Hashimoto *et al*. 1999). However, the substrate for this enzyme is not known in the harvested *A. bisporus* sporophore. Interestingly, Umar & van Griensven (1997) observed by electron microscopy a loss of extracellular matrix in mushrooms after harvest.

Cell wall synthesis

The transcript level of a gene encoding β(1-6) glucan synthase (SHS 4) increases after harvest and storage. The upper stipe region of *A. bisporus* elongates greatly during postharvest growth and development (Braaksma *et al*. 1998). Since (1-6)β-linked glucan side branches increase in the cell wall of the upper stipe during rapid elongation (Mol & Wessels 1990),

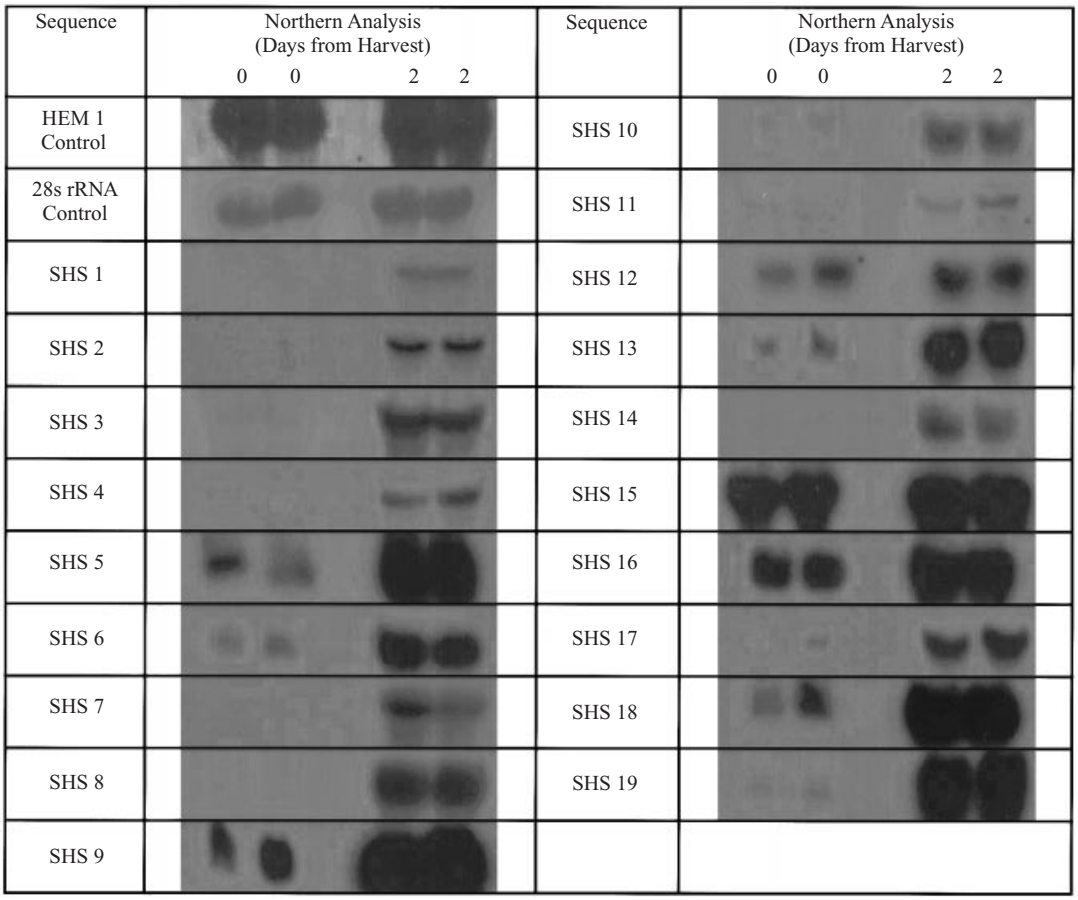

Fig. 2. Northern analysis of gene transcripts with increased levels after harvest and storage from *Agaricus bisporus* sporophores. Two independent RNA extractions were analysed for each time point; day 0 represents extracts from sporophores immediately after harvest, day 2 sporophores were stored for 2 d after harvest.

the β(1-6) glucan synthase may possibly be the enzyme responsible for the changes in cell wall composition.

Stress tolerance

Two genes often associated with stress tolerance had increased transcription levels following harvest, a superoxide dismutase (SHS 5) and a class II metallothionein-like protein (SHS 6). Superoxide dismutase catalyses the reaction of superoxide radicals with protons to produce hydrogen peroxide (Nelson *et al*. 1996). Two distinct families of superoxide dismutase enzymes have been described and they are considered to have separate evolutions (Smith & Doolittle 1992). The superoxide dismutases from *Agaricus bisporus* characterised here belongs to the iron/manganese-type of superoxide dismutases, which are usually located in the mitochondrial matrix (Natvig *et al*. 1996). Superoxide radicals are highly reactive, non-reduced forms of oxygen which can cause severe disruption to cell function and to membranes (Farr & Kogoma 1991). Superoxide radicals can be produced enzymatically by NADPH oxidase, NADH dehydrogenase, succinate dehydrogenase, lactate dehydrogenase, glutathione reductase and cytochrome P450; and non-enzymatically by the autoxidation of cellular components including ubiquitols, catechols, thiols, flavins and quinones (Farr & Kogoma 1991, Kawasaki *et al*. 1999). The activity of NADPH oxidase increases in the mushroom postharvest (Hammond 1978). The product of superoxide dismutase catalysis, hydrogen peroxide, is also potentially highly reactive and damaging, however the activity of the hydrogen peroxide-scavenging enzyme, catalase, is high in the mushroom sporophore (data not shown).

Metallothioneins are low molecular weight, cysteine-rich metal binding proteins often associated with stress responses and developmental processes (Borghesi & Lyres 1996). The metallothionein identified in this study contained an open reading frame of 71 amino acids with a region containing 14 metal-binding cysteine residues. Previously, Münger & Lerch (1985) described a 25 amino acid peptide fragment from *A. bisporus* as a metallothionein protein, but its protein sequence was distinct from the derived protein sequence of the gene identified here. The precise function of the metallothioneins has not been fully established, but they appear to play a role in binding metal ions which can be toxic to a cell at high concentrations (Robinson *et al*. 1993). In addition to the presence of heavy metal cations, metallothioneins may be induced by starvation or the presence of free radicals, although the induction by free radicals appears to be mediated by secondary events (Borghesi & Lyres 1996). The level of soluble protein falls in the detached *A. bisporus* sporophore by 90% in the stipe and 80% in the cap (Burton *et al*. 1993).

Therefore, the function of metallothionein may be to bind free metal ions released from the degradation of metal-binding proteins.

Cytochrome P450

The genes for two separate cytochrome P450 (SHS 7 and 8) genes had higher transcript levels in the mushroom after harvest. Cytochrome P450 (CYP) enzymes are a group of proteins involved in the oxidative metabolism of a large number of compounds, they have been classed into 74 families (Nelson *et al*. 1996, van den Brink *et al*. 1998) and have diverse roles in many aspects of cell function (Nelson *et al*. 1996). Previous work identified a cytochrome P450 (CYPA) associated with the early stages of sporophore development in *A. bisporus* and was described as a flavonoid 3′,5′ hydroxylase (de Groot *et al*. 1997). The cytochrome P450 I and II described here were similar to CYPA but were clearly different proteins with similarities to CYPA of 69% and 57% respectively. Similarity and motif searching with cytochrome P450 enzymes of known catalytic function suggested that these cytochrome P450s belong to the class of mono-oxygenase, oxidoreductase enzymes (EC 1.14.14.1), responsible for the metabolism of a wide range of substrates, including fatty acids, steroids and vitamins. Cytochrome P450 II was most similar to a cytochrome P450 isolated from 'dumpy' mutants of the ink cap mushroom, *Coprinus cinereus* (Muraguchi & Kamada 2000). Stipe elongation is blocked in the 'dumpy' mutant as a consequence of changes to the *eln*2 gene encoding the cytochrome P450 enzyme.

DNA binding

Following harvest, increased transcript levels were found of a gene, (SHS 9) encoding a protein which has similarity to the non-histone cruciform DNA binding proteins HMP1 and Gv1, from *Ustilago maydis* and *Glomus versiforme* respectively (Dutta *et al*. 1997, Burleigh & Harrison 1998). Three conserved regions are common to each protein sequence, probably involved in DNA binding function (data not shown). This class of protein has been suggested to be involved in inducing or maintaining the DNA architecture rather than in a specific role associated with recombination (Dutta *et al*. 1997).

Riboflavin aldehyde-forming enzyme

Two clones were similar to a riboflavin aldehyde-forming enzyme (SHS 10) from *Schizophyllum commune* (EMBL AF 005405). In *S. commune*, the enzyme catalyses the formation of the 5′-aldehyde and 5′-acid of riboflavin, both of which are present in sporophores of *Agaricus bisporus* (Chen & McCormick 1997a, Tachibana & Murakami 1983). The physiological role of the enzyme in *S. commune* or *A. bisporus* has yet to be determined (Chen & McCormick 1997b).

Other sequences

Two further sequences of unknown function showed some homology to known genes. SHS 11 contained a leucine zipper motif (L-X(6)-L-X(6)-L-X(6)-L), which is a DNA binding motif commonly associated with transcription factors and with enzymes that undergo protein/protein interactions (Landschulz, Johnson & McKnight 1988, O'Shea, Rutkowski & Kim 1989).

SHS 12 has similiarities (expressed as Blast P probability and percentage similarity) with a number of proteins of unknown function from *Arabidopsis thaliana* ($5.8e^{-16}$, 53%, GenBank O80818), *Sporobolus stapfianus* ($1.0e^{-11}$, 50%, GenBank O04820) and *Saccharomyces cereviseae* ($7.4e^{-11}$, 50%, GenBank P38216). SHS 13 was a serine and glycine-rich protein. SHS 14, SHS 15, SHS 16, SHS 17, SHS 18 and SHS 19 had novel sequences and similarly to other proteins could not be determined. However, SHS 16 contained a number of potential transmembrane domains.

DISCUSSION AND CONCLUSIONS

We identified 19 genes/gene families with elevated transcript levels by differential screening of cDNA derived from freshly harvested (day 0) and detached and stored mushrooms (day 2). The identities of ten of these genes/gene families were determined by sequencing and database comparison. Previously, only one protein, serine proteinase, was known to increase after harvest or during natural senescence (Burton *et al*. 1993). Serine proteinase is considered to have a nutritional function in the harvested mushroom. The serine proteinase gene is abundantly transcribed in stored *Agaricus bisporus* mushrooms (Kingsnorth *et al*. 2001) and we expected to identify it in this study. However, failure to identify the gene may have been due to the lethal nature of the gene when cloned into *E. coli* cells (Kingsnorth *et al*. 2001).

Consideration of the ten identified up-regulated genes suggests that the detached and stored sporophore is undergoing numerous diverse physiological functions simultaneously, e.g. nutritional (polymer degradation and metabolism), stress tolerance and cell wall synthesis (polymer synthesis). These diverse activities are consistent with the harvested mushroom being nutritionally isolated, wound damaged, continuing to develop and composed of distinct tissues with different physiologies. Within a tissue, different physiologies also occur, for example, cell death has been observed to be localised and discrete, with dying cells adjacent to growing cells (Evered & Burton 1995, Umar & van Griensven 1997). Studies of natural senescence in other organisms also have identified an array of 'up-regulated' genes. In *Brassica napus*, genes with increased transcription levels include those involved in protein, nucleic acid and chlorophyll breakdown, lipid and nitrogen mobilisation, cytochrome P450s and genes of unknown function (Buchanan-Wollaston 1997). Six senescence-associated genes have been identified from daylily (*Hemerocallis* hybrid) petals, aspartic proteinase, nuclease, fatty acid elongase, a cytochrome P450-associated fatty acid hydroxylase and a second cytochrome P450 (Panavas *et al*. 1999). It is not known how many of the 19 genes/gene families identified in this paper have increased transcription due specifically to harvest/post-harvest processes. Parallel work (in progress) has shown that at least two of the genes (SHS 10 and SHS 14) also have increased

transcription levels during the natural, non-harvest development of sporophores (data not shown).

It is not clear how gene responses may have evolved in relation to harvesting. There may be a strategy evolved in direct response to sporophore detachment by external factors, e.g. wild animals, wind. However, it is more likely the expression of these genes evolved independently in response to a number of different stimuli. Many of these stimulus-response relationships would have probably arisen in mycelial cells early in their evolution. Therefore, the nutritional genes have increased transcription in response to nutritional limitation, which is distinct from the signal-response relationships of stress adaptation and cellular growth. The physiology of the harvested and stored mushroom consists of a complex of changes which presumably are co-ordinated but originate from different stimuli. Further work is taking place to identify which of these 19 genes/gene families show increased transcription during natural senescence.

The genes with higher transcript levels represent only part of the biology of the harvested mushroom. Genes with reduced or unchanged transcription were not identified in this study, but these would also contribute to the biology of the harvested sporophore. The gene encoding chitin synthase has been shown to have decreased transcription following harvest (Sreenivasaprasad, Burton & Wood 2000). Furthermore, the regulatory steps beyond transcription are also likely to exert major influences on gene expression.

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REFERENCES

Borghesi, L. A. & Lyres, M. A. (1996) Stress proteins as agents of immunological change: some lessons from metallothionein. *Cell Stress and Chaperones* **1**: 99–108.

Braaksma, A., van Doorn, A. A., Kieft, H. & van Aelst, A. C. (1998) Morphometric analysis of ageing mushrooms (*Agaricus bisporus*) during postharvest development. *Postharvest Biology and Technology* **13**: 71–79.

Bradshaw, R. E., Dixon, S. W. C., Raitt, D. C. & Pillar, T. M. (1993) Isolation and nucleotide sequence of the 5-aminolevulinate synthase gene from *Aspergillus nidulans*. *Current Genetics* **23**: 501–507.

Buchanan-Wollaston, V. (1997) The molecular biology of leaf senescence. *Journal of Experimental Botany* **48**: 181–199.

Burleigh, S. H. & Harrison, M. J. (1998) A cDNA from the arbuscular mycorrhizal fungus *Glomus versiforme* with homology to a cruciform DNA-binding protein from *Ustilago maydis*. *Mycorrhiza* **7**: 301–306.

Burton, K. S., Partis, M. D., Wood, D. A. & Thurston, C. F. (1997) Accumulation of serine proteinase in senescent sporophores of the cultivated mushroom, *Agaricus bisporus*. *Mycological Research* **101**: 146–152.

Burton, K. S., Wood, D. A., Thurston, C. F. & Barker, P. J. (1993) Purification and characterisation of a serine proteinase from senescent sporophores of the commercial mushroom, *Agaricus bisporus*. *Journal of General Microbiology* **139**: 1379–1386.

Chen, H. Y. & McCormick, B. B. (1997a) Fungal riboflavin 5′-hydroxymethyl dehydrogenase catalyses formation of both the aldehyde (riboflavinal) and the acid (riboflavinoic acid). *Biochimica et Biophysica Acta* **1342**: 116–118.

Chen, H. Y. & McCormick, B. B. (1997b) Riboflavin 5′ hydroxymethyl oxidation – molecular cloning, expression and glycoprotein nature of the 5′-aldehyde-forming enzyme from *Schizophyllum commune*. *Journal of Biological Chemistry* **272**: 20077–20081.

de Groot, P. W. J., Schaap, P. J., van Griensven, L. J. L. D. & Visser, J. (1997) Isolation of developmentally regulated genes from the edible mushroom *Agaricus bisporus*. *Microbiology* **143**: 1993–2001.

Donker, H. C. W. & Braaksma, A. (1999) Changes in metabolite concentrations detected by ^{13}C-NMR in the senescing mushroom (*Agaricus bisporus*). *Postharvest Biology and Technology* **10**: 127–134.

Donker, H. C. W. & van As, H. (1999) Cell water balance of white button mushrooms (*Agaricus bisporus*) during its postharvest lifetime studied by quantitative magnetic resonance imaging. *Biochimica et Biophysica Acta* **1427**: 287–297.

Dutta, S., Gerhold, D. L., Rice, M., Germann, M. & Kmiec, E. B. (1997) The cloning and over expression of a cruciform binding protein from *Ustilago maydis*. *Biochimica et Biophysica Acta* **1352**: 258–266.

Evered, C. E. & Burton, K. S. (1995) Cryo SEM study of changes in tissue anatomy of harvested sporophores of *Agaricus bisporus*. *Mushroom Science* **14**: 717–721.

Farr, S. B. & Kogoma, T. (1991) Oxidative stress responses in *Escherichia coli* and *Salmonella typhimurium*. *Microbiological Review* **55**: 561–585.

Hammond, J. B. W. (1978) Carbohydrate catabolism in harvested mushrooms. *Phytochemistry* (*Oxford*) **17**: 1717–1719.

Hammond, J. B. W. (1979) Changes in composition of harvested mushrooms (*Agaricus bisporus*). *Phytochemistry* **18**: 415–418.

Hammond, J. B. W. & Nichols, R. (1975) Changes in respiration and carbohydrates during the post-harvest storage of mushrooms (*Agaricus bisporus*). *Journal of the Science of Food and Agriculture* **26**: 835–842.

Hashimoto, W., Kobayashi, E., Nankai, H., Sato, N., Miya, T., Kawai, S. & Murata, K. (1999) Unsaturated glucuronyl hydrolase of *Bacillus* sp. GL1: Novel enzyme prerequisite for metabolism of unsaturated oligosaccharides produced by polysaccharide lyases. *Archives of Biochemistry and Biophysics* **368**: 367–374.

Henrissat, B., Callebaut, I., Fabregas, S., Lehn, P., Mornon, J.-P. & Davies, G. (1995) Conserved catalytic machinery and the prediction of a common fold for several families of glycosyl hydrolases. *Proceeding of the National Academy of Sciences, USA* **92**: 7090–7094.

Kawasaki, T., Henmi, K., Ono, E., Hatakeyama, S., Iwano, M., Satoh, H. & Shimamoto, K. (1999) The small GTP-binding protein Rac is a regulator of cell death in plants. *Proceedings of the National Academy of Sciences, USA* **96**: 10922–10926.

Kingsnorth, C. S., Eastwood, D. C. & Burton, K. S. (2001) Cloning and post-harvest expression of serine proteinase transcripts in the cultivated mushroom *Agaricus bisporus*. *Fungal Genetics and Biology* **32**: 135–144.

Landschulz, W. H., Johnson, P. F. & McKnight, S. L. (1988) The leucine zipper – a hypothetical structure common to a new class of DNA-binding proteins. *Science* **240**: 1759–1764.

McGarry, A. & Burton, K. S. (1994) Mechanical properties of the mushroom, *Agaricus bisporus*. *Mycological Research* **98**: 241–245.

Mol, P. C. & Wessels, J. G. H. (1990) Differences in the wall structure between substrate hyphae and hyphae of fruit-body stipes in *Agaricus bisporus*. *Mycological Research* **94**: 472–479.

Moore, D. (1998) *Fungal Morphogenesis*. Cambridge University Press, Cambridge, UK.

Morton, N., Dickerson, A. G. & Hammond, J. W. B. (1985) Mannitol metabolism in *Agaricus bisporus*: purification and properties of mannitol dehydrogenase. *Journal of General Microbiology* **131**: 2885–2890.

Münger, K. & Lerch, K. (1985) Copper metallothionein from the fungus *Agaricus bisporus*: chemical and spectroscopic properties. *Biochemistry* **24**: 6751–6756.

Muraguchi, H. & Kamada, T. (2000) A mutation in the *eln*2 gene encoding a cytochrome P450 of *Coprinus cinereus* affects mushroom morphogenesis. *Fungal Genetics and Biology* **29**: 49–59.

Natvig, D. O., Sylvester, K., Dvorachek, W. H. & Baldwin, J. L. (1996) Superoxides and catalases. In *The Mycota*. Vol. III. *Biochemistry and Molecular Biology* (R. Brambl & G. Marzluf, eds): 191–209. Springer-Verlag, Berlin.

Nelson, D. R., Koymans, L., Kamataki, T., Stegeman, J. J., Feyereisen, R., Waxman, D. J., Waterman, M. R., Gotoh, O., Coon, M. J., Eastabrook, R. W., Gunsalus, I. C. & Nebert, D. W. (1996) P450 superfamily: update on new sequences, gene mapping, accession numbers and nomenclature. *Pharmacogenetics* **6**: 1–42.

O'Shea, E. K., Rutkowski, R. & Kim, P. S. (1989) Preferential heterodimer formation by isolated leucine zippers from FOS and JUN. *Science* **243**: 538–542.

Panavas, T., Pikula, A., Reid, P. D., Rubinstein, B. & Walker, E. L. (1999) Identification of senescence-associated genes from daylily petals. *Plant Molecular Biology* **40**: 237–248.

Robinson, N. J., Tommey, A. M., Kuske, C. & Jackson, P. J. (1993) Plant Metallothioneins. *Biochemistry Journal* **295**: 1–10.

Rosen, K. M. & Villa-Kormaroff, L. (1993) An alternative method for the visualisation of RNA in formaldehyde agarose gels. *Focus* **12**: 23–24.

Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular cloning: a laboratory manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Sreenivasaprasad, S., Burton, K. S. & Wood, D. A. (2000) Cloning and characterisation of a chitin synthase gene from the cultivated mushroom *Agaricus bisporus* and its expression during morphogenesis. *FEMS Microbiology Letters* **189**: 73–77.

Smith, M. W. & Doolittle, R. F. (1992) A comparison of evolutionary rates of the two major kind of superoxide dismutases. *Journal of Molecular Evolution* **34**: 175–184.

Tachibana, S. & Murakami, T. (1983) Metabolism of riboflavin by *Schizophyllum commune*. *Molecular and Cellular Biochemistry* **51**: 149–160.

Umar, M. H. & van Griensven, L. J. L. D. (1997) Morphological studies on the life span, developmental stages, senescence and death of fruitbodies of *Agaricus bisporus*. *Mycological Research* **101**: 1409–1422.

van den Brink, H. J. M., van Gorcom, R. F. M., van den Hondel, C. A. M. J. J. & Punt, P. J. (1998) Cytochrome P450 enzyme systems in fungi. *Fungal Genetics and Biology* **23**: 1–17.

Yagüe, E., Mehak-Zunic, M., Morgan, L., Wood, D. A. & Thurston, C. F. (1997) Expression of CEL2 and CEL4, two proteins from *Agaricus bisporus* with similarity to fungal cellobiohydrolase I and β-mannanase, respectively, is regulated by the carbon source. *Microbiology* **143**: 239–244.

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