Different temporal and spatial expression of two hydrophobin-encoding genes of the edible mushroom *Agaricus bisporus*

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In a search for genes that are only expressed in fruit bodies of the basidiomycete *Agaricus bisporus*, two cDNAs, *hypA* and *hypB* that encode hydrophobins have been isolated previously. In this study, the structure of *hypB* is resolved and it is shown that the two genes are differentially expressed, indicating that the encoded hydrophobins serve different functions in *A. bisporus* mushrooms. *hypB* encodes a polypeptide (HYPB) of 119 aa that shows little sequence identity with HYPA apart from the characteristic arrangement of eight cysteines found exclusively in hydrophobins. The temporal and spatial expression of the two hydrophobin-encoding genes during fruit body development was compared using Northern analysis and in situ hybridization. Accumulation of *hypA* mRNA was found in tissue fractions consisting of undifferentiated white hyphae. In situ hybridization showed that the highest *hypA* mRNA levels are not found in the outermost cell layers of the pileipellis but in the cell layers adjacent to that. The highest level of expression of *hypB* occurs early in development when the primordium differentiates into densely packed, randomly oriented cap hyphae and loosely packed, vertically oriented stipe hyphae. In mature mushrooms, a strong accumulation of *hypB* transcripts was found only in the transitional zone between cap and stipe tissue, demonstrating that transcription regulation of *hypB* is clearly distinct from *hypA*.

Keywords: *hypA*, *hypB*, *Agaricus bisporus*, fruit body development, hydrophobin

INTRODUCTION

The edible fungus *Agaricus bisporus* is commercially cultivated on a large scale for mushroom production. The differentiation process leading to these reproductive structures is influenced by available nutrients and abiotic factors such as temperature, humidity and the carbon dioxide concentration (Flegg & Wood, 1985). By manipulating environmental conditions during cultivation, the onset and further development of fruit body formation can be controlled to optimize mushroom yields. At present, however, it is unclear what molecular mechanisms program the vegetatively growing mycelium to start differentiation.

For a better understanding of this process, we have previously isolated cDNAs specifying genes that were either strongly induced or specifically expressed in fruit bodies (De Groot et al., 1996, 1997). From various filamentous fungi, structural genes associated with cellular differentiation processes have been isolated and amongst them genes encoding hydrophobins are often found (reviewed by Wessels, 1997). Hydrophobins are small cell wall proteins of about 110 aa that have eight cysteine residues at characteristic positions, a signal sequence for secretion and a specific hydrophobicity pattern. By interfacial self-assembly, hydrophobins form amphipathic films that provide aerial structures with a hydrophobic surface. Hydrophobin aggregates are normally not dissolved in solutions of 2% SDS but can be...
dissociated into monomers using trifluoroacetic acid or formic acid (Wessels, 1997).

One of the fruit-body-specific genes isolated from A. bisporus, hypA, was previously shown to encode a hydrophobin. Accumulation of hypA mRNA was found in the pileipellis (peel) of mushroom caps, constituting more than 60% of the total mRNA population in that tissue. Treatment of pileipellis tissue with SDS followed by dissociation of the hydrophobin aggregates by trifluoroacetic acid extraction resulted in isolation of the corresponding HYP A protein (De Groot et al., 1996). The self-assembling nature of this protein was shown by in vitro studies, in which HYP A (also named ABH1) protein extracts adhered strongly to hydrophobic surfaces, thereby forming hot-SDS-resistant monolayers (Lugones et al., 1996; Gunning et al., 1998) as has also been shown for the well-studied Sc3p hydrophobin from the basidiomycete Schizophyllum commune (Wosten et al., 1994b). HYP A was therefore proposed to be the major constituent of the protective hydrophobic layer surrounding mushroom caps.

In addition to hypA, other hydrophobin-encoding genes were discovered in A. bisporus. The genomic region immediately downstream of hypA contains hypC, a fruit-body-specific gene for which only low levels of expression were detected (De Groot et al., 1996); hypC and its immediate surroundings are 84% identical at the nucleotide level to the hypA region and hypC is therefore probably the result of a duplication event (De Groot et al., 1996; Lugones et al., 1996). Recently, a hydrophobin (ABH3) specifically secreted by vegetatively growing hyphae of A. bisporus was isolated (Lugones et al., 1998).

In an effort to elucidate the function of more fruit-body-specific genes of A. bisporus, we analysed the sequence of a number of the corresponding cDNAs. In this study, we show that pDG47 specifies a protein that also has all the characteristics typical of fungal hydrophobins but is distinct from HYP A. This hydrophobin was designated HYP B. Because a workable transformation system is not yet available for A. bisporus, the regulation of the two genes was compared by performing detailed analyses of temporal and spatial expression of both hydrophobins using conventional Northern analysis and in situ hybridization. We show that hypA and hypB are differentially regulated and probably serve disparate functions in developing mushrooms.

METHODS

A. bisporus strains and culture conditions. Cultivated Agaricus bisporus strain Horst U1 and one of its homokaryotic constituents, H39, were grown on cellophane sheets in Petri dishes containing DT80 medium (Sonnenberg et al., 1988) supplemented with either 3% (w/v) glucose or 7.5% (w/v) powered and sterilized compost (Van Gils, 1988) and solidified with 1.5% (w/v) agar. Colonies were collected after 10 d growth at 24 °C and frozen in liquid nitrogen.

A. bisporus Horst U1 fruit bodies were obtained using a 10 d flushing cycle (Van Gils, 1988). Primordia and mushrooms were harvested from the first appearance of fruit body initials (5 d after fruit body initiation) until spore-bearing mature mushrooms developed (10 d after fruit body initiation). Some mature mushrooms were separated into different fruit body parts before freezing in liquid nitrogen.

Escherichia coli strains, recombinant DNA techniques and enzymes. E. coli strain LE392 (Promega) was used for plasmid amplification and DNA isolation. E. coli DH5α (BRL) was used for plasmid transformation and propagation. Standard DNA manipulations were carried out as described by Sambrook et al. (1989). Restriction enzymes and other enzymes used for DNA manipulations were purchased from BRL and used according to the supplier’s instructions. Plasmid pUC19 (Yanisch-Perron et al., 1985) was used as cloning vector for genomic DNA fragments. Cloned hybridizing fragments and cDNA clones were sequenced by the method of Sanger et al. (1977) using a Thermo Sequenase fluorescent labelled primer cycle sequencing kit with 7-deaza-dGTP (Amersham) and an ALF automated sequencer (Pharmacia).

Isolation of total genomic DNA, Southern blot analysis and cloning of the genomic sequence of hypB. For Southern analysis, total DNA of strain H39 was isolated according to the method of De Graaff et al. (1988), digested with various restriction enzymes, separated on 0.8% agarose gels and transferred onto Hybond-N membranes (Amersham). The membranes were hybridized overnight at 65 °C by standard methods with a hypB cDNA probe labelled with [32P]dATP by random priming (Feinberg & Vogelstein, 1983). Cloning and selection of hypB cDNA plasmid pDG47 has been described by De Groot et al. (1996). Genomic sequences of hypB were obtained by screening a λ EMBL3 genomic library of A. bisporus strain H39 by standard methods using the hypB cDNA probe. Seven λ clones were isolated of which four comprised the complete hypB gene and surrounding regions. Relevant parts of one of these λ clones were subcloned and sequenced.

Nucleotide and protein sequence comparisons. Database searches were performed with the University of Wisconsin Genetics Computer Group (GCG) sequence analysis software package. Homology calculations and amino acid alignments were made by using the GCG programs BESTFIT, PRETTY and PILEUP (Devereux et al., 1984). Hydrophobicity plots were generated with the program DNA Strider Version 1.1 (Marck, 1988) based on the Kyte–Doolittle algorithm with a window size of seven (Kyte & Doolittle, 1982).

Total RNA isolation, Northern analysis and primer extension. Total RNA for Northern analysis was isolated from fruit body and mycelium samples using TRIZol reagent (BRL). The concentration of the RNA samples was determined spectro photometrically and equal amounts of RNA were denatured in 10× SSC (1× SSC is 0.15 M NaCl, 0.015 M trisodium citrate, pH 7.0), 6×15 M formaldehyde and spotted on Hybond-N membrane, or denatured by glyoxal using standard techniques, separated on a 16% (w/v) agarose gel and transferred to Hybond-N membrane. Hybridization of RNA probes was executed at 42 °C in standard hybridization buffer (6× SSC, 5× Denhardt’s solution, 0.5% SDS and 100 mg denatured herring sperm DNA ml−1), to which 10% (w/v) dextran sulphate and 50% (w/v) formamide were added, using hypA and hypB cDNAs as probes. Washing was performed at 65 °C to a final stringency of 0.1× SSC, 0.1% SDS. To provide a loading control, Northern blots were rehybridized with an A. bisporus 28S rDNA fragment (Schaap et al., 1996). Signal intensities were quantified by scanning the autoradiograms with an Ultrascan XL laser densitometer (LKB). Transcription
Fig. 1. Comparison of the amino acid sequence of HYPB with other basidiomycetous class I hydrophobins. (a) Hydrophobicity plots of HYPB, HYPA and Sc3p. Points above zero on the x axes indicate hydrophobic regions. (b) Alignment of the HYPB amino acid sequence to those of HYP/ABH1 (De Groot et al., 1996; Lugones et al., 1996), ABH3 (Lugones et al., 1998), S. commune Sc3p (de Vocht et al., 1998), P. tinctorius HydPt-1 (Tagu et al., 1996) and C. cinereus CoH1 (EMBL accession no. Y10627). The consensus sequence is composed of residues shared by at least five of the proteins. The N-terminal amino acids of the mature HYPB, ABH3 and Sc3p proteins and the putative N-terminal end of mature HYPB are underlined. The eight conserved cysteine residues which were used as fixed coordinates are marked by asterisks.

**RESULTS**

**hypB encodes a fruit-body-specific hydrophobin**

In a previous study, we screened a cDNA library constructed from RNA isolated from primordia for genes that were strongly expressed during fruit body development but not in vegetatively grown mycelium (De Groot et al., 1996, 1997). One of the isolated cDNA clones, pDG47, harbouring a cDNA of 481 bp, hybridized only in fruit bodies with an mRNA of ~500 nt (De Groot et al., 1997). Sequence analysis of this cDNA revealed only one large ORF which would encode a protein of 11,940 Da. The predicted peptide sequence contains eight cysteine residues arranged in a characteristic way with the conserved cysteine residues hydrophobins generally have low levels of sequence identity. However, they display similar patterns of hydrophobicity. Based on small differences in these patterns and solubility character-
Bodies. Stages of development are according to De Groot et al. (1997). In the stage 4 mushroom the morphologically distinct tissues that are used for analysis of spatial expression patterns of hypA and hypB are indicated. Abbreviations: ba, stipe basis; mi, mid-stipe; tz, transitional zone; an, annulus; gi, gills; ic, inner cap; pv, partial veil; pi, pileipellis; cz, connecting zone.

By comparison with protein database sequences we found that the polypeptide translated from hypB has significant homology with only some of the other class I hydrophobins. The highest levels of sequence identity with HYPB were found for hydrophobins of three other basidiomycetes: Sc3p of S. commune, HydPt-1 of the ectomycorrhizal fungus Pisolithus tinctorius and CoH1 of Coprinus cinereus. When the cysteine residues are used as fixed coordinates in an alignment, the remaining amino acids can be aligned in such a way that they show 37–39% identity (Fig. 1b). Excluding the eight cysteine residues, the previously isolated A. bisporus hydrophobins HYPA, HYPC and ABH3 show only 25–31% identity with HYPB.

A genomic library of the homokaryotic strain H39 was then screened. Southern analysis of isolated λ clones and genomic DNA resulted in the restriction map presented in Fig. 2. Comparison of the genomic sequence with the sequence of the cDNA clone showed that the hypB ORF is interrupted by two intervening sequences of 79 and 71 bp with normal splice junctions. The sequence surrounding the start codon conforms to the consensus sequence for translation initiation of highly expressed genes (Kozak, 1989). The transcriptional start of hypB was determined by primer extension analysis using RNA isolated from pin stage mushrooms as template. The major transcription start point in this tissue is located at position -97 relative to the inferred start codon and a minor start point was found further upstream at position -296. Fifty-three base pairs downstream of the stop codon the transcript ends in a poly(A) tail and this site is preceded by an AT-rich sequence, which is often found in eukaryotic genes (Humphrey & Proudfoot, 1988). In the hypB promoter there is a TATA-box (Bucher, 1990), starting 33 bp upstream of the major transcription initiation site.

A number of gene duplications have been found in A. bisporus genes (Harmsen et al., 1992; De Groot et al., 1996; Van de Rhee et al., 1996; Smith et al., 1998) and in all cases the duplicated copies are immediately adjacent and in the same orientation. This led us to analyse the regions surrounding hypB by hybridization and sequence analysis. A second copy of hypB was not found in the A. bisporus genome. However, 2.5 kb upstream of hypB a large ORF was found (Fig. 2) and a cDNA corresponding to this ORF was isolated. Analysis of this cDNA suggests that it encodes the Fe–S protein subunit of succinate dehydrogenase, as there is 85% sequence identity with the Fe–S protein subunit of the oyster mushroom Pleurotus ostreatus (EMBL accession no. AB007361) and 74% identity with the same protein subunit of the basidiomycete yeast Ustilago maydis (Broomfield & Hargreaves, 1992). We have called this gene sudA. sudA is expressed in vegetatively grown mycelium and thus is regulated independently from hypB (results not shown).

Temporal and spatial expression of hypA and hypB

The transcriptional regulation of the two fruit-body-specific hydrophobin genes was compared by determining the mRNA levels of hypA and hypB in total fruit body tissue during development and in various parts of mature mushrooms by Northern analysis. Developing fruit bodies were obtained from a 10 d flushing cycle using commercial cultivation conditions. Fruit bodies were picked from the first visual appearance of primordia, 5 d after initiation of fruit body formation, until the mature mushrooms had their typical button-like appearance and exposed their spore-bearing gills by breaking the veil (see Fig. 3 for details). The temporal expression of the two genes normalized to the signal
obtained by hybridization with a 28S rDNA probe (Schaap et al., 1996) during fruit body development is presented in Fig. 4(a). hypA mRNA remains at a more or less constant level during mushroom development and maturation. hypB mRNA levels are highest during the early stages of fruit body development and decrease threefold after 8 d of development to the levels observed in mature mushrooms.

For determination of the mRNA levels of hypA and hypB in various parts of mature fruit bodies, caps and stipes of mushrooms were separated and further fractionated (Fig. 3). From caps we isolated the peel (pileipellis), inner cap tissue (plectenchyma), a zone between the pileipellis and the plectenchyma (the connecting zone), partial veil tissue and gill tissue (lamellae). The interconnected tissues of the partial veil and the pileipellis both consist of white hyphae (Umar & Van Griensven, 1997) and are difficult to separate, making isolation of only partial veil tissue technically difficult. The partial veil sample therefore contained both partial veil and pileipellis tissue. From the stipes, we isolated the annulus as a separate fraction and the stipes were then sectioned horizontally into 3 mm slices. Of these, three sections were analysed: the transitional zone (or upper part), comprising the physical connection of the stipe with the cap, a mid-stipe section and the stipe basis that connects the mushroom with its mycelial cord. Northern analysis was done with size-fractionated RNA from the various fruit body parts except for RNA from the stipe basis which did not fractionate properly on agarose gels and was therefore analysed by slot blot techniques (not shown).

Fig. 4(b) shows that in mature fruit bodies hypA mRNA accumulates in the annulus, the partial veil, pileipellis tissue and in the zone connecting the pileipellis and the plectenchyma, which is in agreement with previous results (De Groot et al., 1996). The hypA mRNA level in the transitional zone was more than threefold lower, whereas hypA is expressed at relatively low levels in inner cap tissue, in gills and in stipe tissue.

For hypB the distribution of mRNA in mature mushrooms is completely different. A high concentration of hypB mRNA is found in the transitional zone and an approximately fivefold lower hypB mRNA level is detected in inner cap tissue. In all other fractions, hypB mRNA levels were significantly lower than in the transitional zone and the inner cap tissue. In gills, hypB mRNA was below the detection level. Furthermore, within and between assayed fruit body parts, hypB mRNAs of different sizes are found (Fig. 4b). In accordance with the primer extension experiment, this again indicates that hypB transcription can start at different positions.

For both hydrophobin genes, no transcripts were found in compost-grown mycelium. The distribution of hypA and hypB mRNAs in mature mushrooms was compared with the expression pattern obtained with tefA encoding translation elongation factor 1α. As was found previously (Schaap et al., 1997), tefA shows equal transcript levels in all assayed parts of the fruit body and in vegetative mycelium. This suggests that all assayed fruit body parts are metabolically active.

Spatial expression of hypA and hypB during development

The previous experiment using conventional Northern analysis techniques showed that in mature mushrooms the highest expression of hypB is found in the transitional zone. It also shows that hypB transcription is more prominent during the early stages of development.
and declines thereafter. To study the spatial expression of the hydrophobin genes at the early stages of fruit body development we applied in situ hybridization techniques to primordia. Fig. 5(a) shows that with this technique differentiation into cap and stipe tissue can already be observed in pin-shaped primordia that appear 5 d after initiation of fruit body formation. At this stage of development hypA mRNA can be detected in the universal veil covering the whole primordium. However, the highest hypA mRNA levels are found in the outer regions of future cap tissue. For hypB, the highest mRNA levels are also found in the future cap, but the expression is concentrated in other, more internal cell layers. In the future stipe hypB mRNA is also clearly present in the inner regions but at a lower level, whereas hypA mRNA in the stipe is hardly detectable. As negative controls, primordia slides were probed with the sense strand of hypA and hypB and no hybridization signals were obtained (not shown). At stage 2, hypA mRNA clearly accumulates in the pileipellis and veil.
hydrophobins contribute to surface hydrophobicity of the basidiomycetes which often produce large and complex developmental structures, the presence of multiple surface-active hydrophobins seems necessary. For example, of the four hydrophobin-encoding genes discovered in *S. commune*, *sc3* was only expressed in vegetatively growing mycelium of both mono- and dikaryons, whereas *sc1*, *sc4* and *sc6* were dikaryon-specific and associated with fruit body formation. Expression patterns obtained with in situ hybridization of the dikaryon-specific genes indicate that regulation of *sc4* differs from *sc1* and *sc6* (Ruiters & Wessels, 1989). Separate functions were also suggested by the low level of sequence identity between the *S. commune* hydrophobins and from the fact that the secondary mycelium of *sc3* deletion strains produced aerial hyphae that were hydrophilic (Van Wetter *et al*., 1996). The expression patterns observed with Northern analysis of *hypA* and *hypB* show that the two genes are differentially regulated in time and space and indicate that each of these *A. bisporus* hydrophobins has its specific role in development.

The highest transcript levels of *hypB* are found during the early stages of fruit body development. During these stages the primordium differentiates into a distinctive cap and stipe. Using in situ Northern hybridization techniques it was shown that in primordia the highest levels of *hypB* mRNA are found in the inner regions of the future cap structure. In mature mushrooms that show a threefold overall decrease in *hypB* mRNA levels, a strong accumulation of *hypB* mRNA was found only in the transitional zone using conventional Northern analysis. These results suggest that the HYPB hydrophobin is instrumental in the differentiation process and that HYPB could be part of a mono- or bilayer that is formed between fruit body caps and stipes. This might relate to the fact that bacterial infections, starting in peel tissue, are often unable to invade the mushroom caps (Umar & Van Griensven, 1995). Another possibility is that, analogous to the proposed function of the Sc4p hydrophobin of *S. commune* (Wessels *et al*., 1995), HYPB is functional in coating air channels in the (stipe of the) fruit body.

*hypA* mRNA levels during development are more or less constant. At the primordial stage of development, high levels of expression of *hypA* are observed in the universal veil covering the whole ‘undifferentiated’ primordium. In mature fruit bodies, *hypA* mRNA is mainly found in the pileipellis, the partial veil and the annulus. These fruit body parts consist of so-called white hyphae, which are suggested to represent a lower grade of differentiation than the hyphae of the cap, stipe and gills (Umar & Van Griensven, 1997).

In a previous paper (De Groot *et al*., 1996) in which the cloning and regulation of *hypA* was described, elevated expression of this gene was also observed in gill tissue. However, the gill fraction used in that paper was probably contaminated with partial veil tissue in which *hypA* is highly expressed. After improving our method of isolating spore-bearing gills, we have now obtained a purer gill fraction that showed low expression of *hypA* and no expression of *hypB*, whereas *tefA* mRNA levels in gills were equal to the mRNA levels in other parts of mature fruit bodies. Whether this is a general phenomen-
enon in basidiomycetes is unknown. The hydrophobins
discovered in the ascomycetes A. nidulans and Neuro-
spora crassa are part of the rodlet layer surrounding
conidiophores and contribute to the hydrophobicity of
the spore surface. Using a different approach of in situ
hybridization on fixed tissue prior to embedding in the
glycol methacrylate resin Technovit 7100, we could
analyse expression of hypA in gill tissue. In preliminary
experiments, we detected hypA mRNA only in sub-
hymenal cells (results not shown), which could indicate
that these cells produce HYP A monomers that may be
part of a hydrophobic layer surrounding the basidio-
spores. However, it should be noted that Lugones et al.
(1996) found that the only fruit body part that was
completely unreactive to anti-ABH1/HYP A were the
gills which also contain (immature) spores.

The in situ Northern analysis also showed that the hypA
mRNA levels in the outermost layer of hyphae are low
compared to cell layers located more internally (Fig. 5a,
c). When the longitudinal sections were stained for total
RNA content of the various cell layers, the outermost
layer of hyphae showed less staining than the more
internally located cell layers (R. T. P. Roeven, unpub-
lished results) and this suggests that these cells are simply
less active. It has been shown that the HYP A rodlet layer
is the outermost stratum of the fruit body pileipellis
(Lugones et al., 1996). If we assume a direct correlation
between hypA mRNA and HYP A protein expression,
this implies that the HYP A hydrophobin is mainly
produced by these inner layers of hyphae and self-
assembly of HYP A monomers does not take place before
these molecules have reached the interface
between the mushroom surface and the air. This
supports the idea that in vivo HYP A specifically self-
assembles at hydrophobic–hydrophilic interfaces.
In agreement with these results, hot-SDS-resistant HYP A
layers are formed when aqueous solutions of purified
HYP A monomers are brought into contact with hydro-
phobic surfaces (Lugones et al., 1996; Gunning et al.,
1998) and this behaviour is similar to the interfacial
self-assembly observed for Sc3p (Wösten et al., 1993,
1994a, b).

ACKNOWLEDGEMENTS

The authors thank Drs J. Baars and A. Sonnenberg of the
Mushroom Experimental Station for their expertise in cul-
turing the A. bisporus strains and for fruitful discussions.

REFERENCES

Bochenek, B. & Hirsch, A. M. (1990). In-situ hybridisation of
nodulin mRNAs in root nodules using non-radioactive probes.
Plant Mol Biol Rep 8, 237–248.

acid change in the iron-sulphur protein subunit of succinate
dehydrogenase confers resistance to carboxin in Ustilago maydis.
Curr Genet 22, 117–121.

Bucher, P. (1990). Weight matrix descriptions of four eukaryotic
RNA polymerase II promoter elements derived from 502 unre-

gene transcripts by nuclease protection assays and cDNA primer

and expression of the Aspergillus nidulans pyruvate kinase gene.
Curr Genet 11, 499–503.

encodes a hydrophobin and specifically accumulates in peel
tissue of mushroom caps during fruit body development. J Mol
Biol 257, 1008–1018.

from the edible mushroom Agaricus bisporus. Microbiology 143,

set of sequence analysis programs for the VAX. Nucleic Acids Res
12, 387–395.

De Vocht, M. L., Scholtmeijer, K., van der Vegte, E. W., de Vries,
O. M. H., Sonveaux, N., Wösten, H. A. B., Ruyschaert, J. M.,
Structural characterization of the hydrophobin SC3, as a mono-
mer and after self-assembly at hydrophobic/hydrophilic inter-
faces. Biophys J 74, 2059–2068.

labeling DNA restriction endonuclease fragments to high specific

Biology and Technology of the Cultivated Mushroom, pp.
Chichester: Wiley.

Atomic Force Microscopy of a hydrophobin protein from the edible
mushroom Agaricus bisporus. J Colloid Interface Sci 201,
118–126.

Harmsen, M. C., Schuren, F. H. J., Moukha, S. M., Van Zuilen,
glyceraldehyde-3-phosphate dehydrogenase genes from the ba-
sidiozymetes Schizopyllum commune, Phanerochaete chryso-


Lugones, L. G., Bosscher, J. S., Scholtmeijer, K., De Vries, O. M. H.
forms hydrophobic rodlet layers in Agaricus bisporus fruiting
bodies. Microbiology 142, 1321–1329.

hydrophobin (ABH1) specifically secreted by vegetatively growing
hyphae of Agaricus bisporus (common white button mushroom).
Microbiology 144, 2345–2353.

analysis of DNA and protein sequences for the Apple Macintosh

Nakari-Setälä, T., Aro, N., Kalkkinen, N., Alatalo, E. & Penttilä, M.
(1996). Genetic and biochemical characterisation of the Tricho-

Nakari-Setälä, T., Aro, N., Ilämä, M., Muñoz, G., Kalkkinen, N.
& Penttilä, M. (1997). Differential expression of the vegetative and


Received 28 September 1998; revised 4 December 1998; accepted 2 February 1999.