Note

Distribution of Hydrophobin 1 Gene Transcript in Developing Fruiting Bodies of Lentinula edodes

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Results of in situ RNA-RNA hybridization showed the presence of transcripts of the Lentinula edodes hydrophobin 1 gene, Le.hyd1, everywhere in the mycelial tissues of developing fruiting bodies except for the top parts of the pileus (cap) and for the prehymenophore. A high level of the transcript was detected in the parts surrounding the prehymenophore.

Key words: hydrophobin gene expression; fruiting body; hymenophore; in situ RNA-RNA hybridization; Lentinula edodes

Hydrophobins are moderately hydrophobic small proteins (100–150 amino acid residues) containing eight cysteine residues in a conserved pattern.1) These compounds appear to be unique to mycelial fungi such as basidiomycetes and ascomycetes, where they probably act in morphogenesis and pathogenesis.2,3) They were discovered in Schizophyllum commune as the products of genes expressed highly during the formation of aerial hyphae and fruiting bodies.2,3) S. commune contains at least four hydrophobin genes, SC1, SC3, SC4, and SC6.4) SC3 hydrophobin coats aerial hyphae of monokaryons and dikaryons with a hydrophobic layer, and mediates the attachment of hyphae to hydrophobic surfaces.5) The SC1, SC4, and SC6 genes are expressed in dikaryons only, at the time of fruiting-body formation.5) Immunoelectron microscopy showed that SC4, the most abundant hydrophobin of the three gene products, is secreted into the mucilage that surrounds hyphae of the plec-tenchyma (fungal tissues) of the fruiting bodies, lining air channels within them with a hydrophobic membrane.5) Subsequently, hydrophobins were isolated from many fungi: the compounds include COH1 and COH2 in Coprinus cinereus,6) ABH1, ABH2, and ABH3 in Agaricus bisporus,7-9) and PHO1, PHO2, and PHO3 in Pleurotus ostreatus.10,11) Recently, two hydrophobin genes, Le.hyd1 and Le.hyd2, were isolated by Ng et al. from Lentinula edodes strain L54.11) Levels of the transcripts of the genes were analyzed in vegetatively growing mycelia, primordia, and fruiting bodies. The transcript level of Le.hyd1 was high in primordia, the initial stage of fruiting, and that of Le.hyd2 was high in dikaryotic vegetative mycelial tissues.11) The two genes were expressed little in monokaryotic mycelial cells. Independently, we isolated two hydrophobin genes from L. edodes dikaryotic strain FMC212) that were identical to Le.hyd1 and Le.hyd2.13) In this paper, we examined details of the expression of Le.hyd1 during the formation of fruiting bodies and in parts of the fruiting bodies of L. edodes FMC2. Northern blotting showed that immature small fruiting bodies (in developmental stage I14) that had just developed from primordia (pileus and stipe do not yet develop) contained the highest level of the transcript. Enlarged immature fruiting bodies (developmental stages II and III15) also contained high levels of the transcript, but vegetatively growing mycelia or primordia and mature fruiting bodies contained no or a little Le.hyd1 transcript. The transcript level of Le.hyd1 in the primordia found by Ng et al. and by us was different. The definition of primordia used may have differed; if the primordia used by Ng et al. correspond to the immature small fruiting bodies in developmental stage I used by us, the difference could be explained. In situ RNA-RNA hybridization showed that the Le.hyd1 gene was expressed everywhere in the mycelial tissues of immature fruiting bodies except for the top parts of the pileus and for the prehymenophores. This is the first report of the distribution of hydrophobin transcripts on the basis of in situ RNA-RNA hybridization.

The Le.hyd1 cDNA coding sequence (384 bp) with 8-bp BamHI restriction sites at both ends was prepared as follows. Total cellular RNA was isolated from immature small fruiting bodies of developmen-
tal stage I of *L. edodes* FMC2\(^{12}\) by the method of Han *et al.*\(^{15}\) Poly(A)\(^+\) RNA was prepared from the total cellular RNA with an Oligoex-T30 (Super) (Takara Shuzo) and treated by RT (reverse transcriptase) PCR. The primers F1 and R1 were used. The former (35-mer) was the sense sequence at nucleotides 1–27 of *Le.hyd1* cDNA\(^{11,15}\) and an 8-nucleotide sequence containing a BamHI restriction site to facilitate the cloning of the amplified DNA. The latter (32-mer) is the antisense sequence at nucleotides 361–384 and an 8-nucleotide sequence containing a BamHI site. The *Le.hyd1* cDNA sequence and the two 8-nucleotide BamHI sequence were amplified by RT (Super Script II RNase H\(^-\) (GIBCO-BRL)) and DNA polymerase (KOD dush (TOYOBO), and digested with BamHI, and the resulting DNA was inserted into the BamHI site of pSPT18 vector (Roch Diagnostics). The recombinant plasmid obtained was designated pSPT18-*Le.hyd1c*(1–384), and its sequence was checked by the chain-termination method of Sanger *et al.*\(^{16}\)

To investigate the expression of the *Le.hyd1* gene during fruiting-body formation of *L. edodes*, northern blotting was done of total cellular RNAs isolated from vegetatively growing mycelia of FMC2 and from FMC2-derived primordia, immature fruiting bodies, and mature fruiting bodies by the method of Han *et al.*\(^{15}\) The stages of immature fruiting bodies were divided into developmental stages I, II, and III as before.\(^{14}\) The pileus and stipe do not develop in primordia, but they can be observed in developmental stage I (when the fruiting body is small). In developmental stage II, the edge of the pileus curls downward to the stipe and adheres to it. In developmental stage III, the edge of the pileus detaches from the stipe and the fruiting body itself enlarges. In the mature stage, the edge of the pileus is straight and the fruiting body grows to maximum size. The RNA samples (25 μg each) were size-fractionated, transferred to a nylon filter, hybridized with \(^{32}\)P-labeled probes of the aforementioned *Le.hyd1* cDNA sequence (nucleotides 1–384), and autoradiographed as described previously.\(^{17}\) Strong signals of about 1 kb (the *Le.hyd1* gene band) were observed with the RNAs of immature fruiting bodies of developmental stages I, II, and III (Fig. 1, lanes 3, 4, and 5, respectively). The intensity of the band was highest with the immature fruiting bodies of developmental stage I. Very weak signals were detected with the RNAs of primordia (lane 2) and mature fruiting bodies (lane 6). No signal was observed with the RNA of vegetatively growing mycelia (lane 1). These results suggest that the *Le.hyd1* gene acts mainly in the stage of development and enlargement of fruiting bodies.

Next, in situ RNA-RNA hybridization was done to investigate the expression of the *Le.hyd1* gene in parts of immature small fruiting bodies of developmental stage I. The samples were fixed with 4% paraformaldehyde in phosphate-buffered saline at 4°C for 4 h, and cut into 10-μm thin longitudinal cryosections as described previously.\(^{10}\) Sense and antisense RNA probes were prepared by the *in vitro* transcription of pSPT18-*Le.hyd1c*(1–384) with T7 or SP6 RNA polymerase and digoxigenin-UTP (Roche Diagnostics) according to the manufacturer's instructions. Treatment of the thin sections before hybridization, hybridization with RNA probes, and washing of the hybridized sections were done basically as reported by Bohchenek and Hirsch.\(^{18}\) Immunological detection of the hybridized probes was done with the digoxigenin-nucleic acid detection kit (Roche Diagnostics). In the experiments, total RNA in the fixed thin sections was stained shocking pink by methylgreen pyronin stain.\(^{10}\) The *Le.hyd1* antisense strand probe showed the transcript everywhere in the mycelial tissues of immature small fruiting bodies except for the top parts of pileus (cap) (Fig. 2, panel B1); the distribution was similar to that of total RNA except for the top parts of the pileus (panel A). The *Le.hyd1* sense strand probe gave no signal (panel B2).

The distribution of *Le.hyd1* transcript around the prehymenophore (which looks like eyes in Fig. 2) was analyzed for immature fruiting bodies of developmental stages I and II (Fig. 3). The prehymenophore develops into the hymenophore on which a large number of basidia and basidiospores are formed (see Ref. 14). Intense signals were detected in the surrounding parts of prehymenophores of developmen-
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Fig. 2. Distribution of the *Le.hyd1* Transcript in Immature Small Fruiting Bodies of *L. edodes* Seen by *in situ* RNA-RNA Hybridization. Fixed longitudinal thin sections of immature small fruiting bodies of developmental stage I were hybridized with a digoxigenin-labeled *Le.hyd1* antisense strand probe (panel B1) or sense strand probe (panel B2) and were stained shocking pink by methylgreen pyronin stain (total RNA was stained) (panel A). Bar, 2 mm.

Fig. 3. Distribution of the *Le.hyd1* Transcript in Mycelial Tissues Around the Prehymenophore Seen by *in situ* RNA-RNA Hybridization. Fixed longitudinal thin sections of mycelial tissues around the prehymenophore of immature small fruiting bodies of developmental stage I (panels A, B1, and B2) and immature fruiting bodies of developmental stage II (panels C1 and C2) were hybridized with a digoxigenin-labeled *Le.hyd1* antisense strand probe (panels B1 and C1) or sense strand probe (panels B2 and C2) and were stained shocking pink by methylgreen pyronin stain (total RNA was stained) (panel A). Bar, 200 μm.

As mentioned earlier, hydrophobins seem to be involved in the formation in the extracellular matrix of lined air channels with a hydrophobic membrane.
These channels may help to provide gas exchange during respiration in mycelial tissues of developing fruiting bodies. Our results suggested that hydrophobin-mediated air channels may be formed all over the mycelial tissues of developing fruiting bodies except for the top parts of the pileus (cap) and for the prehymenophore. In particular, the parts surrounding the prehymenophore may come to have a large number of air channels.

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References