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Comparative Proteomic Analysis of Mushroom Cell Wall Proteins among the Different Developmental Stages of *Pleurotus tuber-regium*

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Supporting Information

ABSTRACT: Cell wall proteins (CWPs) play a vital role in the development of the different morphological stages including mycelium, fruiting body, and sclerotium in mushrooms which are important human food sources. Using fractionation by detergents and reducing agents, mushroom cell wall fractions from the different developmental stages of *Pleurotus tuber-regium* (PTR) were prepared. Using one-dimensional gel electrophoresis coupled with LC−MS, there were 103, 91, and 48 noncovalently linked CWPs identified in the cell wall fractions of the PTR mycelium, fruiting body, and sclerotium, respectively. Comparing the CWPs in these cell wall fractions, 19 of them were in common, among which 17 belonged to the functional categories of translation, ribosomal structure, and biogenesis. This is the first study to provide important biochemical insights into the different developmental stages of PTR mediated by CWPs, and the identified CWPs helped to explain the morphological changes of PTR mushrooms during cultivation.

KEYWORDS: *mushroom, cell wall proteins, proteomic analysis, Pleurotus tuber-regium*

INTRODUCTION

Mushrooms which belong to higher or macro fungi (Basidiomycetes) have a life cycle that can involve different developmental stages or morphotypes (morphogenesis) including germination of spores, growing of mycelial hyphae, and formation of fruit body and sclerotium.[1] Mushrooms in their different morphological forms have been known for their nutritional/medicinal values and are also important sources of human foods.[2−4] While the detailed mechanism of morphogenesis in lower fungi such as unicellular fungi, yeast (in particular *Saccharomyces cerevisiae*) and filamentous fungi such as *Aspergillus fumigatus* have been well studied,[5,6] the biochemical changes in mushroom morphogenesis are less understood. Among all the changes, the fungal cell wall architecture is subjected to the most drastic ones during morphogenesis in mushrooms.[7]

Fungal cell wall proteins (CWPs) with content ranging from 3 to 20% dry weight of cell wall vary in their chemical composition depending on the different phases of the fungal life cycle, environmental conditions, and developmental stages.[8] Fungal CWPs play a key role in morphogenesis in terms of coordinating various cellular metabolisms necessary for the different developmental stages.[9,10] CWPs can be divided into covalent and noncovalent types depending upon their degree of interaction with the other cell wall components in the structural network.[11] Covalent CWPs perform structural functions via different linkages to the polysaccharide network in the cell wall, whereas noncovalent CWPs are related to the biosynthesis of wall constituents in the building process of the cell wall, transport of substances, and signal transmission.[12]

Proteomics is an excellent tool in profiling, discovering, and identifying proteins produced in response to a changing cellular environment.[13] By a comparative proteomic analysis of the proteins (especially CWPs) in the various developmental stages of mushrooms, a better picture of the biochemical changes occurred during fungal morphogenesis and the related cellular metabolism can be revealed.[6] One-dimensional gel electrophoresis (1-D GE), followed by enzymatic hydrolysis and nanoelectrospray ionization-LC−MS/MS (nESI-LC−MS/MS) analysis, is one of the most common methods in proteomic analysis.[14] Recently, a few systematic proteomic studies of fungi and mushrooms have expanded our understanding on their proteomes.[10,15]

Pleurotus tuber-regium (PTR), an edible/medicinal mushroom that is capable of having all the different developmental stages mentioned above, is an ideal model for the study of biochemical changes in the cellular structure during morphogenesis of Basidiomycetes.[16] Besides, the sclerotium, fruiting body, and mycelium of PTR have a wide range of useful applications as functional food ingredients and nutraceuticals that have human health implications.[4,16−18] Hence, the investigation of CWPs of PTR in its different morphological stages including mycelium, fruiting body, and sclerotium by 1-D GE can contribute to the understanding on the dynamic changes in mushroom life cycle in terms of differential protein

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expression and facilitate the possible improvement of mushroom cultivation at the molecular aspect.

MATERIALS AND METHODS

Sample Preparation. The mycelium of *Pleurotus tuber-regium* (PTR) was purchased from Fungi Perfecti, Ltd. Co. (Olympia, WA, USA). Stock culture was maintained on a potato dextrose agar (PDA) plate and subcultured periodically. The inoculum was prepared by transferring 10 pieces of 7-day-old mycelia each with an area of 1 cm^2 cut from the PDA plate to 100 mL of defined culture medium in a 250 mL conical flask and incubated for 4 days. Shake-flask culture was carried out in 500 mL conical flasks containing 200 mL of defined culture medium after inoculation with 10% (v/v) of the inoculum. The defined medium contained 30 g/L glucose, 4 g/L yeast extract, 1 g/L KH_2PO_4, 0.6 g/L $Mg_2SO_4{\cdot}7H_2O$. The cultures were incubated at 30 °C in a rotary shaker incubator at 180 rpm, and the mycelium was harvested after 7 days by filtration. Sclerotia were obtained from solid-state fermentation of PTR mycelium in substrates containing saw dust (50%), smashed cotton seed hulls (30%), rice bran (20%) and mixed with water (1:1.1 v/v). The mixed substrates were packed in mushroom culture bags made of polypropylene resin, sterilized, and cooled to room temperature. Precultured PTR mycelia were inoculated on the top of the culture bags and were kept at 30 °C, 70−85% relative humidity for about 4 months for the sclerotia to develop. Fruiting body was obtained from PTR sclerotium embedded in soil kept in a greenhouse under humid and warm conditions for 2 months.

Cell Wall Isolation. Samples of mycelium, sclerotium, or fruiting body were lyophilized before being pulverized into powders to pass through a screen with an aperture of 0.5 mm by using a Cyclotech Mill (Tecator, Höganäs, Sweden). The milled powders were suspended in distilled water and ultrasonicated at 300 W with a 30% duty cycle for 10 min (Sonics & Materials Vibracell, model VC 600) for cell breakage. The degree of cell breakage was monitored by a phase contrast microscope (PCM, Eclipse 80i, Nikon). Cleaved mushroom cells were washed sequentially with wash solution A [1 mM phenylmethylsulfonyl fluoride (PMSF)], wash solution B [5% (w/v) NaCl, 1 mM PMSF], wash solution C [2% (w/v) NaCl, 1 mM PMSF], wash solution D [1% (w/v) NaCl, 1 mM PMSF], and then wash solution A again to remove intracellular contaminants. Each step was repeated three times. The washed residues were lyophilized to give the isolated cell wall fraction.

Protein Extraction and Fractionation. Total proteins in the mushroom samples and their corresponding isolated cell wall fractions were extracted using the Tris (pH 8.8) buffered phenol (TBP) method.[15]

Extraction of the noncovalent CWPs was performed using a mixture of detergent and reducing agent [DRA] consisting of 50 mM Tris-HCl, pH 8.0, 0.1 M ethylenediaminetetraacetic acid (EDTA), 2% (w/v) sodium dodecyl sulfate (SDS), and 10 mM dithiothreitol (DTT) according to the CWP fractionation protocol used in yeast and filamentous fungi.[19] The combined use of SDS and DTT facilitated the solubilization of noncovalent CWPs and increased the cell wall porosity to enhance the action of wall degrading enzymes. The purified cell wall fractions (1 g dry weight) were resuspended in 200 mL of ice-cold wash buffer (50 mM Tris-HCl, pH 8.0, 1 mM PMSF) and centrifuged at 4000*g* for 10 min at 4 °C. After decanting the supernatants, the residues were suspended in 5 mL of DRA and boiled at 100 °C for 10 min. The extraction was repeated twice. After centrifugation, the supernatants were pooled, while the pellets were stored at −80 °C. The solubilized proteins in the supernatants were precipitated by adding an ice-cold 100% trichloroacetic acid (TCA) solution to give a final concentration of 10% TCA. The suspensions were mixed thoroughly and incubated on ice for 30 min. After centrifugation (at 4000*g* for10 min at 4 °C), the supernatant was discarded and the pellet was washed twice with cold acetone to remove residual TCA. The precipitated protein pellet was then dried by vacuum-drying using Speed-Vac (LABCONCO) for 15 min and stored at −80 °C. These proteins were redissolved in 0.5 mL of rehydration solution [8 M urea, 2% (w/v) CHAPS, 0.002% (w/v) bromophenol blue and 2% (v/v) immobilized pH gradient (IPG) buffer], followed by centrifugation at 4000*g* for 15 min at 4 °C. The clear supernatant containing the soluble proteins was used for protein quantification by the PlusOne 2D Quant Kit (GE Healthcare) and stored in aliquots at −80 °C.

Protein Quantification. Total proteins of mushroom samples, total CWPs, noncovalent CWPs, and covalent CWPs (residual proteins bound to cell walls after the fractionation using DRA) were measured using the modified biuret method.[20] A standard curve was prepared for calibration using the 2 mg/mL bovine serum albumin (BSA) standard solution. A sample of about 0.1 g of protein was mixed with 0.25 mL of CCl_4 and 10 mL of biuret solution in a tube. After 10 min of vortex-mixing, the suspension was placed at room temperature for 1 h. The absorbance of the supernatant was measured at 550 nm, and the protein concentration of the samples was determined using the standard curve.

Before the one-dimensional gel electrophoresis, the concentration of extracted protein was measured using the PlusOne 2D Quant Kit (GE Healthcare), and this assay has a linear response to protein in the range of 0 to 50 μg.

One-Dimensional Gel Electrophoresis (1-D GE) and In-Gel Digestion. Before electrophoresis, the extracted proteins (40 μg) in rehydration solution were mixed with 1/3 volume of loading buffer [16% (w/v) SDS, 48% (v/v) glycerol, 2.4% (w/v) Tris base, 8% (v/v) β-mercaptoethanol, and 0.1% (w/v) bromophenol blue] and heated for 4 min at 94 °C. The proteins were then separated by SDS−polyacrylamide gel electrophoresis (PAGE) using gels (4% stacking gel and 12% separating gel) on a vertical electrophoresis unit at constant voltage of 110 V. The running buffer was composed of 25 mM Tris base, 192 mM glycine, and 0.1% (w/v) SDS. About 5 μL of the commercially available broad range SDS−PAGE standards (pre-stained, Bio-Rad, Hercules, CA) was loaded at the same time in the well next to the samples.

After the SDS−PAGE run, the gel was washed with Milli-Q water and then stained with 50 mL of Coomassie staining solution containing 45% (v/v) methanol, 10% (v/v) acetic acid, and 0.15% (w/v) Coomassie Brilliant Blue R350 for 1 h. The gels were finally destained for 1−1.5 h using 100 mL of destaining solution containing 40% (v/v) methanol and 10% (v/v) acetic acid in Milli-Q water.

As a comparison, the other gel under the same electrophoresis conditions was stained to determine the sugar in the sample according to periodic acid Schiff's reaction (PAS). After electrophoresis, the gel was transferred to the development Petri dish with the fixing solution (10% (v/v) TCA in water) for shaking 5 min at 60 rpm on a plate shaker. The gel was washed twice for 2 min in washing solution (5% (v/v) acetic acid in water) and then submerged in the oxidizing solution (1% (w/v) periodic acid in water) for shaking 15 min at 60 rpm. After washing with distilled water for three times, the washed gels were stained with Schiff's reagent in the dark under the same shaking conditions for 30 min. After that, the gel was washed with reducing solution (0.5% (w/v) sodium metasulfitein water) three times for 5 min and stored in 7% (v/v) acetic acid solution at 4 °C.

For the gel stained by Coomassie solution, each lane of sample was sliced into four pieces of gel matrix and further cut into about 1 mm cubes using a clean razor blade on a clean glass surface. The cubes were transferred into Eppendorf tubes and digested with trypsin. In-gel digestion with trypsin and extraction of the in-gel tryptic fragments were performed. About 25−35 μL of acetonitrile (ACN) was added to each tube to cover the gel pieces and incubated for 10 min at room temperature to dehydrate and shrink the gel pieces. After vacuum-drying using Speed-Vac, protein gel pieces were swollen by 150 μL of 10 mM DTT in 100 mM NH_4HCO_3 and incubated for 1 h at 56 °C. The mixture was cooled to room temperature. Then the DTT solution was replaced by 150 μL of 55 mM iodoacetamide (IAA) in 100 mM NH_4HCO_3 and incubated for 45 min at room temperature in the dark with occasional vortexing. The solution was removed, and 150 μL of ACN was added to dehydrate gel pieces followed by 10-min incubation. After vacuum-drying using Speed-Vac, the gel pieces were incubated in 25−35 μL of digestion buffer [12.5 ng/μL trypsin

(sequencing grade modified trypin, Promega) in 50 mM NH_4HCO_3] for 45 min in an ice water bath. The trypsin-containing buffer was removed. Ten microliters of 50 mM NH_4HCO_3 was added to keep gel pieces wet during cleavage, and the mixture was incubated overnight at 37 °C. The mixture was centrifuged at 10000*g*, and the supernatant was stored in a new Eppendorf tube. Twenty microliters of 20 mM NH_4HCO_3 was added to the gel pieces, followed by incubation for 10 min, and the supernatant was removed and combined with the previous extracts. About 25 μL of extraction buffer (50% ACN, 5% formic acid) was added to the gel pieces and incubated for 20 min. This supernatant was combined with the previous ones. Finally, the extracted sample in the pooled supernatants was completely dried by Speed-Vac and store at −80 °C until MS analysis.

nESI-LC−MS/MS Analysis. Just before MS analysis, the trypsin-digested peptides were desalted by using the $ZipTip_{u\text{-}C18}$ (Millipore) treatment. Then the desalted peptides were separated by a C18 reverse-phase column and analyzed on a nanoelectrospray ionization mass spectrometer (nESI-LC−MS/MS). An Ultimate 3000 (Dionex, USA) nanoLC system, combined with the Ultimate 3000 autosampler, was used. The samples were loaded onto a precolumn (5 mm × 300 μm i.d.; Acclaim@PepMap100 C18, Dionex, USA) coupled with an analytical column (15 cm ×75 μm i.d.; Acclaim@PepMap100 C18, Dionex, USA). The nanoflow was eluted at a flow rate of 300 nL/min, where solvent A was 2% ACN with 0.1% formic acid and solvent B was 80% ACN with 0.1% formic acid. LC analyses were performed using a 60 min staged gradient elution program: 0−8 min (0% B), 8−38 min (0−55% B), 38−39 min (55−80% B), 39−44 min (80% B), 44−45 min (80−0% B), 45−60 min (0% B). The column outlet was coupled directly to the high voltage ESI source, which was interfaced to the Fourier transform ion cyclotron resonance (FTICR) mass spectrometer (Apex Ultra 7.0, Bruker, USA). The nanospray voltage was typically 1.7 kV in the nESI-LC−MS/MS mode. The m/z range was 200−2200 Da.

Acquired data were searched against the National Center for Biotechnology Information (NCBI) nonredundant protein database (fungi) using the MASCOT software package (Version 2.3, Matrix Science, U.K.; www.matrixscience.com). The peptide mass and MS/MS tolerance were both 0.05 Da. The peptides have the allowance of one tryptic missed cleavage, one fixed modification with carbamidomethyl (C) and one variable modification by oxidation (M). The peptides could be charged at (1+, 2+, and 3+). A protein hit with a confidence level higher than 95% ($p < 0.05$) was considered as an identified protein.

RESULTS AND DISCUSSION

Cell Wall Content of PTR. Among the three mushroom morphological stages in PTR, sclerotium had the highest yield of cell wall content of 78.42% dry weight (DW), followed by 46.56% DW in fruiting body and 20.91% DW in mycelium. These results reflected the different morphological features of PTR, which include sclerotium as a compact mass of dense hyphae; mycelium as a mass of loosely associated hyphae; and fruiting body as a fleshy basidioma or sporocap. The present results were also consistent with previous findings that the extremely high level of cell wall materials in the sclerotial stage of PTR had the food application of a rich source of dietary fiber.[21]

Protein Content of Cell Wall Fractions. The amount of total proteins (intracellular and CWPs) in fruiting body was the highest (21.44% DW) among the three morphological stages of PTR (Table 1). However, the amount of CWPs in fruiting body was only 15.24% of the total proteins, which was much less than that of mycelial CWPs (21.26%) and sclerotial CWPs (76.97%). Compared to the other morphological stages in PTR, sclerotium had the smallest amount of intracellular proteins.

Table 1. Protein Content of Different Morphological Stages and Cell Wall Fractions of PTR[a]

	mycelium	sclerotium	fruiting body
total proteins (% dry wt)	17.81 ± 0.07 a	9.08 ± 0.06 b	21.44 ± 0.19 c
CWPs (% of the total protein)	21.26 ± 0.27 a	76.97 ± 0.08 b	15.24 ± 0.01 c
noncovalently linked CWPs (% of CWPs)	90.72 ± 2.70 a	84.34 ± 3.50 b	90.59 ± 1.05 a
covalently linked (% of CWPs)		6.66 ± 0.02	

[a]Data are mean ± SD (n = 3). Different letters (a, b, c) denote significant difference between means in the same row evaluated by ANOVA followed by Tukey multiple comparison test with $p < 0.05$.

After the extraction of CWPs using DRA, DRA-extractable noncovalent linked CWPs were the major fraction obtained from all the cell wall samples in PTR (Table 1). No residual proteins were detected in the cell wall fraction of both mycelium and fruiting body, while small amount of proteins (6.66% of the CWPs) was found in sclerotial cell wall of PTR (Table 1). According to these results, most proteins in the cell wall of PTR mycelium and fruiting body were noncovalently linked with other cell wall components, while some covalently linked proteins might exist in the sclerotial cell wall even after the DRA extraction.

One-Dimensional Gel Profile. From the 1-D GE, the profile of the proteins extracted by DRA from PTR sclerotium was very different from that of the mycelium and fruiting body (Figure 1A). Most of the sclerotial proteins were found to have low molecular weight (less than 20 kDa), which might be coupled with sugar according to the result of 1-D GE stained by PAS (Figure 1B). Since almost all the CWPs could be extracted by DRA (except for sclerotial cell wall) (Table 1), we focused on unraveling the noncovalent cell wall proteome by identifying CWPs in protein extracts from the isolated mushroom cell walls in the present study.

Proteins Identified by 1-D GE Gel and nESI-LC−MS/MS. By use of 1-D GE analysis in combination with LC−MS/MS, a total of 48, 91, and 103 nonredundant proteins were identified, respectively, from the cell wall of sclerotium, fruiting body, and mycelium of PTR (Table 2 and Table 3) where there were only 19 common proteins found in the data sets of all these three cell wall fractions (Figure 2). Seventeen of these common proteins are from the functional categories of translation, ribosomal structure, and biogenesis (Table 3). Although PTR sclerotial cell wall fraction had a higher CWP content (Table 1), the number of its identified CWPs (48) was much smaller than that of mycelial (103) and fruiting body cell wall (91).

On the basis of annotations from Genetic sequence database at the NCBI and Universal Protein Resource Knowledgebase (UniProtKB), the identified proteins were functionally categorized according to their biological processes as shown in Table 3. The importance and relevance of these identified proteins to the different morphological stages of PTR mushroom are discussed below.

Proteins of Translation, Ribosomal Structure, and Biogenesis. Biosynthesis of the cell wall polysaccharides and proteins and regulation of the cell wall architecture are very important in the life cycle of mushroom. Therefore, CWPs of translation, ribosomal structure, and biogenesis, which contribute significantly to the biosynthesis and morphogenesis of the

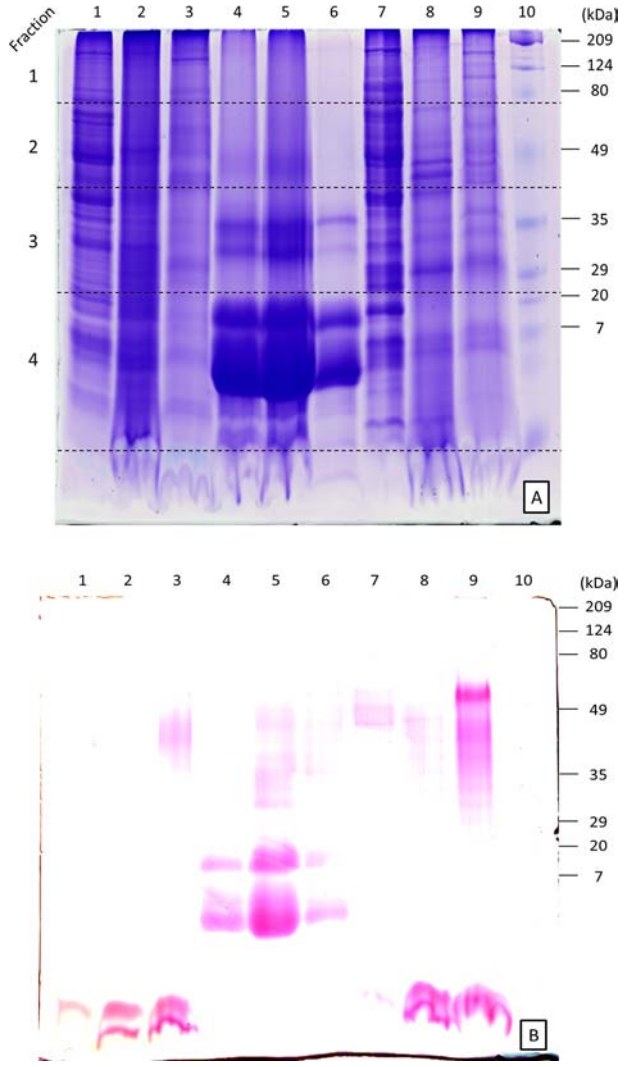

Figure 1. 1-D GE profile of the proteins in PTR mycelium, sclerotium, and fruiting body. (A) Stained by Coomasie Blue R 350. (B) Stained by periodic acid schiff (PAS) reagent: (1) mycelial proteins; (2) mycelial CWPs; (3) noncovalent mycelial CWPs; (4) sclerotial proteins; (5) sclerotial CWPs; (6) noncovalent sclerotial CWPs; (7) fruiting body proteins; (8) fruiting body CWPs; (9) noncovalent fruiting body CWPs; (10) molecular weight markers.

fungal cell wall, were widely found in the cell walls of all three morphological stages of PTR mushroom.

Although the amounts of total CWPs were different, proteins involved in translation, ribosomal structure, and biogenesis were predominant in all the three cell wall samples. They accounted for 73.79%, 60.42%, and 61.54% of the total identified proteins in each cell wall sample, respectively (Table 3). Translation elongation factor proteins such as EF1A, EF1B, and EF2 are associated with ribosomes cyclically during the elongation phase of protein synthesis, and catalyze formation of the acyl bond between the incoming amino acid residue and the peptide chain.[22] They play two important roles during the elongation cycle of protein biosynthesis on the ribosome. First, translation elongation factors are involved in bringing aminoacyl-tRNA (aa-tRNA) to the ribosome during protein synthesis. Second, an elongation factor is involved in translation during the step of elongation at which the peptidyl-tRNA is moved from one ribosomal site to another as the mRNA moves through the ribosome. Both steps result in the hydrolysis of guanosine triphosphate (GTP), and the conformation of the elongation factors changes depending on whether they are bound to GTP or to guanosine diphosphate (GDP).[22] According to a previous study of *Escherichia coli*, both GDP and GTP are associated with the biosynthesis of capsular polysaccharide.[23] In many fungi, a predominantly β-1,3 glucan has been reported to be a major component of the cell wall.[24,25] During cell growth and division, cell wall morphology must keep pace with the other anabolic processes that take place during the cell cycle and with their changes in rate.[26] Studies with *S. cerevisiae*[27,28] and several other fungi[29] showed that the activity of β-1,3-D-glucan synthase (UDP-glucose: β-1,3-D-glucan 3-β-D-glucosyltransferase, EC 2.4.1.34) was stimulated by nucleoside triphosphates, with guanosine derivatives being the most efficient. GTP-binding protein that modulates the biosynthesis of β-1,3-D-glucan of fungal cell walls has also been reported to play a major role in the regulation of cell wall morphogenesis.[30] Therefore, the high level of translation elongation factor proteins (Table 2 and Table 3) found in all three samples (73.79%, 60.42%, and 61.54% of cell walls from three samples, respectively) might be attributed to the biosynthesis and morphogenesis of mushroom PTR cell wall.

As the early stage of the life cycle of PTR, mycelium is the vegetative part of the mushroom, consisting of a mass of branching, threadlike hyphae. For supporting the growth of the mycelium and the mushroom, enzymes are secreted from the tips of mycelial hyphae for digesting the food outside the cells. With a fast growth rate at this stage, more proteins which are related to translation and biogenesis for synthesizing the cell wall components should be transported to the cell wall. Similar to mycelium, the development of fruiting body also requires massive proteins with these similar functions. However, sclerotium, as a hypopus and mycoma, is in an inactive stage to keep survival in the extreme environments. Consequently, the number of proteins under this category found in the PTR sclerotial cell wall was much less than that found in the PTR cell wall of mycelium and fruiting body (Table 3).

Ribosomal proteins such as 60S ribosomal protein family and 40S ribosomal protein family with differential expression levels were found in this category among the three different morphological stages (Table 2). In general, ribosomal proteins are involved in ribosome biogenesis and/or for different stages of the translation process.[31] Accordingly, ribosomal proteins might be very important in transport of the ribosomal precursors, RNA folding, protein assembly, rRNA processing, stabilization of the subunit structure, and/or interaction with other factors required for either ribosome biogenesis or translation. Furthermore, they can also play a key role in cotranslational processes like cotranslational translocation or the interaction with protein folding factors at the exit tunnel of the ribosome.[31] Although not much direct evidence on the direct relationship between cell wall and ribosomal proteins has been found, Ras2 identified in mycelial cell wall has been reported to regulate cell wall architecture by inhibiting GPI-anchor protein synthesis and perhaps by increasing chitin production.[32] The resulting changes in cell wall structure could be important during the growth of mycelium. Although the full mechanism has not been elucidated out, this crosstalk between Ras signaling, GPI-anchor synthesis, and cell wall structure adds an exciting new perspective to Ras signal transduction.

Proteins of Post-Translational Modification, Protein Turnover, and Chaperones. The percentage of mycelial CWPs related to post-translational modification, protein

Table 2. The Functional Categories of Proteins from PTR Mycelial, Sclerotial, and Fruiting Cell Wall Identified by 1-D GE and nESI-LC−MS/MS

accession no.	protein name	organism	theor M_w	source[a]
	Chromatin Structure and Dynamics			
gil50400217	histone H4	*Mortierella alpina*	11402	M
gil188595808	histone H2B	*Saccharomyces cerevisiae* (baker's yeast)	21026	M
gil19112358	histone H4 h4.2	*Schizosaccharomyces pombe 972h-*	11416	M
gil23476924	histone H4	*Glomerella cingulata*	5700	M
gil2495140	histone H2B	*Agaricus bisporus*	15156	F
gil39942996	histone H2B	*Magnaporthe oryzae 70-15*	14845	F
	Translation, Ribosomal Structure, and Biogenesis			
gil336374137	hypothetical protein SERLA73DRAFT_175951	*Serpula lacrymans var. lacrymans S7.3*	50406	M, S, F
gil3023693	elongation factor 1-alpha	*Aureobasidium pullulans*	50031	M, S, F
gil119141	elongation factor 1-alpha	*Absidia glauca*	50139	M, S, F
gil66731352	translation elongation factor 1-alpha	*Aureoboletus thibetanus*	45558	M, S, F
gil6015061	elongation factor 1-alpha	*Schizophyllum commune*	50444	M, S, F
gil212527772	translation elongation factor EF-1 alpha subunit, putative	*Penicillium marneffei ATCC 18224*	50388	M, F
gil39943122	elongation factor 1-alpha	*Magnaporthe oryzae 70-15*	51543	M, S, F
gil6319594	Tef2p	*Saccharomyces cerevisiae S288c*	50400	M
gil283975525	elongation factor 1-alpha	*Pseudozyma flocculosa*	55200	M
gil11078140	translation elongation factor 1-alpha	*Chaetocladium brefeldii*	45284	M, S, F
gil209402353	translation elongation factor 1 alpha	*Absidia psychrophilia*	39947	M
gil210161792	translation elongation factor 1alpha	*Dichotomocladium elegans*	39844	M
gil73990901	translation elongation factor 1-alpha	*Hanseniaspora clermontiae*	33610	M
gil328850299	hypothetical protein MELLADRAFT_73378	*Melampsora larici-populina 98AG31*	50422	M, S, F
gil32563298	translation elongation factor 1-alpha	*Zygosaccharomyces mrakii*	41463	M
gil296825910	elongation factor 1-alpha	*Arthroderma otae CBS 113480*	50150	M, S, F
gil83863541	translation elongation factor EF1-alpha	*Fomitopsis pinicola*	44018	M
gil11078188	translation elongation factor 1-alpha	*Gamsiella multidivaricata*	47044	M, F
gil121702563	translation elongation factor EF-1 alpha	*Aspergillus clavatus NRRL 1*	50451	M, S, F
gil110666941	translation elongation factor 1 alpha	*Echinoplaca strigulacea*	28830	M
gil82792152	elongation factor 1-alpha	*Cladochytrium replicatum*	43530	M, F
gil32563364	translation elongation factor 1 alpha	*Candida castellii*	41345	M, F
gil320590768	translation elongation factor 1 alpha	*Grosmannia clavigera kw1407*	50173	M, S, F
gil148788090	elongation factor 1-alpha	*Tricholoma subaureum*	27065	M
gil134284926	elongation factor 1-alpha	*Dactylellina parvicollis*	27758	M
gil2367625	elongation factor 1-alpha	*Rhodotorula mucilaginosa*	44220	M, S, F
gil37730263	translation elongation factor EF1-alpha	*Pichia angusta*	50370	M
gil330894885	elongation factor 1-alpha	*Rhizomucor pusillus*	34061	M
gil9230401	translation elongation factor 1 alpha	*Gibberella fujikuroi*	9709	M, S, F
gil32563328	translation elongation factor EF1-alpha	*Kluyveromyces yarrowii*	41540	M, F
gil170092519	40S ribosomal protein S0	*Laccaria bicolor S238N-H82*	31115	M
gil242211468	40S ribosomal protein S18	*Postia placenta Mad-698-R*	18042	M
gil170092941	predicted protein	*Laccaria bicolor S238N-H82*	18011	M
gil148788088	elongation factor 1-alpha	*Tricholoma portentosum*	27106	M
gil159895354	translation elongation factor EF1-alpha	*Pichia methanolica*	33937	M, F
gil159895314	translation elongation factor EF1-alpha	*Pichia minuta var. nonfermentans*	33919	M, F
gil156099538	translation elongation factor EF1-alpha	*Pichia canadensis*	31408	M, F
gil156099632	translation elongation factor EF1-alpha	*Nakazawaea holstii*	33995	M
gil300394642	translation elongation factor 1-alpha	*Sarcomyxa serotina*	32875	M, S, F
gil60115834	translation elongation factor EF1-alpha	*Cotylidia sp. MB5*	44904	M, F
gil302698065	hypothetical protein SCHCODRAFT_73326	*Schizophyllum commune H4-8*	94282	M
gil156099362	translation elongation factor 1 alpha	*Candida abiesophila*	34023	M, S, F
gil116687341	translation elongation factor 1-alpha	*Hypocrea microcitrina*	30894	M, S, F
gil11078248	translation elongation factor EF1-alpha	*Rhizopus stolonifer*	45735	M
gil238572113	hypothetical protein MPER_14287	*Moniliophthora perniciosa FA553*	13791	M
gil159895262	translation elongation factor EF1-alpha	*Ambrosiozyma ambrosiae*	33634	M
gil66473239	elongation factor 1-alpha	*Uromyces appendiculatus*	44525	M
gil32563370	translation elongation factor EF1-alpha	*Hanseniaspora guilliermondii*	41491	M
gil148788042	elongation factor 1-alpha	*Lyophyllum connatum*	26601	M
gil299745325	40S ribosomal protein S4	*Coprinopsis cinerea okayama7#130*	29492	M, F
gil169858544	40S ribosomal protein S20	*Coprinopsis cinerea okayama7#130*	13661	M
gil169864162	Ras2	*Coprinopsis cinerea okayama7#130*	40999	M

Table 2. continued

accession no.	protein name	organism	theor M_w	source[a]
		Translation, Ribosomal Structure, and Biogenesis		
gil87280970	translation elongation factor 1 alpha	*Hypocrea rogersonii*	7658	M
gil238608211	hypothetical protein MPER_02448	*Moniliophthora perniciosa FA553*	27692	M
gil169862655	60s ribosomal protein l27	*Coprinopsis cinerea okayama7#130*	15893	M
gil169848048	40S ribosomal protein S0	*Coprinopsis cinerea okayama7#130*	32184	M
gil19114383	60S ribosomal protein L3	*Schizosaccharomyces pombe 972h*	43946	M
gil169856964	40S ribosomal protein S14	*Coprinopsis cinerea okayama7#130*	16049	M
gil170088466	Predicted protein	*Laccaria bicolor S238N-H82*	28595	M, S
gil242206289	60S ribosomal protein L18A	*Postia placenta Mad-698-R*	20198	M
gil71010845	60S ribosomal protein L6	*Ustilago maydis 521*	25834	M
gil170095049	predicted protein	*Laccaria bicolor S238N-H82*	29347	M
gil293491188	translation elongation factor 1 alpha	*Sporobolomyces ruberrimus*	22977	M
gil242208525	60S ribosomal protein L9	*Postia placenta Mad-698-R*	21287	M
gil238566324	hypothetical protein MPER_15891	*Moniliophthora perniciosa FA553*	20427	M, S, F
gil169862772	ribosomal protein L31e	*Coprinopsis cinerea okayama7#130*	14487	M
gil85719133	ribosomal protein L31	*Pichia jadinii*	13716	M
gil164658722	hypothetical protein MGL_2282	*Malassezia globosa CBS 7966*	27601	M
gil169856609	60S ribosomal protein L24	*Coprinopsis cinerea okayama7#130*	16858	M
gil299755587	60S ribosomal protein L32	*Coprinopsis cinerea okayama7#130*	14572	M
gil50554395	YALI0E30811p	*Yarrowia lipolytica*	14812	M
gil169847119	40S ribosomal protein S5-1	*Coprinopsis cinerea okayama7#130*	22584	M
gil212539748	40S ribosomal protein S13	*Penicillium marneffei ATCC 18224*	16919	M
gil170111669	predicted protein	*Laccaria bicolor S238N-H82*	22768	M
gil169854505	elongation factor 1-gamma	*Coprinopsis cinerea okayama7#130*	45860	M
gil302674577	hypothetical protein SCHCODRAFT_86168	*Schizophyllum commune H4-8*	29838	M
gil66473253	translation elongation factor 1-alpha	*Phaeolus schweinitzii*	39018	S, F
gil11078158	translation elongation factor 1-alpha	*Dichotomocladium elegans*	45806	S, F
gil224223902	transcription elongation factor 1-alpha	*Gyroporus castanaeus*	31866	S, F
gil224223890	transcription elongation factor 1-alpha	*Astraeus hygrometricus*	33323	S, F
gil11078162	translation elongation factor 1-alpha	*Dissophora decumbens*	46907	S, F
gil67043894	translation elongation factor 1-alpha	*Rhodocollybia maculata*	31710	S, F
gil113958648	elongation factor 1 alpha	*Pseudocyphellaria anomala*	28474	S, F
gil113958668	elongation factor 1 alpha	*Lobariella pallida*	28687	S, F
gil154819731	translation elongation factor 1 alpha	*Lecanicillium psalliotae*	36280	S, F
gil58268102	60S ribosomal protein L6	*Cryptococcus neoformans var. neoformans JEC21*	25405	S
gil58260012	60S ribosomal protein l11	*Cryptococcus neoformans var. neoformans JEC21*	19770	S, F
gil1136783	elongation factor 1 alpha-A	*Schizosaccharomyces pombe*	50128	F
gil346977583	elongation factor 1-alpha	*Verticillium dahliae VdLs.17*	51393	F
gil82792178	elongation factor 1-alpha	*Phycomyces blakesleeanus*	40296	F
gil20137974	elongation factor 1-alpha	*Piriformospora indica*	50305	F
gil58618691	elongation factor 1-alpha	*Hygrophoropsis aurantiaca*	45786	F
gil156558207	elongation factor 1-alpha	*Hebeloma cylindrosporum*	50418	F
gil345562374	elongation factor 1-alpha	*Arthrobotrys oligospora ATCC 24927*	50438	F
gil58758727	translation elongation factor 1-alpha	*Grifola frondosa*	44499	F
gil222476495	translation elongation factor 1 alpha	*Arthonia caesia*	25193	F
gil156099482	translation elongation factor 1-alpha	*Pichia salicaria*	31455	F
gil148788076	elongation factor 1-alpha	*Termitomyces sp. IE-BSG-BSIsp.1*	27115	F
gil242784621	translation elongation factor EF-1 alpha subunit	*Talaromyces stipitatus ATCC 10500*	50309	F
gil258564078	translation elongation factor EF-1, subunit alpha	*Uncinocarpus reesii 1704*	50369	F
gil11078128	translation elongation factor 1-alpha	*Amylomyces rouxii*	45494	F
gil336373180	hypothetical protein SERLA73DRAFT_176829	*Serpula lacrymans var. lacrymans S7.3*	20959	F
gil32563284	translation elongation factor 1-alpha	*Kazachstania viticola*	41103	F
gil1168489	ADP-ribosylation factor	*Candida albicans*	20277	F
gil1173217	40S ribosomal protein S22	*Agaricus bisporus*	14815	F
gil254578586	ZYRO0B07590p	*Zygosaccharomyces rouxii*	14643	F
		Post-Translational Modification, Protein Turnover, and Chaperones		
gil172713	70 kDa heat shock protein	*Saccharomyces cerevisiae*	37593	M
gil255712457	KLTH0C06556p	*Lachancea thermotolerans*	70557	M, F
gil53829560	HSP70	*Rhizophlyctis rosea*	49976	M
gil255941672	Pc16g13060	*Penicillium chrysogenum Wisconsin 54-1255*	18109	M
gil4917	BiP	*Schizosaccharomyces pombe*	73093	M

Table 2. continued

accession no.	protein name	organism	theor M_w	source[a]
		Post-Translational Modification, Protein Turnover, and Chaperones		
gil170087204	predicted protein	*Laccaria bicolor S238N-H82*	71300	M
gil260940130	hypothetical protein CLUG_05851	*Clavispora lusitaniae ATCC 42720*	74220	M
gil4098191	catalase	*Pleurotus djamor*	30935	S, F
gil299753594	heat shock cognate 70	*Coprinopsis cinerea okayama7#130*	70717	S
gil170097613	predicted protein	*Laccaria bicolor S238N-H82*	73590	F
gil67537918	heat shock 70 kDa protein	*Aspergillus nidulans FGSC A4*	70045	F
gil45198632	AFR114Wp	*Ashbya gossypii ATCC 10895*	69783	F
gil407521	chaperone	*Saccharomyces cerevisiae*	68617	F
		Energy Production and Conversion		
gil336363900	hypothetical protein SERLA73DRAFT_191389	*Serpula lacrymans var. lacrymans S7.3*	58894	M
gil82400263	eukaryotic ADP/ATP carrier	*Cryptococcus neoformans var. grubii*	34158	M
gil50308493	ATP synthase subunit alpha, mitochondrial	*Kluyveromyces lactis NRRL Y-1140*	59124	M
gil170087348	ATP synthase subunit beta	*Laccaria bicolor S238N-H82*	57818	M, F
gil170083897	predicted protein	*Laccaria bicolor S238N-H82*	33356	M, F
gil50543574	YALI0A10659p	*Yarrowia lipolytica*	32967	M, F
gil169865690	malate dehydrogenase	*Coprinopsis cinerea okayama7#130*	35160	M
gil67537828	ATP synthase subunit alpha	*Aspergillus nidulans FGSC A4*	73664	M
gil71023159	hypothetical protein UM05662.1	*Ustilago maydis 521*	31952	M, S, F
gil170096999	NAD-malate dehydrogenase	*Laccaria bicolor S238N-H82*	34836	M, S
gil116204743	ATP synthase beta chain, mitochondrial precursor	*Chaetomium globosum CBS 148.51*	55682	S
gil1827812	ATP synthase subunit beta, mitochondrial	*Bos taurus*	51673	S, F
gil299743469	voltage-dependent ion-selective channel	*Coprinopsis cinerea okayama7#130*	31090	S, F
gil71018215	ATP synthase subunit beta	*Ustilago maydis 521*	55695	F
gil170086766	ATP synthase subunit alpha	*Laccaria bicolor S238N-H82*	58421	F
gil68466091	malate dehydrogenase	*Candida albicans SC5314*	34821	F
gil3549613	ADP/ATP carrier protein	*Candida parapsilosis*	32914	F
gil169856054	aldehyde dehydrogenase	*Coprinopsis cinerea okayama7#130*	54634	F
gil164657588	ATP synthase subunit beta	*Malassezia globosa CBS 7966*	47384	F
gil158251738	cytochrome *c* oxidase subunit 2	*Pleurotus ostreatus*	27741	F
		Carbohydrate Transport and Metabolism		
gil299740078	adenosylhomocysteinase	*Coprinopsis cinerea okayama7#130*	47514	M
gil336375198	hypothetical protein SERLA73DRAFT_158148	*Serpula lacrymans var. lacrymans S7.3*	48118	M
gil169845435	enolase	*Coprinopsis cinerea okayama7#130*	47418	M, S, F
gil225562322	enolase	*Ajellomyces capsulatus G186AR*	47379	M
gil417016	glyceraldehyde-3-phosphate dehydrogenase 1	*Agaricus bisporus*	36954	S, F
gil30038515	glyceraldehyde-3-phosphate dehydrogenase	*Coprinopsis cinerea*	36756	S, F
gil1730152	glyceraldehyde-3-phosphate dehydrogenase	*Amanita muscaria*	31245	S
gil47779437	glyceraldehyde 3-phosphate dehydrogenase	*Leccinum monticola*	25253	S
gil170096919	glycoside hydrolase family 3 protein	*Laccaria bicolor S238N-H82*	81144	F
gil238617024	hypothetical protein MPER_00065	*Moniliophthora perniciosa FA553*	13258	F
gil170100663	predicted protein	*Laccaria bicolor S238N-H82*	52096	F
gil320592453	phosphoglycerate mutase family protein	*Grosmannia clavigera kw1407*	33637	F
		Amino Acid Transport and Metabolism		
gil642360	peptidyl-prolyl cis-trans isomerase	*Tolypocladium inflatum*	19495	S
gil19113718	agmatinase	*Schizosaccharomyces pombe 972h-*	43024	F
gil238580962	hypothetical protein MPER_11413	*Moniliophthora perniciosa FA553*	23457	F
gil58258949	endopeptidase	*Cryptococcus neoformans var. neoformans JEC21*	47745	F
gil58268812	proteasome component pts1	*Cryptococcus neoformans var. neoformans JEC21*	33052	F
		Cytoskeleton		
gil1703157	actin	*Xanthophyllomyces dendrorhous*	41938	S
gil50980792	actin	*Paxillus involutus*	41881	S
gil113307	actin	*Thermomyces lanuginosus*	41837	S
gil31581482	actin	*Vanderwaltozyma polyspora*	33651	S
		Signal Transduction		
gil6692796	14-3-3	*Lentinula edodes*	29009	S
gil164657714	hypothetical protein MGL_2969	*Malassezia globosa CBS 7966*	30426	S
		Unclassified		
gil336371680	hypothetical protein SERLA73DRAFT_122112	*Serpula lacrymans var. lacrymans S7.3*	23905	M
gil46125031	hypothetical protein FG06893.1	*Gibberella zeae PH-1*	20604	M
gil336368415	hypothetical protein SERLA73DRAFT_93339	*Serpula lacrymans var. lacrymans S7.3*	109879	F

Table 2. continued

accession no.	protein name	organism	theor M_w	source[a]
		Unclassified		
gil336365874	hypothetical protein SERLA73DRAFT_115180	*Serpula lacrymans var. lacrymans S7.3*	96108	F
gil170086626	linoleate diol synthase	*Laccaria bicolor S238N-H82*	118658	F

[a]M: mycelial cell wall. S:sclerotial cell wall. F: fruiting body cell wall.

Table 3. Functional Category List of Proteins (PTR) Identified by 1-D GE and nESI-LC−MS/MS[a]

functional category	MCW (%)	SCW (%)	FCW (%)
chromatin structure and dynamics	4 (3.88)[b]		2 (2.20)
translation, ribosomal structure, and biogenesis	76 (73.79)	29 (60.42)	56 (61.54)
post-translational modification, protein turnover, and chaperones	7 (6.80)	2 (4.17)	6 (6.59)
energy production and conversion	10 (9.71)	5 (10.42)	13 (14.29)
carbohydrate transport and metabolism	4 (3.88)	5 (10.42)	7 (7.69)
amino acid transport and metabolism		1 (2.08)	4 (4.40)
cytoskeleton		4 (8.33)	
signal transduction		2 (4.17)	
unclassified	2 (1.94)		3 (3.30)
total no. of CWPs	103	48	91

[a]FCW: cell wall of fruiting body. MCW: cell wall of mycelium. SCW: cell wall of sclerotium. [b]Figures in parentheses represent the relative percentage of the number of proteins under that functional category compared to the number of total proteins found in the cell wall fraction.

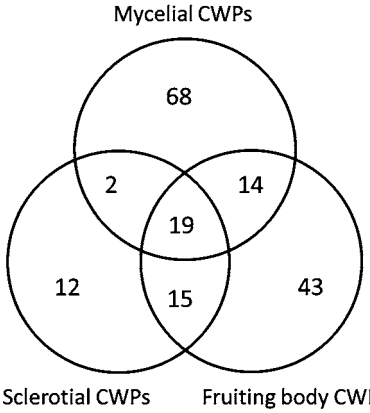

Figure 2. Venn diagram analysis of differentially expressed CWPs in different morphological stages of PTR.

turnover, and chaperones (6.80%) was higher than that in the cell walls of sclerotium (4.17%), but close to that of fruiting body (6.59%) (Table 3). Most proteins in this category belong to the heat shock protein family. Heat shock proteins are either constitutively expressed or activated when exposed to elevated temperatures or other environmental stress conditions, such as contact with toxins, infection, starvation, or other nutrition deficiency.[33] The mechanism by which environmental stress induces the heat shock factor is not clearly understood. Apart from the stress response ability, HSP70 was found to play essential roles under normal conditions including assisting folding of newly synthesized proteins, guiding translocation of proteins across organelle membrane, disassembling oligomeric protein structures, and facilitating proteolytic degradation of unstable proteins.[34]

During the active growing stage of mushroom including mycelium and fruiting body, mushroom cell wall should perform the function to cope with the external stress around them. Consequently, the demand for heat shock proteins had to be increased to provide additional repair of misfolded proteins, protecting cellular proteins, and maintaining cellular viability under conditions of intensive stress. While in the dormant stage, sclerotium does not need to deal with the change of the external environment, so the number of CWPs under this category in this morphological stage was less than that in the mycelial and fruiting body stages (Table 3).

Proteins of Energy Production and Conversion. Thirteen proteins (14.29%) in this category were identified in the cell wall of PTR fruiting body (Table 3). During the development from a massive hypha in the PTR sclerotial stage to becoming a huge carp in the fruiting body stage, much more energy is necessary for the conversion of ADP into ATP. As another active growing stage, PTR mycelial cell wall contained 10 proteins on energy production and conversion which was more than that in sclerotial cell wall (Table 3). As mentioned before, sclerotium is an inactive stage in the mushroom life cycle, and its energy demand should not be as great as that required in mycelium and fruiting body.

Proteins of Carbohydrate Transport and Metabolism. Enolase was identified in all the CWPs of the three different morphological stages of PTR mushroom (Table 2). Enolase, which is also known as phosphopyruvate dehydratase, is a glycolytic enzyme. Glycolysis is an anaerobic process by which glucose is broken down into two molecules of pyruvic acid, involving two phases: the energy-investment phase and the energy-yielding phase.[35] Cells consume ATP in the energy-investment phase, while ATP is produced via phosphorylation in the energy-yielding phase. Specific enzymes are involved in the catabolic pathway of glycolysis with each one being substrate-specific and produce an intermediate product in the conversion of glucose to pyruvate. Enolase is one of the enzymes involved in the energy-yielding phase of glycolysis. The function of enolase is to convert the compound 2-phosphoglycerate to phosphoenolpyruvate. Accordingly, enolase has been found in the noncovalently attached or soluble CWPs of *Saccharomyces cerevisiae*.[35] Moreover, glucan-associated enolase has been identified as a main CWP of *Candida albicans*,[36] which located in the inner layer of the cell wall. Enolase had also been identified in *Coprinopsis cinerea*,[37] but its role played in mushroom cell wall has not been reported.

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was the other main CWP identified under this category (Table 2). The cell wall-associated GAPDH of *Candida albicans* has been reported to be a fibronectin and laminin binding protein.[38] The protein was clearly detected at the outer surface of the cell wall, particularly on blastoconidia, as well as in the cytoplasm of *Candida albicans*. GAPDH catalyzes the conversion of glyceraldehyde 3-phosphate as its name indicates. This is the

sixth step of the breakdown of glucose (glycolysis), which is an important pathway of energy and carbon molecule supply located in the cytosol of eukaryotic cells.[39] GAPDH gene has also been characterized from higher fungi, including *Lentinus polychrous*[40] and *Ganoderma lucidum*.[41] However, GAPDH has been suggested to be a heat shock protein which might be involved in the developmental phase of the *L. polychrous*.[40]

Other Proteins. Three new categories of proteins on amino acid transport and metabolism, cytoskeleton, and signal transduction were identified in the PTR sclerotial cell wall, while proteins on amino acid transport and metabolism were also identified in the cell wall of PTR fruiting body (Table 3). As an important cytoskeleton protein, actin is demonstrated to be associated with initiation of cell wall formation, the proper deposition of cell wall materials, and maintaining the normal morphology of reverting protoplasts in yeast *Schizosaccharomyces pombe*.[42,43] Although cytoskeleton is important to the cell of mycelium and fruiting body as well as to sclerotium,[44] this protein was only identified in the cell wall of PTR sclerotium (Table 2 and Table 3). This might be related to the special function and rigid structure of sclerotium, which may require actin to deposit the cell wall materials and maintain the normal morphology. However, the likely differences caused by the method of extraction (such as DRA) and detection method of LC−MS could not be ruled out when explaining the differences found in these PTR CWPs.

In general, mycelium will eventually form a hyphal knot which grows and develops into a sclerotium before developing into a mushroom fruiting body to restart the cycle again. Sclerotium is a morphologically variable, nutrient-rich, multihyphal aggregated structure that can remain dormant or quiescent when the environment is unfavorable.[45] To survive in environmentally extreme conditions, sclerotial cell wall should be hard and inactive. Moreover, in the sclerotial stage, mushrooms do need to perceive the environmental changes to know whether it is suitable for developing into fruiting body for reproduction. Therefore, proteins on cytoskeleton and signal transduction exist in the sclerotial cell wall. On the other hand, sclerotium has the energy reserve to developing into a fruiting body, so the presence of proteins related to amino acid transport and metabolism is essential for this stage.

This study had identified the mushroom noncovalent CWPs fractionated by DRA from three morphological stages of PTR for the first time through the method of 1-D GE coupled with nESI-LC−MS/MS analysis. Differing from yeasts and filamentous fungi, noncovalently linked proteins were the dominant proteins in cell walls of PTR found among its three morphological stages. Although the total protein content in sclerotium was the least compared with that of mycelium and fruiting body, the proportion of sclerotial CWPs was much higher than that of mycelium and fruiting body. Moreover, almost all CWPs of mycelium and fruiting body were noncovalently linked proteins, while a small amount of residual proteins that were possibly covalently linked was found in sclerotial cell wall.

Based on the comparative proteomic analysis of the CWPs found in the different morphological stages of PTR, it is obvious that PTR mushroom can regulate its protein expression levels to initiate appropriate changes in its cellular metabolisms to cope with the demand in individual developmental stages. Although the genome of PTR is not known yet and the molecular aspect of its cell wall biosynthesis remains largely unknown, the results of differential protein expression in this study could provide some important insights into the differences of the biochemical and physiological changes occurring in the various stages of development of PTR. Further investigations on the functional analysis of the proteome of PTR can lead to more detailed understanding of a mushroom life cycle at the molecular level and improve agricultural production of mushrooms. Studies to unravel the functional relevance of some of these proteins in fungal morphogenesis (especially those for cell wall biogenesis) are underway.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Tables of the functional categories of proteins from PTR mycelial cell wall and PTR sclerotial cell wall. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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