Differential Proteomic Profiles of *Pleurotus ostreatus* in Response to Lignocellulosic Components Provide Insights into Divergent Adaptive Mechanisms

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**INTRODUCTION**

To adapt to changing environments, fungi have developed mechanisms to sense and respond to a multitude of environmental factors such as different carbon sources (Akai, 2012; Kües, 2015). *P. ostreatus* is a white-rot fungus that can be easily cultivated on a variety of lignocellulosic substrates, owing to its ability to degrade cellulose, lignin, and hemicellulose through the action of complex oxidative and hydrolytic enzymatic systems (Fernández-Fueyo et al., 2016). However, lignin does not act as the sole source of carbon and energy; the degradation of lignin by white-rot fungi enables access to holocellulose, which is the carbon and energy source for this species. Presumably, cellulose and hemicellulose provide...
carbon and energy sources for growth, whereas lignin serves a barrier to prevent *P. ostreatus* from attacking polysaccharides. Lignin likely acts as the target for enzymes participating in degradation. Manganese peroxidase (MnP) and laccase are the major oxidative enzymes secreted by *P. ostreatus* that are responsible for the oxidation of lignin and a wide range of lignin-analogous compounds (Wan and Li, 2012). In addition, various auxiliary enzymes generate hydrogen peroxide, which is required for oxidation of lignin. During the lignin degradation process, aromatic radicals are produced that catalyze subsequent degradation, generating potentially toxic molecules that trigger a defense response to protect the fungus from harmful environments (Li et al., 2015b). Primary mycelial enzymes play important roles in cellular processes involving utilization of lignocellulose; earlier studies revealed that the use of conditional transitions in biological pretreatment would affect the expression of the white rot fungi genes encoding ligninolytic enzymes at the transcriptional level (Sindhu et al., 2016).

After the lignin barrier is broken, *P. ostreatus* attacks lignocellulosic polysaccharides. The most abundant hemicellulose is xylan, which is composed of pentoses such as xylose, whereas the most abundant form of cellulose is glucose. The degradation of hemicellulose and cellulose is dependent on carbohydrate-active enzymes, whose functions do not overlap (Lombard et al., 2014); therefore, a large number of different enzymes is required for hemicellulose and cellulose degradation.

Flavin adenine dinucleotide (FAD)-dependent proteins are a current research focus, as these enzymes play important roles in lignocellulose oxidation (Levasseur et al., 2013). Flavin-mediated oxidation, which involves dioxygen as the electron acceptor, is thermodynamically favorable (Hamdane et al., 2015). Previous studies of the response of flavoproteins to lignin have focused on the role of extracellular flavoprotein during lignocellulose degradation (Hernández-Ortega et al., 2012); however, there have been few reports on the role of intracellular flavoproteins in lignocellulose degradation.

In addition, the molecular mechanisms underlying the mycelial response to hemicellulose, cellulose, and lignin remain poorly understood. Recent studies have shown that cellular responses to lignin derivatives are critical for optimization of ligninolytic conditions in fungal cells (Simon et al., 2014). Therefore, elucidation of the catalytic functions of lignin-responsive enzymes is necessary.

The degradation of lignocellulose by *P. ostreatus* plays a role in the acclimation of this fungus to the environment. Adaptation to the specific environment is mediated via profound changes in the expression of genes, which leads to changes in the composition of the fungal transcriptome, proteome, and metabolome (Gaskell et al., 2016). On the basis of their activity, proteins are traditionally classified as catalysts, signaling molecules, or building blocks in cells and microorganisms. Therefore, researchers have attempted to explore the mechanism underlying the interaction between fungi and lignocellulose by proteomics. Proteomics analysis of the filamentous fungus *Trichoderma atroviride* grown on cell walls identified 24 upregulated proteins, including fungal cell wall-degrading enzymes such as *N*-acetyl-β-D-glucosaminidase and the 42-kDa protein endochitinase (Taniguchi et al., 2005). Proteome analysis of *Botrytis cinerea* revealed that proteins such as malate dehydrogenase or peptidyl-prolyl cis–trans isomerase from the mycelium were differentially expressed among strains when using CMC as the sole carbon source; these proteins are involved in host-tissue invasion, pathogenicity, and fungal development (González-Fernández et al., 2014). These studies attempted to elucidate the effects of plant cell wall composition on microbes by mixing lignocellulose or cellulose as substrates; however, they only provide limited evidence that the main components of the plant cell wall alter the gene expression in fungal cells, and that lignin and hemicellulose might also affect the growth and protein expression of fungal cells. To date, few studies have been published regarding the intracellular proteomics of the white-rot fungal response to lignocellulose.

In this work, we performed two-dimensional protein fractionation coupled with mass spectrometry to analyze the potential biological differences among *P. ostreatus* cells grown on different lignocellulose media. *P. ostreatus* was grown in Kirk’s medium to which lignin, xylan, and CMC were added; this medium is commonly used in studies of the response of white-rot fungus to lignocellulose. We compared the biomass and FAD concentration in cells during cultivation. Next, proteomic profiles of *P. ostreatus* under lignocellulose culture conditions were obtained. The 2-DE expression profiles were used to analyze the intracellular proteins differentially expressed in various substrates, and differentially expressed proteins were identified by MALDI-TOF-MS. Finally, the metabolic pathways involving in the lignocellulose response in *P. ostreatus* were examined according to the differentially expressed proteins in the various substrates.

**MATERIALS AND METHODS**

**Microorganism and Cultivation**

*P. ostreatus* isolate BP2 obtained from the Culture Collection Center, Huazhong Agriculture University (Hubei, China) was used in this study. The strain was maintained on potato dextrose agar (PDA) slants at 4°C and activated for 1 week on new PDA slant before use, then transferred into potato dextrose broth (PDB) medium for 7 days at 28°C as inoculum.

In order to exclude influence of other organics, the strain was inoculated into a 250 ml flask with 100 ml modified Kirk’s liquid medium which just contain basal salt component as basic medium (Taniguchi et al., 2005). The Kirk’s liquid medium contained: 9 × 10−3 mol/L KH₂PO₄, 3 × 10−3 mol/L MgSO₄.7H₂O, 2 × 10−5 mol/L ammonium tartrate, 3 × 10−4 mol/L CaCl₂.2H₂O, 5 × 10−2 mol/L glucose, and 10 ml/L trace element contained: 7.8 × 10−3 mol/L amino acetic acid, 1.2 × 10−3 mol/L MgSO₄.7H₂O, 2.9 × 10−3 mol/L MnSO₄.7H₂O, 1.7 × 10−2 mol/L NaCl, 3.59 × 10−4 mol/L FeSO₄.7H₂O, 7.75 × 10−4 mol/L CoCl₂, 9.0 × 10−4 mol/L CaCl₂, 3.48 × 10−4 mol/L ZnSO₄.7H₂O, 4 × 10−5 mol/L CuSO₄.5H₂O, 2.1 × 10−5 mol/L AlK(SO₄)₂.12H₂O, 1.6 × 10−4 mol/L H₃BO₃, 4.1 × 10−5 mol/L NaMoO₄.2H₂O or 100 ml Kirk’s liquid medium supplemented with 0.5 g lignin (Sigma), xylan (Sigma), or cellulose (Sigma). All
experiments were accompanied by controls that lacked the lignocellulose amendment. The mycelia were collected after 7 days incubation in dark at 28°C with continuous stirring at 120 r/min, and the cultures were centrifuged for collection washed with sterilized MilliQ water for several times to separate from medium, then kept at −80°C for use.

Growth Measurement
The mycelial dry weight was used to characterize *P. ostreatus* growth condition. Base on the method reported before (Taniwaki et al., 2006), the mycelium cultured in lignin as mentioned before was weighed after cultured for 0, 3, 5, 7, 9, 11 days. Three individual cultures of the mycelium were weighed at every time point.

Analysis of FAD Concentration during *P. ostreatus* Growth
Mycelium proteins were obtained using a dynamic high pressure homogenizing (GEA Niro Soavi S.p.A), and proteins were quantified by BCA method. Intracellular FAD concentration was measured using an FAD Colorimetric/Fluorometric Assay Kit (BioVision). Experimental methods refer to product description.

Laccase Activity Assays
Laccase activity was determined spectrophotometrically as previous study described by with 14°C days incubation in dark at 28°C. The mycelial dry weights were determined at pH 3.0, the optimum pH for laccase of *P. ostreatus* with ABTS as the substrate (Srinivasan et al., 1995). All the assays were done at pH 3.0, the optimum pH for laccase of *P. ostreatus* with ABTS as the substrate.

2-De Analysis of Mycelia Protein
Frozen mycelia were used to extract total mycelia proteins by the TCA-acetone precipitation method (Rabilloud et al., 2010). Mycelia (dry weight of 1 g) was ground to a fine powder under liquid nitrogen and was collected into 50 ml microcentrifuge tubes. Three individual cultures of the mycelium were harvested and extracted separately. Twenty milliliters cold acetone (−20°C, 10% w/v trichloroacetic acid (TCA), 0.1% w/v diithiothreitol (DTT, Bio-Rad), 1 mmol/L phenylmethanesulfonyl fluoride (PMSF, Sigma) was added into the tube. After the samples were resuspended totally, the tube was incubated at −20°C for more than 12 h, and then the samples were centrifuged for 20 min at 14,000 r/min. The resulting pellet was washed with 15 mL cold acetone (0.1% w/v DTT, 1 mmol/L PMSF), then centrifuged at 14,000 r/min for 20 min. This washing procedure was repeated twice and final pellet was resuspended. The pellet was vacuum-dried and solubilized with lysis buffer containing 7 mmol/L urea, 2%CHAPS (Sigma), 10 mmol/L DTT and 0.5% biolytes (Bio-Rad). After fully dissolved, the samples were stored at −80°C for 2-DE analysis. Protein concentration was determined using Bradford’s method with bovine serum albumin as standards (Fernández and Novo, 2013). Ready strip IPG strips (18 cm, 4–7 linear pH gradient, Bio-Rad) were rehydrated for 12 h with 800 μg of protein sample as most mycelial proteins were in this range according to previous studies (Jami et al., 2010). Then the IPG were carried out for the first electrophoretic dimension in a Protean IEF-Cell (Bio-Rad). The isoelectric focusing was performed with a limiting current of 50 μA/strip following the program setting: (i) 250 v, rapid, 0.5 h. (ii) 1,000 v, rapid, 0.5 h. (iii) 9,000 v, liner, 4.5 h. (iv) 9,000 v, rapid, 75,000 vh(v) 500 v, rapid, 1 h. The IPG strips were treated twice for at least 30 in with SDS equilibration buffer (6 mmol/L urea, 1.5 mmol/L Tris-Cl with pH 8.8, 30% v/v glycerol, 2% (w/v) SDS, 0.001% bromophenol blue). Ten milligrams per milliliters DTT was added to the equilibration buffer in the first step, and 25 mg/mL iodoacetamide was added in the second step. The second dimensional SDS-polyacrylamide electrophoresis (SDS-PAGE) was performed on v/v 12.5% acrylamide gel (v/v 2% SDS) by using a Protean II xi Cell system (Bio-Rad). Coomassie PAGE Blue (Bio-Rad) was used to stain the gels. The finished gels were scanned with GE Gel Scan system (GE) and analyzed with PDQuest software (7.0.1 version, Bio-Rad). In order to verify the significant change of protein/spot, three replicate 2-DE gels were visually compared by using PDQuest software. The spots/proteins appeared in all three biological replicates could be considered the infallible spots/proteins. Finally, only differences with a ratio lignocellulose/control (R) 0.5 > R or R > 2 (CV < 25%), and with a t-test (p < 0.05), were considered as significant. The theoretical ps were calculated using the ExPASy Compute pi/Mw tool (http://web.expasy.org/compute_pi/).

ESI-MS/MS of 2-De Spots
Then, we performed MALDI-TOF/TOF to identify significantly changed spots in one or two cultures compared with that in the control. Spots from 2-DE gels were excised and digested with trypsin for 20 h. The resulting peptide mixtures were desalted using ZipTips C18 (Millipore), and eluted onto a 96-well MALDI target plate. Then, 2 mL samples on the plate were mixed with 1 mL supersaturated CHCA solution with 0.1% TFA and 50%ACN. Mass spectrometric analysis were measured on 5800 MALDI-TOF/TOF (AB SCIEX). Briefly, mass data acquisitions were piloted by 4000 Series Explorer Software v3.0 using batched-processing and automatic switching between MS and MS/MS modes. The PMF data were collected and blasted in JGI database using Mascot software (http://matrixscience.com).

RESULTS
Lignocellulose Components Influence the Growth of Mycelium
*P. ostreatus* grown in Kirk's medium supplemented with lignin, xylan, and CMC was used to study the relative intensity of proteins affected by lignocellulose, and Kirk’s medium without lignocellulose was used as control. The mycelial dry weights of colonies grown on lignocellulose significantly differed from those of the control (Figure 1). The growth of fungal mycelium was suppressed on lignin relative to other cultures. Xylan and CMC served as slow-acting carbon resources; accordingly, the biomass of mycelium in xylan and CMC accumulated slowly at first, and then began to surpass that of the control 7 days after inoculation. Compared with the control, lignocellulose supplementation suppressed mycelial growth for the first 7 days of culture; subsequently, mycelia underwent adaptation to xylan.
and CMC, resulting in rapid growth of *P. ostreatus* in these medium.

**Lignocellulose Components Influence the FAD Levels of Mycelia**

FAD is a redox cofactor that plays an important role in metabolism ([Figure 2](#)). The primary sources of reduced FAD levels during eukaryotic metabolism are the citric acid cycle and beta oxidation reaction pathways. FAD accumulates with time, especially during growth on lignin. After inoculation for 7 days, the FAD concentration was higher in fungi grown in lignin than in other cultures.

**Lignin Influence Laccase Activity**

Since laccase is the most important extracellular enzyme responsible for lignin modification, we examined its activity in the lignin group ([Figure 3](#)). After inoculation, the laccase activity in this group was lower than that in the control for the first 5 days; however, after culturing for 7 days, laccase activity in the lignin group was higher than that in the control.

**Differences between the Mycelial Proteomes during Growth in Lignocellulose and in the Control Medium**

Three biological replicates for each mycelial protein of *P. ostreatus*, grown in Kirk's medium and in Kirk's medium supplemented with lignin, were separated by 2-DE. Total 531 ± 23, 496 ± 19, 567 ± 38, and 601 ± 27 protein spots were detected in the control, lignin, xylan, and CMC conditions, respectively ([Figure 4](#)). Proteins that were differentially expressed under various culture conditions were divided into categories according to their molecular functions and involvement in biological processes, based on the JGI database and GO (http://geneontology.org/) classification system ([Table 1](#), [Figures 5, 6](#)). For proteins lacking exact functional annotations in this database, we used family and domain databases (Inter Pro and Pfam) to reveal annotations of their conserved domains. Identified proteins included those involving in (i) redox processes and (ii) stress response. The stress-response group included antioxidation proteins and proteins involving in the response to toxic stress that are considered to play a role in the protection of cells from damage. The intensity of four spots (6, 7, 19, 20) for proteins involving in the stress response and three spots (58, 85, 112) for proteins involving in redox processes show a significant increase in all fungi grown in the three substrates relative to the control. The identified proteins also included proteins involving in (iii) carbohydrate metabolism and energy metabolism; these proteins are involved in the conversion of carbohydrates into energy to support cell processes. [Figure 5](#) show that the intensity of 15 spots representing proteins involving in this process was significantly decreased for fungi grown on lignin, whereas five spots representative of proteins related to carbohydrate metabolism exhibited an increase for those grown on xylan and CMC. The identified proteins also included proteins involving in (iv) protein and amino acid synthesis, (v) nucleotide metabolism, and (vi) others. Proteins in the “others” group were related to other types of metabolism or considered to have unknown functions.
Lignin-Responsive Proteins

Based on the result of proteomics, the intensity of 36 spots was found to be significantly increased (fold > 2) and that of 71 spots significantly decreased (fold < 0.5; Table 1, Figure 6). Eight spots only increased or detected for fungus grown on lignin, whereas the intensity of spot 9 (oxidation-resistance protein), spot 11 (10-kDa heat shock protein), spot 28 (superoxide dismutase [Cu-Zn]), spot 29 (14-3-3 protein), and spot 30 (glutathione-S-transferase), representing proteins involving in the stress response in lignin, was 3.5-, 2.5-, 2.6-, 2.4-, and 2.5-fold higher than that of the control, respectively. Among proteins related to the redox process, the intensity of spot 10 (cytochrome c oxidase copper chaperone) increased by 2.5-fold in lignin compared to that in the control, whereas spot 22 (putative oxidoreductase) was only detected for fungus grown on lignin. Notably, spot 30 and spot 62 both corresponded to glutathione-S-transferase; however, spot 62 was not detected for the lignin group, probably because subunits of the same protein would separate during the focusing process. The intensity of 26 proteins related to carbohydrate metabolism was significantly decreased for the lignin group. Most of these proteins participate in six types of carbohydrate metabolism. Interestingly, the intensity of the carbohydrate metabolism-related protein adenylylate kinase (spot 15) was 6.3-fold higher than that in the control.

Polysaccharide-Responsive Proteins

Xylan and cellulose, which are the main polysaccharides present in lignocellulose, are the primary carbon sources for fungi. In this study, CMC was used as a substitute for cellulose to study...
### TABLE 1 | List of proteins identified by ESI-MS/MS from *P. ostreatus* growing in lignocellulose.

| Spot no. | Biological process | JGI ID | Description | MW(kDa)/pI | Mascot score | Fold change (treat/control) |
|----------|--------------------|--------|-------------|------------|--------------|-----------------------------|
|          | Carbohydrate metabolism and energy metabolism |        |             |            |              |                             |
| 3        | Carbohydrate metabolism and energy metabolism | jgi1043453 | Mannose-6-phosphate isomerase | 32.9/3.91 | 98           | a b a                       |
| 99       | Carbohydrate metabolism and energy metabolism | jgi1036636 | Phospholipid:serine deacylase proenzyme 2 | 54.4/6.19 | 85           | 0.4 0.64 2.18               |
| 109      | Carbohydrate metabolism and energy metabolism | jgi1070334 | Pyruvate kinase 1 | 61.6/7.47 | 151          | 0.25 0.75 1.53              |
| 100      | Carbohydrate metabolism and energy metabolism | jgi1011623 | Phosphoglycerate kinase | 41.8/3.67 | 169          | 0.96 a 1.95                 |
| 76       | Carbohydrate metabolism and energy metabolism | jgi109049 | Triosephosphate isomerase | 27.4/6.85 | 109          | 0.48 2.03 2.67             |
| 106      | Carbohydrate metabolism and energy metabolism | jgi1070334 | Pyruvate kinase 1 | 45.6/7.69 | 84           | 0.47 1.53 3.16              |
| 95       | Carbohydrate metabolism and energy metabolism | jgi1032066 | Phosphoglycerate kinase | 45.0/4.4 | 115          | a 0.49 4.25                 |
| 86       | Carbohydrate metabolism and energy metabolism | jgi1019376 | Xylose kinase | 28.9/4.01 | 87           | a b a                       |
| 93       | Carbohydrate metabolism and energy metabolism | jgi1102061 | NADPH-dependent D-xylose reductase | 34.7/6.69 | 99           | 1.22 6.41 3.1               |
| 96       | Carbohydrate metabolism and energy metabolism | jgi1114405 | 6-phosphogluconate dehydrogenase, deoxyxylating | 62.3/3.9 | 75           | 0.3 0.37 1.54              |
| 55       | Carbohydrate metabolism and energy metabolism | jgi1068318 | Ribose-5-phosphate isomerase | 31.4/6.08 | 90           | 0.34 1.59 a                |
| 69       | Carbohydrate metabolism and energy metabolism | jgi109004 | 6-phosphogluconate dehydrogenase | 53.7/6.23 | 147          | 0.14 0.17 0.69             |
| 81       | Carbohydrate metabolism and energy metabolism | jgi108563 | Glucose-6-phosphate dehydrogenase | 16.4/7.9 | 106          | 0.42 a 1.14                |
| 75       | Carbohydrate metabolism and energy metabolism | jgi1090672 | Glycerolaldehyde-3-phosphate dehydrogenase | 34.6/6.63 | 135          | 0.44 0.72 1.51             |
| 77       | Carbohydrate metabolism and energy metabolism | jgi1037028 | Phosphogluconate dehydrogenase | 26.6/7.02 | 126          | 0.11 2.62 3.55             |
| 54       | Carbohydrate metabolism and energy metabolism | jgi1101576 | Ribulose-phosphate 3-epimerase | 25.9/5.5 | 219          | 0.13 1.17 1.26             |
| 38       | Carbohydrate metabolism and energy metabolism | jgi1018327 | Glycosyl transferase family 4 | 81.8/6.11 | 76           | 0.514 0.88 1.13            |
| 71       | Carbohydrate metabolism and energy metabolism | jgi1096444 | Glucose-6-phosphate 1-dehydrogenase | 58.5/6.55 | 140          | a 0.14 3.85                |
| 70       | Carbohydrate metabolism and energy metabolism | jgi1064981 | Glucose-1-phosphate uridylyltransferase | 58.7/6.26 | 154          | 0.43 0.44 1.23             |
| 72       | Carbohydrate metabolism and energy metabolism | jgi48499 | Pyruvate dehydrogenase | 44.9/7.7 | 163          | 0.41 0.57 0.67             |
| 73       | Carbohydrate metabolism and energy metabolism | jgi48714 | Isocitrate dehydrogenase | 47.5/5.93 | 209          | 0.13 1.11 0.72             |
| 56       | Carbohydrate metabolism and energy metabolism | jgi1094663 | Malate dehydrogenase | 34.2/6.12 | 139          | 0.3 0.35 1.23             |
| 60       | Carbohydrate metabolism and energy metabolism | jgi1075656 | Malate dehydrogenase | 34.1/6.09 | 91           | 0.34 2.06 1.87             |
| 23       | Carbohydrate metabolism and energy metabolism | jgi1097340 | Pyruvate carboxylase 2 | 18.0/4.34 | 87           | a 7.57 2.16                |
| 78       | Carbohydrate metabolism and energy metabolism | jgi1113799 | Pyranomodalacturonan acetylene isomerase | 25.6/6.02 | 181          | a a a                     |
| 94       | Carbohydrate metabolism and energy metabolism | jgi1082594 | Carbon catabolite derepressing protein kinase | 35.0/5.7 | 89           | a 4.82 0.96                |
| 36       | Carbohydrate metabolism and energy metabolism | jgi1053965 | ATPase | 100.3/5.27 | 239          | 0.23 2.58 1.15             |
| 40       | Carbohydrate metabolism and energy metabolism | jgi1044485 | Hydrogen-transporting ATPase | 57.0/5.24 | 204          | a 0.77 1.25                |
| 115      | Carbohydrate metabolism and energy metabolism | jgi109099 | Glutamine synthetase | 22.9/3.98 | 148          | a 1.3 2.13                |
| 15       | Carbohydrate metabolism and energy metabolism | jgi108799 | Adenylyl kinase | 27.7/6.77 | 104          | 6.29 2.33 2.26             |
| 13       | Nucleotide metabolism | jgi1105829 | GDP-mannose transporter | 23.7/4.26 | 92           | a b a                     |
| 88       | Nucleotide metabolism | jgi1099408 | Suppressor of kinetochore protein 1 | 17.4/3.63 | 76           | a b a                     |
| 84       | Nucleotide metabolism | jgi47308 | Alpha-1,3/1,6-mannosyltransferase ALG2 | 49.7/4.09 | 64           | 0.45 2.35 a               |
TABLE 1 | Continued

| Spot no. | Biological process          | JGI ID    | Description                                           | MW(kDa)/pI | Mascot score | Fold change (treat/control) |
|----------|-----------------------------|-----------|-------------------------------------------------------|------------|--------------|-----------------------------|
|          |                             |           | Lignin Xylan CMC                                      |            |              |                             |
| 79       | Nucleotide metabolism       | jgi1037108 | Scavenger mRNA decapping enzyme                        | 25.4/6.11  | 204          | 0.19                        | a | a |
| 46       |                             | jgi1054232 | Ribonuclease T2                                       | 41.5/5.92  | 198          | 0.17                        | 0.27 | 0.62 |
| 47       |                             | jgi1083505 | 4OS ribosomal protein                                  | 32.1/5.21  | 72           | 0.38                        | 0.15 | 0.94 |
| 41       |                             | jgi1056351 | cysteine-type endopeptidase                            | 55.0/5.81  | 88           | a                           | a | 1.19 |
| 44       |                             | jgi1088444 | GTP binding                                           | 42.0/5.11  | 109          | a                           | 1.31 | 1.23 |
| 57       |                             | jgi1037683 | Ribosomal                                             | 33.6/5.99  | 95           | 0.3                         | 0.92 | 1.23 |
| 68       |                             | jgi1054296 | GTPase                                                | 93.4/6.27  | 136          | 0.28                        | 0.22 | 1.4 |
| 103      | Glucosamine 6-phosphate N-acetyltransferase | jgi1090777   |                                                   | 30.9/3.91  | 99           | 0.45                        | 0.45 | 1.55 |
| 59       |                             | jgi1095212 | Endo/exonuclease                                      | 34.7/5.25  | 92           | 0.33                        | 0.76 | 1.76 |
| 113      |                             | jgi106249  | Tethering factor for nuclear proteasome st5           | 24.0/8.28  | 88           | 2.23                        | 1.28 | 2.16 |
| 101      |                             | jgi1075990 | cAMP-dependent protein kinase regulatory subunit       | 34.1/6.25  | 78           | a                           | 1.63 | 2.19 |
| 108      |                             | jgi101333  | 4OS ribosomal protein S29                             | 45.2/7.36  | 144          | 0.46                        | 0.61 | 2.63 |
| 24       |                             | jgi1066340 | 6OS ribosomal protein L43                             | 14.7/4.98  | 106          | 2.15                        | 7.78 | 3.34 |
| 27       |                             | jgi1047882 | Dimethyl adenosine transferase                         | 36.1/9.0   | 79           | 2.32                        | 0.464 | 4.64 |
| 90       |                             | jgi185993  | 6OS ribosomal protein L23-B                            | 14.3/4.26  | 83           | b                           | b | b |
| 89       | Other metabolism            | jgi1114368 | Calmodulin                                            | 31.3/3.57  | 100          | a                           | b | a |
| 63       |                             | jgi52279   | Formamidase                                           | 42.9/5.22  | 137          | 0.3                         | 0.67 | 0.93 |
| 39       |                             | jgi1098883 | Amidase                                               | 55.7/5.09  | 188          | 0.27                        | a | 1.12 |
| 64       |                             | jgi1093313 | Delta-aminolevulinic acid dehydratase                 | 35.8/5.79  | 151          | a                           | 1.03 | 1.52 |
| 65       |                             | jgi1054502 | Enolase                                               | 47.1/5.55  | 157          | 0.15                        | 1.17 | 1.74 |
| 66       |                             | jgi1107810 | CoA-transferase                                       | 41.8/5.93  | 218          | 0.28                        | 1.21 | 1.99 |
| 97       |                             | jgi155235  | Mitochondrial import inner membrane translocase       | 115.6/4.7  | 100          | a                           | 0.44 | 2.26 |
| 61       |                             | jgi1067896 | Putative hydroxylase                                  | 40.5/6.32  | 157          | 0.47                        | 1.74 | 2.65 |
| 25       |                             | jgi1056645 | Putative Sugar transporter                            | 57.5/3.97  | 83           | 2.31                        | 21.19 | 4.62 |
| 114      |                             | jgi1094266 | Diphosphoinositol polyphosphate phosphohydrolase       | 20.7/4.77  | 82           | 1.76                        | 4.91 | 4.86 |
| 35       |                             | jgi1107810 | Putative CoA-transferase                              | 41.9/5.93  | 256          | 4.29                        | 4.78 | 8.18 |
| 102      |                             | jgi1101425 | Sterol 3-beta-glucosyltransferase                     | 32.6/4.24  | 86           | b                           | b | b |
| 83       | Protein and amino acid synthesis | jgi1089644    | Leucine carboxyl methyltransferase                    | 50.0/3.47  | 76           | a                           | b | a |
| 45       |                             | jgi1076233 | 3-isopropylmalate dehydrogenase                        | 40.7/5.56  | 182          | a                           | 0.39 | 0.67 |
| 48       |                             | jgi176309  | Glucosamine-6-phosphate isomerase                     | 32.8/5.81  | 136          | 0.47                        | a | 0.68 |
| 74       |                             | jgi1094925 | Aconitase                                             | 47.3/5.93  | 141          | a                           | 1.3 | 0.85 |
| 111      |                             | jgi1010364 | SWI5-dependent HO expression protein 3                | 60.2/7.74  | 95           | a                           | a | 0.88 |
| 37       |                             | jgi1091537 | Aniline–RNA ligase                                    | 106.3/5.52 | 116          | a                           | 2.54 | 1.21 |
| 49       |                             | jgi1099302 | Methyltransferase                                     | 31.0/5.7   | 206          | 0.37                        | a | 1.23 |
| 67       |                             | jgi1060865 | Aspartate aminotransfer                               | 45.4/4.54  | 176          | 0.21                        | 0.37 | 1.28 |

(Continued)
TABLE 1 | Continued

| Spot no. | Biological process | JGI ID       | Description                                                      | MW(kDa)/pI   | Mascot score | Fold change (treat/control) |
|----------|--------------------|--------------|-----------------------------------------------------------------|--------------|--------------|-----------------------------|
|          |                    |              |                                                                 |              |              | Lignin | Xylan | CMC  |
| 107      | Redox processes    | jgi|1112899 | Phenylalanine ammonia-lyase | 33.4/7.08 | 96            | a     | 0.75 | 1.59 |
| 104      |                    | jgi|1027245 | E3 ubiquitin-protein ligase TOM1 | 31.6/4.64 | 102           | 1.21  | 1.18 | 2.17 |
| 110      |                    | jgi|1039251 | Serine/threonine-protein kinase MEC1 | 61.4/6.65 | 79            | 2     | 0.53 | 2.57 |
| 98       |                    | jgi|48252  | Glycylpeptide N-tetradecanoyltransferase | 85.2/6.66 | 80            | a     | a    | a    |
| 22       |                    | jgi|1066477 | Putative oxidoreductase | 22.8/3.22 | 72            | b     | a    | a    |
| 10       |                    | jgi|1027050 | Cytochrome c oxidase copper chaperone | 13.9/4.93 | 74            | 2.55  | 0.51 | 0.26 |
| 112      |                    | jgi|1098138 | Aldehyde dehydrogenase | 21.9/6.37 | 94            | 0.37  | 0.32 | 1.49 |
| 34       |                    | jgi|1045076 | Putative oxidoreductase | 32.8/5.56 | 147           | 2.24  | 2.24 | 1.79 |
| 58       |                    | jgi|1061301 | Putative Glucose/ribitol dehydrogenase | 33.1/6.17 | 184           | 0.42  | 1.42 | 1.89 |
| 105      |                    | jgi|1023062 | Isocitrate dehydrogenase [NAD] subunit 1 | 28.3/6.19 | 90            | 1.68  | 1.63 | 2.1  |
| 31       |                    | jgi|1086766 | Putative oxidoreductase | 26.3/5.69 | 146           | 4.24  | 2.97 | 2.24 |
| 2        |                    | jgi|19749  | Cytochrome c oxidase assembly protein | 31.1/3.55 | 108           | 2.98  | 2.19 | 2.32 |
| 8        |                    | jgi|1099797 | 3-isopropylmalate dehydrogenase | 15.5/6.24 | 82            | 3.26  | 6.52 | 2.6  |
| 22       | Stress response    | jgi|1081338 | Potassium-activated aldehyde dehydrogenase | 35.4/4.65 | 63            | a     | 3.38 | 3.24 |
| 21       |                    | jgi|1015418 | 3-hydroxyanthranilate 3,4-dioxygenase | 18.6/4.14 | 80            | 2.67  | 4.22 | 3.76 |
| 14       |                    | jgi|1030848 | Putative ary-alkyl dehydrogenase | 31.4/4.96 | 95            | 9.25  | 11.13 | 4.63 |
| 33       |                    | jgi|1076970 | Putative oxidoreductase | 32.3/5.5 | 133           | b     | b    | b    |
| 5        |                    | jgi|1015750 | Cytochrome c oxidase assembly protein | 14.6/4.65 | 88            | a     | a    | a    |
| 16       |                    | jgi|1087944 | Putative nitrate monooxygenase | 19.2/7.34 | 69            | b     | b    | b    |
| 29       |                    | jgi|1064479 | 14-3-3 protein homolog | 23.0/8.26 | 109           | 2.37  | a    | a    |
| 30       |                    | jgi|1077356 | Glutathione-S-Transferase | 26.9/5.77 | 131           | 2.53  | 0.52 | 0.78 |
| 9        |                    | jgi|1077250 | Oxidation resistance protein 1 | 14.4/6.17 | 105           | 3.52  | 0.69 | 0.84 |
| 11       |                    | jgi|185767  | 10 kDa heat shock protein, mitochondrial | 14.4/6.17 | 64            | 4.23  | 1.26 | 0.85 |
| 28       |                    | jgi|1113505 | Superoxide dismutase [Cu-Zn] | 28.1/8.26 | 100           | 2.6   | 0.52 | 1.38 |
| 87       |                    | jgi|1022101 | Thiamine thiazole synthase | 22.8/3.52 | 79            | 0.42  | 2.19 | 1.66 |
| 62       |                    | jgi|1058013 | Glutathione-S-Transferase, C-like | 42.1/6.0 | 98            | a     | 1.28 | 2.56 |
| 19       |                    | jgi|108100  | Inheritance of peroxisomes protein 1 | 40.0/5.42 | 71            | 5.78  | 4.56 | 4.79 |
| 6        |                    | jgi|1032742 | Oxidant-induced cell-cycle arrest protein 5 | 16.6/3.58 | 97            | b     | b    | b    |
| 7        |                    | jgi|1025559 | Monothiol glutaredoxin-5 | 13.5/4.34 | 100           | b     | b    | b    |
| 20       |                    | jgi|106868  | Alternative oxidase, mitochondrial | 45.4/4.61 | 90            | b     | b    | b    |
| 91       | Unknown function   | jgi|1048439 | Uncharacterized protein C6B12.14c | 15.4/4.17 | 111           | a     | b    | 0    |
| 50       |                    | jgi|1080037 | RLC-like phosphodiesterase | 32.7/5.0 | 89            | 0.14  | 0.2  | 0.41 |
| 26       |                    | jgi|1022166 | Purine phosphoribosyltransferase | 43.7/7.33 | 120           | 2.32  | 2.78 | 0.7  |
| 51       |                    | jgi|1097135 | Calcium ion binding protein | 25.4/5.32 | 194           | a     | 0.7  | 1.23 |
| 43       |                    | jgi|1048986 | Predicate protein | 46.5/5.34 | 182           | a     | a    | 1.24 |

(Continued)
TABLE 1 | Continued

| Spot no. | Biological process | JGI ID | Description | MW(kDa)/pI | Mascot score | Fold change(treat/control) |
|----------|--------------------|--------|-------------|------------|--------------|----------------------------|
| 1        | Lignin             | jgi|1075945 | Hypothetical protein | 34.9/3.58 | 69 | 4.47 | 2.77 | 1.52 |
| 2        | Lignin             | jgi|1048238 | Hypothetical protein | 21.4/6.89 | 91 | 4.45 | 2.77 | 1.52 |
| 3        | Lignin             | jgi|1007364 | Hypothetical protein | 18.8/6.03 | 91 | 4.45 | 2.77 | 1.52 |
| 4        | Lignin             | jgi|1064298 | Hypothetical protein | 15.4/6.89 | 91 | 4.45 | 2.77 | 1.52 |

a, particularly low; b, particularly high.

FIGURE 5 | Venn diagram representing the distribution of number and function of validated and significantly changed proteins according to proteome. The numbers in parentheses indicate the amount and percentage (the percentage of the proteins in increased or decreased proteins in different treatments) of protein in this section. (A) increased proteins (B) decreased proteins. Abbreviations refer to different metabolic processes: RP, redox process; NM, nucleotide metabolism; PS, protein and amino acid synthesis; SR, stress response; CM, Carbohydrate metabolism and energy metabolism; OTHER, other metabolism and unknown function.

Differentially expressed proteins displayed similar expression patterns in xylan and CMC; for both substrates, most proteins showing an increase in abundance were associated with carbohydrate metabolism. Ten carbohydrate metabolism-related proteins showed higher abundance in the two substrates than in the control. Table 1 show that the intensity of spot 95 (phosphoglycerate kinase) and spot 106 (pyruvate kinase), which represented proteins involving in the glycolysis/gluconeogenesis pathway, was 4.3- and 3.2-fold higher in the CMC group than in the control. Spot 71 (glucose-6-phosphate 1-dehydrogenase) and spot 77 (phosphogluconate dehydrogenase), which represented proteins involving in the pentose phosphate pathway, had 3.9- and 3.6-fold higher abundance in the CMC group than in the control. However, these spots showed lower abundance in the xylan group. In addition, the intensity of spot 93 (NADPH-dependent D-xylose reductase) was 6.4-fold higher in the xylan group and 3.1-fold higher in the CMC group than in the control. Spot 86, which was identified as a xylulose kinase, was only detected in the xylan group. D-xylose reductase and xylulose kinase are
both involving in the pentose and glucuronate interconversion pathway. In other species, these two proteins are involving in xylan degradation and energy release. The intensity of spot 23 (pyruvate carboxylase) was 7.6-fold higher in the xylan group than in the control group, but only 2.2-fold higher in the CMC group than in the control group.

**DISCUSSION**

Lignocellulose is the main substrate used for cultivation of edible fungi. Hemicellulose and cellulose are carbon sources for fungal growth; however, another main component of lignocellulose, lignin, affects the degradation of fiber by fungi. The presence of lignin limits the access of cellulolytic enzymes to cellulose, that may influence the efficiency of enzymatic hydrolysis of cellulose and hemicellulose (Kumar et al., 2012). This effect is not observed in white-rot fungus, in which lignin is degraded by the extracellular oxidative system. However, the growth of this fungus is affected by a series of lignin derivatives; previous studies have shown that various lignin-related para-phenolic benzoic acids, para-phenolic cinnamic acids, and para-phenolic phenolpropionic acids elicit increased inhibition of growth in white-rot fungus (Buswell and Eriksson, 1994). In addition, higher concentrations of aromatic aldehydes were shown to be more toxic than the corresponding carboxylic acid (Dekker et al., 2002). These findings are consistent with those of the previous work showing that the growth of *P. ostreatus* is inhibited by lignin (Barakat et al., 2012). In the present study, although the fungus was still able to grow on lignin, the relative growth rate increased 7 days after inoculation. The rapid growth of mycelia in the control group was presumably related to the rapid consumption of nutrients. An alternative explanation for this observation is that the fungus began to adapt to the lignin-based medium. To date, little is known about the effects of lignin on mycelial growth and the stress response in fungi.

Lignin degradation is an extracellular oxidative process, and the production of H$_2$O$_2$ is temporarily related to lignin degradation (Achyuthan et al., 2010). *P. ostreatus* has a range of extracellular enzymes that generate H$_2$O$_2$ for utilization by ligninolytic enzymes (Akpinar and Urek, 2014). Superoxide dismutase, ascorbate peroxidases, and glutathione reductase are key enzymes involving in reducing H$_2$O$_2$ in the ascorbate-glutathione cycle in cells (Yousuf et al., 2012; Choudhury et al., 2013; Yang et al., 2013). These proteins, which are induced in response to numerous environmental stresses, mediate the detoxification of reactive oxygen species. The enzymes related to the oxidative stress response were more abundant in the lignin condition, indicating a better response to H$_2$O$_2$ in the mycelium of *P. ostreatus* when compared to that in other culture conditions. These proteins, which are expressed in response to increased concentrations of extracellular H$_2$O$_2$, scavenge excess intracellular reactive oxygen species to protect cells from oxidative damage.

Inhibition of the transformation of carbon sources is another effect of oxidative stress on *P. ostreatus* (Filomeni et al., 2015). In the present study, most proteins involving in carbohydrate and energy metabolism were less abundant in the lignin group. This suggests that the inhibition of energy metabolism in response to lignin restricts mycelial growth. In the present study, as the adaptability of fungi to lignin increased, this restriction was gradually lifted, allowing slow accumulation of mycelial biomass to occur.

Recent research has suggested that laccase may play an important role in the fungal defense against oxidative stress, which acts as an element of the stress response (Giardina et al., 2010). It has been observed that oxidative stress induces the expression of ligninolytic enzymes in some basidiomycetes (Viswanath et al., 2014). In our study, the activity of laccase increased with time in the lignin group, and the increase in laccase expression appeared to increase the resistance of *P. ostreatus* to oxidative stress. The increase in laccase activity was therefore considered to enhance the adaptability of *P. ostreatus* to lignin in a gradual manner.

Interestingly, we found that the intensity of a 14-3-3 protein was significantly increased in the lignin group. The 14-3-3 proteins, which are rarely reported in fungi, are known to be upregulated in plants in response to pathogenic fungi. Previous studies have suggested that 14-3-3 proteins may control a negative feedback loop to prevent harmful overactivation of defense responses in plants (Lozano-Durán and Robatzek, 2015). Our results suggest a prominent role for 14-3-3 proteins in the fungal response to stress; however, it is not clear how lignin regulates the expression of this protein. The question of whether the expression of this protein relates to lignin needs further study.

The present results elucidate the relationship of the expression of antioxidative intracellular proteins and laccase with the defense response to exogenous H$_2$O$_2$—induced oxidative stress in fungi grown on lignin (Strong and Claus, 2011). Although the expression of these proteins promoted the adaptability of *P. ostreatus* to lignin, it is possible that alternative stress response mechanisms may additionally be associated with adaptation to growth in such environments.

Cellulose and hemicellulose in lignocellulose are the main nutrient sources for *P. ostreatus*. In fungi, the cAMP–PKA and TOR pathways respond to carbon and nitrogen signals to regulate a myriad of functions, including protein synthesis, ribosome biogenesis, autophagy, polarized cellular growth, cell-cycle progression, and filamentation (Liu et al., 1993). TOR signaling activates the expression of genes required for ribosome biogenesis, including those encoding ribosomal proteins, ribosomal RNA (rRNA), and tRNA (Dobrenel et al., 2016). In our findings additionally showed that cAMP-dependent protein kinase and three ribosomal proteins involving in sugar sensing were significantly upregulated in fungi grown on xylan and CMC. Furthermore, xylan and CMC regulate the adaptation of the fungus to the environment via their signaling pathways. Therefore, after inoculation for 7 days, the mycelial growth rate was observed to increase rapidly.

In a previous study, sensing of glucose as the preferred carbohydrate source was extensively studied in the yeast model organism (Braunsdorf et al., 2016). In the presence of glucose, genes required for growth on alternative carbon sources are repressed (Bahn et al., 2007). For *P. ostreatus*, the natural growth
environment lacks glucose; accordingly, this fungus has evolved an effective method for regulation of natural polysaccharides. Various filamentous fungi, including Neurospora crassa, are capable of growth on pentose (Li et al., 2014). The genomes of pentose-utilizing fungi are a useful resource for mining novel gene elements, such as D-xylose transporters for metabolic engineering in S. cerevisiae. The xylose metabolism pathway consists of three enzymes, namely xylose reductase, xylitol dehydrogenase, and xylulokinase, which have been studied in relation to the metabolic engineering of S. cerevisiae for xylose fermentation (Farwick et al., 2014). This has been a subject of great interest over the past decade, as xylose is easier to obtain in nature (Li et al., 2015a). Despite these endeavors to improve xylose fermentation, the yields and productivity for ethanol obtained from xylose, using engineered S. cerevisiae, are much lower than those for ethanol obtained by glucose fermentation (Kurosawa et al., 2013). The high intensity of D-xylose reductase and xylulose kinase in P. ostreatus grown on xylan may be related to increased xylose metabolism under xylan regulation. However, this is not the only carbon metabolism pathway that is enhanced under xylan regulation; the expression of malate dehydrogenase, pyruvate carboxylase, ATPase, and adenylate kinase, which are involved in TCA metabolism, is also increased on xylan. The enhancement of xylose metabolism and other carbohydrate metabolism pathways greatly promotes the utilization of polysaccharides by P. ostreatus.

The hydrolysis product of CMC is glucose; therefore, the response mechanism of P. ostreatus for CMC is similar to that for glucose. Previous studies proved that GTPase activity may be indicative of the activation of signaling pathways in the presence of glucose as a carbon source, and almost half of the identified signaling-related proteins are G-protein coupled receptors or small GTPases (Post and Brown, 1996; Gancedo, 2008). GTPases are present at high levels in CMC, suggesting that it activates this signaling pathway. Addition of glucose to cells growing on non-fermentable carbon sources, or to stationary-phase cells, triggers a wide variety of regulatory processes directed toward the exclusive and optimal utilization of the preferred carbon source (Gancedo, 2008). Pyruvate kinase, phosphoglycerate kinase, triosephosphate isomerase, and phosphoglycerate kinase are upregulated in fungi growing on CMC, suggesting that glycolysis is activated by glucose. When glucose influx and utilization through glycolysis are stimulated, gluconeogenesis is inhibited, and there is a drastic increase in growth rate, which is preceded by a characteristic upshift in ribosomal RNA and protein synthesis.

Sugars such as xylan and cellulose are the primary fuel for most fungi (de Souza et al., 2014). The amount of available sugar may fluctuate widely, necessitating a mechanism for sensing available amounts and responding appropriately. In most organisms, this response involves changes in gene expression. Studies of the yeast glucose repression system have provided novel insights into the signaling pathway that responds to sugar. When yeast cells growing on high levels of sugar obtain most of their energy via fermentation, large amounts of sugar are metabolized through glycolysis (Johnston, 1999; Kim et al., 2013). Our findings suggest that addition of CMC and xylan to the medium significantly enhances the ability of P. ostreatus to transform sugars via different metabolic

FIGURE 6 | Heatmap of the fold changes of differential proteins related to carbohydrate metabolism, stress response and redox process. (A) Fold change of proteins related to carbohydrate metabolism and energy metabolism. (B) Fold change of proteins response to stress. (C) Fold change of proteins related to redox process. The data are presented in matrix format in which rows represent the individual proteins and the columns represent each culture. Each cell in the matrix represents the fold change of a protein at an individual substrate compared with control. The red and green colors in cells reflect low and high change fold, respectively.
pathways, and improves the adaptability of *P. ostreatus* to the environment.

Alcohol oxidation is critical for lignocellulose degradation. In our study, aryl-alcohol dehydrogenase enzymes showed higher abundance in all of the lignocelloses substrates. Moreover, aryl-alcohol dehydrogenase coupled with NADPH as a cofactor constitutes a redox system involving in aryl-alcohol/aryl-aldehyde production in the fungus that ensures steady availability of H$_2$O$_2$ for ligninolytic activities (Yang et al., 2012). Recent studies have shown that aryl-alcohol oxidases and dehydrogenase are induced by lignin derivatives and are involving in their metabolism *in vitro* (Feldman et al., 2015). Our results suggest that aryl-alcohol dehydrogenase is induced by lignin as well as lignocellulosic polysaccharides, and regulated by lignocellulose.

Flavin-containing oxidases catalyze a wide variety of different oxidation reactions; in the last decade, many flavoprotein oxidases with varied substrate specificities and reactivities have been discovered (Dijkstra et al., 2011). Glucose oxidase, the best-known flavoprotein, is involving in lignocellulose degradation (Hernández-Ortega et al., 2012). To date, few studies have focused on the correlation between flavoprotein and lignocellulose degradation in plants. The only flavoproteins known to be involving in this process are the flavin-containing monooxygenases, which are widely distributed within living organisms and involving in various biological processes such as the detoxification of drugs, biodegradation of environmental aromatic compounds, and biosynthesis of antibiotics (Nakamura et al., 2012). In our study, the level of FAD increased with time; the level of FAD in fungus grown on lignin was higher than that in fungus grown in the control medium, and highest in fungus grown on lignin. This indicates that the expression of FAD is regulated by lignocellulose, and that flavoprotein in cells plays an important role in the response to lignocellulose. Although it was not possible to determine which proteins are specifically regulated by lignocellulose, our findings provide novel insights into the roles of intracellular flavoproteins in the response to lignocellulose.

Some studies have shown that *P. ostreatus* selectively degrades hemicellulose when cultured with solid biomass (Ander and Eriksson, 1977; Chandra et al., 2007). This implies that *P. ostreatus* favors the use of hemicellulose as a carbon source. In our study, xylan had a certain effect on the accumulation of mycelial biomass, and we believe that xylan plays a key role in the regulation of genes related to the metabolism of xylulose. We speculate that the selective degradation of hemicellulose when *P. ostreatus* is cultured in solid biomass occurs because xylan is a carbon source that is beneficial for the growth of *P. ostreatus* (Dwivedi et al., 2011), and xylan activates the expression of genes in the xylene-related metabolic pathway, which allows *P. ostreatus* to use hemicellulose as a carbon source. There are some reports that lignin in natural lignocellulose limits the growth of fungi, that because of the structural limitation of mycelial invasion and the use of other polysaccharides (Sattler and Funnell-Harris, 2013). Our results suggest that this restriction may also be due to the inhibition of mycelial growth by lignin and the effect of lignin on carbon metabolism in *P. ostreatus* hyphae. Our results provide further understanding of the solid-state culture of *P. ostreatus*.

Elucidation of lignocellulose–fungal interactions is important for understanding fungal ecology and for the maintenance of the delicate balance of fungal symbionts in our ecosystem. Understanding the mechanism of the fungal response to lignocellulose will facilitate its application in metabolic engineering of biotechnology to optimize the bioconversion of biomass resources in the future.

**AUTHOR CONTRIBUTIONS**

XZ and CL designed the experiments. QX, FM, and HY wrote the manuscript. QX conducted most of the experimental work and performed analysis of data. YL assisted with experiments. All authors discussed the results and reviewed the manuscript.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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