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Comparative secretomic analysis of lignocellulose degradation by *Lentinula edodes* grown on microcrystalline cellulose, lignosulfonate and glucose

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ABSTRACT

Lentinula edodes has the potential to degrade woody and nonwoody lignocellulosic biomass. However, the mechanism of lignocellulose degradation by L. *edodes* is unclear. The aim of this work is to explore the profiling of soluble secreted proteins involved in lignocellulose degradation in L. *edodes*. For that, we compared the secretomes of L. *edodes* grown on microcrystalline cellulose, cellulose with lignosulfonate and glucose. Based on nanoliquid chromatography coupled with tandem mass spectrometry of whole-protein hydrolysate, 230 proteins were identified. Label-free proteomic analysis showed that the most abundant carbohydrate-active enzymes involved in polysaccharide hydrolysis were *endo*-β-1,4-glucanase, α-galactosidase, polygalacturonase and glucoamylase in both cellulosic secretomes. In contrast, enzymes involved in lignin degradation were most abundant in glucose culture, with laccase 1 being the predominant protein (13.13%). When the cellulose and cellulose with lignosulfonate secretomes were compared, the abundance of cellulases and hemicellulases was higher in cellulose with lignosulfonate cultures, which was confirmed by enzyme activity assays. In addition, qRT-PCR analysis demonstrated that the expression levels of genes encoding cellulases and hemicellulases were significantly increased (by 32.2- to 1166.7-fold) when L. *edodes* was grown in cellulose with lignosulfonate medium.
Biological significance: In this article, the secretomes of L. *edodes* grown on three different carbon sources were compared. The presented results revealed the profiling of extracellular enzymes involved in lignocellulose degradation, which is helpful to further explore the mechanism of biomass bioconversion by L. *edodes*.

1. I

Lentinula edodes (order Agaricales), commonly known as Xianggu or shiitake, is the second most commercially produced mushroom species, surpassed only by the white button mushroom (*Agaricus bisporus* [Lange] Imbach) [1]. In addition to its commercial value, *L. edodes* shows potential in biotransformation and fiber bleaching as well as bioremediation [2–4]. In nature, *L. edodes* is a saprobic, wood-colonizing white-rot species that degrades lignocellulose in wood. Therefore, its cultivation materials have been dead trees or wood chips in China for the last eight centuries [5]. Currently, sawdust-based cultivation of L. *edodes* consumes forest resources, preventing ecological conservation. Replacing wood or wood chips with other lignocellulose biomass such as agricultural straws is necessary for cultivation of this species. Moreover, the biological efficiency of L. *edodes* could improve with growth on new cultivated materials. Understanding the mechanism of lignocellulose degradation by L. *edodes* is critical.

The wood decay mechanism of white-rot fungi has been investigated largely in Polyporales species because of the availability of genomic information, often combined with transcriptomic and secretomic analyses. Lignin degradation relies on a wide array of extracellular oxidoreductases produced by white-rot fungi. Of these enzymes, fungal class-II lignin modifying peroxidases (CAZy auxiliary activity family 2, AA2) including lignin peroxidases (LiPs), manganese peroxidases (MnPs), and versatile peroxidases (VPs) play major roles in lignin modification [6,7]. Class-II peroxidases require hydrogen peroxide as an oxidant, which may be generated by copper radical oxidases (CROs, AA5) including glyoxal oxidase (GLOX), glucose–methanol–choline oxidoreductases (GMCs, AA3) such as aryl-alcohol oxidase, methanol oxidase, and pyranose oxidase [8,9]. In addition to peroxidases, laccases have been implicated in lignin degradation [10]. Laccases are phenol-oxidizing multicopper oxidases (MCOs, AA1) that may act on lignin substructures with the aid of aromatic mediator compounds [6].

Crystalline cellulose is depolymerized by white-rot fungi with the aid of lytic polysaccharide monooxygenase (LPMO, CAZy family AA9) enzymes [11,12]. Depolymerized cellulose is completely digested by cellobiohydrolases (CAZy glycoside hydrolase family GH6 and GH7) and endoglucanases (GH5, GH9, GH12, GH44 and GH45), in conjunction with β-glucosidases (GH1 and GH3) [6,13]. In addition, a wide array of

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other carbohydrate-active enzymes such as carbohydrate esterases and polysaccharide lyases (PLs) degrade plant cell wall components including hemicelluloses and pectins [13]. Interestingly, the lignin degradation process may involve modulation of the hydrolytic enzymes commonly associated with cellulose and hemicellulose [11]. The genes expressed and proteins produced during growth on plant material reflect the specific lifestyle of each fungal species and its strategy for lignocellulose conversion [6].

While Polyporales species are generally grown on woody substrates, members of the Agaricales order such as *Pleurotus* naturally grow on wood, leaf litter, and other lignocellulosic agricultural wastes, suggesting diverse strategies for lignocellulose conversion. The diversity of strategies could be reflected in the differences among the secretomes. For example, *Pleurotus ostreatus* secretomes shared only 39% of proteins when grown on polar wood or wheat straw [11,14]. A number of studies have revealed that the composition of the secretome changes in response to different carbon sources, as reviewed by McCotter et al. [15]. In general, the availability, quality, and complexity of the carbon source influence the secretome composition [15]. This suggests that various carbon sources from lignocellulosic biomass can be used as inducers to investigate the mechanism of lignocellulose degradation of L. *edodes* using secretome analysis.

Several (hemi)cellulolytic enzymes including xylanase, endoglucanases (GH12) and cellobiohydrolases (GH6 and GH7) and enzymes involved in lignin degradation (laccases and MnP) in L. *edodes* have been biochemically characterized [16–22]. Recently, the genome of strain W1–26, a monokaryon from a commercial dikaryon of L. *edodes*, was sequenced in our lab (*L. edodes* genome database website: http://LEgdb.chenlianfu.com; [23]), and the annotated information is available in the NCBI database (GenBank accession number LDAT00000000). According to the genome sequence, selective ligninolysis by L. *edodes* [24] is probably attributable to the presence of an arsenal of lignin-degrading enzymes, including 14 MCOs, 2 MnPs, 1 VP and a set of MCOs and GMCs [23]. Moreover, secretomic analysis combined with genomic information will offer insight into the enzymatic mechanism involved in the decay of not only lignins but also other lignocellulose constituents by saprotrophic fungi (reviewed in reference [25]).

The present work aims to characterize potentially important enzymes involved in degradation of lignocellulose and to provide insight into extracellular enzyme gene expression patterns in L. *edodes*. This will reveal additional reagents to increase the efficiency of biomass conversion to useful chemical feedstocks and will lead to improvements in mushroom crop yields. Here, we use glucose, microcrystalline cellulose, and sodium lignosulfonate (a natural chemically modified product of lignin and a byproduct from pulping spent liquor, usually used as anionic polymeric surfactant) as carbon sources to investigate the unique enzyme system of L. *edodes* and to evaluate its capacity for bioconversion of agricultural materials.

2. M

2.1. Fungal strain and culture conditions

The L. *edodes* commercial heterokaryon strain WX1 (ACCC 50926) has been deposited in the Agricultural Culture Collection of China (Wuhan). Stock cultures were activated on complete yeast extract medium (CYM) plates with 2% glucose, 0.2% yeast extracts, 0.2% peptone, 0.1% K_2HPO_4, 0.05% $MgSO_4$, 0.046% KH_2PO_4, and 2% agar.

For preparation of extracellular enzymes, 9-mm-diameter agar plugs from the leading mycelia edge of the stock cultures were transferred to 25 mL liquid modified CYM containing 2% (wt/vol) glucose (glucose), 2% microcrystalline cellulose (cellulose) or 1.9% microcrystalline cellulose plus 0.1% sodium lignosulfonate (cellulose-SLS) as the major carbon source. The cultures (triplicate for each condition) were incubated at 25 °C under stationary conditions for 20 days.

For the three media conditions, culture supernatants were collected, centrifuged twice at 10,000 ×*g* for 15 min to remove the mycelia and then clarified by filtration through a 0.45 μm filter. 10 mL of filtrate from each condition was extracted for enzyme activity assays, and the rest used for proteomic analysis.

2.2. Secreted protein preparation

Triplicate filtrates for each condition were combined equally [11]. Next, 5 mL of saturated Tris-phenol was added to each 25 mL filtrate sample in a 50 mL centrifuge tube; the samples were mixed by inversion and then held at room temperature for 10 min. Following centrifugation at 10,000 ×*g* for 10 min at 4 °C, 1 mL of phenol solution containing the dissolved proteins (at the bottom of the centrifuge tube) was collected and placed in a new centrifuge tube, in which proteins were precipitated via the addition of five volumes of cold 0.1 M sodium acetate in pure methanol. The sample was centrifuged, and after decanting the supernatant, the pellet was washed several times with cold 80% acetone. Pelleted proteins were re-solubilized and denatured in 40 μL of a 9 M urea solution (9 M urea, 2% CHAPS, 1 mM PMSF, 50 mM DTT). The Bradford method was employed to determine the protein concentration using bovine serum albumin (BSA) to generate a standard curve. Protein solutions were diluted to the same concentration, and 200 μL (100 μg) of each sample was pipetted and purified using a readyprep 2-D Cleanup kit (BIO-RAD, USA) according to the manufacturer's instructions. Purified proteins were re-solubilized in 25 mM NH_4HCO_3 at pH 8.2 for tryptic digestion (100 ng/μL Trypsin Gold from PROMEGA Corp. in 25 mM NH_4HCO_3).

2.3. Secretomic analysis

Mass spectrometric analysis of soluble extracellular proteins was performed as described for *P. ostreatus* [11]. Digestion was conducted overnight at 34.1(t)-7447.p eren (eror9.8(e)-177.1(mi)26.3(xe)29(m)19.7(ex5

calculation (label-free quantification), mass spectrometric signal intensities (MaxQuant) of peptide precursor ions belonging to each protein were divided by the total abundance of all detected proteins in each culture condition. Protein abundance value was calculated from the normalized values of the three technical replicates. The presence or absence of a signal peptide was predicted with SignalP 4.1 [26].

2.4. Enzyme activity assays

Enzymatic hydrolyses of the polysaccharides were performed in a sodium acetate buffer solution (SABF, 0.2 M, pH 4.8). The filter paper activity (FPA) and endoglucanase (CMCase), xylanase, cellobiohydrolase (*p*NPCase), and β-glucosidase (*p*NPGase) activities of the culture supernatants were assayed according to the methods reported by Li et al. [27], with a few differences. The control to account for the presence of any reducing sugars in the crude enzyme samples was not boiled, but rather digested with proteinase K (PROMEGA). The SABF used for the FPA assay included 1% β-glucosidase (Sigma # C6105). Independent triplicate cultures were sampled and analyzed.

2.5. Real time quantitative PCR analysis

L. edodes mycelia grown in liquid CYM for 20 days were washed twice with sterilized water and transferred to glucose, cellulose or cellulose-SLS medium. Cultures were inoculated at 25 °C and sampled at 1, 3 and 5 d, respectively. Mycelia that were not transferred served as controls. All samples were centrifuged twice at 10,000 × g to remove supernatants, washed and stored at −80 °C for total RNA preparation.

For total RNA isolation, approximately 100 mg of L. *edodes* mycelia was ground in liquid nitrogen and extracted using RNAiso plus (TaKaRa, Japan) according to the manufacturer's instructions. RNA integrity and quantity were checked using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) and the RNA6000 Nano Assay, respectively. First-strand cDNA was synthesized with 2 μg of RNA and 0.5 μg of oligo(dT) in 20-μL reactions using TranScript One-Step gDNA Removal and cDNA Synthesis SuperMix (TransGen, Beijing, China) according to the manufacturer's instructions. The primer sequences of the tested genes and the reference gene are listed in Table S3. Quantitative RT-PCR was performed using a CFX Connect real-time PCR system (BIO-RAD). Each reaction consisted of 1 μL each of the forward and reverse primers (10 mM), 30 ng of sample cDNA, 10 μL of AceQ qPCR SYBR Master Mix (Vazyme, China) and water to a final volume of 20 μL. The cycling parameters were 95 °C for 2 min followed by 40 cycles of 95 °C for 15 s and 60 °C for 20 s. A dissociation curve was generated to verify that a single product was amplifi

1

Relative abundance of the individual proteins involved in cellulose, hemicellulose, pectin and lignin degradation.

Protein ID[a]	CAZy Family[b]	Protein description[a]	Protein abundance (% of total MS intensity)[c]			SP[d]
			Glucose	Cellulose	Cellulose SLS	
Cellulose degrading enzymes						
04541	GH12	endo-β-1,4-glucanase	[e]	0.09	0.21	Y
04829	GH7 CBM1	1,4-β-D-glucan cellobiohydrolase	0.03	0.02	0.18	Y
10050	GH6 CBM1	1,4-β-D-glucan cellobiohydrolase	0.01	0.03	0.11	Y
08136	GH5	endo-1,4-β-glucanase	0.00	0.02	0.10	Y
12864	GH7 CBM1	1,4-β-D-glucan cellobiohydrolase	0.00	0.01	0.07	Y
10512	GH3	β-glucosidase	0.29	0.00	0.06	Y
07961	GH7 CBM1	1,4-β-D-glucan cellobiohydrolase	0.01	0.00	0.02	Y
10266	AA9	lytic cellulose monooxygenase			0.02	Y
04089	GH7 CBM1	1,4-β-D-glucan cellobiohydrolase			0.02	Y
10634	GH3 CBM1	β-glucosidase		0.00	0.01	Y
07574	GH3	β-glucosidase	0.02	0.00	0.00	Y
Hemicellulose degrading enzymes						
06823	GH27 CBM35	α-galactosidase	0.46	0.98	0.74	Y
05323	GH27	α-galactosidase	0.00	0.10	0.69	Y
06046	GH51	α-L-arabinofuranosidase	0.11	0.27	0.66	Y
00068	GH43 CBM35	galactan-1,3-beta-galactosidase	0.00	0.11	0.33	Y
03223	GH10 CBM1	endo-1,4-β-xylanase	0.10	0.10	0.30	Y
05156	GH3	exo-1,4-β-xylosidase	0.01	0.05	0.19	Y
10175	GH43	galactan-1,3-β-galactosidase		0.04	0.14	N
09625	GH27	α-galactosidase	0.13	0.12	0.14	N
12913	GH2	β-mannosidase	0.20	0.14	0.14	Y
05961	GH35	β-galactosidase	0.03	0.07	0.12	Y
09692	GH51	α-L-arabinofuranosidase	0.07	0.08	0.12	Y
11487	CE15 CBM1	glucuronoyl esterase	0.13	0.07	0.09	Y
06248	CE3	acetylxylan esterase	0.02	0.01	0.08	Y
04279	GH95	α-fucosidase	0.04	0.06	0.04	N
05655	GH115	xylan α-glucuronidase	0.00	0.02	0.04	Y
02639	GH10	endo-1,4-β-xylanase		0.00	0.03	Y
01882	GH27 CBM1	α-galactosidase			0.01	Y
07273	GH74 CBM1	xyloglucanase			0.01	Y
07975	GH10 CBM1	endo-1,4-β-xylanase			0.01	Y
00726	GH5	mannan endo-1,4-β-mannosidase	0.04	0.01	0.00	Y
13906	GH37	trehalase	0.01			N
05191	GH95	α-L-fucosidase	0.00	0.00	0.00	Y
Lignin degrading enzymes						
08330	AA1	laccase 1	13.13	7.92	1.81	Y
13044	AA1	laccase 6	0.05	0.00	0.91	Y
13975	AA7	glucose-methanol-choline oxidoreductase	0.64	0.06	0.73	Y
00046	AA7	glucose-methanol-choline oxidoreductase	0.57	0.35	0.53	Y
01372	AA1	laccase 5	0.00	0.04	0.09	Y
02763	AA7	glucose-methanol-choline oxidoreductase	0.11	0.05	0.08	Y
08997	AA2	mannan endo-1,4-β-mannosidase 2	0.01	0.00	0.06	Y
03487	AA3	aryl alcohol oxidoreductase	0.03	0.06	0.02	Y
04633	AA5	glyoxal oxidoreductase	0.02	0.01	0.01	Y
02931	AA7	glucose-methanol-choline oxidoreductase	0.02	0.01	0.01	Y
04793	AA5	cytokinin dehydrogenase	0.05	0.01	0.00	Y
09425	AA3	glucose oxidase	0.01	0.00	0.00	Y
02092	AA5	glyoxal oxidase		0.01	0.00	N
07621	AA3	glucose oxidase		0.00	0.00	Y
04660	AA1	laccase 8	0.00		0.00	Y
13226	AA3	cytokinin dehydrogenase			0.00	Y
04737	AA	laccase 13		0.00		Y
12439	AA3	glucose oxidase	0.00	0.01		Y
04836	AA3	aryl alcohol oxidase	0.02			N

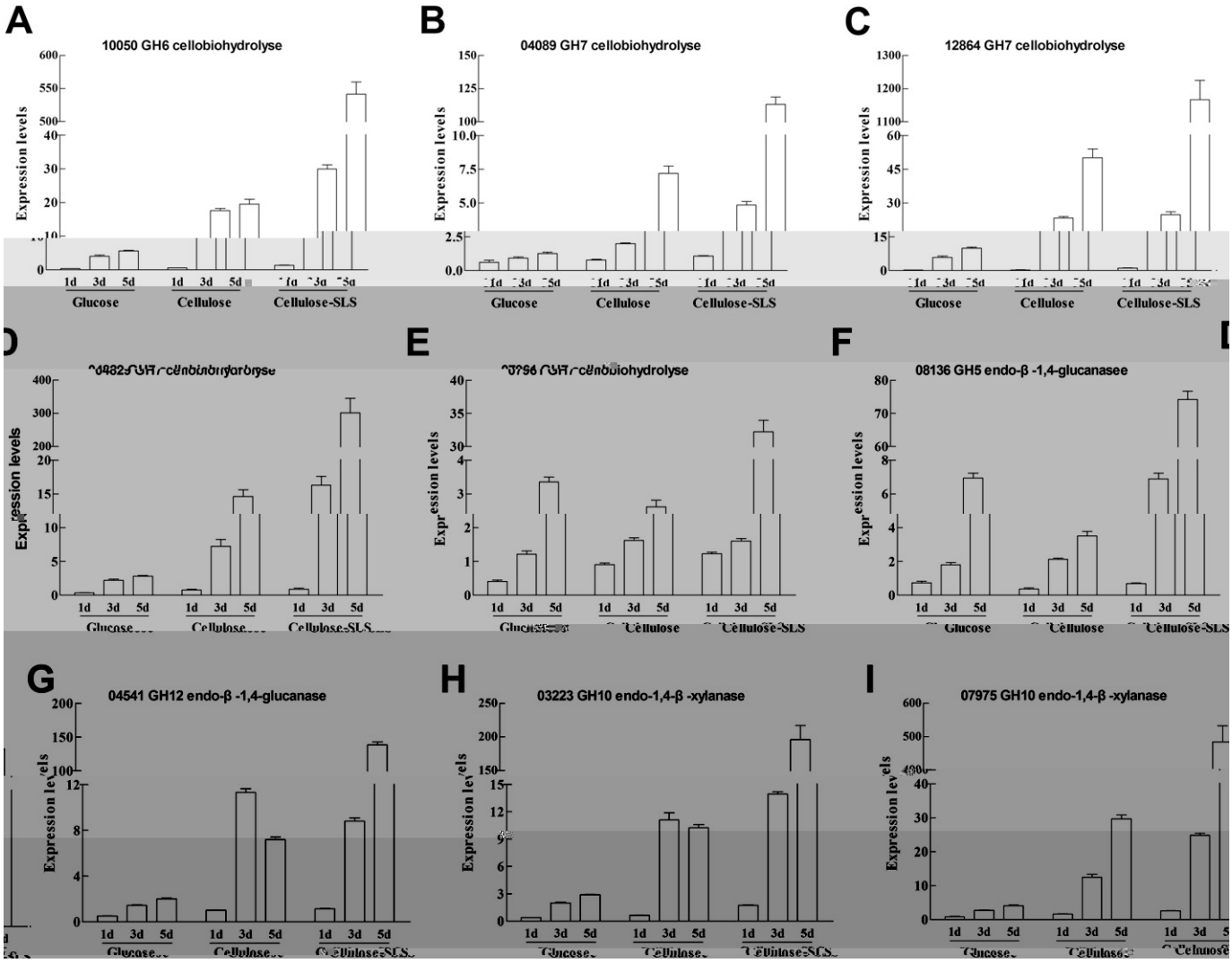
(continued on next page)

cellulases was lowest in the cellulose medium (0.16%; Fig. 3). As shown in Tables 1, 5 CBHs (GH6 and GH7) were significantly abundant (0.40%)

an aryl-alcohol oxidase (AA3) and a glucose oxidase (AA3) were upregulated either in the cellulose or cellulose-SLS cultures.

3.3. Determination of the activities of several extracellular enzymes

The activities of xylanase, endoglucanase (CMCase), cellobiohydrolase (*p*NPCase) and β-glucosidase (*p*NPGase) and FPA were evaluated. As shown in Fig. 4A, L. *edodes* produced higher levels of FPA, CMCase activity and xylanase activity in cellulose and cellulose-SLS media than in the glucose medium. *p*NPCase and *p*NPGase activities showed no significant differences between cellulose and glucose media (Fig. 4



F . 5. Quantitative RT-PCR analysis of 9 selected cellulase and hemicellulase-encoding genes. Total RNA was isolated from mycelia of L. *edodes* following transfer to glucose, cellulose or cellulose-SLS for 1, 3, or 5 days. The relative expression levels were normalized to the expression level of the actin gene (ID: LE01Gene04556). The values shown are the means of three replicates, and the *error bars* indicate standard deviations from the mean values.

Two class-II peroxidase genes have been isolated and characterized as MnPs [16,18,29], and one was predicted to be a VP [23]. A MnP was identified in present study, with relatively higher protein abundance in cellulose-SLS cultures. This finding was consistent with a previous study reporting that L. *edodes* produces manganese peroxidase as its main lignolytic enzyme [16].

Laccases can strongly degrade non-phenolic lignin in lignocellulosic materials in the presence of redox mediators [30–32]. Three laccases, LACC1 [19,33], LACC4 (previously designated LACC2; [22]), and LACC6 [34] have been purified from L. *edodes*. And the corresponding genes of LACC1 and LACC4 have been cloned [35,36]. Additional laccase-encoding genes (lacc7–11) were reported in L. *edodes* strain L45A, and LACC1, LACC4, LACC5, and LACC7 were found to be expressed in *Pichia pastorisfor* [21]. Three novel laccases (lacc12-lacc14) were found in L. *edodes* through genomic sequencing [23,37]. At least five laccases were secreted by L. *edodes* when grown on cellulose or cellulose-SLS, as shown in the present secretomic study. LACC1 is the most highly expressed protein of the lignin-degrading protein category in the cellulose-SLS secretome (1.81%); however, its expression was much higher in the glucose secretome (13.13%). This indicates that LACC1 may have a protective role rather than a direct role in lignocellulose deconstruction. A rich available glucose in the medium leads to oxidative stress cellular. It has been observed that oxidative stress can stimulate the extracellular laccase activity of some white rot basidiomycetes such as *Fomes fomentarius*, *Tyromyces pubescens*, *Trametes versicolor*, and *Abortiporus biennis* [38,39]. The expression of the laccase gene from white rot fungus *Trametes* sp. 5930 in *Pichia pastoris* can enhance the resistance of yeast to H_2O_2-mediated oxidative stress by stimulating the glutathione-based antioxidative system to protect the cell from oxidative damage [40]. LACC1 might increase fungal resistance against oxidative stress. Additionally, the *lacc1* RNAi mutants in L. *edodes* caused morphological phenotypes including not forming a thick aerial mycelium mat on agar medium, with many short branches with low mycelial density, a thin cell wall, and few fibrous layers [20]. LACC6 and LACC5 were upregulated in the cellulose-SLS culture, emphasizing their roles in the L. *edodes* secretome regarding processing of the growth substrate and carbon source. LACC8 and LACC13 were also produced by L. *edodes* in cellulose or cellulose-SLS cultures, although at low protein abundance. The above results support the findings of a previous transcriptional analysis of multicopper oxidases in the same fungus, except for LACC8, whose encoding gene was not identified in the genome [37].

Laccases and MnPs have been reported in the secretomes of several white-rotting basidiomycetes, including the Agaricales species *Agaricus bisporus*, *P. ostreatus*, *P. eryngii*, and *Trametes trogii* [11,41–43], the Polyporales species *Ceriporiopsis subvermispora*, *Phlebia radiata*, and *Ganoderma lucidum* [44–47], and the *Schizophyllum commune* [48].

Interestingly, in another Agaricales species, *P. ostreatus*, laccases (LACC10, LACC2, LACC9 and LACC6) are also the main proteins in the polar wood and wheat straw secretomes [11]. However, the model ligninolytic basidiomycetes *P. chrysosporium* completely lacks laccase-encoding genes in its genome [49]. The presence of at least six different *P. chrysosporium* LiPs and MnPs has been reported in carbon- or nitrogen-limited glucose and lignocellulose-containing cultures [50–52], although some studies failed to detect any LiPs in lignocellulose-containing media [53,54]. In this respect, different white-rot fungi demonstrate distinct non-LiP-dependent or non-laccase-dependent strategies for lignin degradation.

GMCs from the CAZy family AA3, together with CROs/GLOXs from the CAZy family AA5, may supply extracellular hydrogen peroxide for fungal lignin modification and class-II peroxidases, and AA3 oxidoreductases may also couple activities with aryl-alcohol dehydrogenases [43]. Both AA3 and AA5 proteins were detected in L. *edodes* secretomes and were relatively less abundant in cellulose-SLS cultures than in glucose cultures. These proteins may be as helpful as LACC1 for increasing fungal resistance to oxidative stress.

4.2. CAZy proteins involved in polysaccharide hydrolysis in the L. edodes secretomes

In addition to lignin attack, cellulose degradation by white-rot fungi occurs through the activity of a combination of several divergent protein families: cellulases of the GH5, GH6, GH7, GH9, GH11, GH12, GH44 and GH45 families [6,55,56], LPMOs and CDH [11,57]. The cellulases identified in the present study were upregulated in cellulose media, especially in cellulose-SLS media, compared to control media, as validated by qRT-PCR analysis and enzyme activity assays. The components and expression patterns of GH family cellulases identified in the secretome of L. *edodes* are similar to those in *P. chrysosporium* cultured in cellulosic medium [54]. The GH12 protein was the most abundantly and specifically expressed in the cellulosic conditions, which illustrated a dominant '*endo*-' enzyme activity. Three GH12 family enzymes in L. *edodes* were characterized and found to be upregulated in cellulose media [58]. The L. *edodes* genome contains one GH6- and four GH7-encoding genes [23], all of which were identified in the secretome of L. *edodes* grown on cellulose-SLS. The GH6-encoding gene and one of the four GH7-encoding genes have been isolated and characterized as cel6B and cel7A, respectively, and the transcript levels of both were significantly upregulated when L. *edodes* was cultured in the presence of crystalline cellulose [59]. In addition, the transcript abundance of both genes and one GH5 encoding gene, which was also identified in the present secretomic analysis, greatly increased when the formation of brown film by L. *edodes* was induced by light during solid state fermentation [60]. This suggests that these cellulases participate in polysaccharide degradation and are involved in life cycles. LPMOs from CAZy family AA9 play important roles in cellulose and hemicellulose degradation, with cellobiose dehydrogenase (CDH) acting as a source of electrons for LPMOs [12]. An LPMO enzyme (LE01Gene10266) and a CDH were identified only in the cellulose-SLS conditions, in line with the report of Harris et al. [61] that a protein belonging to the AA9 family enhances the activity of cellulose hydrolysis in the presence of lignocellulose but not pure cellulose. Although cellulases were upregulated in the cellulose-SLS conditions, the overall abundance of cellulases was much lower than that of lignin-degrading proteins, which could be related to the selective degradation of lignin by L. *edodes* [24].

Hemicellulose in hardwood mainly contains xylan, and its degradation requires xylanase, β-xylosidase, glucuronidase, α-L-arabinofuranosidase and acetyl xylan esterase [62], all of which were identified in the L. *edodes* secretome except for glucuronidase. In this work, hemicellulose main chain-cleaving enzymes, including a GH10 xylanase and a CE3 acetyl xylan esterase, showed significant upregulation in cellulose-SLS medium. In addition, two GH2 and GH5 β-mannosidases in low abundance and the absence of mannanase (hydrolyzing the backbone of mannan) encoding genes in the genome [23] suggested a low softwood-hydrolyzing potential of L. *edodes*, since mannan is the main hemicellulose in softwood fiber. Moreover, it is well known that the production of mannanase can be induced by cellulose in many fungi, including *P. chrysosporium* [54]. The L. *edodes* hemicellulose degradation system has been less extensively studied, with only a purified xylanase reported [17]. The pectin-degrading enzymes of L. *edodes* have rarely been documented, but this study indicated the expression and regulation of polygalacturonase (the most abundant pectinase in the three secretomes), pectin esterase, rhamnogalacturonase, and *endo*-β-1,4-galactanase in cellulose and cellulose-SLS media. Now that the L. *edodes* genome has been sequenced, transcript and protein expression profiling studies of lignocellulose-degrading genes should be performed to clarify the role of each protein in this fungus.

In this work, we found that SLS greatly enhanced the expression of cellulase- and hemicellulase-encoding genes in L. *edodes* at both the transcriptional and secretory levels. It has been reported that SLS enhanced the biodegradation of polychlorinated biphenyls by *Pseudomonas* sp. 7509 [63]. SLS and lignin-based polyoxyethylene ether enhance enzymatic hydrolysis of lignocellulose by dispersing cellulase aggregates, thereby significantly reducing nonproductive adsorption of cellulase on lignin [64–68]. However, the mechanism by which SLS enhances the expression of cellulase- and hemicellulase-encoding genes at the transcript level is still unclear. Future studies will clarify this mechanism as well as the mechanism for the transcriptional regulation of cellulase- and hemicellulase-encoding genes in L. *edodes*.

In addition to extracellular enzymes, different culture substrates affected the taste component content and taste quality of L. *edodes* [69]. Accordingly, quality characters including organoleptic and medical characteristics of fruiting bodies should be considered when L. *edodes* is cultivated on woody and nonwoody lignocellulosic substrates.

5. C

Our integrative analysis of the data set obtained from L. *edodes* grown on glucose, cellulose or cellulose-SLS demonstrated the polysaccharide and lignin degradation capacities of L. *edodes*. Under lignocellulose-free conditions (glucose medium), *L. edodes* produces only a small amount of essential lignocellulose-degrading enzymes (GH5, GH6, and GH7 for cellulose degradation; GH10 for hemicellulose degradation; GH28 for pectin degradation; and AA2 for lignin degradation). During the degradation of cellulose or cellulose-SLS, *L. edodes* secretes large amounts of lignocellulose-degrading enzymes, especially under cellulose-SLS conditions. Additionally, the transcript expression levels of genes encoding cellulases and hemicellulases were significantly increased by SLS.

Supplementary data to this article can be found online at http3(med)-0TnGH

GH	glycoside hydrolase
GLOX	glyoxal oxidase
GMC	glucose-methanol-choline oxidases/dehydrogenases
LACC	laccase
LiP	lignin peroxidase
LPMO	lytic polysaccharide monooxygenase
MnP	manganese peroxidase
*p*NPC	*p*-nitrophenol-D-cellobioside
*p*NPG	*p*-Nitrophenyl β-D-glucopyranoside
SLS	sodium lignosulfonate
	qRT-PCR Real time quantitative PCR
SABF	sodium acetate buffer solution
VP	versatile peroxidase

The Transparency document associated with this article can be found, in the online version.

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