Journal of Bioscience and Bioengineering
VOL. 128 No. 5, 529–536, 2019

ELSEVIER

www.elsevier.com/locate/jbiosc

De novo transcriptome analysis of *Pleurotus djamor* to identify genes encoding CAZymes related to the decomposition of corn stalk lignocellulose

Yanli Li,[1,2,3,*] Jiahao Liu,[1,2] Gang Wang,[2] Meiying Yang,[1] Xue Yang,[1] Tongbing Li,[2] and Guang Chen[2]

Engineering Research Center of the Chinese Ministry of Education for Bioreactor and Pharmaceutical Development, College of Life Science, Jilin Agricultural University, Xincheng Street 2888, Nanguan District, Changchun 130118, Jilin, China,[1] Innovation Platform of Jilin Province for Straw Comprehensive Utilization Technology, Jilin Agricultural University, Xincheng Street 2888, Nanguan District, Changchun 130118, Jilin, China,[2] and Engineering Research Center of the Chinese Ministry of Education for Edible and Medicinal Fungi, Jilin Agricultural University, Xincheng Street 2888, Nanguan District, Changchun 130118, Jilin, China[3]

Received 21 October 2018; accepted 15 April 2019
Available online 27 May 2019

CAZymes play a very important role in the biotransformation of corn stalk biomass, which is an important resource for sustainable development. *Pleurotus djamor* can produce CAZymes related to the decomposition of corn stalk lignocellulose biomass in sole corn stalk substrate; however, little is known about their encoding genes. In order to identify CAZymes encoding genes, RNA high-throughput sequencing of *P. djamor* was performed in this study. The results showed that a core set of 70 upregulated genes encoding putative CAZymes were revealed. They encode 19 kinds of CAZymes in total, of which there are 4 EGLs, 8 CBHs, 5 BGLs, and 12 LPMOs related to cellulose degradation, 8 XYNs, 1 XYL, 2 AGUs, 3 ABFs, 2 AGLs, and 2 AXEs related to hemicellulose degradation, and 5 LACCs, 2 MnPs, 5 VPs, 3 CDHs, 1 AAO, 1 GOX, 1 AOX, 2 GAOXs, and 3 GLOXs related to lignin degradation. This variety suggests that CAZymes may play a very important role in decomposing the lignocellulose biomass of corn stalk. This is the first study to report the *de novo* transcriptome sequencing of *P. djamor*, which will produce a dataset of genes encoding CAZymes, thereby laying the foundation to elucidate the degradation mechanism of corn stalk biomass and boost the biotransformation of corn stalk biomass resources.

[**Key words:** *Pleurotus djamor*; Corn stalk biomass; Transcriptome; Carbohydrate-active enzymes; Real-time quantitative PCR]

Plant biomass is the most abundant renewable carbon source on earth; it is of great significance to sustainable development of human society (1). Biotransformed plant biomass serves as not only the raw material of the pulp and paper industry, but also as a promising feed stock for biofuels (2,3) and newly derived value-added products (4,5). In Northeast China, corn stalk biomass is quite abundant. However, over the past few years, the low utilization rate of corn stalk, has led to the abandonment of its use or its disposal by incineration. This has resulted in not only a severe waste of corn stalk biomass resources, but also environmental pollution (6). Hence, it is imperative to boost the biotransformation of corn stalk biomass.

Plant cell walls comprise cellulose, hemicellulose, lignin, and some other polymers in various ratios (1,7–9). Naturally, cellulose aggregates together to form microfibrils or microcrystalline structures. The surfaces of the recalcitrant amorphous regions of cellulose bind tightly with hemicellulose (10). Lignin consists of a largely amorphous phenyl-propane polymer that acts like glue by filling the space around the cellulose and hemicellulose structures, binding them together (11). Therefore, complex and recalcitrant structures in plant cell walls are obstacles for the biotechnological exploitation of plant-based biomass resources (12).

White rot fungi from *Basidiomycetes* are the only organisms known to degrade plant cell walls on a global scale by producing large amounts of plant biomass-degrading enzymes (4). These enzymes have been categorized in the carbohydrate-active enzymes database (CAZy; http://www.cazy.org/), as glycoside hydrolases (GHs), glycosyl transferases (GTs), polysaccharide lyases (PLs), carbohydrate esterases (CEs), carbohydrate-binding modules (CBMs), and auxiliary activities (AAs) (13). The carbohydrate-active enzymes (CAZymes) act synergistically on plant biomass-based substrates.

Most cellulose-degrading enzymes are categorized into GHs, including endo-β-1,4-glucanase (EGL, EC3.2.1.4), cellobiohydrolase (CBH, EC3.2.1.91), and β-glucosidase (BGL, EC3.2.1.21) (4,14,15). They decompose the plant biomass through the hydrolyzation of the glycosidic bond. An exception to these GHs is lytic polysaccharide monooxygenase (LPMO), which can degrade cellulose by oxidation (16–19); it was formerly classified into the GH61 family owing to its weak EGL activity, but is now classified into the AA9 family (13,20,21).

Like cellulose-degrading enzymes, most hemicellulose-degrading enzymes, mainly including endo-β-1,4-xylanase (XYN, EC3.2.1.8), β-xylosidase (XYL, EC3.2.1.37), α-glucuronidase (AGU, EC3.2.1.131), α-L-arabinofuronosidase (ABF, EC3.2.1.55), and α-galactosidase (AGL, EC3.2.1.22), have also been categorized as GHs, while acetyl xylan esterase (AXE, EC3.2.1.72), and feruloyl esterase

* Corresponding author at: Engineering Research Center of the Chinese Ministry of Education for Bioreactor and Pharmaceutical Development, College of Life Science, Jilin Agricultural University, Xincheng Street 2888, Nanguan District, Changchun 130118, Jilin, China. Tel.: +86 431 84532886; fax: +86 431 84532887.
E-mail address: liyanlijlau@yahoo.com (Y. Li).

1389-1723/$ – see front matter
https://doi.org/10.1016/j.jbiosc.2019.04.011

(FAE, EC3.2.1.73), which can also act on hemicellulose, are categorized as CEs (4,10).

With regards to lignin-degrading enzymes, AAs play key roles; they are classified into lignin oxidases (LOs) and lignin-degrading auxiliary enzymes (LDAs) (13). LOs take responsibility for generating highly reactive non-specific free radicals to cleave carbon−carbon and ether inter-unit bonds of lignin (14). LOs include laccase (LACC, EC 1.10.3.2, AA1) and class II heme peroxidases (class II PODs, AA2), which mainly consist of lignin peroxidase (LiP, EC 1.11.1.14, AA2_1), manganese peroxidase (MnP, EC 1.11.1.13, AA2_2), and versatile peroxidase (VP, EC 1.11.1.16, AA2_3). LDAs include glucose/methanol/choline oxidases (GMCs, AA3) and copper radical oxidases (CROs, AA5) (22,23); these can generate the hydrogen peroxide required for the function of class II PODs (24) and Fenton chemistry, in which Fe (II) and hydrogen peroxide react to form hydroxyl radicals, highly reactive oxidants capable of depolymerizing cellulose (25,26). The GMCs mainly consist of cellobiose dehydrogenase (CDH, EC 1.1.99.18, AA3_1), aryl alcohol oxidase (AAO, EC 1.1.3.7, AA3_2a), glucose oxidase (GOX, EC 1.1.3.4, AA3_2b), alcohol oxidase (AOX, EC 1.1.3.13, AA3_3), and pyranose oxidase (PYO, EC 1.1.3.10, AA3_4), and the CROs mainly consist of galactose oxidase (GAOX, EC 1.1.3.9, AA5_1) and glyoxal oxidase (GLOX, EC 1.2.3.15, AA5_2) (12,13).

Pleurotus djamor, belonging to the class Basidiomycetes, is a pink oyster mushroom cultivated widely in China; it can grow on different agro-waste substrates (27,28). It is also a valuable and important mushroom because it possesses a variety of physiological activities such as antitumor properties (29), antioxidant properties (30), antimicrobial properties (31), and hepatoprotective activities (32). Furthermore, *P. djamor* has potential for bioremediation due to its ability to decolorize the Benazol Black ZN textile dye (33) and degrade olive mill wastewater (34). Our research has found that *P. djamor* can grow well solely on corn stalk medium by producing lignocellulose-degrading enzymes. However, only one complete gene sequence encoding CAZymes of *P. djamor*, i.e., the *lac1* sequence (GI: 566037481), has been reported thus far in the NCBI. Thus, the degradation mechanism of corn stalk biomass has remained largely unknown.

Here, we carried out the transcriptome sequencing of *P. djamor* that grew solely in corn stalk medium using Illumina HiSeq™ 4000. A core set of genes of *P. djamor* encoding putative CAZymes related to the decomposition of corn stalk cellulose, hemicellulose, and lignin was studied.

MATERIALS AND METHODS

Fungal strain and culture conditions *P. djamor* CM13 was stored on Potato Dextrose Agar (PDA) plates at 4 °C. For pre-culturing, the strain was incubated at 28 °C for 6–7 days on PDA plates. Three mycelia-covered agar plugs (diameter = 1 mm) from the PDA plates were inoculated into PDA liquid medium (PDL), and then incubated at 28 °C with shaking at 150 rpm for eight days. Then, 10 mL of the mycelia suspension from the PDL was transferred into 90 mL of corn stalk liquid medium (CSL, 30 g/L corn stalk powder with a particle size of less than 1 mm) in a 250-mL Erlenmeyer flask. The flasks were incubated at 28 °C with shaking at 120 rpm for different time periods. Each experiment was performed in triplicate.

Enzyme extraction and enzymatic activity assay Crude enzymes were extracted after filtering the fermentation broth using 0.45-μm sterile membranes. Cellulase and xylanase activities were measured according to previously described methods (35,36); the LACC and MnP activities were measured according to previously described methods (37,38).

RNA extraction, cDNA library preparation and RNA sequencing The mycelia incubated in CSL for 12 days were used as the samples in the treatment group (T1), and those incubated for 0 days, as the samples in the control group (CK). Mycelia were collected quickly by filtering the broth through one layer of gauze and washing the mycelia with sterile water; next, the mycelia were suspended in liquid nitrogen. Total RNA was extracted from the mycelia using Trizol reagent. mRNA was enriched by Oligo (dT) beads and was then reverse transcribed into cDNA using random primers. Second-strand cDNA was synthesized using DNA polymerase I, RNase H, dNTPs. The cDNA fragments were purified with 1.8× Agencourt AMPure XP Beads, and subjected to end repairing and poly (A) addition, after which they were ligated to Illumina sequencing adapters. The ligation products were size selected by agarose gel electrophoresis, PCR amplified, and then sequenced using Illumina HiSeq 4000 by Gene Denovo Biotechnology Co. (Guangzhou, China). This Transcriptome Shotgun Assembly project has been deposited at DDBJ/EMBL/GenBank under the accession number GHEA00000000. The version described in this paper is the first version, GHEA01000000.

Filtering the raw reads and the *de novo* assembly of the reads To obtain high-quality clean reads, the raw reads were further filtered by removing the adapters, more than 10% of unknown nucleotides (N), and more than 40% of low-quality (Q-value ≤ 20) bases. The *de novo* assembly of the reads to obtain unigenes was carried out using the short-read assembling program Trinity (39).

Relationship analysis between samples Correlation analyses of triplicate experiments were performed to evaluate the reliability of the experimental results as well as the operational stability of the experiments. The correlation coefficient was computed to evaluate the repeatability between the samples.

Functional annotation of the unigenes To annotate the unigenes, we used the BLASTx program (http://www.ncbi.nlm.nih.gov/BLAST/) on the NCBI non-redundant protein (Nr) database (http://www.ncbi.nlm.nih.gov), with an E-value threshold of 1e-5 to, the Swiss-Prot protein database (http://www.expasy.ch/sprot), the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (http://www.genome.jp/kegg), and the Clusters of Orthologous Groups (COG/KOG) database (http://www.ncbi.nlm.nih.gov/COG). Protein functional annotations could then be obtained according to the best alignment results.

Quantification of gene abundance The gene abundance was calculated and normalized to the reads per kb per million reads (RPKM) (40), and the formula for this calculation is as follows: $RPKM = 10^6 \times C/(NL/10^3)$, where RPKM (A) is the expression of gene A, C is the number of reads that were uniquely aligned to gene A, N is the total number of reads that were uniquely aligned to all genes, and L is the number of bases on gene A.

Analysis of differentially expressed genes In order to identify differentially expressed genes across samples or groups, the edgeR package (http://www.r-project.org/) was used. Genes with a fold change of ≥2 and a false discovery rate (FDR) of ≤ 0.05 were identified as significant differentially expressed genes (DEGs).

KEGG pathway enrichment analysis KEGG is the major public pathway-related database (41). Pathway enrichment analysis identified significantly enriched metabolic pathways or signal transduction pathways in DEGs when compared with the whole genomic background. The calculated *p*-value was subjected to FDR correction, setting an FDR threshold value of ≤ 0.05. Pathways fulfilling this condition were defined as significantly enriched pathways in DEGs.

Real-time quantitative PCR experiments For real-time quantitative PCR (RT-qPCR) analysis, cDNA was synthesized from the total RNA using a PrimeScript RT-PCR Kit (Takara, Dalian, China) in accordance with the instructions. All quantitative PCR procedures were performed in triplicate on an Agilent M x 3000P system using SYBR Premix ExTaq Kit (Takara). The glyceraldehyde-3-phosphate dehydrogenase gene (*gapdh*) was chosen as the reference gene (42). We selected 13 DEGs in the transcriptome of *P. djamor*, and their relative expression levels were normalized to the *gapdh* expression levels to validate the reliability of our RNA-Seq data. Specific primers were synthesized (Sangon Biotech, Shanghai, China); The sequences of these primers are given in Table S1. The total volume of the reaction mixture was 20 μL. The reaction mixture comprised 0.5 μM of the forward and reverse primers, 1× SYBR Green qPCR master mix, and 30 ng of the cDNA template; an appropriate amount of H_2O was added to attain the final volume of 20 μL. The cycling protocol was performed as follows: initial denaturation at 95 °C for 10 min, followed by 40 cycles of (i) denaturation at 95 °C for 30 s, (ii) annealing at 60 °C for 1 min, and (iii) extension at 72 °C for 30 s. Fluorescence data acquisition was performed during the extension step. To confirm the specificity of the RT-qPCR primers, melting curves were obtained and inspected for the presence of a single peak. The relative expression levels were calculated by $2^{-\Delta\Delta Ct}$ method for the *gapdh*-normalized cycle threshold (Ct) values, and the results are reported as the relative fold changes.

RESULTS AND DISCUSSION

Lignocellulose-degrading enzymes activities of *P. djamor* Cellulase and xylanase activities of *P. djamor* growing in CSL increased with the prolongation of the incubation time, reaching the highest activity on the 16th day, and then decreasing (Fig. 1A). Laccase and MnP activities reached their peak values on the 4th day and then decreased rapidly; however, they were still maintained at low levels (Fig. 1B). Considering that the apoenzyme expression occurs after RNA transcription and the

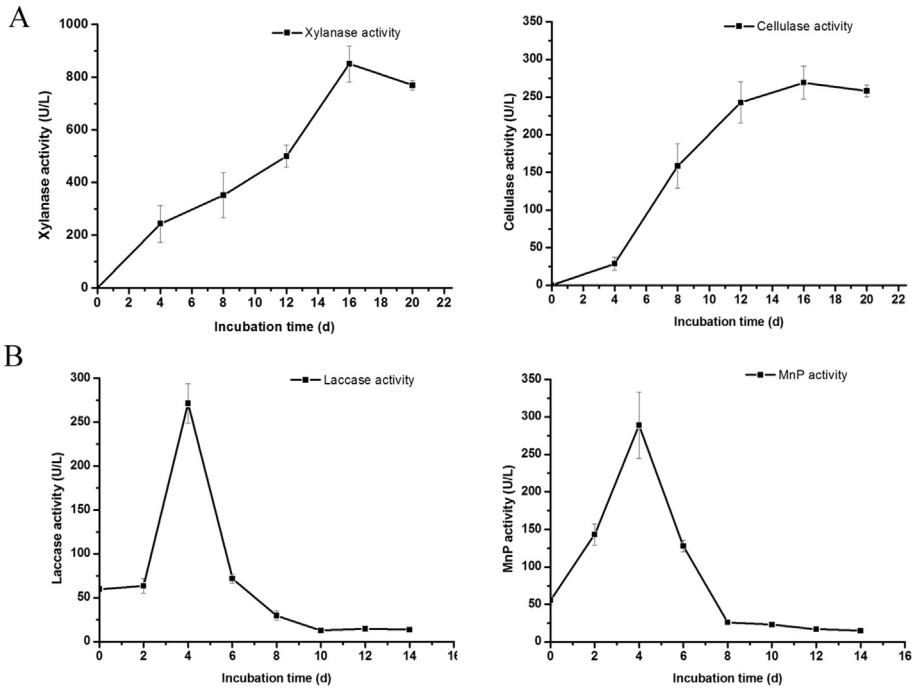


FIG. 1. Lignocellulose-degrading enzyme activities of *P. djamor* CM13 cultured in CSL under submerged fermentation conditions at 28 °C with shaking at 120 rpm. (A) Cellulase activity and xylanase activity after incubation for 0, 4, 8, 12, 16, and 20 days. (B) Laccase activity and MnP activity after incubation for 0, 2, 4, 6, 8, 10, 12, and 14 days. The experiment was performed in triplicate. The error bars indicate the standard deviation. MnP, manganese peroxidase.

action of cellulase and xylanase, the mycelia cultured in CSL for 12 days were selected as samples in the treatment group (T1), and those cultured for 0 days, as samples in the control group (CK), to perform the RNA-Seq of *P. djamor* CM13.

Illumina sequencing and *de novo* assembly A total of 45.4 and 46.4 million clean reads were obtained after 46.5 and 47.4 million raw reads were filtered from the *P. djamor* transcriptome samples in the CK and T1 groups, respectively. They were successfully *de novo* assembled to obtain a total of 17,207 unigenes, with an average length of 1889 nt, maximum length of 16,640 nt, and minimum length of 201 nt. The GC percentage of 50.8% and N50 length of 3304 nt indicate a good assembly quality (Table 1).

Relationship analysis of samples Correlation analysis of triplicate biological experiments of each sample including those from the CK and T1 groups was performed to evaluate the repeatability of the experiments. As shown in Fig. S1, a closer correlation between samples indicates a highly repeatability of the experiments. This indicates that the datasets of RNA-Seq in the present study are reliable.

Functional annotations of unigenes Among the 17,207 assembled unigenes, a total of 11,054 unigenes were annotated after searching the Nr, Swiss-Prot, COG/KOG, and KEGG databases. The numbers of unigenes with significant similarity to sequences in Nr, Swiss-Prot, COG/KOG, and KEGG databases are 10,991 (99.0%), 6379 (57.9%), 5372 (48.6%), and 3639 (32.9%), respectively. The number of interactions that the unigenes share with different databases is shown in Fig. S2.

TABLE 1. Assembly results of the *P. djamor* transcriptome.

Gene number	GC percentage (%)	N50 (nt)	Max length (nt)	Min length (nt)	Average length (nt)
17207	50.8	3304	16640	201	1889

Validation of the RNA-Seq results by RT-qPCR RT-qPCR analysis of 13 selected DEGs was performed to validate the *P. djamor* transcriptome in the present study (Table S1). The RT-qPCR results correspond well with the gene expression data obtained from *P. djamor* transcriptome (Fig. 2). This suggests that the dataset obtained from the RNA-Seq is reliable.

Analysis of CAZy DEGs related to the decomposition of corn stalk biomass To gain insights into the gene expression of *P. djamor* cultured in CSL under submerged fermentation conditions, we analyzed the DEGs between the *P. djamor* transcriptome samples from the T1 and CK groups. A total of 3322 DEGs were obtained, of which, 1913 showed upregulated expression and 1409 showed downregulated expression (Fig. S3). A total of 675 CAZy DEGs related to the decomposition of corn stalk biomass were obtained, among which GHs (37.9%) were the dominant group, followed by CBMs (33.0%), AAs (11.8%), GTs (11.7%), CEs (3.8%), and PLs (1.8%). Compared to the CK samples, 448 (66.4%) CAZy DEGs in the T1 samples showed upregulated expression (Fig. S4). In the transcriptome of *Lentinula edodes* that was cultured using glucose or cellulose as the carbon sources, only 461 CAZymes were annotated, for which the number of upregulated DEGs is only 23 (43). This is probably due to the difference in substrates and fungal species.

Genes encoding putative CAZymes related to the decomposition of corn stalk lignocellulose biomass Among 675 CAZy DEGs, a total of 87 putative CAZymes related to lignocellulose degradation were identified. Of these, 32 (36.8%), 21 (24.1%), and 34 (39.1%) CAZymes were involved in cellulose, hemicellulose, and lignin degradation, respectively (Table S2). They belong to different CAZy families with varying gene numbers; among these, GHs were still the dominant group (Fig. 3). This indicates that *P. djamor* can express a large amount of specific extracellular hydrolytic enzymes to cooperatively decompose corn stalk lignocellulose biomass. Similar results were obtained in

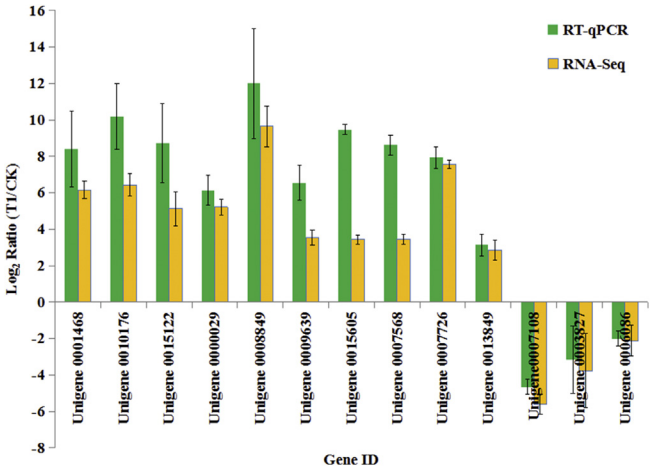
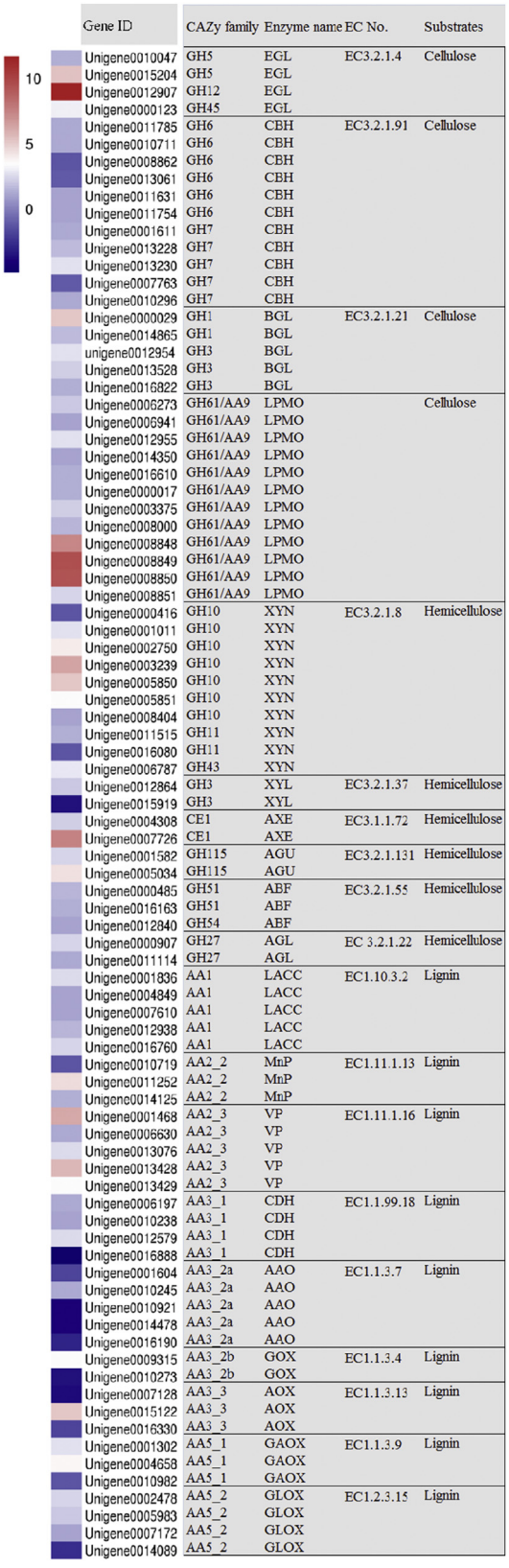


FIG. 2. Validation of the RNA-Seq of 13 selected DEGs in the *P. djamor* transcriptome by RT-qPCR. *gapdh* was chosen as the reference gene. The green column represents the $\log_2$ Ratio (T1/CK) of DEGs, as calculated by the RT-qPCR analysis, and the orange column represents the $\log_2$ Ratio (T1/CK) of DEGs, as calculated by the RNA-Seq analysis. The error bars represent the standard deviation of three biological replicates and three replicates of RT-qPCR runs.

case of 22 transcriptome datasets from 10 basidiomycete species growing on several different substrates (5).

Cellulose is the most abundant plant biomass component, and is a highly stable polymer comprising many glucose units attached by β-1,4 linkages. The hydrolysis of cellulose requires many types of enzymes that work in combination (44). A total of 32 CAZymes that are related to cellulose biodegradation have been found, comprising 4 EGLs, 11 CBHs, 5 BGLs, and 12 LPMOs in the transcriptome of *P. djamor* (Fig. 3). This indicates that *P. djamor* can produce many types of cellulases that work in combination to degrade corn stalk cellulose. With the exception of three CBH-encoding genes, the other (twenty-nine) genes showed upregulated expression in the T1 samples, when compared to that in the CK *P. djamor* transcriptome samples (Fig. 3, Table S3). Interestingly, some upregulated genes showed a high fold change (≥10), such as unigene 0012907 ($2^{11.7}$) encoding EGL (GH12), unigene 0015204 ($2^{5.3}$) encoding EGL (GH5), unigene 0008849 ($2^{9.5}$), unigene 0008850 ($2^{9.3}$), and unigene 0008848 ($2^{7.4}$) encoding LPMOs (GH61/AA9), and unigene 0000029 ($2^{5.2}$) encoding BGL (GH1) (Table S3). These results suggest that EGL, BGL, and LPMO play more important roles in the decomposition of corn stalk cellulose than CBH in *P. djamor*. EGL acts on cellulose, and the resulting oligosaccharides are usually processed by BGL to glucose in fungi (4,10,15). LPMO is widely distributed among fungi and has changed the paradigm of cellulose conversion in the past few years as the key factor for lignocellulose breakdown (16,19). Recent work has shown that LPMO can deconstruct other components besides cellulose in plant cells by oxidizing the carbon of sugar rings, leading to chain cleavage, thus, boosting the activity of classical GHs by introducing new chain-ends, which are targets for enzyme action (20). Hence, LPMO must be a very important factor during the process of decomposition of corn stalk biomass; its function is very worthy of being a subject for further research.

Hemicellulose is a heterogenous polymer comprising pentoses, hexoses, sugar acids, and acetyl groups (4,10). Decomposition of hemicellulose is very complicated and requires a cocktail of hemicellulases. In the *P. djamor* transcriptome, there are 21 CAZymes related to hemicellulose biodegradation, comprising 10 XYNs, 2 XYLs, 2 AGUs, 3 ABFs, 2 AGLs, and 2 AXEs (Fig. 3). In the present research, FAE were absent. With the exception of two XYN-encoding genes and one XYL-encoding gene, the other (eighteen)



Gene ID	CAZy family	Enzyme name	EC No.	Substrates
Unigene0010047	GH5	EGL	EC3.2.1.4	Cellulose
Unigene0015204	GH5	EGL		
Unigene0012907	GH12	EGL		
Unigene0000123	GH45	EGL		
Unigene0011785	GH6	CBH	EC3.2.1.91	Cellulose
Unigene0010711	GH6	CBH		
Unigene0008862	GH6	CBH		
Unigene0013061	GH6	CBH		
Unigene0011631	GH6	CBH		
Unigene0011754	GH6	CBH		
Unigene0001611	GH7	CBH		
Unigene0013228	GH7	CBH		
Unigene0013230	GH7	CBH		
Unigene0007763	GH7	CBH		
Unigene0010296	GH7	CBH		
Unigene0000029	GH1	BGL	EC3.2.1.21	Cellulose
Unigene0014865	GH1	BGL		
unigene0012954	GH3	BGL		
Unigene0013528	GH3	BGL		
Unigene0016822	GH3	BGL		
Unigene0006273	GH61/AA9	LPMO		Cellulose
Unigene0006941	GH61/AA9	LPMO		
Unigene0012955	GH61/AA9	LPMO		
Unigene0014350	GH61/AA9	LPMO		
Unigene0016610	GH61/AA9	LPMO		
Unigene0000017	GH61/AA9	LPMO		
Unigene0003375	GH61/AA9	LPMO		
Unigene0008000	GH61/AA9	LPMO		
Unigene0008848	GH61/AA9	LPMO		
Unigene0008849	GH61/AA9	LPMO		
Unigene0008850	GH61/AA9	LPMO		
Unigene0008851	GH61/AA9	LPMO		
Unigene0000416	GH10	XYN	EC3.2.1.8	Hemicellulose
Unigene0001011	GH10	XYN		
Unigene0002750	GH10	XYN		
Unigene0003239	GH10	XYN		
Unigene0005850	GH10	XYN		
Unigene0005851	GH10	XYN		
Unigene0008404	GH10	XYN		
Unigene0011515	GH11	XYN		
Unigene0016080	GH11	XYN		
Unigene0006787	GH43	XYN		
Unigene0012864	GH3	XYL	EC3.2.1.37	Hemicellulose
Unigene0015919	GH3	XYL		
Unigene0004308	CE1	AXE	EC3.1.1.72	Hemicellulose
Unigene0007726	CE1	AXE		
Unigene0001582	GH115	AGU	EC3.2.1.131	Hemicellulose
Unigene0005034	GH115	AGU		
Unigene0000485	GH51	ABF	EC3.2.1.55	Hemicellulose
Unigene0016163	GH51	ABF		
Unigene0012840	GH54	ABF		
Unigene0000907	GH27	AGL	EC 3.2.1.22	Hemicellulose
Unigene0011114	GH27	AGL		
Unigene0001836	AA1	LACC	EC1.10.3.2	Lignin
Unigene0004849	AA1	LACC		
Unigene0007610	AA1	LACC		
Unigene0012938	AA1	LACC		
Unigene0016760	AA1	LACC		
Unigene0010719	AA2_2	MnP	EC1.11.1.13	Lignin
Unigene0011252	AA2_2	MnP		
Unigene0014125	AA2_2	MnP		
Unigene0001468	AA2_3	VP	EC1.11.1.16	Lignin
Unigene0006630	AA2_3	VP		
Unigene0013076	AA2_3	VP		
Unigene0013428	AA2_3	VP		
Unigene0013429	AA2_3	VP		
Unigene0006197	AA3_1	CDH	EC1.1.99.18	Lignin
Unigene0010238	AA3_1	CDH		
Unigene0012579	AA3_1	CDH		
Unigene0016888	AA3_1	CDH		
Unigene0001604	AA3_2a	AAO	EC1.1.3.7	Lignin
Unigene0010245	AA3_2a	AAO		
Unigene0010921	AA3_2a	AAO		
Unigene0014478	AA3_2a	AAO		
Unigene0016190	AA3_2a	AAO		
Unigene0009315	AA3_2b	GOX	EC1.1.3.4	Lignin
Unigene0010273	AA3_2b	GOX		
Unigene0007128	AA3_3	AOX	EC1.1.3.13	Lignin
Unigene0015122	AA3_3	AOX		
Unigene0016330	AA3_3	AOX		
Unigene0001302	AA5_1	GAOX	EC1.1.3.9	Lignin
Unigene0004658	AA5_1	GAOX		
Unigene0010982	AA5_1	GAOX		
Unigene0002478	AA5_2	GLOX	EC1.2.3.15	Lignin
Unigene0005983	AA5_2	GLOX		
Unigene0007172	AA5_2	GLOX		
Unigene0014089	AA5_2	GLOX		

FIG. 3. Expression of putative CAZyme DEGs related to the decomposition of corn stalk lignocellulose in the *P. djamor* transcriptome. The dark red strip represents significantly upregulated expression, and the dark blue strip represents significantly downregulated expression in the T1 *P. djamor* transcriptome samples, when compared to that in the CK *P. djamor* transcriptome samples. The terms 10, 5, and 0 represent the $\log_2$ ratio (T1/CK) value, and the higher the numerical value, the more obvious is the upregulated expression. Abbreviations are presented in Table S5.

genes showed an upregulated expression in the T1 samples, compared to that in the CK samples (Fig. 3, Table S3). Of these genes, unigene 0007726 ($2^{7.6}$) encoding AXE (CE1), unigene 0005034 ($2^{4.4}$) encoding AGU (GH115), and unigene 0003239 ($2^{6.4}$), unigene 0005850 ($2^{5.2}$), and unigene 0002750 ($2^{4.0}$) encoding XYNs (GH10) showed a high fold change (≥10) (Table S3). These CAZymes may play more important role in the decomposition of corn stalk hemicellulose. AXE and AGU can remove acetyl group and 4-*O*-methyl-D-glucuronic acid in the branch of hemicellulose, respectively, then XYN can break the exposed xylan chains into monomer molecules (10).

The lignin in plant cell walls is firstly decomposed by oxidation or modification, so that cellulose and hemicellulose can be exposed for further utilization by fungi (10,23). *P. djamor* can produce 34 CAZymes including 13 LOs and 21 LDAs under liquid fermentation culture conditions using CSL as the substrate, to degrade lignin (Fig. 3). 13 LOs include 5 LACCs (AA1), 3 MnPs (AA2_2) and 5 VPs (AA2_3). With the exception of one gene encoding MnP, the other (twelve) LO-encoding genes were upregulated in the T1 *P. djamor* transcriptome samples, when compared to that in the CK *P. djamor* transcriptome samples (Fig. 3, Table S3). Our results prove that the decomposition of lignin by LOs is still indispensable for *P. djamor* to utilize corn stalk polysaccharides biomass. Some genes showed a high fold change (≥10), such as unigene 0011252 ($2^{4.5}$) encoding MnP, unigene 00001468 ($2^{6.2}$), and unigene 0013428 ($2^{5.6}$) and unigene 0013429 ($2^{3.4}$) encoding VPs (Table S3). Hence, the key LOs may be VPs during the decomposition of corn stalk lignin in this research. VPs are less common in white-rot fungi (23), which exhibit dual activities of both LiP and MnP, to degrade the phenol and non-phenol substrates of lignin (45,46). Due to their applicability with a wide range of substrates, VPs have a great potential to be used as catalysts for various biotechnological applications, such as the decolorization of the azo dyes (45), delignification of eucalypt kraft pulp (47), and degradation of phenolic and non-phenolic aromatic pollutants (48). Therefore, the VPs of *P. djamor* have a great potential for applications in the field of environmental protection. In the present study, LiPs and PYOs are absent and may not be necessary for *P. djamor* to decompose corn stalk lignin. In the genome of some fungi including several *Pleurotus* species, there is a lack of LiP- and PYO-encoding genes (43). There may be a lack of LiP and PYO in the *P. djamor* genome.

21 LDAs comprise: 14 GMCs, including 4 CDHs, 5 AAOs, 2 GOXs, and 3 AOXs; and 7 CROs, including 3 GAOXs and 4 GLOXs (Fig. 3). Among 21 LDAs, 6 GMCs (3 CDHs, 1 AAO, 1 GOX, and 1 AOX) and 5 CROs (2 GAOXs and 3 GLOXs) showed upregulated expression in the T1 *P. djamor* transcriptome samples, when compared to that in the CK samples (Fig. 3, Table S3). Only two genes showed a high fold change (≥ 10), and they are unigene 0015122 ($2^{5.3}$) encoding AOX (AA3_3) and unigene 0004658 ($2^{3.7}$) encoding GAOX (AA5_1) (Table S3). These results suggest that these CAZymes play an important role in the decomposition of corn stalk lignin by providing hydrogen peroxide for class II PODs (24) and Fenton chemistry (25,26). Thus, these CAZymes are imperative for the exploitation and utilization of corn stalk polysaccharides.

In summary, our results showed that *P. djamor* expressed a complete repertoire of genes encoding CAZymes for the degradation of cellulose, hemicellulose, and lignin in corn stalk, similar to the results obtained from the white-rot fungus *Obba rivulosa* on spruce (12). A core set of 70 upregulated genes encoding putative CAZymes was obtained. These genes encode 19 kinds of CAZymes in total, which comprise 4 EGLs, 8 CBHs, 5 BGLs, and 12 LPMOs related to cellulose degradation, 8 XYNs, 1 XYL, 2 AGUs, 3 ABFs, 2 AGLs, and 2 AXEs related to hemicellulose degradation, and 5 LACCs, 2 MnPs, 5 VPs, 3 CDHs, 1 AAO, 1 GOX, 1 AOX, 2 GAOXs, and 3 GLOXs related to lignin degradation (Fig. 3, Table S3). Some genes showed a high fold change (≥ 10). The maximum $\log_2$ Ratio (T1/CK) of each upregulated CAZyme encoding gene ranged from 1.2 to 11.7 (Fig. 4). They may play a more important role in synergistically decomposing lignocellulose of corn stalk.

KEGG pathway enrichment analysis to reveal CAZyme DEGs The KEGG database has been a useful tool in identifying candidate genes in some biological processes (49–52). To gain a better understanding of the decomposition of corn stalk lignocellulose in DEGs in the T1 *P. djamor* transcriptome samples compared to that in the CK *P. djamor* transcriptome samples, the CAZyme DEGs were analyzed using the KEGG tool. Among 104 pathways that were found to be enriched, 11 significantly enriched KEGG pathways were obtained (Fig. S5). However, the only pathway relevant to our research was carbohydrate metabolism, which consists of three metabolic pathways, namely glycolysis/gluconeogenesis (ko00010), pentose and glucuronate interconversions (ko00040), and starch and sucrose metabolism (ko00500).

In glycolysis/gluconeogenesis (Fig. S6), unigene 0011954 encoding aldose 1-epimerase (EC 5.1.3.3), unigene 0013086 encoding aldehyde dehydrogenase (EC 1.2.1.3), and unigene 0016571 encoding alcohol dehydrogenase (EC 1.1.1.1) were upregulated in the T1 *P. djamor* transcriptome samples, when compared to that in the CK *P. djamor* transcriptome samples. In pentose and glucuronate interconversions (Fig. S7), unigene 0014312 and

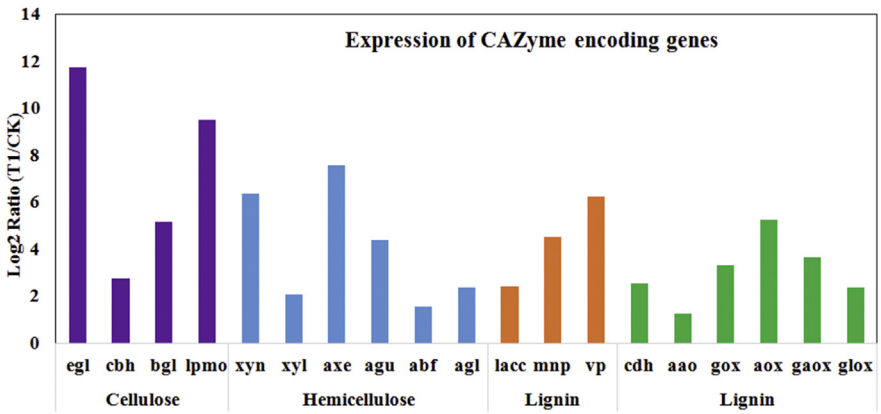


FIG. 4. The maximum $\log_2$ Ratio (T1/CK) of upregulated gene encoding each putative CAZyme related to the decomposition of corn stalk lignocellulose. Corresponding substrates of each CAZyme are indicated in the figure. Enzyme abbreviations are presented in Table S5.

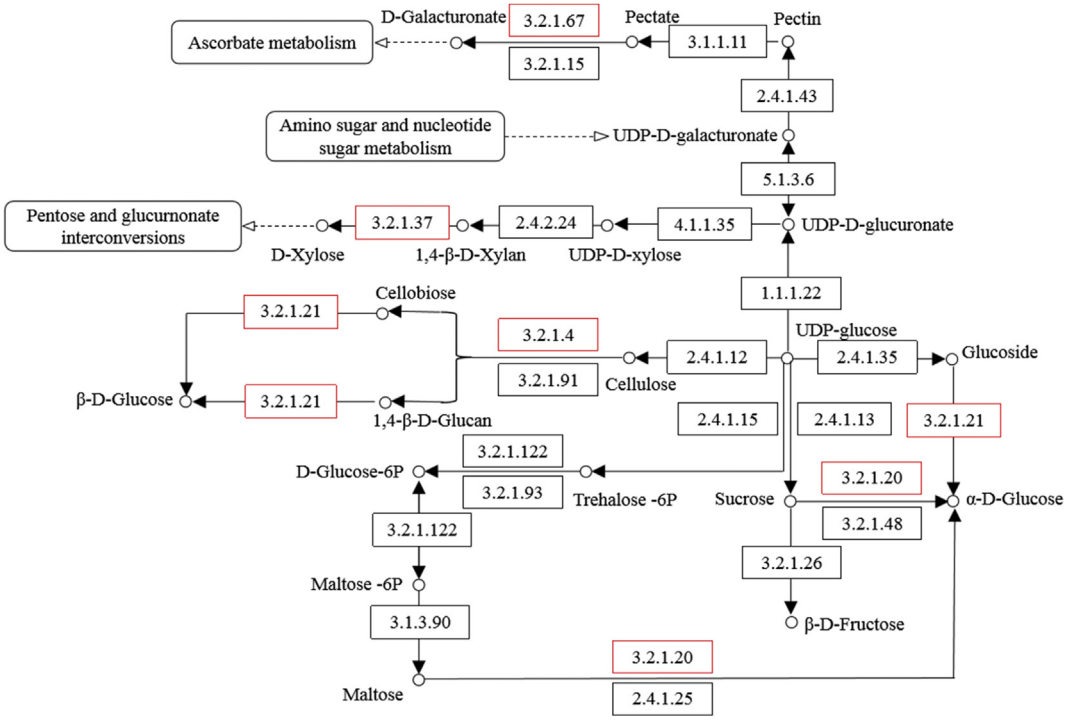


FIG. 5. The reaction in the starch and sucrose metabolism. The number in the frame represents the enzyme number, which is stipulated by the Enzyme Commission. The red frame indicates that the enriched genes are upregulated. The circle represents the metabolite, and the arrow represents the enzymatic reaction.

unigene 0014505 encoding pectate lyases (EC 4.2.2.2), unigene 0013391 encoding galacturan 1,4-alpha-galacturonidase (EC 3.2.1.67), unigene 0012501 encoding D-galacturonate reductase (EC 1.1.1.-, GaaA), unigene 0012307 encoding L-threo-3-deoxy-hexylosonate aldolase (EC 4.1.2.54), and unigene 0004068 encoding L-glyceraldehyde reductase (EC 1.1.1.372) were upregulated; unigene 0010310 encoding D-xylose reductase (EC 1.1.1.307), unigene 0010819 encoding L-iditol 2-dehydrogenase (EC 1.1.1.14), and unigene 0008789 encoding D-arabinitol dehydrogenase (EC 1.1.1.287) were upregulated in the T1 *P. djamor* transcriptome samples, when compared to that in the CK samples. These upregulated DEGs from the two above mentioned pathways are involved in carbohydrate metabolism necessary for mycelial growth and development; however, they are not classified as CAZymes.

In starch and sucrose metabolism (Fig. 5), we found that unigene 0015646 encoding α-glucosidase (EC 3.2.1.20) and unigene 0013391 encoding galacturan 1,4-α-galacturonidase (EC 3.2.1.67) were upregulated in this pathway, in the T1 *P. djamor* transcriptome samples, when compared to that in the CK samples. However, they do not belong to the group of CAZymes. We found that 15 candidate CAZyme DEGs were upregulated in this pathway, comprising 9 unigenes encoding EGLs (EC 3.2.1.4) that catalyze the conversion of cellulose to cellobiose or 1,4-β-D-glucan, 5 unigenes encoding BGLs (EC 3.2.1.21) that catalyze the conversion of cellobiose or 1,4-β-D-glucan or glucoside to β-glucose, and 1 unigene encoding XYL (EC 3.2.1.37) that catalyzes the conversion of 1,4-β-D-xylan to β-xylose, in the T1 *P. djamor* transcriptome samples, when compared to that in the CK *P. djamor* transcriptome samples (Table S4). These results are consistent with our above results (Fig. 3, Table S3). Here, 8 out of 9 EGLs are LPMOs categorized into the GH61/AA9 family. This is the first time that LPMOs were obtained by using KEGG pathway enrichment analysis. These findings prove that LPMOs play important roles in the decomposition of corn stalk lignocellulose (Table S4). Nevertheless, the CAZyme DEGs in starch and sucrose metabolism are related to the decomposition of corn stalk lignocellulose, which further confirms their roles in the decomposition of corn stalk lignocellulose biomass.

With the reduction in costs over the past few years, the next-generation sequencing (NGS) technology has had more extensive applications with regards to plant (49,50,53,54), animal (55,56), and fungal (51,52,57,58) samples. This is the first study to report the *de novo* RNA-Seq of *P. djamor*. These results will produce a dataset of genes encoding CAZymes, thereby laying the foundation to illustrate the degradation mechanism of corn stalk biomass and boost the biotransformation of corn stalk resources.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jbiosc.2019.04.011.

ACKNOWLEDGMENTS

This work was supported by Science and Technology Department of Jilin Province, China [20180101248JC] and Education Department of Jilin Province, China [JJKH20180681KJ].

References

1. **Himmel, M. E., Ding, S. Y., Johnson, D. K., Adney, W. S., Nimlos, M. R., Brady, J. W., and Foust, T. D.:** Biomass recalcitrance: engineering plants and enzymes for biofuels production, Science, **31**, 804–807 (2007).
2. **Isikgor, F. H. and Becer, C. R.:** Lignocellulosic biomass: a sustainable platform for the production of bio-based chemicals and polymers, Polym. Chem., **6**, 4497–4559 (2015).
3. **Sticklen, M. B.:** Plant genetic engineering for biofuel production: towards affordable cellulosic ethanol, Nat. Rev. Genet., **9**, 433–443 (2008).
4. **Alfaro, M., Castanera, R., Lavín, J. L., Grigoriev, I. V., Oguiza, J. A., Ramírez, L., and Pisabarro, A. G.:** Comparative and transcriptional analysis of the predicted secretome in the lignocellulose-degrading basidiomycete fungus *Pleurotus ostreatus*, Environ. Microbiol., **18**, 4710–4726 (2016).
5. **Peng, M., Aguilar-Pontes, M. V., Hainaut, M., Henrissat, B., Hildén, K., Mäkelä, M. R., and de Vries, R. P.:** Comparative analysis of basidiomycete transcriptomes reveals a core set of expressed genes encoding plant biomass degrading enzymes, Fungal Genet. Biol., **112**, 40–46 (2018).
6. **Liu, J., Ju, M., Wu, W., Liu, B., Zhan, L., Wu, M., Wu, P., Liu, Y., and Tong, S.:** Lignocelluloytic enzyme production in solid-state fermentation of corn stalk

with ammoniation pretreatment by *Lentinus edodes* L-8, BioResources, **9**, 1430–1444 (2014).
7. **Miao, Y., Liu, D., Li, G., Li, P., Xu, Y., Shen, Q., and Zhang, R.:** Genome-wide transcriptomic analysis of a superior biomass-degrading strain of *A. fumigatus* revealed active lignocellulose degrading genes, BMC Genomics, **16**, 459–478 (2015).
8. **Mäkelä, M. R., Donofrio, N., and de Vries, R. P.:** Plant biomass degradation by fungi, Fungal Genet. Biol., **72**, 2–9 (2014).
9. **Patyshakuliyeva, A., Post, H., Zhou, M. M., Jurak, E., Heck, A. J. R., Hildén, K. S., Kabel, M. A., Mäkelä, M. R., Altelaar, M. A. F., and de Vries, R. P.:** Uncovering the abilities of *Agaricus bisporus* to degrade plant biomass throughout its life cycle, Environ. Microbiol., **17**, 3098–3109 (2015).
10. **Adebayo, E. A. and Martínez-Carrera, D.:** Oyster mushrooms (*Pleurotus*) are useful for utilizing lignocellulosic biomass, Afr. J. Biotechnol., **14**, 52–67 (2015).
11. **Hamelinck, C. N., Hooijdonk, G. V., and Faaij, A. P. C.:** Ethanol from lignocellulosic biomass:techno-economic performance in short-, middle- and long term, Biomass Bioenergy, **28**, 384–410 (2005).
12. **Marinović, M., Aguilar-Pontes, M. V., Zhou, M. M., Miettinen, O., de Vries, R. P., Mäkelä, M. R., and Hildén, K.:** Temporal transcriptome analysis of the white-rot fungus *Obba rivulosa* shows expression of a constitutive set of plant cell wall degradation targeted genes during growth on solid spruce wood, Fungal Genet. Biol., **112**, 47–54 (2018).
13. **Lombard, V., Golaconda Ramulu, H., Drula, E., Coutinho, P. M., and Henrissat, B.:** The carbohydrate-active enzymes database (CAZy) in 2013, Nucleic Acids Res., **42**, D490–D495 (2014).
14. **Rytioja, J., Hildén, K., Hatakka, A., and Mäkelä, M. R.:** Transcriptional analysis of selected cellulose-acting enzymes encoding genes of the white-rot fungus *Dichomitus squalens* on spruce wood and microcrystalline cellulose, Fungal Genet. Biol., **72**, 7291–7298 (2014).
15. **Baldrian, P. and Valásková, V.:** Degradation of cellulose by *basidiomycetous* fungi, FEMS Microbiol. Rev., **32**, 501–521 (2008).
16. **Monclaro, A. V. and Filho, E. X. F.:** Recent developments and application in lignocelullose breakdown, Int. J. Biol. Macromol., **102**, 771–778 (2017).
17. **Liu, B., Olson, Å., Wu, M., Broberg, A., and Sandgre, M.:** Biochemical studies of two lytic polysaccharide monooxygenases from the white-rot fungus *Heterobasidion irregulare* and their roles in lignocellulose degradation, PLoS One, **12**, e0189479 (2017).
18. **Kracher, D., Scheiblbrandner, S., Felice, A. K. G., Breslmayr, E., Preims, M., Ludwicka, K., Haltrich, D., Eijsink, V. G. H., and Ludwig, R.:** Extracellular electron transfer systems fuel cellulose oxidative degradation, Science, **352**, 1089–1101 (2016).
19. **Hemsworth, G. R., Johnston, E. M., Davies, G. J., and Walton, P. H.:** Lytic polysaccharide monooxygenases in biomass conversion, Trends Biotechnol., **33**, 747–761 (2015).
20. **Hemsworth, G. R., Davies, G. J., and Walton, P. H.:** Recent insights into copper-containing lytic polysaccharide mono-oxygenases, Curr. Opin. Struct. Biol., **23**, 660–668 (2013).
21. **Levasseur, A., Drula, E., Lombard, V., Coutinho, P. M., and Henrissat, B.:** Expansion of the enzymatic repertoire of the CAZy database to integrate auxiliary redox enzymes, Biotechnol. Biofuels, **6**, 41–54 (2013).
22. **Bourbonnais, R. and Paice, M. G.:** Oxidation of non-phenolic substrates an expanded role for lactase in lignin biodegradation, FEBS Lett., **267**, 99–102 (1990).
23. **Knop, D., Yarden, O., and Hadar, Y.:** The ligninolytic peroxidases in the genus *Pleurotus*:divergence in activities, expression, and potential applications, Appl. Microbiol. Biotechnol., **99**, 1025–1038 (2015).
24. **Fernández-Fueyo, E., Ruiz-Duenas, F. J., López-Lucendo, M. F., Pérez-Boada, M., Rencoret, J., Gutiérrez, A., Pisabarro, A. G., Ramírez, L., and Martínez, A. T.:** A secretomic view of woody and nonwoody lignocellulose degradation by *Pleurotus ostreatus*, Biotechnol. Biofuels, **9**, 49–66 (2016).
25. **Martínez, D., Challacombe, J., Morgenstern, I., Hibbett, D., Schmoll, M., Kubicek, C. P., Ferreira, P., Ruiz-Duenas, F. J., Martinez, A. T., Kersten, P., and other 43 authors:** Genome, transcriptome, and secretome analysis of wood decay fungus *Postia placenta* supports unique mechanisms of lignocellulose conversion, Proc. Natl. Acad. Sci. USA, **106**, 1954–1959 (2009).
26. **Daniel, G., Volc, J., Filonova, L., Plíhal, O., Kubátová, E., and Halada, P.:** Characteristics of *Gloeophyllum trabeum* alcohol oxidase, an extracellular source of H_2O_2 in brown rot decay of wood, Appl. Environ. Microbiol., **73**, 6241–6253 (2007).
27. **Lechner, B. E. and Alberto, E.:** Search for new naturally occurring strains of *Pleurotus* to improve yields: *Pleurotus albidus* as a novel proposed species for mushroom production, Rev. Iberoam. Micol., **28**, 148–154 (2011).
28. **Periasamy, K. and Natarajan, K.:** Role of lignocellulosic enzymes during basidiomata production by *Pleurotus djamor* var. *roseus*, Indian J. Biotechnol., **3**, 577–583 (2004).
29. **Borges, G. M., De Barba, F. F., Schiebelbein, A. P., Pereira, B. P., Chaves, M. B., Silveira, M. L., Pinho, M. S., Furlan, S. A., and Wisbeck, E.:** Extracellular polysaccharide production by a strain of *Pleurotus djamor* isolated in the south of Brazil and antitumor activity on Sarcoma 180, Braz. J. Microbiol., **44**, 1059–1065 (2013).
30. **Mishra, K. K., Pal, R. S., Arunkumar, R., Chandrashekara, C., Jain, S. K., and Bhatt, J. C.:** Antioxidant properties of different edible mushroom species and increased bioconversion efficiency of *Pleurotus eryngii* using locally available casing materials, Food Chem., **138**, 1557–1563 (2013).
31. **Ragasa, C. Y., Tan, M. C. S., Ting, J., Reyes, R. G., Brkljača, R., and Urban, S.:** Chemical constituents of *Pleurotus djamor*, Der. Pharma. Chemica., **8**, 343–346 (2016).
32. **Zhang, J., Liu, M., Yang, Y., Lin, L., Xu, N., Zhao, H., and Jia, L.:** Purification, characterization and hepatoprotective activities of mycelia zinc polysaccharides by *Pleurotus djamor*, Carbohydr. Polym., **136**, 588–597 (2016).
33. **Kalmis, E., Azbar, N., and Kalyoncu, F.:** Evaluation of two wild types of *Pleurotus ostreatus* (MCC07 and MCC20) isolated from nature for their ability to decolorize Benazol Black ZN textile dye in comparison to some commercial types of white rot fungi: *Pleurotus ostreatus*, *Pleurotus djamor*, and *Pleurotus citrinopileatus*, Can. J. Microbiol., **54**, 366–370 (2008).
34. **Koutrotsios, G. and Zervakis, G. I.:** Comparative examination of the olive mill wastewater biodegradation process by various wood-rot macrofungi, BioMed Res. Int., **2014**, 482937 (2014).
35. **Xiao, Z., Storms, R., and Tsang, A.:** Microplate-based filter paper assay to measure total cellulase activity, Biotechnol. Bioeng., **88**, 832–837 (2008).
36. **Bailey, M. J., Biely, P., and Poutanen, K.:** Interlaboratory testing of methods for assay of xylanase activity, J. Biotechnol., **23**, 257–270 (1992).
37. **Coconi-Linares, N., Magaña-Ortíz, D., Guzmán-Ortiz, D. A., Fernández, F., Loske, A. M., and Gómez-Lim, M. A.:** High-yield production of manganese peroxidase, lignin peroxidase, and versatile peroxidase in *Phanerochaete chrysosporium*, Appl. Microbiol. Biotechnol., **98**, 9283–9294 (2014).
38. **Ruiz-Dueñas, F. J., Guillén, F., Camarero, S., Pérez-Boada, M., Martínez, M. J., and Martínez, A. T.:** Regulation of peroxidase transcript levels in liquid cultures of the ligninolytic fungus *Pleurotus eryngii*, Appl. Environ. Microbiol., **10**, 4458–4463 (1999).
39. **Grabherr, M. G., Haas, B. J., Yassour, M., Levin, J. Z., Thompson, D. A., Amit, I., Adiconis, X., Fan, L., Raychowdhury, R., Zeng, Q. D., and other 11 authors:** Full-length transcriptome assembly from RNA-Seq data without a reference genome, Nat. Biotechnol., **29**, 644–668 (2011).
40. **Mortazavi, A., Williams, B. A., Mccue, K., Schaeffer, L., and Wold, B.:** Mapping and quantifying mammalian transcriptomes by RNA-Seq, Nat. Methods, **5**, 621–628 (2008).
41. **Kanehisa, M., Araki, M., Goto, S., Hattori, M., Hirakawa, M., Itoh, M., Katayama, T., Kawashima, S., Okuda, S., Tokimatsu, T., and Yamanishi, Y.:** KEGG for linking genomes to life and the environment, Nucleic Acids Res., **36**, D480–D484 (2008).
42. **Hirano, T., Sato, T., Okawa, K., Kanda, K., Yaegashi, K., and Enei, H.:** Isolation and characterization of the glyceraldehyde-3-phosphate dehydrogenase gene of *Lentinus edodes*, Biosci. Biotechnol. Biochem., **63**, 1223–1227 (1999).
43. **Chen, L., Gong, Y., Cai, Y., Liu, W., Zhou, Y., Xiao, Y., Xu, Z., Liu, Y., Lei, X., Wang, G., and other 3 authors:** Genome sequence of the edible cultivated mushroom *Lentinula edodes* (Shiitake) reveals insights into lignocellulose degradation, PLoS One, **11**, e0160336 (2016).
44. **Wilson, D. B.:** Three microbial strategies for plant cell wall degradation, Ann. NY Acad. Sci., **1125**, 289–297 (2008).
45. **Salame, T. M., Knop, D., Levinson, D., Mabjeesh, S. J., Yarden, O., and Hadar, Y.:** Release of *Pleurotus ostreatus* versatile-peroxidase from Mn^{2+} repression enhances anthropogenic and natural substrate degradation, PLoS One, **7**, e52446 (2012).
46. **Perez-Boada, M., Ruiz-Duenas, F. J., Pogni, R., Basosi, R., Choinowski, T., Martinez, M. J., Piontek, K., and Martinez, A. T.:** Versatile peroxidase oxidation of high redox potential aromatic compounds: site-directed mutagenesis, spectroscopic and crystallographic investigation of three long-range electron transfer pathways, J. Mol. Biol., **354**, 385–402 (2005).
47. **Marques, G., Gamelas, G. A. F., Ruiz-Dueñas, F. J., del Rio, J. C., Evtuguin, D. V., Martínez, A. T., and Gutiérrez, A.:** Delignification of eucalypt kraft pulp with manganese-substituted polyoxometalate assisted by fungal versatile peroxidase, Bioresour. Technol., **101**, 5935–5940 (2010).
48. **Rodríguez, E., Nuero, O., Guillén, F., Martínez, A. T., and Martínez, M. J.:** Degradation of phenolic and non-phenolic aromatic pollutants by four *Pleurotus* species: the role of laccase and versatile peroxidase, Soil Biol. Biochem., **36**, 909–916 (2004).
49. **Luo, Q., Bian, C., Tao, M., Huang, Y., Zheng, Y., Lv, Y., Li, J., Wang, C., You, X., Jia, B., and other 5 authors:** Genome and transcriptome sequencing of the astaxanthin-producing green microalga, *Haematococcus pluvialis*, Genome Biol. Evol., **11**, 166–173 (2018).
50. **Garcia-Jimenez, P., Llorens, C., Roig, F. J., and Robaina, R. R.:** Analysis of the transcriptome of the red seaweed *Grateloupia imbricata* with emphasis on reproductive potential, Mar. Drugs, **16**, 490–502 (2018).
51. **Wang, Y., Zeng, X., and Liu, W.:** De novo transcriptomic analysis during *Lentinula edodes* fruiting body growth, Gene, **641**, 326–334 (2018).
52. **Zou, L., Sun, T., Li, D., Tan, Y., Zhang, G., Wang, F., and Zhang, J.:** De novo transcriptome analysis of *Inonotus baumii* by RNA-seq, J. Biosci. Bioeng., **121**, 380–384 (2016).

53. **Jia, X., Wang, G., Xiong, F., Yu, X., Xu, Z., Wang, F., and Xiong, A.:** De novo assembly, transcriptome characterization, lignin accumulation, and anatomic characteristics: novel insights into lignin biosynthesis during celery leaf development, Sci. Rep., **5**, 8259 (2015).
54. **Tsanakas, G. F., Manioudaki, M. E., Economou, A. S., and Kalaitzis, P.:** De novo transcriptome analysis of petal senescence in *Gardenia jasminoides* Ellis, BMC Genomics, **15**, 554–570 (2014).
55. **Meng, X., Liu, P., Jia, F., Li, J., and Gao, B.:** De novo transcriptome analysis of *Portunus trituberculatus* ovary and testis by RNA-Seq: identification of genes involved in gonadal development, PLoS One, **10**, e0133659 (2015).
56. **Wang, G., Zhang, J., Shen, Y., Zheng, Q., Feng, M., Xiang, X., and Wu, X.:** Transcriptome analysis of the brain of the silkworm *Bombyx mori* infected with *Bombyx mori* nucleopolyhedrovirus: a new insight into the molecular mechanism of enhanced locomotor activity induced by viral Infection, J. Invertebr. Pathol., **128**, 37–43 (2015).
57. **Graz, M., Jarosz-Wilkołazka, A., Janusz, G., Mazur, A., Wielbo, J., Koper, P., Zebracki, K., and Kubik-Komar, A.:** Transcriptome-based analysis of the saprophytic fungus *Abortiporus biennis*-response to oxalic acid, Microbiol. Res., **199**, 79–88 (2017).
58. **Daly, P., van Munster, J. M., Kokolski, M., Sang, F., Blythe, M. J., Malla, S., de Castro Oliveira, J. V., Goldman, G. H., and Archer, D. B.:** Transcriptomic responses of mixed cultures of ascomycete fungi to lignocellulose using dual RNA-seq reveal inter-species antagonism and limited beneficial effects on CAZyme expression, Fungal Genet. Biol., **102**, 4–21 (2017).