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Transcriptome analysis of *Termitomyces albuminosus* reveals the biodegradation of lignocellulose

Fang Yang[1], Bo Xu[1,2,3,4], Junjun Li[1,2,3,4], Zunxi Huang[1,2,3,4*]

[1] School of Life Sciences, Yunnan Normal University, Kunming 650500, China

[2] Engineering Research Center of Sustainable Development and Utilization of Biomass Energy, Ministry of Education, Kunming 650500, China

[3] Key Laboratory of Yunnan for Biomass Energy and Biotechnology of Environment, Kunming 650500, China

[4] Key Laboratory of Enzyme Engineering, Yunnan Normal University, Kunming 650500, China

Abstract: **[Objective]** To study whether *Termitomyces albuminosus* can degrade lignocelluloses and to understand the symbiotic relationship between termite mushroom and fungus-growing termite. **[Methods]** cDNA library of *T. albuminosus* was sequenced by the Roche 454 GS FLX Titanium platform, and the diverse enzymes relevant to degradation of cellulose and lignin of symbiotic fungus *T. albuminosus* were analyzed. **[Results]** Eighth sequencing run resulted in a total of 82386 reads (express sequence tags, EST). After removing the vector and primer sequences, the remained 54410 reads were assembled into 3301 contigs and 3193 singletons. Comparing sequence similarity with known proteins, these sequences, representing approximately 2681 unique genes, were successfully annotated using BLAST searches (E-value $\leq 1e^{-10}$) against the Nr, SwissProt and CDD databases. The *T. albuminosus* transcripts included 33 enzymes putatively involved in cellulose and hemicelluloses biodegradation. 5 enzymes could hydrolyze cellulose and others had catalytic activities for degradation of hemicelluloses, starch and glycogen and chitin. Moreover, four genes encoding laccases and a single aryl-alcohol oxidase which could degrade lignin were also identified. These results revealed symbiosis fungus *T. albuminosus* had many laccases and possibly decomposed phenolic compounds from plant litter. **[Conclusions]** Data presented in this study indicated that *T. albuminosus* had the ability to degrade lignin, which made cellulose more easily degraded by the cellulase produced by fungus-growing termite.

Keywords: *Termitomyces albuminosus*, enzymes, lassaces, Lignocellulose degradation

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Termite mushroom, *Termitomyces albuminosus*, is a member of Agaricales, Ttricholomaceae. There are twenty-six species of the genus *Termitomyces* distributed over the world; about fifteen are mainly distributed across Yunnan, Guizhou and Sichuan provinces of China [1-2]. *T. albuminosus* lives in a unique habitat

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* Corresponding author. Fax: +86-871-5920952; E-mail: huangzunxi@163.com

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and has an interesting symbiotic relationship with fungus-growing termites, *Odontotermes formosanus*, belonging to an evolutionarily related group of higher termite (Termitidae: Macrotermitinae) [3-4]. The symbiotic fungi grow on a special substrate composed of dead plant material known as fungus comb or fungus garden in termites' nest. The fungus comb is made from partly digested plant litter which passes rapidly through the termite's gut. The resulting fecal pellets are pressed together to form a sponge-like structure. As the fungus comb ages, mycelium forms and produces fungus nodules, and moreover, the aged part of the fungus comb is eaten by the host termites [5]. The termite nest is a favorable environment for the growth of *Termitomyces* as its humidity and temperature are controlled [6]. The termite mushroom is unique in nature, growing only in the termite nests and is commercially interesting due to their prized edibility [6-7].

Bignell deems that there have been several suggestions for the roles of the symbiotic fungi in termite nutrition: (1) decomposition of lignin; (2) supply of cellulase and xylanase to work synergistically with the native enzymes produced by the termite; and (3) concentration of nutrients such as nitrogen for the termite [8]. Many works are involved in studies of the second suggestion, also known as the "acquired enzyme hypothesis", but some researchers questioned a part of this hypothesis. Since an endogenous cellulase, an enzyme produced by the termite, has recently been recognized in wood-and litter-feeding termites, it is difficult to make generalizations on the significance of the "acquired" fungal cellulase in cellulose digestion in fungus-growing termite [6-7].

The well-coordinated cooperation between the termites and the fungi enables efficient utilization of lignocelluloses [9]. It has been reported that the lignin content in the fungus comb progressively decreases as the fungus comb matures [5]. Young workers usually consume fungus nodules, whereas old workers consume old senescent combs to produce final feces. However, final feces are rarely found in the nest of fungus-growing termites, suggesting the highly efficient decomposition and the complete mineralization of plant litter. These observations support the finding that symbiotic fungi have the ability to degrade lignin, which makes cellulose more easily degraded by the native cellulase of the termite [6].

Basidiomycetes, which cause white-rot decay, are able to degrade lignin in wood efficiently. These fungi are called white-rot fungi [7]. *Termitomyces* are classified into white-rot fungi. Lignin degradation by white-rot fungi has been extensively studied, and the results revealed that three kinds of extracellular phenoloxidases, namely, lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase (Lac), are responsible for initiating the depolymerization of lignin [10]. The expression pattern of these enzymes depends on the organism [11]. It is necessary to characterize ligninolytic activity of *Termitomyces* in order to understand the symbiotic relationship in nature. The prior studies detected cellulase activity in *O. formosanus* and its symbiotic fungus *T. albuminosus* [12-13], which suggested *O. formosanus* had an independent cellulose-digesting system but the symbiotic fungus had a synergistic activity with the cellulase of *O. formosanus* [14]. Pan et al. found that there was laccase activity in both the symbiotic fungus *T. albuminosus* and the fungus combs [15]. Zhou et al. isolated a new laccase from the fungus combs in the nest of *O. formosanus* [16]. These results suggest that the symbiotic fungus may participate in the lignin-degradation process of *O. formosanus*.

In this study, we sequenced the transcripts of *T. albuminosus* and analyzed the diverse enzymes relevant to degradation of cellulose and lignin. The result indicated that *T. albuminosus* had the capability to degrade lignin, which made cellulose more easily degraded by the native cellulase of the fungus-growing termite.

1 Materials and methods

1.1 Fungus and RNA isolation

T. albuminosus was purchased from a local

agricultural and trade market in August 2010. The fruiting body of mushroom was cut into many pieces and grinded in liquid nitrogen. Total RNA was extracted from 1 g of fruiting body using Universal Plant Total RNA Rapid Extraction Kit (BioTeke Co., Beijing, China) according to the manufacturer's instructions.

1.2 cDNA library construction and 454 pyrosequencing

About 3 μg of total RNA extracted from the linkage site between the stipe and lamella of termite mushroom was converted into cDNA using SMART cDNA synthesis protocol (Clontech, CA, USA). Double-stranded (ds) cDNA was purified with a QIAquick PCR Purification Kit (Qiagen, Germany). About 5 μg of ds cDNA sample was cut into 500 – 800bp fragments, and the adaptors were then ligated on to the fragment ends. After emPCR (emulsion-based clonal amplification), the amplified emPCR products were sequenced using the Roche 454 GS FLX Titanium System (Roche, Basel, Switzerland) according to the manufacturer's instructions.

1.3 Sequence assembly

Quality clipping of the 454 sequencing reads of *T. albuminosus* and screening for primer and vector sequences were performed by lucy [17] (http://lucy.sourceforge.net). Potential primer and vector relicts were removed using the program SeqClean (http://sourceforge.net/projects/seqclean/). Reads shorter than 50 bases after trimming were then excluded. After trimming, the removal of multiple read attempts, and the exclusion of overly-short reads, the pool of data available for the assembly consisted of the remaining reads. The remaining reads were assembled by CAP3 [18] applying the "accurate" mode, as defined in the program. Sequences after assembly comprised contigs and singletons. Contigs based on at least two reads, singletons refer to single reads that clustered with other reads, but were eventually excluded from the final assembly.

1.4 Sequence prediction and annotation

Contigs and singletons of *T. albuminosus* were compared with the Swiss-Prot protein database (http://www.uniprot.org/), Non-redundant protein sequences database at NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and Conserved Domains Database (http://www.ncbi.nlm.nih.gov/cdd) using the BlastX algorithm as implemented in the Blastall program package. We used two different stringencies for cut-off e-value < 0.001 and similar percentage > 30% in the Blast analysis.

1.5 Detection and modular annotation of putative CAZymes

The search for catalytic modules specific to CAZYmes, glycoside hydrolases (GH), glycosyltransferases (GT), polysaccharide lyases (PL), carbohydrate esterases (CE) and their ancillary carbohydrate-binding modules (CBMs) in *T. albuminosus* was performed exactly as for the daily updates of the Carbohydrate-Active enZymes (CAZy) database (http://www.cazy.org). The resulting fragments are assembled and formatted as module families sequence libraries for BLAST searches. Accordingly, each protein model from *T. albuminosus* was queried using BLAST against the libraries of approximately 75000 individual modules using a database size parameter identical to that of the NCBI non-redundant database.

1.6 Multiple sequence alignment

Multiple sequence alignments are an essential tool for protein structure and function prediction [19]. Amino acid sequences of laccase found in *T. albuminosus* and LCC1-2, LCC2-5, TLCC and CbLCC originating from *Termitomyces* strain, *T. albuminosus* (mycelium pellet) and *Cyathus bulleri*, respectively, were analyzed and alignments of multiple amino acid sequences were generated with Vector NTI 10.3 (InforMax, Maryland, USA).

1.7 Phylogenetic analysis

Nucleotide sequences of the laccase gene fragments were aligned at the DNA level with known sequences in the GenBank database with ClustalW. The nucleotide sequence similarities were assessed by using the BLASTn programs (http://www.ncbi.nlm.

nih.gov/BLAST/). Phylogenetic trees were constructed with MEGA 5.0 software using the maximum likelihood method [20]. Confidence for tree topologies was estimated by bootstrap values based on 1000 replications. Sixteen representative sequences originating from the *Termitomyces* sp. and uncultured *Termitomyces* were selected and used as references for tree construction.

2 Results

2.1 454 sequencing of ESTs from *T. albuminosus*

A cDNA library was constructed from mRNA of *T. albuminosus* and was used in 454 pyrosequencing. Eighth sequencing run resulted in a total of 82386 reads. Quality clipping and removal of vector and primer sequences reduced the number to 54410 reads (mean length: 231 bp, median: 212 bp). Clustering of the sequences resulted in 3301 contigs based on at least two reads (mean length: 444 bp, median: 474 bp). In addition, the assembly resulted in 3193 singletons (mean length: 248 bp, median: 52 bp). In all, all remained sequences were assembled into 6494 unigenes.

2.2 Functional annotation of unigenes

All unique sequences obtained from *T. albuminosus* sequencing were compared with the sequences in the three major public protein databases (listed in the Methods section) using the BlastX algorithm. When the E-value cutoff was set at 10^{-10}, a total of 2681 unigenes were annotated, which accounted for 41.28% of the total unigenes. Under a more stringent condition (cutoff = 10^{-20}), 1700 unique sequences were annotated, which accounted for 26.18% of the total unigenes.

2.3 Genes putatively relevant to degradation of cellulose and hemicellulose in *T. albuminosus*

A large number of models relevant to carbohydrate-and lignin-active enzymes were identified in this research (Table 1). *T. albuminosus* partly degraded major components of plant cell walls including cellulose and hemicellulose. The transcriptome harbored the genetic information encoding 49 putative carbohydrate-active enzymes including 33 glycoside hydrolases, 9 glycosyltransferases, 5 carbohydrate esterases and 2 polysaccharide lyases, comprising 24 distinct families (Table 1). The cellulases in *T. albuminosus* included two cellobiohydrolases and three β-glucosidases, and no endoglucanases included. The two cellobiohydrolase genes were encompassed a single member of type GH6. Among the multiple β-glucosidase genes, two code for the enzymes in family GH3 and one member of GH30 were also found. In addition to the enzymes responsible for hydrolysis of cellulose, numerous other polysaccharide-degrading enzymes were predicted in the transcriptome of *T. albuminosus*, including catalytic activities for degradation of hemicelluloses, starch and glycogen and chitin (Table 1).

2.4 Genes putatively relevant to degradation of lignin in *T. albuminosus*

Lignin biodegradation by white-rot fungi is an oxidative process with the secretion of oxidases and peroxidases [10,21], such as lignin peroxidase (EC 1.11.1.14), manganese peroxidase (EC 1.11.1.13), and laccase (EC 1.10.3.2). Interestingly in the transcriptome of *T. albuminosus*, it included only four genes encoding enzymes homologous with laccase and one genes encoding putative aryl-alcohol oxidase. But the genes putatively relevant to lignin peroxidase and manganese peroxidase were absent (Table 1). This result is distinct from the basidiomycete *Phanerochaete chrysosporium* [22]. This result was possible because of no peroxidases expressed in the transcriptome, so genome sequence analysis could only detect whether there were peroxidases in the mushroom genome.

2.5 Nucleotide sequence accession numbers

cDNA sequences of enzymes putatively relevant to cell wall degradation were deposited into GenBank under the accession numbers: JO124030-JO124084.

2.6 Amino acid sequences of laccase from *T. albuminosus* and their phylogenetic analysis

From the previous studies, we have known that genus *Termitomyces* symbiosis fungi can produce and secrete laccase, which can degrade lignin component

Table 1 Putative cell-wall-degrading enzymes identified in *T. albuminosus*.

Gene product	Model	Length(bp)[a]	E-Value	Identity (%)[b]	Accession[c]
GH family 2 (β-mannosidase)	contig2459	70	3e-25	52	XP_001877546
GH family 3 (1,4-β-xylosidase)	singletonND4D	127	8e-52	678	XP_003031187
GH family 3 (1,4-β-xylosidase)	contig2881	187	6e-84	56	XP_002473890
GH family 3 (β-glucosidase)	contig2191	77	8e-30	53	XP_001879679
GH family 3 (β-glucosidase)	singletonVJ20	73	9e-28	55	XP_002475642
GH family 6 (cellobiohydrolase)	contig2137	130	3e-58	88	AAT64008
GH family 6 (cellobiohydrolase)	singleton6SML	51	2e-18	53	AAT64008
GH family 13 (branching enzyme)	contig1319	247	2e-120	76	XP_001883734
GH family 13 (α-amylase)	contig2335	118	9e-72	59	EGO22943
GH family 15 (glucoamylase)	contig2173	84	1e-27	73	BAL43555
GH family 15 (α,α-trehalase)	contig531	37	8e-13	85	XP_001884761
GH family 16 (endo-1,3-β-glucanase)	contig1441	67	8e-39	83	XP_001886052
GH family 16 (endo-1,3-β-glucanase)	contig2358	101	1e-57	73	XP_001873806
GH family 16 (endo-1,3-β-glucanase)	contig1960	96	2e-41	78	XP_001876573
GH family 16 (endo-1,3-β-glucanase)	contig3278	108	1e-63	69	XP_001873316
GH family 16 (endo-1,3-β-glucanase)	contig1871	144	2e-73	61	XP_001882200
GH family 17 (endo-1,3-β-glucosidase)	contig2348	125	2e-74	73	XP_001880852
GH family 28 (polygalacturonase)	singletonGU7R	48	2e-12	76	XP_001881961
GH family 30 (β-glucosidase)	contig1480	142	1e-75	64	XP_001890272
GH family 30 (β-1,6-glucanase)	contig2081	131	2e-57	77	XP_001890272
GH family 47 (α-mannosidase)	contig3	104	4e-37	79	XP_001874173
GH family 55 (exo-β-1,3-glucanase)	contig3138	238	2e-129	76	BAE20245
GH family 55 (exo-β-1,3-glucanase)	contig1754	118	4e-61	72	BAE20245
GH family 55 (exo-β-1,3-glucanase)	singleton2Y7B	140	8e-82	84	BAE20245
GH family 63 (α-glucosidase)	singletonMKDY	117	2e-78	93	XP_001877857
GH family 63 (α-glucosidase)	contig2681	194	1e-110	75	ENG96016
GH family 71 (α-1,3-glucanase)	contig1785	128	5e-69	69	XP_001875860
GH family 72(β-1,3-glucanosyltransglycosylase)	contig2787	136	7e-60	68	XP_001873929
GT family 2 (chitin synthase)	singletonG061	124	5e-57	88	XP_001876655
GT family 2 (chitin synthase)	contig541	100	2e-48	76	XP_001874156
GT family 2 (chitin synthase)	singleton2YHM	90	3e-33	86	XP_001876655
GT family 2 (dolichyl-phosphate β-D-mannosyltransferase)	singleton3HTW	71	2e-26	91	XP_001881381
GT family 2 (chitin synthase)	contig26	64	1e-21	59	XP_001889277
GT family 4 (lipopolysaccharide N-acetylglucosaminyltransferase)	singletonIG87	89	6e-31	87	XP_001884804
GH family 13 and GT family 5 (α-1,3-glucan synthase)	contig1322	136	3e-79	78	XP_001875405
GH family 13 and GT family 5 (α-1,3-glucan synthase)	contig1693	149	2e-79	69	XP_001875405
GH family 13 and GT family 5 (α-1,3-glucan synthase)	contig2469	79	5e-41	89	XP_001875405
GH family 13 and GT family 5 (α-1,3-glucan synthase)	contig1085	142	1e-56	72	XP_001875405
GH family 13 and GT family 5 (α-1,3-glucan synthase)	singletonZ1YD	72	3e-23	93	XP_001875405
GH family 13 and GT family 5 (α-1,3-glucan synthase)	singletonRAFT	92	1e-37	81	XP_001875405
GT family 8 (galactinol synthase)	contig380	56	1e-16	68	XP_001873854
GT family 20(α,α-trehalose-phosphate synthase)	contig2265	127	1e-71	77	XP_001885618
GT family 32 (α-1,4-N-acetylglucosaminyltransferase)	contig583	68	5e-32	64	XP_001874244
PL family 3 (pectate lyase)	singleton VJSE	87	2e-35	82	XP_001836194
PL family 14 (alginate lyase)	contig3126	190	1e-103	70	XP_001877361
CE family 4 (chitin deacetylase)	contig3053	154	2e-86	66	XP_001877121
CE family 4 (chitin deacetylase)	contig2907	215	6e-148	83	XP_001873628
CE family 4 (chitin deacetylase)	contig1227	135	1e-75	61	XP_001878127
CE family 9 (N-acetylglucosamine 6-phosphate deacetylase)	contig1207	100	3e-24	71	XP_001889881
CE family 9 (N-acetylglucosamine 6-phosphate deacetylase)	contig2041	48	2e-16	58	XP_001880103
Laccase	singletonAB6H	65	1e-36	78	AAG27436
Laccase	contig2330	109	9e-41	81	BAE53767
Laccase	singletonHVBX	89	2e-49	73	XP_001831045
Laccase	singleton EXQX	58	9e-23	64	BAE53732
Aryl-alcohol oxidase	contig3244	141	3e-56	49	ADD14021

[a] Alignment length in amino acids. [b] Percentage of identity of the aligned region. BLASTX search results with Non-redundant protein database. [c] Accession is subject protein which is similar with query in NCBI.

from plant litter. Laccase sequences (LCC1-2, LCC2-5 and TLCC) from other *Termitomyces* fungi have been published [15,23]. We specifically analyzed the 454 data for laccase cDNA sequences. In this study, we have

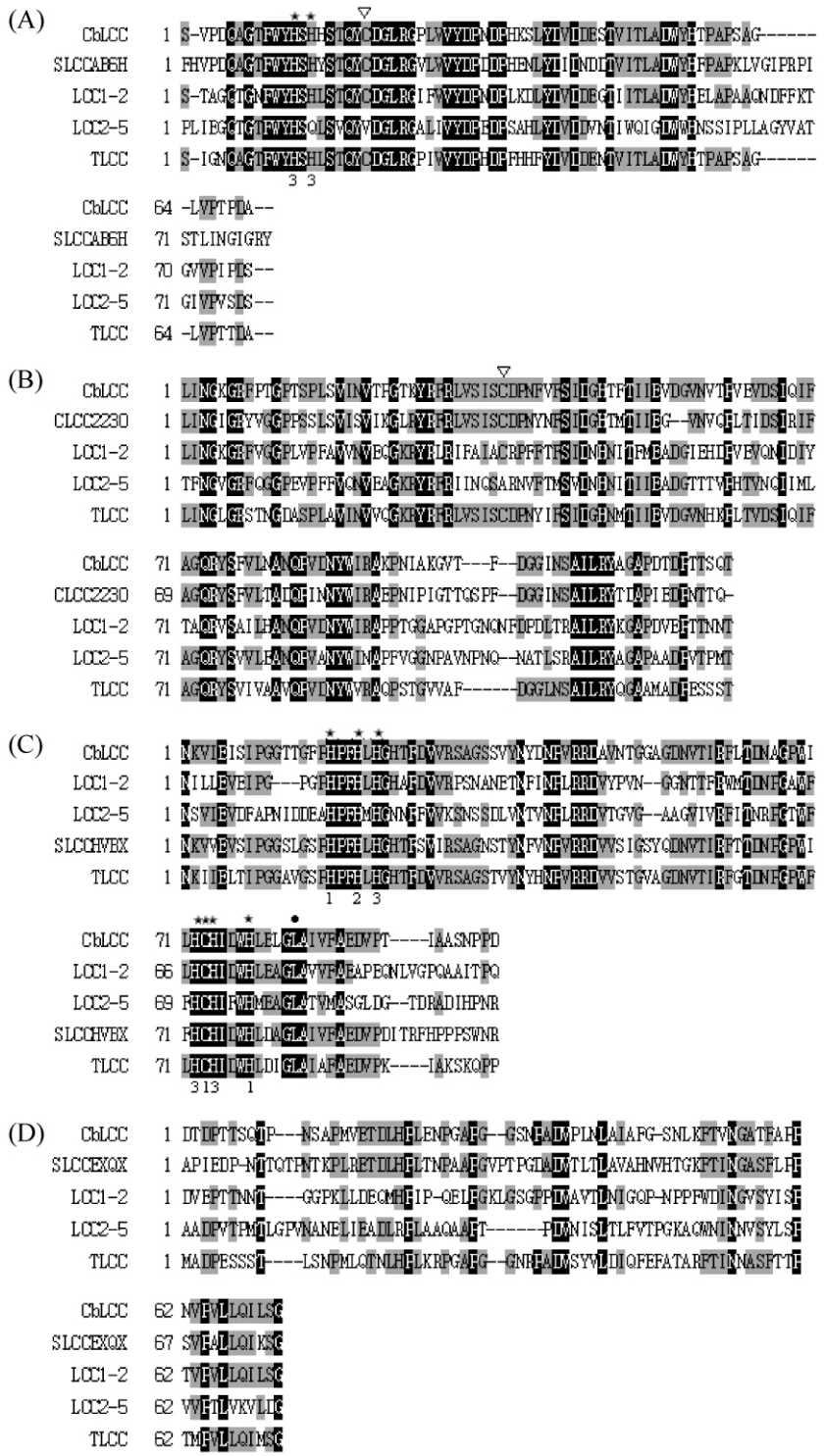

Fig. 1　Sequence comparison among *Cyathus bulleri* CbLCC (ABW75771), *Termitomyces* strain NS/Mg LCC1-2 (BAE53769), LCC2-5 (BAE53770) and SLCCAB6H (or CLCC2230, SLCCHVBX, SLCCEXQX). SLCCAB6H, CLCC2230, SLCCHVBX and SLCCEXQX represent singleton AB6H, contig2230, singletonHVBX and singletonEXQX, respectively. Identical residues are shaded in black, and conserved residues are shaded in gray. The conserved amino acid residues potentially involved in copper ion binding are marked by asterisks, Numbers under the His and Cys residues indicate types of copper ions that bind to each residue. The Cys residues are marked with open inverted triangles. A closed circle indicates the Leu residue that influences a laccase redox potential.

acquired four Laccase cDNA sequence segments and translated into amino acid sequences. After multiple sequence alignment, we found two of the cDNA sequences contained partial amino acid residues that are essential for copper ion binding in laccase (Fig. 1-A and C). However, The CLCC2230 and SLCCEXQX amino acid sequences lacked amino acid residues involved in copper ion binding (Fig. 1-B and D). The Cys residues were also conserved in the SLCCAB6H and CLCC2230 sequence (Fig. 1-A and B), while no Cys residues were present in the SLCCHVBX and SLCCEXQX sequence (Fig. 1-C and D).

A BlastX search showed that best hits for SLCCAB6H, CLCC2230, SLCCHVBX and SLCCEXQX amino acid sequences were laccase 4 (AAG27436) from *Lentinus sajor-caju*, putative laccase (BAE53767) from uncultured *Termitomyces*, laccase 2 (XP_001831045) from *Coprinopsis cinerea* and putative laccase (BAE53732) from *Termitomyces* sp., respectively. Amino acid identities and similarities between SLCCAB6H and laccase 4 were 65 and 77%, and those between CLCC2230, SLCCEXQX and putative laccase were 109 (81%) and 58 (61%), respectively, while those between SLCCHVBX and laccase 2 were 89 and 72% (Table 1).

Phylogenetic analysis showed that four laccase genes from *T. albuminosus* placed dispersive distribution. Every gene appeared more closely related to the best similar laccase gene segment. All of the sequences divided into two clusters and only singletonEXQX was in cluster 2, while other laccase genes from *T. albuminosus* were in cluster 1 (Fig. 2). Interestingly, singletonAB6H was most closely related to the protein reported under accession no. AB201162 (from uncultured *Termitomyces* lccOl6), while it clustered together with that reported under accession no. AB201135 (from *Termitomyces* sp. Group5).

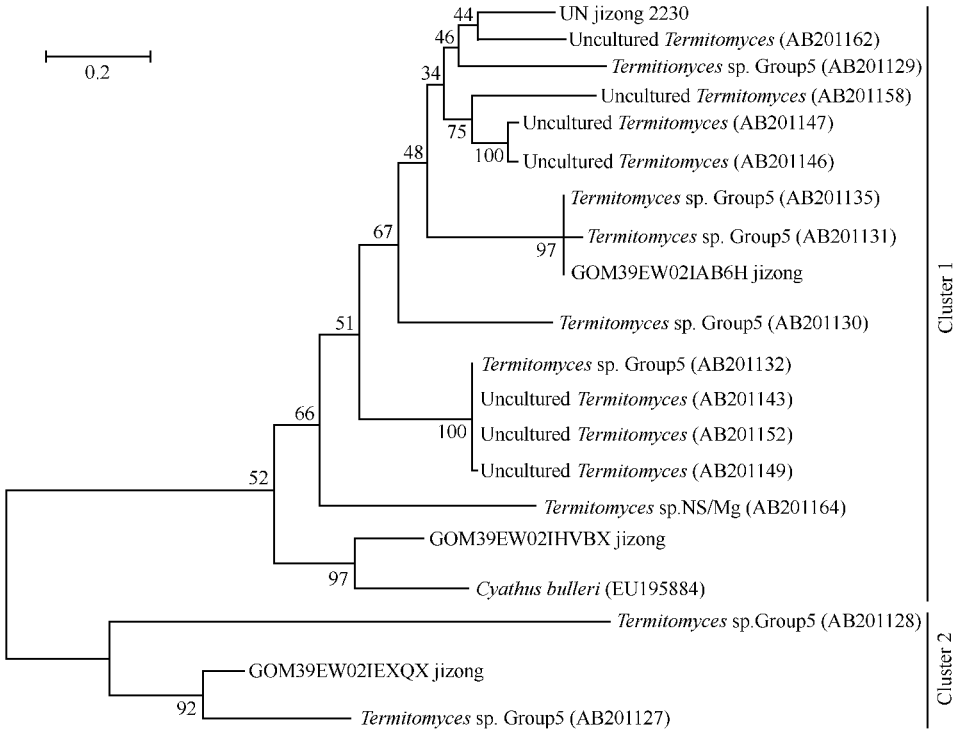

Fig. 2 Maximum likelihood tree for nucleotide sequences of putative laccases identified from *T. albuminosus* and *Termitomyces sp.* Group5 and related DNA segments. The scale bar represents 0.2 nucleotide substitutions per position. The reliability value of each internal branch indicates in present how often the corresponding cluster was found among the 1000 intermediate trees. Accession numbers are shown after the names of organisms. Partial gene designations are as follow: GOM39EW021AB6Hjizong, singletonAB6H; UNjizong2230, contig2230; GOM39EW021HVBXjizong, singletonHVBX; GOM39EW021EXQXjizong, singletonEXQX.

3 Discussions

cDNA collections allowed us to sequence a small fraction of the *T. albuminosus* transcriptome using 454 pyrosequencing. We derived 6494 unigenes from 82386 high-quality reads obtained from *T. albuminosus* cDNA. In addition, our contigs (mean: 444bp) were on average larger than those assembled in previous studies that used earlier 454 technologies with shorter reads (e. g., 197bp, [24]; 247bp, [25]; 440bp, [26]). This data produced here represented a substantial sequence resource for *T. albuminosus* and could contribute to genomic data available for the genus *Termitomyces*.

The large number of sequences that matched in BLAST searches to unique proteins indicated that our 454 sequencing reads identified a substantial portion of the genes in *T. albuminosus*. Furthermore, lack of annotation for many of the unigenes in our data was the result of short length.

Lignocellulose degradation required a broad array of enzymes and associated proteins [27]. In the transcriptome of *T. albuminosus*, there were restricted numbers and specificity cellulose-and hemicellose-degrading enzymes. The genus *Odontotermes* had a close phylogenetic relationship with the genus *Nasutitermes* [28]. Different numbers of CAZy families between *T. albuminosus* and termite gut community from *Nasutitermes* [29] were clearly observed at the family level. Previous study showed that termite gut community identified many genes homologous with more than 700 GHs corresponding to 45 different CAZy families, including a rich diversity of putative cellulases and hemicellulases. It was obvious that termite gut had richer diversity of cellulases and hemicellulases than that of *T. albuminosus*, so we presumed that the fungus-growing termite *O. formosanus* had unique cellulose-degrading system, consisting with the past studies [12-13]. GH families 5, 8, 9, 44, 45 and 74 relevant to cellulose hydrolysis were not identified in our data, but they were present in the termite hindgut community, whereas GH6 family was detected in *T. albuminosus* but was not found in the termite hindgut community. The result investigated that the gut of fungus-growing termite *O. formosanus* probably produced many cellulolytic enzymes and degraded cellulose effectively cooperating with GH6 family cellobiohydrolases produced by *T. albuminosus*. The result was consistent with other researches, which showed that the symbiotic fungus had a synergistic activity on the cellulase of *O. formosanus* [8,14]. A large number of GH10, GH11, GH26 and GH43 hemicellulases were also identified in the termite hindgut community, but none of above-mentioned hemicellulases were identified in *T. albuminosus*. In addition to hemicellulases noted above, β-xylosidase was identified to involve in hemicelluloses-decomposing process in *T. albuminosus*. These results presumed that the symbiosis fungus *T. albuminosus* had capability to degrade cellulose and hemicellulose. Fungus-growing termite *O. formosanus is* a higher termite, and then higher termites harbor a dense and diversity array of gut bacteria which can secret a large number of cellulolytic enzymes [30]. To conclude, there wasn't enough evidence to support 'acquired enzyme hypothesis.

Higher plants synthesize and accumulate a variety of phenolic compounds as secondary metabolites. Although physiological functions of plant phenolic compounds are not yet fully understood, it is thought that they contribute to plant defenses against pests and pathogens [31]. Consequently, phenol degradation in the fungus comb is considered to be important for improving palatability of termite food, especially that containing high phenol content such as fallen leaves and bark. To our knowledge, it has been shown that the extracellular phenol-oxidizing enzymes such as lignin peroxidase, manganese peroxidase and laccase are responsible for the depolymerization of lignin [32]. In this study, we identified four genes encoding putative laccases, whereas no homologs for lignin or manganese peroxidase were identified in this study (Table 1). This result was consistent with a previous viewpoint that laccase had been reported as an essential

enzyme for lignin degradation in fungi without peroxidases [33]. Additionally, one gene encoding oxidase was aryl-alcohol oxidase (AAO), which was a rare enzyme involved in lignin degradation. At present, aryl-alcohol oxidase was found in *Pleurotus eryngii* [34] and in *P. chrysosporium* [22]. Consequently, laccases and aryl-alcohol oxidase produced in *T. albuminosus* explained that the symbiotic fungus participated in the lignin-degradation process of *O. formosanus*. Though an early study by Kato *et al.* has shown that gut micro flora from a higher termite *Nasutitermes* are able to degrade lignin in an in vitro experiment [35]. However, a metagenomic study of hindgut microflora from the same termite has found large numbers of genes for cellulose hydrolysis, but apparently no genes for lignin degradation [29]. The gut microflora of termites help to digest the lignocellulose content of wood, but the role of microorganism in the degradation of lignin has been the subject of debate [36]. No conclusive evidence has been produced up to now on whether the higher termite *O. formosanus* gut harbors microorganism in lignin biodegradation. All the evidences confirmed the 'lignin degradation hypothesis' [5] that the role of the mutualistic fungi was to degrade lignin and enhanced the digestibility of cellulose for the termites, suggesting the ability of the termite-fungus association to make extremely efficient use of plant material. This result was consistent with the early research in fungus-growing termite *Macrotermes gilvus* and the mutualistic fungus [5].

Partial SLCCHVBX amino acid sequence contained seven conserved His and Cys residues required for copper ion binding and an additional conserved Leu residue affecting the redox potential of laccase (Fig. 1C). Partial SLCCAB6H amino acid sequence contained two conserved His residues and CLCC2230 amino acid sequence only contained Cys residues (Fig. 1A and B). However, SLCCEXQX amino acid sequence was partial laccase segment excluding any conserved residues (Fig. 1-D). These results were owing to contigs or singletons fragment obtained by sequencing too short. Probably because of different conversed residues in different proteins, four laccase genes were divided into two clusters (Fig. 2). Multiple laccase genes are often found in single organisms. *Termitomyces* sp. strain KU418 expressed seven putative laccase genes when the fungus was cultured in KB liquid medium under LN conditions [23]. *L. bicolor* [37] and *C. cinerea* [38] genomes included nine and seventeen laccases, respectively. These observations supported indirectly the idea that *Termitomyces* fungi had multiple laccase genes. In order to investigate farther the character of the laccase in *T. albuminosus*, we should gain total amino acid through other molecular methods. Further functional studies on laccase and laccase-like protein from the symbiotic fungi are necessary to clarify the importance of these enzymes for efficient decomposition of plant material by symbiosis fungus.

In conclusion, the aim of this study was to understand the relationship between symbiotic fungus *T. albuminosus* and fungus-growing termite *O. formosanus*. This study provided partial gene sequences of carbohydrate-active enzymes, of which GH family proteins included cellulolytic and hemicelluloses-biodegrading enzymes putatively relevant to plant cell wall degradation. In addition to cellulolytic enzymes, there were four laccases and a single AAO in the transcriptome of *T. albuminosus*. These results were significant evidence for future research which was about 'lignin degradation hypothesis'.

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鸡枞菌转录组分析揭示其对木质纤维素的降解功能

杨芳[1]，许波[1,2,3,4]，李俊俊[1,2,3,4]，黄遵锡[1,2,3,4*]

[1] 云南师范大学生命科学学院，昆明 650500

[2] 生物能源持续开发利用教育部工程研究中心，昆明 650500

[3] 云南省生物质能与环境生物技术重点实验室，昆明 650500

[4] 云南师范大学酶工程重点实验室，昆明 650500

摘要：【目的】探究鸡枞菌是否能降解木质纤维素成分，并理解其与共生白蚁之间的共生关系。**【方法】**本研究是应用新一代高通量测序技术 454 GS FLX Titanium 对鸡枞菌的转录组进行测序，挖掘鸡枞菌中能参与降解纤维素和木质素等成分的多样性酶系。**【结果】**八分之一的 RUN 测序总共得到了 82386 条表达序列标签，去除引物和载体等序列后，剩余的 54410 条序列被拼接成 3301 条 contigs 以及 3193 条 singletons。根据序列相似性，将这些 unigenes 与三大蛋白数据库(Nr 数据库、SwissProt 数据库、CDD 数据库)中的蛋白序列进行 BLAST 比较，发现有 2681 条基因与其他生物的已知基因有不同程度的相似性。在鸡枞菌的这些转录产物中，有 33 条编码可能参与降解纤维素或半纤维素的酶基因，其中包括 5 种纤维素酶以及 28 种水解半纤维素、淀粉或几丁质等物质的酶类。更重要的是，还发现了 4 种漆酶以及一种芳基乙醇氧化酶基因，这些都是能有效降解木质素的酶类。这些结果揭示了鸡枞菌中存在漆酶并可能有效降解植物残渣中的酚化合物。**【结论】**这些基因的发现说明了鸡枞菌能降解木质素，并能与共生白蚁分泌的纤维素酶协同作用有效降解纤维素。

关键词： 鸡枞菌，酶，漆酶，木质纤维素降解

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* **通信作者**。Fax：+86-871-5920952；E-mail：huangzunxi@163.com

作者简介： 杨芳(1986－)，女，湖北武汉人，硕士研究生，主要从事分子生物学及生物信息学研究。E-mail：fangyang09@163.com

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