Mycologia, 105(6), 2013, pp. 1412–1427. DOI: 10.3852/13-072

Genomewide analysis of polysaccharides degrading enzymes in 11 white- and brown-rot Polyporales provides insight into mechanisms of wood decay

Chiaki Hori

Department of Biomaterials Sciences, Graduate School of Agricultural and Life Sciences, University of Tokyo, l-l-l, Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan, and Institute for Microbial and Biochemical Technology, Forest Products Laboratory, 1 Gifford Pinchot Drive, Madison, Wisconsin 53726

Jill Gaskell

Institute for Microbial and Biochemical Technology, Forest Products Laboratory, 1 Gifford Pinchot Drive, Madison, Wisconsin 53726

Kiyohiko Igarashi
Masahiro Samejima

Department of Biomaterials Sciences, Graduate School of Agricultural and Life Sciences, University of Tokyo, l-l-l, Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan

David Hibbett

Biology Department, Clark University, Worcester, Massachusetts 01610

Bernard Henrissat

Architecture et Fonction des Macromolécules Biologiques, Aix-Marseille University, CNRS, 163 Avenue de Luminy, 13288 Marseille, France

Dan Cullen[1]

Institute for Microbial and Biochemical Technology, Forest Products Laboratory, 1 Gifford Pinchot Drive, Madison, Wisconsin 53726

Abstract: To degrade the polysaccharides, wood-decay fungi secrete a variety of glycoside hydrolases (GHs) and carbohydrate esterases (CEs) classified into various sequence-based families of carbohydrate-active enzymes (CAZys) and their appended carbohydrate-binding modules (CBM). Oxidative enzymes, such as cellobiose dehydrogenase (CDH) and lytic polysaccharide monooxygenase (LPMO, formerly GH61), also have been implicated in cellulose degradation. To examine polysaccharide-degrading potential between white- and brown-rot fungi, we performed genomewide analysis of CAZys and these oxidative enzymes in 11 Polyporales, including recently sequenced monokaryotic strains of *Bjerkandera adusta*, *Ganoderma* sp. and *Phlebia brevispora*. Furthermore, we conducted comparative secretome analysis of seven Polyporales grown on wood culture. As a result, it was found that genes encoding cellulases belonging to families GH6, GH7, GH9 and carbohydrate-binding module family CBM1 are lacking in genomes of brown-rot polyporales. In addition, the presence of CDH and the expansion of LPMO were observed only in white-rot genomes. Indeed, GH6, GH7, CDH and LPMO peptides were identified only in white-rot polypores. Genes encoding aldose 1-epimerase (ALE), previously detected with CDH and cellulases in the culture filtrates, also were identified in white-rot genomes, suggesting a physiological connection between ALE, CDH, cellulase and possibly LPMO. For hemicellulose degradation, genes and peptides corresponding to GH74 xyloglucanase, GH10 endo-xylanase, GH79 β-glucuronidase, CE1 acetyl xylan esterase and CE15 glucuronoyl methylesterase were significantly increased in white-rot genomes compared to brown-rot genomes. Overall, relative to brown-rot Polyporales, white-rot Polyporales maintain greater enzymatic diversity supporting lignocellulose attack.

Key words: carbohydrate active enzymes, genome, proteome, Secretome, wood-rot fungi

Submitted 2 Mar 2013; accepted for publication 8 Jun 2013.
[1] Corresponding author. E-mail: dcullen@wisc.edu

Introduction

Wood-decay fungi are unique organisms capable of degrading lignocellulose, the most abundant source of terrestrial carbon. In addition to their importance in carbon cycling, these fungi have drawn attention for converting plant cell walls to low molecular weight, high-value products. Many efficient wood-decay fungi belong to the Polyporales, including *Phanerochaete chrysosporium*, *Postia placenta* and *Ceriporiopsis subvermispora* (Blanchette et al. 1992, Binder et al. 2005, Hibbett et al. 2007). These can be categorized as white-rot fungi or brown-rot fungi, based on decay patterns (Eriksson et al. 1990, Blanchette 1991, Daniel 1994, Schwarze 2007). White-rot fungi degrade cell wall polysaccharides such as cellulose and hemicellulose as well as the more recalcitrant phenylpropanoid polymer, lignin. In contrast, brown-rot fungi degrade the polysaccharides but lignin remains as a modified residue (Niemenmaa et al. 2008, Yelle et al. 2008, Yelle et al. 2011). A distinctive aspect of brown-rot decay, cellulose depolymerization, occurs rapidly with minimal weight loss (Gilbertson 1981, Kirk et al. 1991, Worrall et al. 1997).

Much research has focused on the mechanisms by which white- and brown-rot fungi degrade polysac-

charides. Proteome studies of white-rot fungi have identified a variety of glycoside hydrolases (GHs), carbohydrate esterases (CEs) and polysaccharide lyases (PLs) involved in plant cell-wall degradation (Vanden Wymelenberg et al. 2005, 2010, 2011; Ravalason et al. 2008; Mahajan and Master 2010; Adav et al. 2011; Hori et al. 2012). These extracellular proteins typically are categorized as carbohydrate-active enzymes (CAZys) in the CAZy database (http://www.cazy.org/). In addition to CAZys, secreted oxidative enzymes, such as cellobiose dehydrogenase (CDH) and lytic polysaccharide monooxygenase (LPMO formerly GH61), also have been implicated in polysaccharide degradation (Henriksson et al. 2000, Harris et al. 2010, Vaaje-Kolstad et al. 2010, Langston et al. 2011, Phillips et al. 2011, Quinlan et al. 2011, Westereng et al. 2011, Bey et al. 2013).

Little was known of the enzymatic repertoire of brown-rot fungi before genomic analysis of *P. placenta* (Martinez et al. 2009). Genes encoding cellulose-binding domains (carbohydrate-binding module family 1; CBM1) and cellulases belonging to GH families 6 and 7 were absent from *P. placenta* but present in almost all cellulose-degrading microbes, including the white-rot fungus *P. chrysosporium* (Martinez et al. 2004). Subsequent transcriptome and secretome analyses showed that *P. placenta* secreted an array of hemicellulases but no cellulases relative to *P. chrysosporium* (Vanden Wymelenberg et al. 2010). These and other studies (Xu and Goodell 2001; Cohen et al. 2002, 2004; Arantes et al. 2011) collectively are consistent with initial cellulose attack by extracellular hydroxyl radicals generated via the Fenton reaction ($H_2O_2 + Fe^{2+} + H^+ \rightarrow H_2O + Fe^{3+} + OH$). Indeed, comparative and functional genome analysis of the brown-rot fungus *Serpula lacrymans*, which belongs to the Boletales, also supported hydroxyl radical generation during early degradation of polysaccharides (Eastwood et al. 2011). Contractions in the number of CBM1, GH6 and GH7 genes were observed broadly in brown-rot genomes by Floudas and coworkers (Floudas et al. 2012).

Comparative analysis of white- and brown-rot gene repertoires and expression profiles have revealed substantial variation, but considerable uncertainty persists with respect to precise mechanisms. Addressing this issue, we performed genomewide analysis of carbohydrate-active enzymes and some oxidative enzymes related to polysaccharide degradation in 11 white- and brown-rot fungi. This analysis included classifying and enumerating genes from three recently sequenced polyporales *Bjerkandera adusta*, *Ganoderma* sp. and *Phlebia brevispora*. Furthermore, comparative secretome analysis of seven Polyporales grown on wood culture was conducted by mass spectrometry-based identification.

MATERIALS AND METHODS

Classifying genes encoding polysaccharide degrading enzymes.—For comparative genome analysis, we studied 11 Polyporales genomes available via the MycoCosm portal of Joint Genome Institute (JGI) website (http://genome.jgi-psf.org/programs/fungi/index.jsf). *Bjerkandera adusta* strain HHB-12826-SP, *Ganoderma sp.* strain 10597 SS1 and *Phlebia brevispora* strain HHB-7030 recently were sequenced, whereas *Ceriporiopsis (Gelatoporia) subvermispora* B (Tello et al. 2001, Fernandez-Fueyo et al. 2012), *Phanerochaete carnosa* HHB-10118-sp (Suzuki et al. 2012), *Phanerochaete chrysosporium* RP-78 (Stewart et al. 2000, Martinez et al. 2004), *Postia placenta* MAD-698 (Martinez et al. 2009), *Dichomitus squalens* LYAD-421 SS1, *Fomitopsis pinicola* FP-58527 SS1, *Trametes versicolor* FP-101664 SS1 and *Wolfiporia cocos* MD-104 SS10 were released previously (Floudas et al. 2012). Annotated gene models of *B. adusta*, *G.* sp. and *P. brevispora* were assigned to CAZy families by semi-automatic modular assignment (Cantarel et al. 2009). Based on these classifications, our analyses focused on polysaccharide-degrading enzymes within carbohydrate esterase families CE1 and CE15, polysaccharide lyase family PL8 and glycoside hydrolase families GH1, GH2, GH3, GH5, GH6, GH7, GH9, GH10, GH11, GH12, GH28, GH30, GH31, GH45, GH53, GH74, GH79 and GH61. The latter family has been reclassified as LPMOs assigned to auxiliary activity family 9 (AA9; Levasseur et al. 2013). We also examined aldose epimerase (ALE), cellobiose dehydrogenase (CDH) and a cellulose-binding cytochrome b_{562} (Yoshida et al. 2005), all of which have been implicated in cellulose degradation.

Phylogenetic analysis.—The polypore sequences belonging to CE1, GH3, GH5, GH10, GH45, LPMO (GH61) and ALE were aligned. In the case of GH45, sequences included members of subgroup A, B and C (Igarashi et al. 2008) obtained from the CAZy database and from the swollenins and expansins in the NCBI database. These multiple alignments were performed with MAFFT 7 (http://mafft.cbrc.jp/alignment/software/) using E-INS-i strategy (Kato et al. 2002, Katto et al. 2005) and the phylogenetic tree file was generated with bootstrap analysis of 100 resampling with the minimum linkage method. To determine the subfamily of the obtained GH5 genes, we prepared a phylogenetic tree of GH5 genes with the amino-acid sequences belonging to Eukaryote subfamilies 1, 2, 4, 5, 7, 8, 9, 11, 12, 15, 16, 22, 23, 24, 27, 30 31, 49, 50 and 51 (Aspeborg et al. 2012). This multiple alignment was performed with MegAlign software (DNASTAR, Madison, Wisconsin) using CLUSTAL W, and phylogenetic trees were supported by bootstrapping. Images were drawn with FigTree 1.3.1 (http://tree.bio.ed.ac.uk/software/figtree/).

Fungal strain and cultivation conditions.—For protein expression analysis, we cultivated *Bjerkandera adusta* strain HHB-12826-SP, *Ganoderma* sp. strain 10597 SS1 and *Phlebia brevispora* strain HHB-7030 on media containing ground aspen as sole carbon source and compared the secretome data with four Polyporales, *Dichomitus squalens*, *Fomitopsis pinicola*, *Trametes versicolor* and *Wolfiporia cocos* (Floudas et

al. 2012), grown under identical conditions. Comparative genome studies also included analyzed isolates of *Ceriporiopsis subvermispora* (Fernandez-Fueyo et al. 2012), *Phanerochaete carnosa* (Mahajan and Master 2010), *Phanerochaete chrysosporium* (Sato et al. 2007, Vanden Wymelenberg et al. 2010, Vanden Wymelenberg et al. 2011a) and *Postia placenta* MAD-698 (Vanden Wymelenberg et al. 2010, 2011a).

The sequenced monokaryotic strains *Bjerkandera adusta* strain HHB-12826-SP, *Ganoderma* sp. strain 10597 SS1 and *Phlebia brevispora* strain HHB-7030 were obtained from US Department of Agriculture Forest Mycology Center, Madison, Wisconsin. They were maintained on potato dextrose agar plates at room temperature (26.5 C) and mycelial plugs used to inoculate 250 mL of Highley's basal salt medium (Highley 1973) containing 0.5% (w/v) wiley-milled bigtooth aspen (*Populus grandidentata*; 1 mm^2 path) as the sole carbon source in 2 L Erlenmeyer flasks. Highley's medium contained these basal salts per liter: 2 g NH_4NO_3, 2 g KH_2PO_4, 0.5g $MgSO_4 \cdot 7H_2O$, 0.1g $CaCl_2 \cdot 2H_2O$ and 1 mg thiamine hydrochloride and 10 mL mineral solution. Mineral solution contained per liter 1.5 g nitrilotriacetic acid, 3 g $MgSO_4 \cdot 7H_2O$, 0.5 g $MnSO_4 \cdot H_2O$, 1 g NaCl, 0.1 g $FeSO_4 \cdot H_2O$, 0.1 g $CoSO_4$, 0.1 g $CaCl_2$, 0.1 g $ZnSO_4 \cdot 7H_2O$, 0.01 g $CuSO_4$, 0.01 g $AlK(SO_4)_2 \cdot 12H_2O$, 0.01 g H_3BO_3 and 0.01 g $NaMoO_4 \cdot 2H_2O$. Cultures were incubated on a rotary shaker (150 rpm) at room temperature 5 d. Filtrates were separated from mycelia and insoluble substrate by filtration through Miracloth (CN Biosciences, La Jolla, California). For mass spectroscopic analysis, culture filtrates were stored at −20 C.

Secretome analysis.—As described by Vanden Wymelenberg et al. (2010) and Floudas et al. (2012), soluble proteins of culture filtrates were precipitated by direct addition of solid trichloroacetic acid to 10% (w/v). After tryptic digestion, the generated peptides were subjected to nano-liquid chromatography separation with an Agilent 1100 nanoflow system (Agilent, Palo Alto, California). The chromatography system was coupled to a hybrid linear ion trap-Orbitrap mass spectrometer (LTQ-Orbitrap; Thermo Fisher Scientific, San Jose, California) equipped with a nano electrospray ion source as described by Vanden Wymelenberg et al. (2010) and Floudas et al. (2012). The acquired MS/MS spectra were analyzed by in-house-licensed Mascot search engine (Matrix Science, London, UK) using annotated gene models from each fungal genome databases in the Joint Genome Institutes (JGI) website (http://genome.jgi-psf.org/programs/fungi/index.jsf). Mascot searches were performed with these parameters: A fragment ion mass tolerance of 0.6 Da, a parent ion tolerance of 15 ppm and methionine oxidation as a variable modification. Scaffold software (Scaffold_3_00_6; Proteome Software Inc., Portland, Oregon) was used to validate MS/MS-based peptide and protein identifications. Protein identifications were accepted if they contained at least two unique peptides and if protein probabilities exceeded 95.0%, as determined by the protein prophet algorithm (Nesvizhskii et al. 2003). Detailed information of each identified protein was accessed via the JGI genome portals (Grigoriev et al. 2012) including gene ontology, InterPro domain and BLASTp search results. For some interesting genes, the deduced amino acid sequences were manually confirmed by BLASTp search with the NCBI database. Function or putative function was assigned when it was supported by direct experimental evidence or when comparisons to known proteins revealed conserved catalytic features and/or significant alignment scores (bit scores >150) to known proteins.

RESULTS

Genome analysis and phylogenetic tree analysis.—We classified CAZy genes in the 11 Polyporales genomes and determined the number of genes encoding GHs, PLs, CEs and CBMs (TABLE I). Overall, the average number of genes encoding CAZys in the genomes of white-rot fungi was 376 ± 53, significantly more than the 286 ± 43 observed in brown-rot fungi. Relative to white-rot fungi, the number of GHs was considerably lower and the total number of PLs, CEs and CBMs slightly decreased in brown-rot fungi.

In addition to CAZys, we determined the number of genes encoding oxidoreductases potentially related to cellulose degradation, such as CDH, LPMO and a cellulose-binding cytochrome, b_{562}. The distribution of these genes among the 11 Polyporales genomes is illustrated in scatter plots with the average gene number in white-rot fungi on the y axes and that in brown-rot fungi on x axes (FIG. 1A, B). These plots illustrate significant differences (below y = 1/2x or above y = 2x) in gene number between white-rot and brown-rot genomes (TABLES II, III). As a result, the genes encoding family GH30 β-glycosidase and trehalase family GH37 were significantly increased in brown-rot fungi (below y = 1/2x), although these genes expanded only in brown-rot *F. pinicola* and *P. placenta*. In contrast, the genes encoding 24 CAZy families and the above-mentioned oxidoreductases increased significantly in white-rot fungi (above y = 2x) while the difference between white- and brown-rot genes were less than twofold in other families.

Family CE1 of white-rot fungi averaged three genes, while brown-rot fungi averaged only one (TABLE II). Phylogenetic analysis of CE1 sequences (FIG. 2) revealed two main clades; one clade contains acetyl xylan esterase-like proteins and other contains conserved hypothetical proteins, among which are several brown-rot CE1s. Of interest, two CE1 proteins have CBM1s at C-termini (Bjead122937 and Phlbr123855) whereas other CBM1-CE1 proteins have these cellulose binding modules at N-termini. In addition, white-rot genes belonging to family CE15 endo β-4-*O*-glucuronoyl methylesterase were more than twice as numerous (1.0 versus 2.3), relative to brown-rot genes. This enzyme likely modifies glucuronoyl acid

TABLE I. Overview of CAZy genes in the 11 Polyporales genomes

	Strain	GH[a]	GT	PL	CE	CBM	Total
BR	*Fomitopsis pinicola*	203	72	3	16	41	335
	Wolfiporia cocos	152	67	2	14	18	253
	Postia placenta	153	72	8	18	20	271
	Average of BR	169	70	4	16	26	286
WR	*Bjerkandera adusta*	208	72	10	26	62	378
	Ganoderma spp	279	68	9	30	57	443
	Phlebia brevispora	224	90	7	19	101	441
	Dichomitus squalens	236	68	11	26	46	387
	Trametes versicolor	237	83	9	20	49	398
	Ceriporiopsis subvermispora	174	67	6	17	37	301
	Phanerochaete carnosa	197	74	8	17	43	341
	Phanerochaete chrysosporium	183	66	4	16	48	317
	Average of WR	218	74	8	21	55	376

[a] Abbreviations: GH = glycoside hydrolase; GT = glycosyltransferases; PL = polysaccharide lyase; CE = carbohydrate esterase; CBM = carbohydrate-binding module; BR = brown-rot; WR = white-rot.

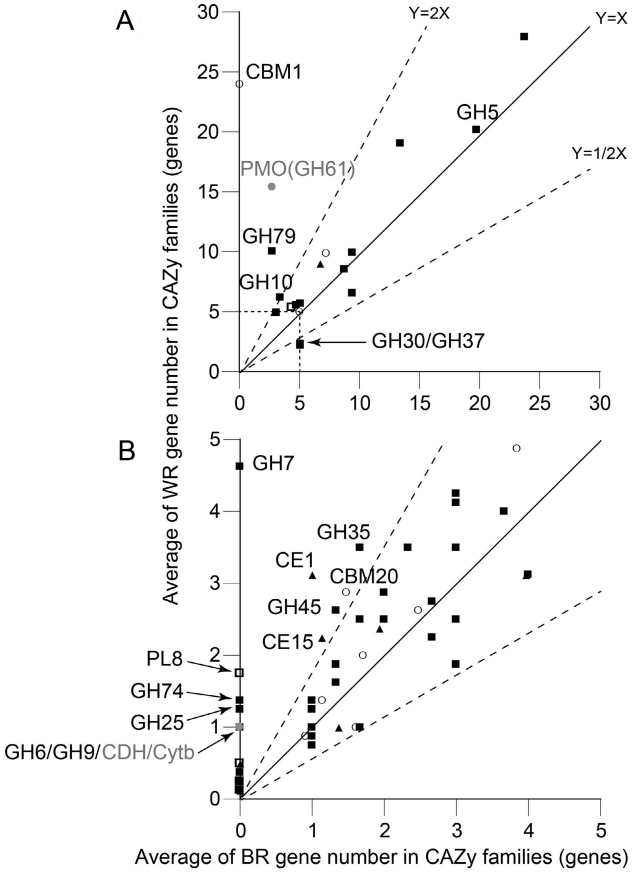

FIG. 1. Relation of average gene number encoding CAZys and oxidoreductases involved in polysaccharides degradation between white- and brown-rot Polyporales genomes. The average number is more than five genes (A) and fewer than than five genes (B). ■ = glycoside hydrolase families; ▲ = carbohydrate esterase families; □ = polysaccharide lyase families; ○ = carbohydrate-binding module families; ● = oxidoreductase genes.

residues in xylan (Spanikova and Biely 2006). Among PL families, genes belonging to PL8 were observed only in the white-rot genomes with an average of 1.9 genes. The role of PL8s in wood degradation, if any, has not been characterized. In the case of CBM families, white-rot fungi have an average of 24 genes encoding proteins with family CBM1 cellulose-binding modules whereas this module is absent from the brown-rot genomes. CBM1 attaches mainly to crystalline cellulose and may serve to concentrate enzymes on cellulose surfaces (Tomme et al. 1988, Reinikainen et al. 1995). Of note, white-rot fungi also have an average number of 2.9 genes containing family CBM20 starch-binding modules, which was significantly greater than 1.3 genes in brown-rot fungi. These binding modules typically are associated with catalytic domains classified as GH13s or GH15s, (SUPPLEMENTARY TABLE I).

Several GH families are significantly increased in white-rot fungi relative to brown-rot fungi (FIG. 1, TABLE II). Fungal cellulase systems generally consist of cellobiohydrolases (CBHs), endo-glucanases (EGs) and β-glucosidases (BGL). White-rot fungi typify this enzymatic strategy with family GH6 and GH7 cellulases, which include CBHs. In contrast, these are lacking in genomes of brown-rot fungi. Among potential EGs, exhaustive BLAST queries of white-rot fungi revealed a single family GH9 cellulase (Gilad et al. 2003). Each GH9 features a unique transmembrane-anchored domain at C-terminal as reported for *P. chrysosporium* (Vanden Wymelenberg et al. 2002) (SUPPLEMENTARY FIG. 1). Furthermore, white-rot genes belonging to family GH45 EGs were twice as common (1.3 versus 2.6) relative to brown-rot genes. The GH45 EG of *P. chrysosporium* was assigned to subfamily C (Igarashi et al. 2008), and all Polyporales GH45 genes

TABLE II. CAZy genes exhibiting significant differences between brown- and white-rot fungi in the 11 Polyporales genomes (referred to FIG. 1) and important for polysaccharides degradation

		GH[a]															PL	CE		CBM	
	Strain	3	5	6	7	9	10	11	12	25	30	35	37	45	74	79	8	1	15	1	20
BR	*Fomitopsis pinicola*	12	19	0	0	0	2	0	2	0	10	2	2	2	0	3	0	1	1	0	2
	Wolfiporia cocos	9	18	0	0	0	4	0	2	0	2	2	4	1	0	3	0	1	1	0	1
	Postia placenta	7	22	0	0	0	4	0	2	0	3	1	9	1	0	2	0	1	1	0	1
	Average of BR	9.3	20	0	0	0	3.3	0	2	0	5	1.7	5	1.3	0	2.7	0	1.0	1.0	0	1.3
WR	*Bjerkandera adusta*	9	19	1	5	1	4	0	2	1	1	4	3	3	2	8	1	2	2	32	2
	Ganoderma spp	13	18	1	3	1	9	0	3	2	3	7	2	3	1	12	3	2	2	18	3
	Phlebia brevispora	8	23	1	4	1	8	0	2	1	2	4	3	4	1	11	2	2	2	28	3
	Dichomitus squalens	8	19	1	4	1[b]	5	0	3	2	2	3	3	2	1	13	3	2	2	17	2
	Trametes versicolor	13	22	1	4	1	6	0	5	1	4	2	2	3	1	11	2	4	2	23	4
	Ceriporiopsis subvermispora	7	18	1	3	1[b]	6	1	3	1	1	1	2	2	1	8	2	4	2	16	4
	Phanerochaete carnosa	11	24	1	7	1	6	1	3	1	3	4	2	2	2	13	1	3	3	27	3
	Phanerochaete chrysosporium	11	19	1	7	1	6	1	2	1	2	3	2	2	2	5	1	5	2	31	2
	Average of WR	10	20	1	4.6	1	6.3	0.4	2.9	1.3	2.3	3.5	2.4	2.6	1.4	10.1	1.9	3.0	2.3	24	2.9

[a] Abbreviations: GH = glycoside hydrolase; PL = polysaccharide lyase; CE = carbohydrate esterase; CBM = carbohydrate-binding module; BR = brown rot; WR = white rot.

[b] Manual BLAST queries predicted that GH9 gene is encoded in genome.

except for Gansp_156501 and Trave_24300 also belong to subfamily C (FIG. 3). The average numbers of EG genes assigned to family GH12 in white- and brown-rot genomes were respectively 2.9 and 2.0. Phylogenetic trees of families GH12 and GH45 genes showed that one clade consisted of genes from all white-rot fungi except for *F. pinicola* (FIGS. 3, 4). Although the actual functions of GH12 and GH45 EG in wood decay remain unclear, these small-molecular weight EGs are thought to somehow swell polysaccharide complexes with low activity toward β-glucan and act on cellulose synergistically with other cellulases (Henriksson et al. 1999, Sandgren et al. 2005, Igarashi et al. 2008).

Members of family GH5 feature significant variation in enzyme specificities, but classification into subfamilies (Aspeborg et al. 2012) can improve confidence in predicting function. Phylogenetic analysis categorized polypore GH5 genes into subfamily 5 endo-β -1,4-glucanase, subfamily 7 β-1,4-mannan-cleaving enzyme including endo-β-1,4-mannanase, subfamily 9 fungal cell wall modifying enzyme

TABLE III. Oxidoreductase and epimerase genes exhibiting significant differences between brown- and white-rot fungi in the 11 Polyporales genomes (referred to FIG. 1) that are potentially important for cellulose degradation

	Strain	LPMO[a] (GH61)	CDH	Cytb_{562}	ALE
BR	*Fomitopsis pinicola*	4	0	0	3
	Wolfiporia cocos	2	0	0	2
	Postia placenta	2	0	0	3
	Average of BR	3	0	0	3
WR	*Bjerkandera adusta*	28	1	1	4
	Ganoderma spp	16	1	1	5
	Phlebia brevispora	12	1	1	3
	Dichomitus squalens	15	1	1	5
	Trametes versicolor	18	1	1	3
	Ceriporiopsis subvermispora	9	1	1	3
	Phanerochaete carnosa	11	1	1	4
	Phanerochaete chrysosporium	15	1	1	4
	Average of WR	16	1	1	4

[a] Abbreviations: GH = glycoside hydrolase; LPMO = lytic polysaccharide monooxygenase; CDH = cellobiose dehydrogenase; Cytb_{562} = cytochrome b_{562} with cellulose-binding module; ALE = aldose-1-epimerase; BR = brown rot; WR = white rot.

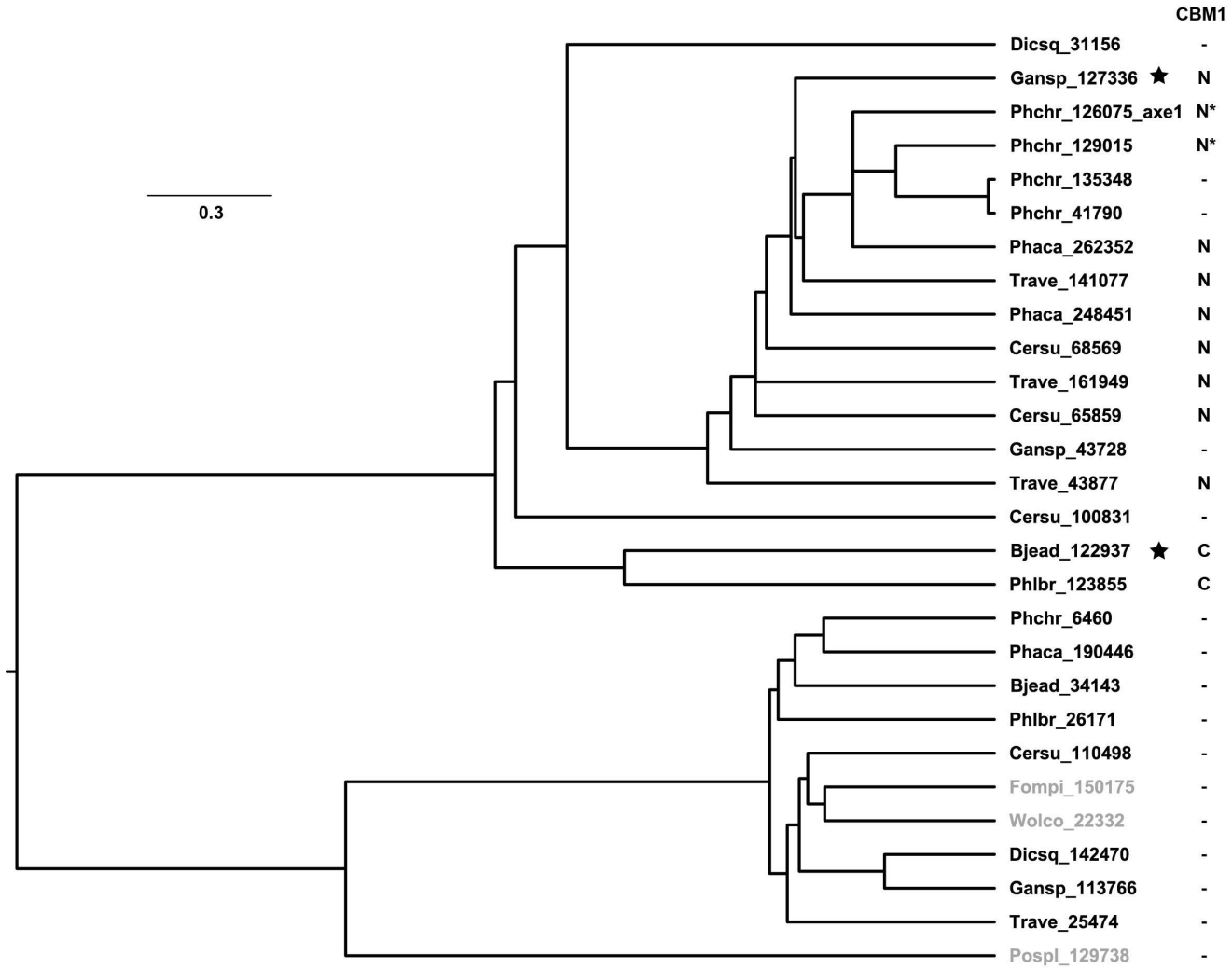

FIG. 2. Phylogenetic tree analysis and secreted protein expression profile of CE1 amino acid sequences in 11 Polyporales genomes. Gray font denotes genes of brown-rot fungi. ★ indicates gene encoding detected peptides. N and C denote CBM1s at N and C terminal end respectively. *Manually annotated CBM1.

including exo-β-1,3-glucanase, subfamily 12 β-glucosylceramidase and (flavonoid) β-glucosidase, subfamily 15 endo-β-1,6-glucanase, subfamily 22 putative endo-β-1,4-glucanase, subfamily 31 putative β-1,3-(gluco)mannanase and subfamily 49/50 uncharacterized protein (FIG. 5). Based on this classification system, polypore genes belonging to subfamily 5 endo-glucanases and subfamily 7 β-mannanases with CBM1 were confined to the white-rot fungi. Family GH3 is also functionally diverse (Harvey et al. 2000) and the phylogenetic distributions of GH3 genes to glucosidase, xylosidase, glycosidase and N-acetylglucosaminidase were equal between white- and brown-rot fungi, although only white-rot BGLs have appended CBM1s (SUPPLEMENTARY FIG. 2).

Various hemicellulases are associated with degradation of xylan, mannan, xyloglucan and other components in wood. The average number of genes encoding family GH10 endo-xylanase in white- and brown-rot genomes was 6.3 and 3.3 respectively, and only white-rot genes encode GH10 proteins with an appended CBM1 (TABLE II, SUPPLEMENTARY FIG. 3). The family GH11 endo-xylanases were represented by a single gene in *P. chrysosporium*, *P. carnosa* and *C. subvermispora*. There was no significant difference between white- and brown-rot fungi in the number of genes belonging to subfamily GH5_7, but these mannan-cleaving enzymes have an appended CBM1 (FIG. 5) in white-rot genomes. Of note, one or two genes belonging to family GH74 xyloglucanase were observed in white-rot genomes but none were observed in brown-rot genomes. Among other GHs probably related to degradation of wood components, white-rot genomes averaged 10 genes encoding family GH79 β -glucuronidase. Likely involved in processing arabinogalactan-protein (Konishi et al. 2008), these

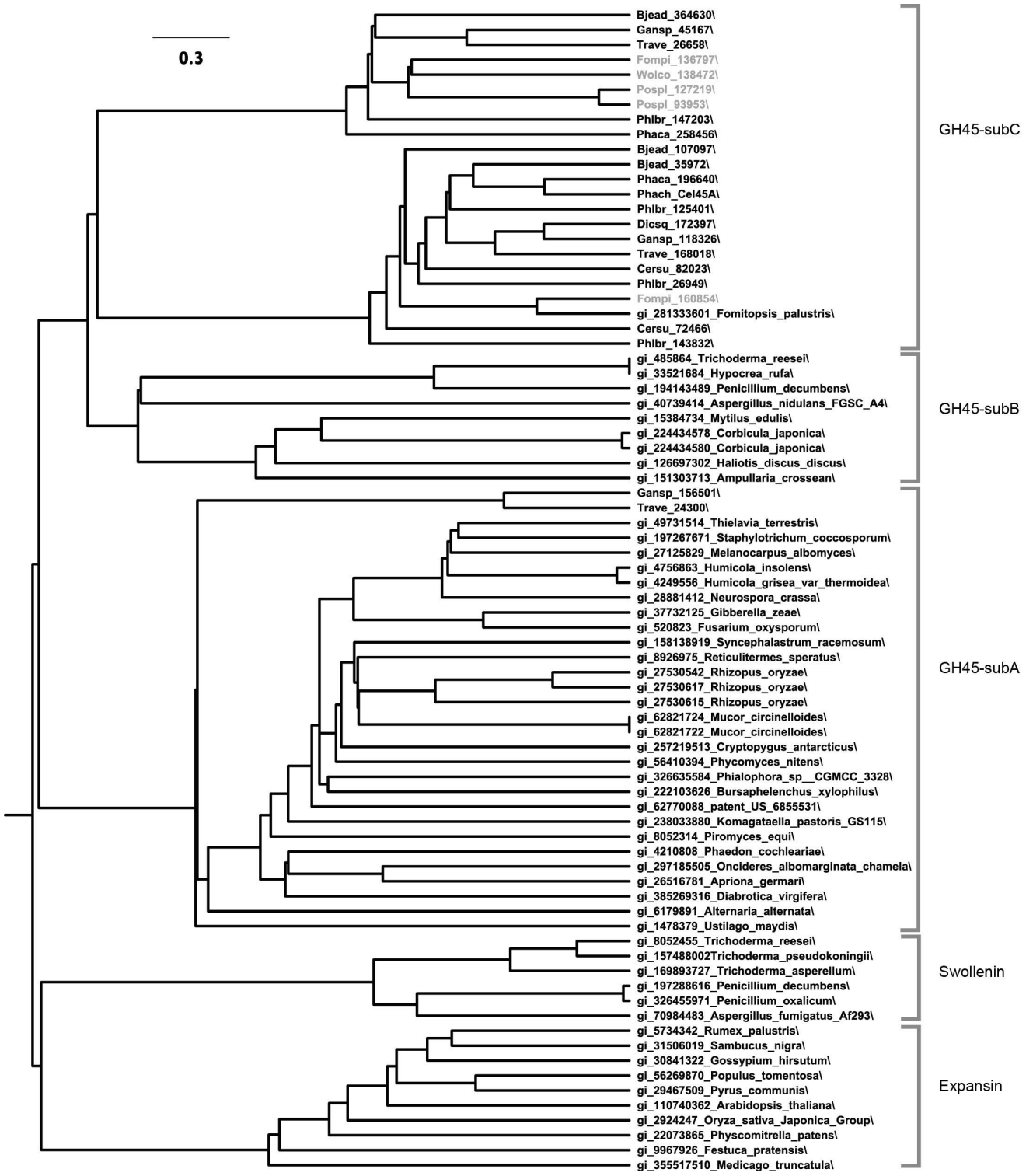

FIG. 3. Phylogenetic tree of GH45 amino acid sequences in the 11 Polyporales. Gray font denotes genes of brown-rot fungi. Phchr_Cel45A was characterized previously.

gene families showed significant expansions, relative to an average of 2.7 genes per brown-rot genome. Only white-rot fungi had genes belonging to lysozyme family GH25 known for microbial cell wall degradation.

In addition to CAZys, several enzymes potentially involved in cellulose degradation were significantly increased (FIG. 1, TABLE III). All white-rot fungi have one gene encoding CDH. Although the precise role

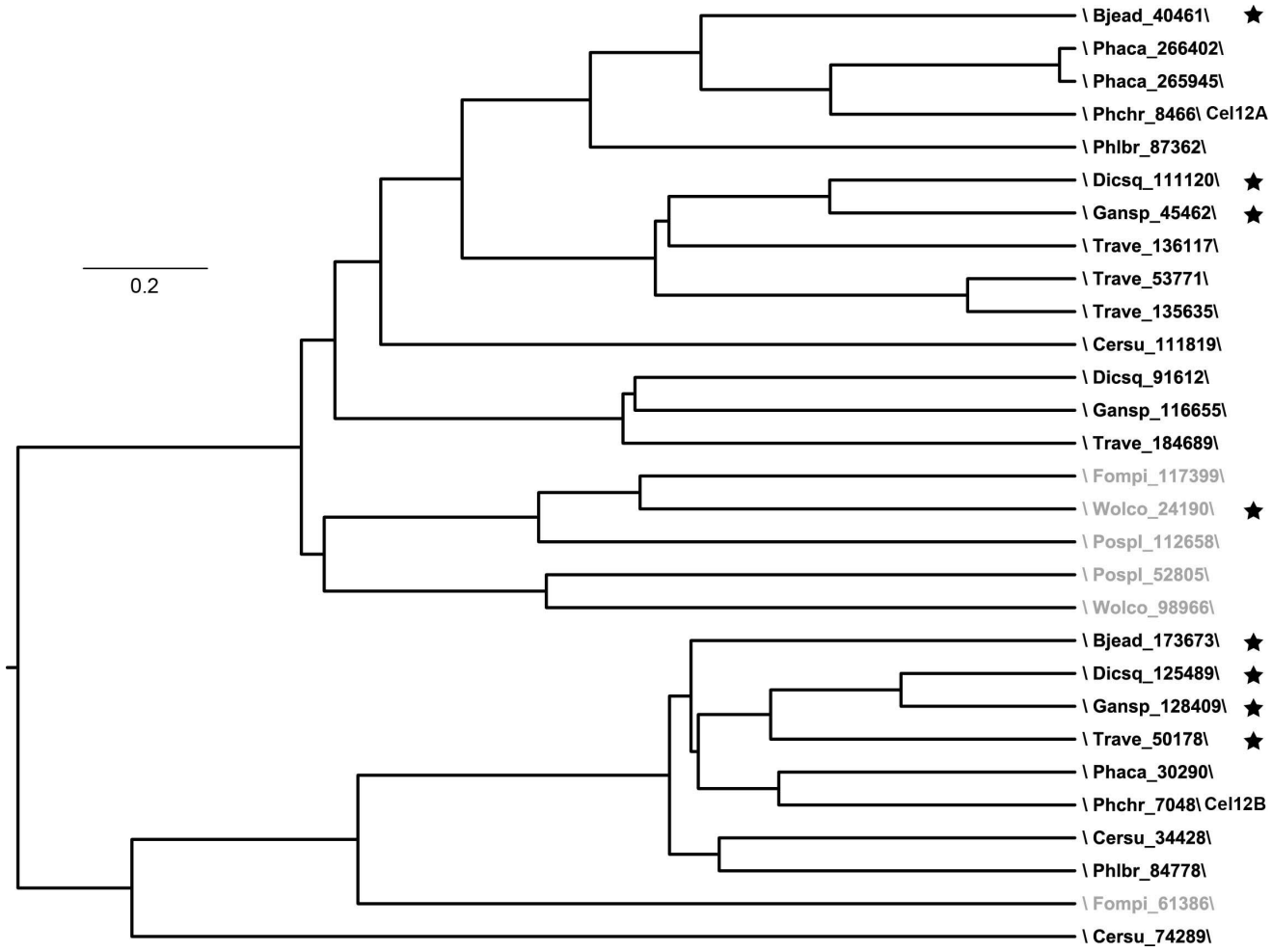

FIG. 4. Phylogenetic tree analysis and secreted protein profile of GH12 amino acid sequences in the 11 Polyporales genomes. Gray font denotes gene of brown-rot fungi. ★ indicates genes encoding detected peptides.

of CDH remains unclear (Henriksson et al. 2000) research has demonstrated oxidative boosting of cellulase preparations containing CDH and GH61 enzymes (Harris et al. 2010, Vaaje-Kolstad et al. 2010, Langston et al. 2011, Phillips et al. 2011, Quinlan et al. 2011, Westereng et al. 2011, Bey et al. 2013). As the name implies, GH61s were considered hydrolytic cellulases but now are understood to be copper-dependent LPMOs (Phillips et al. 2011, Quinlan et al. 2011, Westereng et al. 2011, Bey et al. 2013). These LPMOs are more numerous in white-rot fungi (TABLE III), and phylogenetic analysis of GH61 genes from the brown rots separates them into two clades (FIG. 6). Aldose epimerases (ALEs) also may interact with CDH via generation of the cellobiose β -anomer, the preferred CDH substrate (FIG. 7A; Higham et al. 1994). Although the average number of aldose 1-epimerase was almost the same, one of the clades contained only white-rot genes including *P. chrysosporium*. Ale1 was detected previously in *P. chrysosporium* culture filtrates along with CDH and CBHs (FIG. 7B; Vanden Wymelenberg et al. 2005, 2006; Vanden Wymelenberg 2011). Genes encoding cytochrome b_{562}, a putative iron-reductase with an appended CBM1 domain, were observed only in white-rot genomes, although its function remains uncertain (Yoshida et al. 2005).

Protein expression analysis.—Soluble proteins of *B. adusta*, *Ganoderma* sp. and *P. brevispora* were identified in culture filtrates by LC-MS/MS. In the *P. breviospora* secretome, 178 proteins were identified; 39% and 4% were GHs and other CAZys respectively (FIG. 8A). Among those likely involved in cellulose degradation, GH6, GH7, GH5, GH44, LPMO (GH61) and CDH were detected. Although previously detected in other white-rot fungi, peptides corresponding to GH12 and GH45 were not identified. GH44 was a unique protein that was present only in *P. brevispora*. In the case of *G.* sp., a total of 105 proteins were

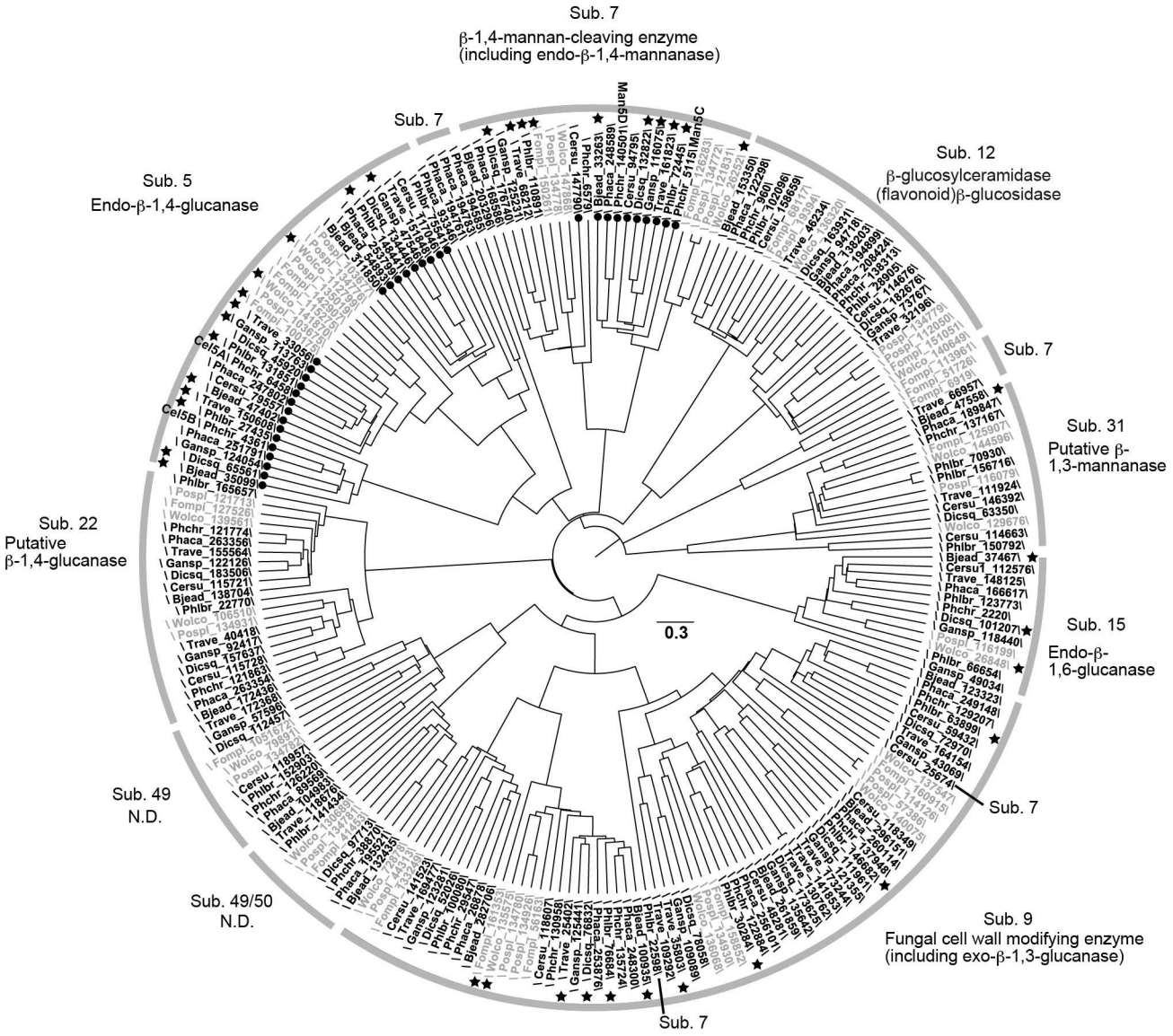

FIG. 5. Phylogenetic tree analysis and secreted protein expression profile of GH5 amino acid sequences in 11 Polyporales genomes. Gray font denotes gene of brown-rot fungi. ★ indicates genes encoding detected peptides. ● signifies CBM-containing gene.

determined, 37% and 6% of which were GHs and other CAZys respectively (FIG. 8A). These included GH6, GH7, GH5, GH12, LPMO (GH61) and CDH but not GH45. In *B. adusta* cultures, 187 proteins were detected where 33% and 6% were GHs and other CAZys respectively (FIG. 8A). Again, GH45 proteins could not be detected.

We compared secretome data from *B. adusta*, *G.* sp., *P. brevispora*, *D. squalens*, *T. versicolor*, *F. pinicola* and *W. cocos* grown under identical conditions (FIG. 8A). The percentage of total proteins categorized as CAZys was 30–43% and 18–21% in white- and brown-rot fungi respectively. Of these CAZy proteins, GHs were more abundant in white-rot fungi. Of interest, GH6, GH7 and the oxidoreductases LPMO (GH61) and CDH were identified only in white-rot fungi (FIG. 8B). Every white-rot fungus except *T. versicolor* secreted GH74 proteins, whereas brown-rot fungi did not secrete this protein. Likewise, PL8 proteins were identified only in white-rot fungi including *B. adusta*, *P. brevispora*, *D. squalens*, *T. versicolor* while CE1 proteins were secreted by white-rot fungi *B. adusta* and *G.* sp. In addition, the number of identified GH10 and GH79 proteins were more abundant in white-rot fungi relative to brown-rot fungi. In contrast, benzoquinone-reductase (quinone-reductase, QRD) was identified only in brown-rot fungi such as *F. pinicola* and *W. cocos*.

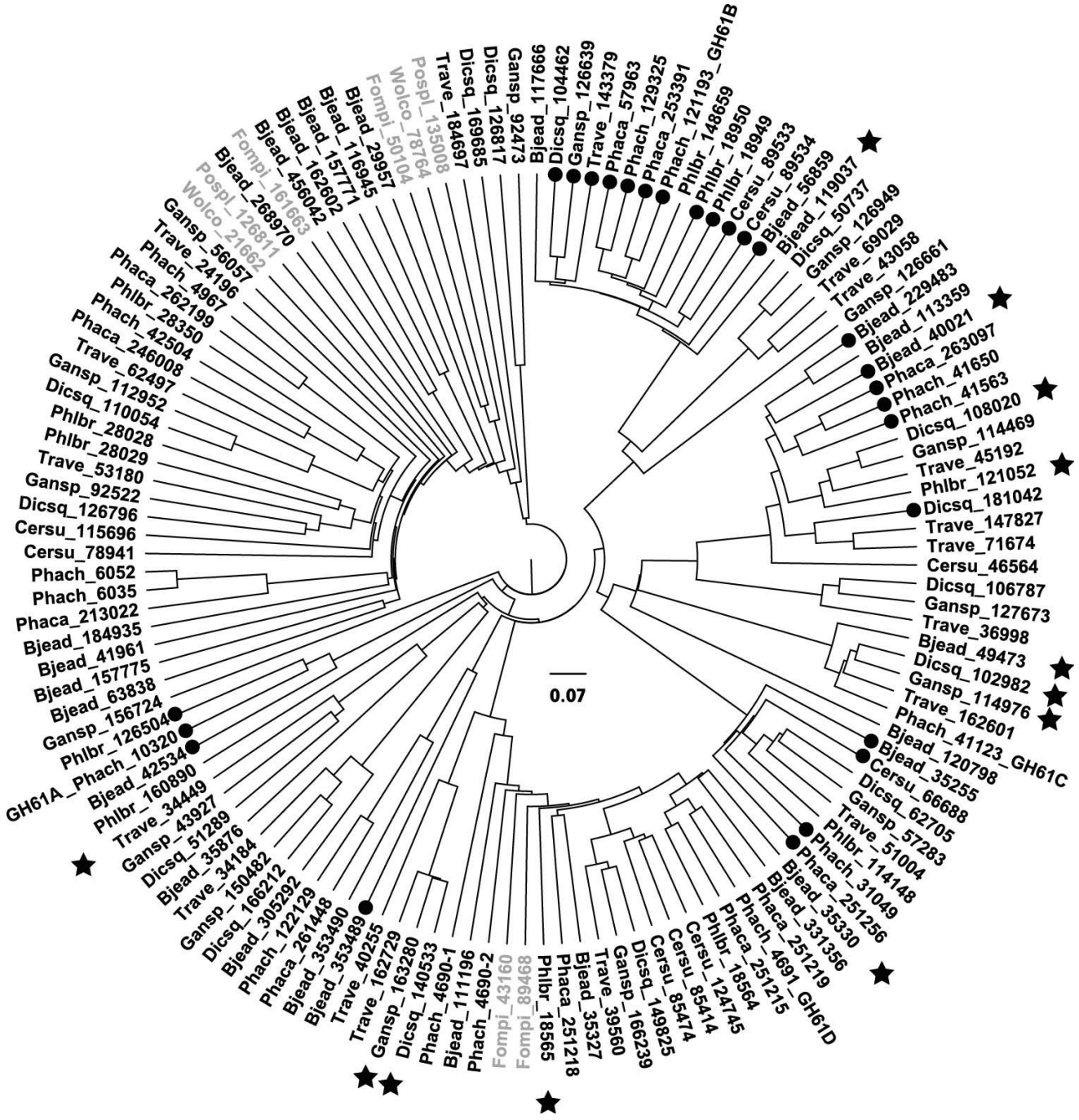

FIG. 6. Phylogenetic tree analysis and secreted protein expression profile of lytic polysaccharide monooxygenase (LPMO, formerly GH61) amino acid sequences in 11 Polyporales genomes. Gray font denotes genes of brown-rot fungi. ★ indicates genes encoding detected peptides. ● signifies CBM-containing gene. *Manually annotated CBM1.

DISCUSSION

We examined polysaccharide-degrading potential of white- and brown-rot Polyporales. Genome analysis revealed that CBM1 and cellulases belonging to GH6 and GH7 were lacking in brown-rot fungal genomes (TABLE II), an observation consistent with Martinez et al. (2009) and Floudas et al. (2012). Furthermore, our analysis showed the absence of genes encoding GH5s with CBM1s and membrane-anchored GH9 as well as a relatively lower numbers of GH12 and GH45 in brown-rot genomes (FIGS. 3–5). Genes encoding oxidoreductases CDH and Cytb_{562}, potentially involved in the oxidative degradation of cellulose, were

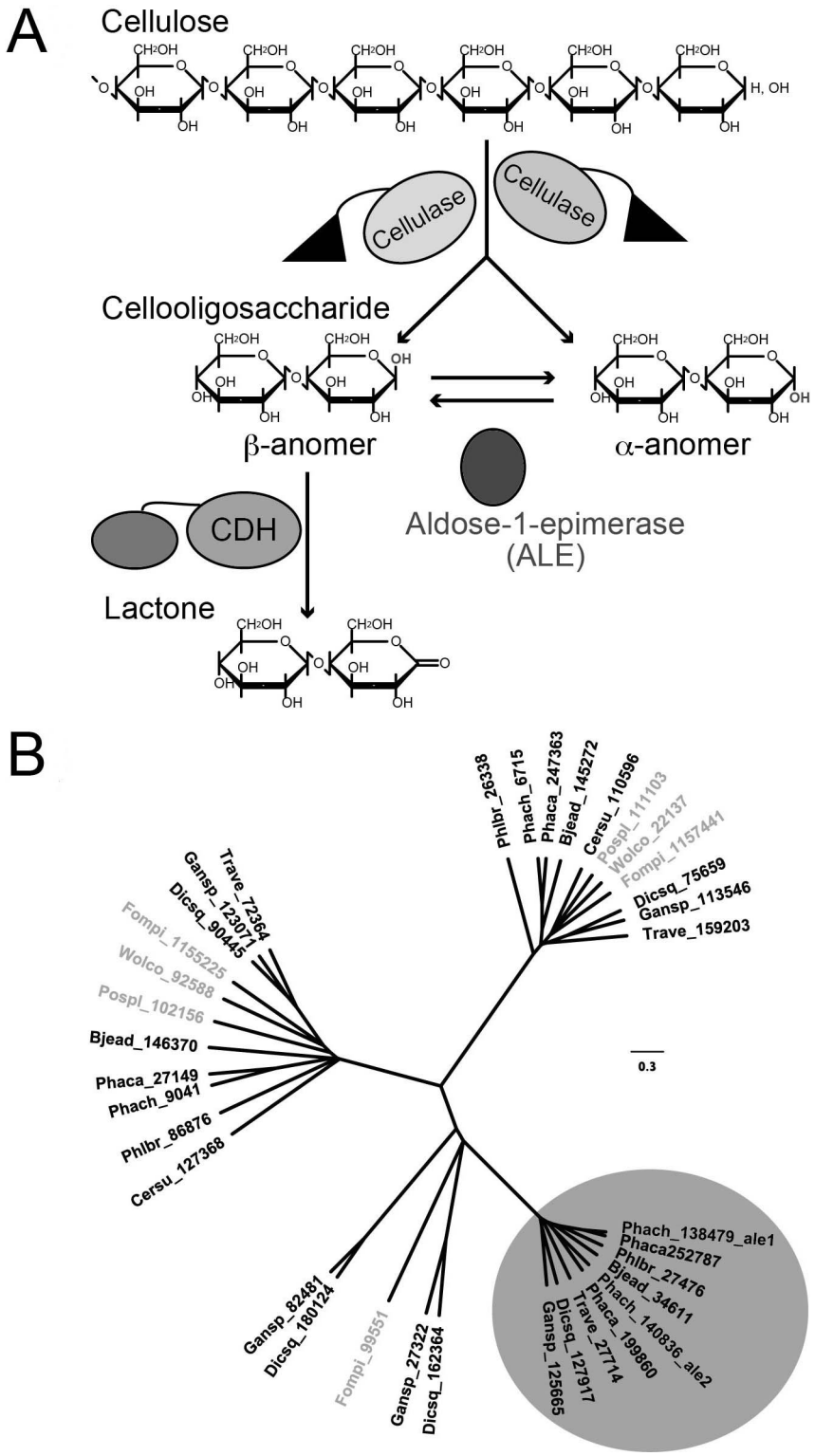

FIG. 7. Schematic participation of aldose-1-epimerase (ALE) in fungal cellulose degradation (A) and phylogenetic tree analysis of ALE amino acid sequences in the 11 Polyporales (B). Gray font denotes genes of brown-rot fungi. *ale1* and *ale2* were detected previously in the culture filtrate of white-rot *Phanerochaete chrysosporium*.

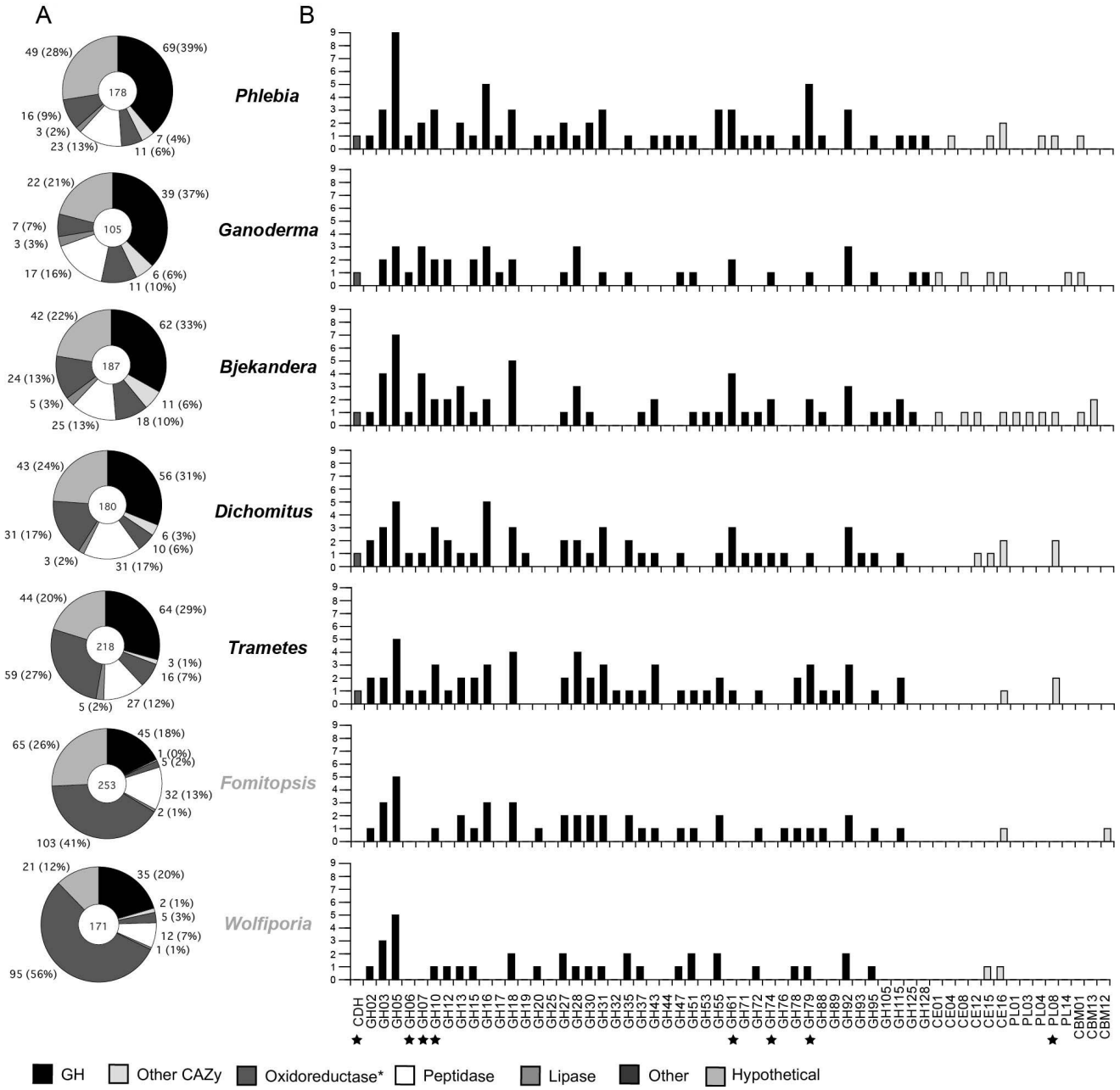

FIG. 8. Distribution of proteins detected among seven Polyporales grown in the media containing aspen wood using LC-MS/MS (A). *Oxidoreductase signifies oxidoreductase potentially related to wood degradation. The number of identified proteins within CAZy families and oxidoreductases potentially related to cellulose degradation (B). ★ indicates significantly increased protein family in white-rot fungi secretomes. Y-axis indicates the number of identified proteins.

found only in white-rot genomes (TABLE III). White-rot fungi have abundant LPMO (GH61) genes relative to brown-rot fungi (FIG. 6). Of note, genes encoding aldose-1-epimerase, detected in culture filtrates of white-rot fungi (Vanden Wymelenberg et al. 2005, 2006), also existed in the *B. adusta*, *Ganoderma* sp, and *P. brevispora* white-rot genomes (FIG. 7), suggesting that this kind of ALE could be physiologically connected to CDH, cellulase and possibly LPMO. Indeed, secretome analysis identified GH6, GH7, CDH and LPMO peptides only in white-rot fungi (FIG. 8). In contrast, peptides corresponding to quinone reductase (QRD) were identified only in brown-rot fungi in this study. This is consistent with studies of the brown-rot fungus *P. placenta* (Martinez et al. 2009, Vanden Wymelenberg et al. 2010) and may constitute a key component of a redox cycle supporting Fenton chemistry (Paszczynski et al. 1999,

Suzuki et al. 2006). These results collectively suggest that white-rot fungi employ both extracellular hydrolytic and oxidative reactions for cellulose degradation, a strategy distinct from Fenton systems of brown-rot fungi.

Nevertheless, the role of LPMOs in brown-rot fungi remains unclear. Although substantially reduced in number, all brown-rot fungi, including non-polypores, feature several LPMO-encoding genes (Floudas et al. 2012). However, none of these genes include cellulose-binding modules and, with the exception of *Gloeophyllum trabeum*, the corresponding proteins have not been detected in lignocellulose-containing media (Martinez et al. 2009, Floudas et al. 2012). The breakdown of crystalline cellulose is stimulated by the combination of CBH, LPMO and CDH (Langston et al. 2011), all of which are absent or barely expressed by brown-rot fungi. Thus, it seems likely that brown-rot depolymerization of cellulose involves alternative mechanisms such as the non-enzymatic generation of hydroxyl radical.

The enzymatic machinery for hemicellulose degradation also appears to be different between white- and brown-rot fungi. The number of genes encoding CE1 acetylxylan esterases, GH10 endo-xylanases and GH79 β-glucuronidases are greater in white-rot fungi relative to brown-rot genomes (TABLE II). GH74-encoding genes were absent in the brown-rot polypores. However, it should be noted that the brown-rot fungus *G. trabeum* (Gloeophyllales) contains at least one GH74 gene that is expressed in wood-containing media (Floudas et al. 2012). Differences in genetic repertoires may be due, in part, to the general substrate preferences, especially the prevalence of glucomannans (conifers) versus glucuronoxylans (hardwood).

Genes encoding CE15 4-*O*-glucuronoyl methylesterase were increased in white-rot fungi, but their role remains unsettled. This enzyme has been proposed to hydrolyze ester linkages between xylan glucuronic acid residues and phenyl propane residues in lignin (Spanikova and Biely 2006). However, it seems unlikely that CE15 activity is unique to lignin mineralization in that both brown- and white-rot fungi secrete this protein. The CE15s conceivably could contribute to cell-wall degradation by enhancing substrate accessibility.

Also unclear is the increased number of genes encoding polysaccharide lyase family 8 (PL8) and family CBM20 starch-binding modules in white-rot fungi. Often associated with a glucoamylase catalytic domain (GH15), the CBM20-containing proteins have been detected in cultures of both white- and brown-rot fungi (FIG. 8; Floudas et al. 2012). Presumably these enzymes are necessary for metabolism of low levels of starch in ground wood. In contrast, PL8 proteins have been identified in cultures of the white-rot fungi *P. brevispora*, *B. adusta* (FIG. 8), *T. versicolor*, *D. squalens* and *P. chrysosporium* but not in brown-rot polypores (Vanden Wymelenberg et al. 2010, 2011a; Floudas et al. 2012). The physiological role is complicated by the functional heterogeneity of the PL8 family, which includes chondroitin AC lyase (EC 4.2.2.5), chondroitin ABC lyase (EC 4.2.2.20), hyaluronate lyase (EC 4.2.2.1) and xanthan lyase (EC 4.2.2.12). These lyases possibly enhance cellulose degradation by complementing the LPMOs secreted in same medium. Establishing precise physiological connections will require detailed enzyme characterization, but the preponderance of genome and expression data support fundamentally different strategies employed by white- and brown-rot fungi.

ACKNOWLEDGMENTS

This research was supported by Technology and a Research Fellowship (No. 233288) to C.H. from the Japan Society for the Promotion of Science (JSPS).

LITERATURE CITED

Adav SS, Ravindran A, Sze SK. 2012. Quantitative proteomic analysis of lignocellulolytic enzymes by *Phanerochaete chrysosporium* on different lignocellulosic biomass. J Proteome 75:1493–1504, doi:10.1016/j.jprot.2011.11.020

Arantes V, Milagres AMF, Filley TR, Goodell B. 2011. Lignocellulosic polysaccharides and lignin degradation by wood decay fungi: the relevance of nonenzymatic Fenton-based reactions. J Ind Microbiol Biotechnol 38: 541–55, doi:10.1007/s10295-010-0798-2

Aspeborg H, Coutinho PM, Wang Y, Brumer H, Henrissat B. 2012. Evolution, substrate specificity and subfamily classification of glycoside hydrolase family 5 (GH5). BMC Evol Biol 12:186, doi:10.1186/1471-2148-12-186

Bey M, Zhou S, Poidevin L, Henrissat B, Coutinho PM, Berrin JG, Sigoillot JC. 2013. Cello-oligosaccharide oxidation reveals differences between two lytic polysaccharide monooxygenases (family GH61) from *Podospora anserina*. Appl Environ Microbiol 79:488–496, doi:10.1128/AEM.02942-12

Binder M, Hibbett DS, Larsson K-H, Larsson E, Langer E, Langer G. 2005. The phylogenetic distribution of resupinate forms across the major clades of mushroom-forming fungi (Homobasidiomycetes). Syst Biodivers 3:1–45, doi:10.1017/S1477200005001623

Blanchette RA. 1991. Delignification by wood-decay fungi. Ann RevP hytopathol 29:381–398, doi:10.1146/annurev.py.29.090191.002121

———, Burnes TA, Eerdmans MM, Akhtar M. 1992. Evaluating isolates of *Phanerochaete chrysosporium* and *Ceriporiopsis subvermispora* for use in biological pulping

processes. Holzforschung 46:109–115, doi:10.1515/hfsg.1992.46.2.109

Cantarel BL, Coutinho PM, Rancurel C, Bernard T, Lombard V, Henrissat B. 2009. The Carbohydrate-Active EnZymes database (CAZy): an expert resource for Glycogenomics. Nucl Acids Res 37:D233–238, doi:10.1093/nar/gkn663

Cohen R, Jensen KA, Houtman CJ, Hammel KE. 2002. Significant levels of extracellular reactive oxygen species produced by brown rot basidiomycetes on cellulose. FEBS Lett 531:483–488, doi:10.1016/S0014-5793(02)03589-5

———, Suzuki MR, Hammel KE. 2004. Differential stress-induced regulation of two quinone reductases in the brown rot basidiomycete *Gloeophyllum trabeum*. Appl Environ Microbiol 70:324–331, doi:10.1128/AEM.70.1.324-331.2004

Daniel G. 1994. Use of electron microscopy for aiding our understanding of wood biodegradation. FEMS Microbiol Rev 13:199–233, doi:10.1111/j.1574-6976.1994.tb00043.x

Eastwood DC, Floudas D, Binder M, Majcherczyk A, Schneider P, Aerts A, Asiegbu FO, Baker SE, Barry K, Bendiksby M et al. 2011. The plant cell wall-decomposing machinery underlies the functional diversity of forest fungi. Science 333:762–765, doi:10.1126/science.1205411

Eriksson K-E, Blanchette R, Ander P. 1990. Microbial and enzymatic degradation of wood and wood components. Berlin: Springer-Verlag.

Fernandez-Fueyo E, Ruiz-Dueñas FJ, Ferreira P, Floudas D, Hibbett DS, Canessa P, Larrondo LF, James TY, Seelenfreund D, Lobos S, et al. 2012. Comparative genomics of *Ceriporiopsis subvermispora* and *Phanerochaete chrysosporium* provide insight into selective ligninolysis. Proc Natl Acad Sci USA 109:8352.

Floudas D, Binder M, Riley R, Barry K, Blanchette RA, Henrissat B, Martínez AT, Otillar R, Spatafora JW, Yadav JS, et al. 2012. The Paleozoic origin of enzymatic lignin decomposition reconstructed from 31 fungal genomes. Science 336:1715–1719, doi:10.1126/science.1221748

Gilad R, Rabinovich L, Yaron S, Bayer EA, Lamed R, Gilbert HJ, Shoham Y. 2003. CelI, a noncellulosomal family 9 enzyme from *Clostridium thermocellum*, is a processive endoglucanase that degrades crystalline cellulose. J Bacteriol 185:391–398, doi:10.1128/JB.185.2.391-398.2003

Gilbertson RL. 1981. North American wood-rotting fungi that cause brown rots. Mycotaxon 12:372–416.

Grigoriev IV, Nordberg H, Shabalov I, Aerts A, Cantor M, Goodstein D, Kuo A, Minovitsky S, Nikitin R, Ohm RA, et al. 2012. The genome portal of the Department of Energy Joint Genome Institute. Nucl Acids Res 40:D26–32, doi:10.1093/nar/gkr947

Harris PV, Welner D, Mcfarland KC, Re E, Navarro Poulsen J-C, Brown K, Salbo R, Ding H, Vlasenko E, Merino S, et al. 2010. Stimulation of lignocellulosic biomass hydrolysis by proteins of glycoside hydrolase family 61: structure and function of a large, enigmatic family. Biochemistry 49:3305–3316, doi:10.1021/bi100009p

Harvey AJ, Hrmova M, de Gori R, Varghese JN, Fincher GB. 2000. Comparative modeling of the three-dimensional structures of family 3 glycoside hydrolases. Proteins 41:257–69, doi:10.1002/1097-0134(20001101)41:2<257::AID-PROT100>3.0.CO;2-C

Henriksson G, Johansson G, Pettersson G. 2000. A critical review of cellobiose dehydrogenases. J Biotechnol 78:93–113, doi:10.1016/S0168-1656(00)00206-6

———, Nutt A, Henriksson H, Pettersson B, Ståhlberg J, Johansson G, Pettersson G. 1999. Endoglucanase 28 (Cel12A), a new *Phanerochaete chrysosporium* cellulase. Eur J Biochem 259:88–95, doi:10.1046/j.1432-1327.1999.00011.x

Hibbett DS, Binder M, Bischoff JF, Blackwell M, Cannon PF, Eriksson OE, Huhndorf S, James T, Kirk PM, Lücking R, et al. 2007. A higher-level phylogenetic classification of the Fungi. Mycol Res 111:509–547, doi:10.1016/j.mycres.2007.03.004

Higham CW, Gordon-Smith D, Dempsey CE, Wood PM. 1994. Direct ^{1}H-NMR evidence for conversion of β-NMR evidence for conversion of MψχoλO*Phanerochaete chrysosporium*. FEBS Lett 351:128–132, doi:10.1016/0014-5793(94)00847-7

Highley T. 1973. Influence of carbon source on cellulase activity of white-rot and brown-rot fungi. Wood Fiber 5:50–58.

Hori C, Igarashi K, Katayama A, Samejima M. 2011. Effects of xylan and starch on secretome of the basidiomycete *Phanerochaete chrysosporium* grown on cellulose. FEMS Microbiol Lett 31:14–23, doi:10.1111/j.1574-6968.2011.02307.x

Igarashi K, Ishida T, Hori C, Samejima M. 2008. Characterization of an endoglucanase belonging to a new subfamily of glycoside hydrolase family 45 of the basidiomycete *Phanerochaete chrysosporium*. Appl Environ Microbiol 74:5628–5634, doi:10.1128/AEM.00812-08

Katoh K, Kuma K, Toh H, Miyata T. 2005. MAFFT 5: improvement in accuracy of multiple sequence alignment. Nucl Acids Res 33:511–518, doi:10.1093/nar/gki198

———, Misawa K, Kuma K, Miyata T. 2002. MAFFT: a novel mothod for rapid multiple sequence alignment based on fast Fourier transform. Nucl Acids Res 30:3059–3066, doi:10.1093/nar/gkf436

Kirk K, Ibach R, Mozuch MD, Conner AH, Highley TL. 1991. Characteristics of cotton cellulose depolymerized by a brown-rot fungus, by acid or by chemical oxidants. Holzforschung 45:239–244, doi:10.1515/hfsg.1991.45.4.239

Konishi T, Kotake T, Soraya D, Matsuoka K, Koyama T, Kaneko S, Igarashi K, Samejima M, Tsumuraya Y. 2008. Properties of family 79 β-glucuronidases that hydrolyze β-glucuronosyl and 4-*O*-methyl-β-glucuronosyl residues of arabinogalactan-protein. Carbohydr Res 343:1191–1201, doi:10.1016/j.carres.2008.03.004

Langston JA, Shaghasi T, Abbate E, Xu F, Vlasenko E, Sweeney MD. 2011. Oxidoreductive cellulose depolymerization by the enzymes cellobiose dehydrogenase and glycoside hydrolase 61. Appl Environ Microbiol 77:7007–7015, doi:10.1128/AEM.05815-11

Levasseur A, Drula E, Lombard V, Coutinho PM, Henrissat B. 2013. Expansion of the enzymatic repertoire of the CAZy database to integrate auxiliary redox enzymes. Biotechnol Biofuels 6:41, doi:10.1186/1754-6834-6-41

Mahajan S, Master ER. 2010. Proteomic characterization of lignocellulose-degrading enzymes secreted by *Phanerochaete carnosa* grown on spruce and microcrystalline cellulose. Appl Microbiol Biotechnol 86:1903–1914, doi:10.1007/s00253-010-2516-4

Martinez D, Challacombe J, Morgenstern I, Hibbett D, Schmoll M, Kubicek CP, Ferreira P, Ruiz-Duenas FJ, Martinez AT, Kersten P. 2009. 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, doi:10.1073/pnas.0809575106

———, Larrondo LF, Putnam N, Gelpke MDS, Huang K, Chapman J, Helfenbein KG, Ramaiya P, Detter JC, Larimer F. 2004. Genome sequence of the lignocellulose-degrading fungus *Phanerochaete chrysosporium* strain RP78. Nat Biotechnol 22:695–700, doi:10.1038/nbt967

Nesvizhskii A, Keller A, Kolker E, Aebersold R. 2003. A statistical model for identifying proteins by tandem mass spectrometry. Anal Chem 75:4646–4658, doi:10.1021/ac0341261

Niemenmaa O, Uusi-Rauva A, Hatakka A. 2008. Demethoxylation of [O14CH3]-labeled lignin model compounds by the brown-rot fungi *Gloeophyllum trabeum* and *Poria* (*Postia*) *placenta*. Biodegradation 19:555–65, doi:10.1007/s10532-007-9161-3

Paszczynski A, Crawford R, Funk D, Goodell B. 1999. De novo synthesis of 4,5-dimethoxycatechol and 2, 5-dimethoxyhydroquinone by the brown-rot fungus *Gloeophyllum trabeum*. Appl Environ Microbiol 65: 674–9.

Phillips CM, Beeson IV WT, Cate JH, Marletta MA. 2011. Cellobiose dehydrogenase and a copper-dependent polysaccharide monooxygenase potentiate cellulose degradation by *Neurospora crassa*. ACS Chem Biol 6: 1399–1406, doi:10.1021/cb200351y

Quinlan RJ, Sweeney MD, Leggio LL, Otten H, Poulsen JCN, Johansen KS, Krogh BRM, Jogensen CI, Tovborg M, Athonsen A, et al. 2011. Insights into the oxidative degradation of cellulose by a copper metalloenzyme that exploits biomass components. Proc Natl Acad Sci USA 108:15079–15084, doi:10.1073/pnas.1105776108

Ravalason H, Jan G, Mollé D, Pasco M, Coutinho PM, Lapierre C, Pollet B, Bertaud F, Petit-Conil M, Grisel S, et al. 2008. Secretome analysis of *Phanerochaete chrysosporium* strain CIRM-BRFM41 grown on softwood. Appl Microbiol Biotechnol 80:719–733, doi:10.1007/s00253-008-1596-x

Reinikainen T, Teleman O, Teeri TT. 1995. Effects of pH and high ionic strength on the adsorption and activity of native and mutated cellobiohydrolase I from *Trichoderma reesei*. Proteins 2:392–403, doi:10.1002/prot.340220409

Sandgren M, Ståhlberg J, Mitchinson C. 2005. Structural and biochemical studies of GH family 12 cellulases: improved thermal stability and ligand complexes. Prog Biophys Mol Biol 89:246–291, doi:10.1016/j.pbiomolbio.2004.11.002

Sato S, Liu F, Koc H, Tien M. 2007. Expression analysis of extracellular proteins from *Phanerochaete chrysosporium* grown on different liquid and solid substrates. Microbiology 153:3023–2033, doi:10.1099/mic.0.2006/000513-0

Schwarze FWMR. 2007. Wood decay under the microscope. Fungal Biol Rev 21:133–170, doi:10.1016/j.fbr.2007.09.001

Spániková S, Biely P. 2006. Glucuronoyl esterase—novel carbohydrate esterase produced by *Schizophyllum commune*. FEBS Lett 580:4597–4601, doi:10.1016/j.febslet.2006.07.033

Stewart P, Gaskell J, Cullen D. 2000. A homokaryotic derivative of a *Phanerochaete chrysosporium* strain and its use in genomic analysis of repetitive elements. Appl Environ Microbiol 66:1629–1633, doi:10.1128/AEM.66.4.1629-1633.2000

Suzuki H, Macdonald J, Syed K, Salamov A, Hori C, Aerts A, Henrissat B, Wiebenga A, Vankuyk PA, Barry K et al. 2012. Comparative genomics of the white-rot fungi, *Phanerochaete carnosa* and *P. chrysosporium*, to elucidate the genetic basis of the distinct wood types they colonize. BMC Genomics 13:444, doi:10.1186/1471-2164-13-444

Suzuki MR, Hunt CG, Houtman CJ, Dalebroux ZD, Hammel KE. 2006. Fungal hydroquinones contribute to brown rot of wood. Environ Microbiol 8:2214–2223, doi:10.1111/j.1462-2920.2006.01160.x

Tello M, Seelenfreund D, Lobos S, Gaskell J, Cullen D, Vicuña R. 2001. Isolation and characterization of homokaryotic strains from the ligninolytic basidiomycete *Ceriporiopsis subvermispora*. FEMS Microbiol Lett 199:91–96, doi:10.1111/j.1574-6968.2001.tb10656.x

Tomme P, Tilbeurgh H, Pettersson G, Damme J, Vandekerckhove J, Knowles J, Teeri T, Claeyssens M. 1988. Studies of the cellulolytic system of *Trichoderma reesei* QM 9414. Analysis of domain function in two cellobiohydrolases by limited proteolysis. Eur J Biochem 170: 575–581, doi:10.1111/j.1432-1033.1988.tb13736.x

Vaaje-Kolstad G, Denman S, Dietrich D, Bassett J, Yu X, Atalla R, Predki P, Rudsander U, Teeri TT, Cullen D. 2002. Transcript analysis of genes encoding a family 61 endoglucanase and a putative membrane-anchored family 9 glycosyl hydrolase from *Phanerochaete chrysosporium*. Appl Environ Microbiol 68:5765–5768, doi:10.1128/AEM.68.11.5765-5768.2002

———, Gaskell J, Mozuch M, Bondurant SS, Sabat G, Ralph J, Skyba O, Mansfield SD, Blanchette RA, Grigoriev IV, et al. 2011. Significant alteration of gene expression in wood-decay fungi *Postia placenta* and *Phanerochaete chrysosporium* by plant species. Appl Environ Microbiol 77:4499–4507, doi:10.1128/AEM.00508-11

———, ———, ———, Sabat G, Ralph J, Skyba O, Mansfield SD, Blanchette RA, Martinez D, Grigoriev I, et al. 2010. Comparative transcriptome and secretome analysis of wood-decay fungi *Postia placenta* and

Phanerochaete chrysosporium. Appl Environ Microbiol 76:3599–3610, doi:10.1128/AEM.00058-10

———, Westereng B, Horn SJ, Liu Z, Zhai H, Sørlie M, Eijsink VGH. 2010. An oxidative enzyme boosting the enzymatic conversion of recalcitrant polysaccharides. Science 330:219–322, doi:10.1126/science.1192231

Vanden Wymelenberg A, Minges P, Sabat G, Martinez D, Aerts A, Salamov A, Grigoriev I, Shapiro H, Putnam N, Belinky P, et al. 2006. Computational analysis of the *Phanerochaete chrysosporium* 2.0 genome database and mass spectrometry identification of peptides in ligninolytic cultures reveal complex mixtures of secreted proteins. Fungal Genet Biol 43:343–356, doi:10.1016/j.fgb.2006.01.003

———, Sabat G, Martinez D, Rajangam AS, Teeri TT, Gaskell J, Kersten PJ, Cullen D. 2005. The *Phanerochaete chrysosporium* secretome: database predictions and initial mass spectrometry peptide identifications in cellulose-grown medium. J Biotechnol 118:17–34, doi:10.1016/j.jbiotec.2005.03.010

Westereng B, Ishida T, Vaaje-Kolstad G, Wu M, Eijsink VGH, Igarashi K, Samejima M, Ståhlberg J, Horn SJ, Sandgren M. 2011. The putative endoglucanase PcGH61D from *Phanerochaete chrysosporium* is a metal-dependent oxidative enzyme that cleaves cellulose. PLoS ONE 6(11):e27807, doi:10.1371/journal.pone.0027807

Worrall JJ, Anagnost SE, Zabel RA. 1997. Comparison of wood decay among diverse lignicolous fungi. Mycologia 89:199–219, doi:10.2307/3761073

Xu G, Goodell B. 2001. Mechanisms of wood degradation by brown-rot fungi: chelator-mediated cellulose degradation and binding of iron by cellulose. J Biotechnol 87:43–57, doi:10.1016/S0168-1656(00)00430-2

Yelle DJ, Ralph J, Lu F, Hammel KE. 2008. Evidence for cleavage of lignin by a brown-rot basidiomycete. Environ Microbiol 10:1844–1849, doi:10.1111/j.1462-2920.2008.01605.x

———, Wei D, Ralph J, Hammel KE. 2011. Multidimensional NMR analysis reveals truncated lignin structures in wood decayed by the brown-rot basidiomycete *Postia placenta*. Environ Microbiol 13:1091–100, doi:10.1111/j.1462-2920.2010.02417.x

Yoshida M, Igarashi K, Wada M, Kaneko S, Suzuki N, Matsumura H, Nakamura N, Ohno H, Samejima M. 2005. Characterization of carbohydrate-binding cytochrome *b*562 from the white-rot fungus *Phanerochaete chrysosporium*. Appl Environ Microbiol 71:4548–4555, doi:10.1128/AEM.71.8.4548-4555.2005