# Comparative genomics of *Ceriporiopsis subvermispora* and *Phanerochaete chrysosporium* provide insight into selective ligninolysis

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**Efficient lignin depolymerization is unique to the wood decay basidiomycetes, collectively referred to as white rot fungi. *Phanerochaete chrysosporium* simultaneously degrades lignin and cellulose, whereas the closely related species, *Ceriporiopsis subvermispora,* also depolymerizes lignin but may do so with relatively little cellulose degradation. To investigate the basis for selective ligninolysis, we conducted comparative genome analysis of *C. subvermispora* and *P. chrysosporium*. Genes encoding manganese peroxidase numbered 13 and five in *C. subvermispora* and *P. chrysosporium*, respectively. In addition, the *C. subvermispora* genome contains at least seven genes predicted to encode laccases, whereas the *P. chrysosporium* genome contains none. We also observed expansion of the number of *C. subvermispora* desaturase-encoding genes putatively involved in lipid metabolism. Microarray-based transcriptome analysis showed substantial upregulation of several desaturase and MnP genes in wood-containing medium. MS identified MnP proteins in *C. subvermispora* culture filtrates, but none in *P. chrysosporium* cultures. These results support the importance of MnP and a lignin degradation mechanism whereby cleavage of the dominant nonphenolic structures is mediated by lipid peroxidation products. Two *C. subvermispora* genes were predicted to encode peroxidases structurally similar to *P. chrysosporium* lignin peroxidase and, following heterologous expression in *Escherichia coli*, the enzymes were shown to oxidize high redox potential substrates, but not $Mn^{2+}$. Apart from oxidative lignin degradation, we also examined cellulolytic and hemicellulolytic systems in both fungi. In summary, the *C. subvermispora* genetic inventory and expression patterns exhibit increased oxidoreductase potential and diminished cellulolytic capability relative to *P. chrysosporium*.**

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Data deposition: The annotated genome is available on an interactive web portal, http://jgi.doe.gov/Ceriporiopsis and at DNA Data Base in Japan/European Molecular Biology Laboratory (DDBJ/EMBL)/GenBank (project accession no. AEOV00000000). The data reported in this paper have been deposited in the Gene Expression Omnibus (GEO) database, www.ncbi.nlm.nih.gov/geo (accession no. GSE34636).

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The most abundant source of photosynthetically fixed carbon in land ecosystems is plant biomass, composed primarily of cellulose, hemicellulose, and lignin. Many microorganisms are capable of using cellulose and hemicellulose as carbon and energy sources, but a much smaller group of filamentous fungi in the phylum Basidiomycota has also evolved with the unique ability to efficiently depolymerize and mineralize lignin, the most recalcitrant component of plant cell walls. Collectively known as white rot fungi, they remove lignin to gain access to cell wall carbohydrates for carbon and energy sources. These wood-decay fungi are common inhabitants of fallen trees and forest litter. As such, white rot fungi play a pivotal role in the carbon cycle. Their unique metabolic capabilities are of considerable recent interest in bioenergy-related processes (1).

White rot basidiomycetes differ in their gross morphological patterns of decay (ref. 2 and refs. therein). *Phanerochaete chrysosporium* simultaneously degrades cellulose, hemicellulose, and lignin, whereas a few others such as the closely related polypore species, *Ceriporiopsis subvermispora*, have the ability to remove lignin in advance of cellulose. The mechanistic basis of this selectivity is unknown.

The roles of *P. chrysosporium* lignin peroxidase [LiP; Enzyme Commission (EC) 1.11.1.14] and manganese peroxidase (EC 1.11.1.13) have been intensively studied (3). Reactions catalyzed by LiP include $C_\alpha$-$C_\beta$ cleavage of propyl side chains in lignin and lignin models, hydroxylation of benzylic methylene groups, oxidation of benzyl alcohols to the corresponding aldehydes or ketones, phenol oxidation, and aromatic ring cleavage in nonphenolic lignin model compounds. In addition to *P. chrysosporium*, multiple ligninolytic peroxidase isozymes and their corresponding genes have been identified in several efficient lignin-degrading fungi (4). In some white rot fungi, such as the oyster mushroom *Pleurotus ostreatus* and related species, LiP is absent, but a third ligninolytic peroxidase type that combines LiP and MnP catalytic properties, versatile peroxidase (VP; EC 1.11.1.16), has been characterized (4, 5) and identified by genome analysis (6). Repeated and systematic attempts have failed to identify LiP (or VP) activity in *C. subvermispora* cultures, but substantial evidence implicates MnP in ligninolysis (e.g., refs 7, 8). First discovered in *P. chrysosporium* cultures, this enzyme oxidizes $Mn^{2+}$ to $Mn^{3+}$, using $H_2O_2$ as an oxidant (9, 10). MnP cannot directly cleave the dominant nonphenolic structures within lignin, but it has been suggested that oxidation may be mediated by lipid peroxidation mechanisms that are promoted by $Mn^{3+}$ (3).

In addition to peroxidases, laccases (EC 1.10.3.2) have been implicated in lignin degradation. Several have been characterized from *C. subvermispora* cultures (11), whereas no genes encoding laccase, in the strict sense, are present in the *P. chrysosporium* genome (12). The mechanism by which laccases might degrade lignin remains unclear, as the enzyme lacks sufficient oxidation potential to cleave nonphenolic linkages within the polymer. However, various mediators have been proposed (13).

Other components commonly ascribed to ligninolytic systems include extracellular enzymes capable of generating hydrogen peroxide. Glucose–methanol–choline oxidoreductases such as aryl-alcohol oxidase, methanol oxidase and pyranose oxidase, together with copper radical oxidases such as glyoxal oxidase, have been characterized in *P. chrysosporium* (14), but none of these activities have been reported in *C. subvermispora* cultures.

Conceivably, selective lignin degradation patterns may involve modulation of the hydrolytic enzymes commonly associated with cellulose and hemicellulose degradation. These systems are well characterized in *P. chrysosporium*, whereas little is known about *C. subvermispora* glycoside hydrolases (GHs) (15).

To further our understanding of selective ligninolysis, we report here initial analysis of the *C. subvermispora* genome. Comparison with the genome, transcriptome, and secretome of *P. chrysosporium* reveal substantial differences among the genes that are likely to be involved in lignocellulose degradation, providing insight into diversification of the white rot mechanism.

## Results

**General Features of *C. subvermispora* Genome.** The 39-Mb haploid genome of *C. subvermispora* monokaryotic strain B (16) (*SI Appendix*, Fig. S1) is predicted to encode 12,125 proteins (*SI Appendix* provides detailed assembly and annotation information). For comparison, the latest release of the related polypore white rot fungus *P. chrysosporium* features 35.1 Mb of nonredundant sequence and 10,048 gene models (12, 17). The overall relatedness of these polypore fungi was clearly evident from the syntenic regions between their largest scaffolds and large number of similar (BLAST E-values $<10^{-5}$) protein sequences, i.e., 74% ($n$ = 9,007) of *C. subvermispora* models aligned with *P. chrysosporium* and 82% ($n$ = 8,258) of *P. chrysosporium* models aligned with *C. subvermispora*. Most ($n$ = 5,443) of these pairs were also reciprocal "best hits" and are thus likely to represent orthologues. Significant expansions compared with *P. chrysosporium* and/or other sequenced Agaricomycetes were observed in transporters, various oxidoreductases including peroxidases, cytochrome p450s, and other gene families discussed here.

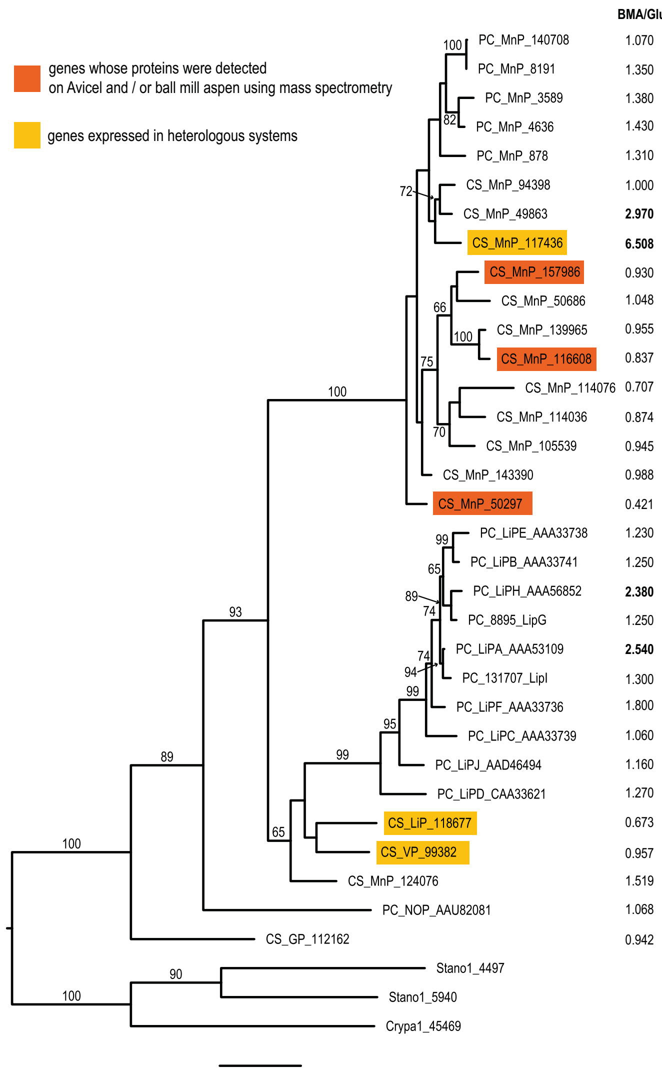


**Fig. 1.** Phylogenetic analysis of selected peroxidases from *C. subvermispora* and *P. chrysosporium*. The analysis was performed in RAxML Blackbox under the model GTRGAMMA, using the substitution matrix WAG with 100 rapid bootstrap replicates. The ascomycete sequences of class II peroxidases were used to root the tree (http://phylobench.vital-it.ch/raxml-bb/) (32). Ball-milled aspen versus glucose transcript ratios (BMA/Glu) are indicated, and complete data are available under Gene Expression Omnibus accession nos. GSE1473 and GSE34636 for *P. chrysosporium* and *C. subvermispora*, respectively.

**Peroxidases.** Twenty-six *C. subvermispora* gene models are predicted to encode heme peroxidases. Fifteen were classified as probable ligninolytic peroxidases, which included 13 MnPs, a VP, and an LiP. These classifications were based on homology modeling (18) with particular attention to conserved $Mn^{2+}$ oxidation and catalytic tryptophan sites (19, 20). Those classified as MnPs include seven typical "long" MnPs specific for $Mn^{2+}$, and a "short" MnP also able to oxidize phenols and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate) in the absence of $Mn^{2+}$, as previously reported in the *P. ostreatus* genome (6). The remaining five could be classified as "extra long" MnPs in view of their long C-termini, as reported for the first time in *Dichomitus squalens* MnPs (21). Only four full-length MnP-encoding genes were previously identified in *C. subvermispora* (GenBank accession nos. AAB03480, AAB92247, AAO61784, and AF161585). Additional class II peroxidases have long been suspected (22, 23), but no LiP/VP-like transcripts or activities have been identified. Thus, the repertoire of *C. subvermispora* peroxidases differs from *P. chrysosporium*, which features 10 LiP and five MnP genes (Fig. 1). Extending comparative analysis to 90 basidiomycete peroxidases (*SI Appendix*, Fig. S3) suggested that the *C. subvermispora* VP and LiP represent divergent proteins, an observation consistent with their catalytic properties (as detailed later).

By using a previously developed *Escherichia coli* expression system including in vitro activation (24, 25), the *C. subvermispora* putative LiP (Cesubv118677) and VP (Cesubv99382) were evaluated for their oxidation of three representative substrates, namely $Mn^{2+}$, the high redox-potential veratryl alcohol (VA), and Reactive Black 5 (RB5) (Table 1). The corresponding steady-state kinetic constants were compared with those of *Pleurotus eryngii* VP (isozyme VPL; AF007244), a *P. chrysosporium* LiP (isozyme H8; GenBank accession no. Y00262), and a conventional *C. subvermispora* MnP (Cesubv117436; Fig. 1) also produced in *E. coli*. The putative *C. subvermispora* LiP (protein model Cesubv118677) was unable to oxidize $Mn^{2+}$, as expected given the absence of a typical manganese oxidation site in its theoretical molecular structure (*SI Appendix*, Fig. S2). A conventional *C. subvermispora* MnP protein (Cesubv117436), also predicted based on structure, and the VP from *P. eryngii* showed $Mn^{2+}$ oxidation. Surprisingly, the *C. subvermispora* protein designated Cesubv99382, which we tentatively classified as a VP, was not able to oxidize $Mn^{2+}$, irrespective of the presence of a putative manganese oxidation site in its structural model (*SI Appendix*, Fig. S2). The catalytic behaviors of Cesubv99382 and Cesubv118677 are very similar. Both enzymes oxidize VA, the typical LiP (and VP) substrate, and also RB5, a characteristic substrate of VP (that LiP is unable to oxidize in the absence of mediators), with similar $K_m$, $k_{cat}$, and $k_{cat}/K_m$ values (Table 1).

Peroxidase expression patterns differed significantly between *C. subvermispora* and *P. chrysosporium*. In medium containing ball-milled *Populus grandidentata* (aspen) as sole carbon source, transcript levels of two *C. subvermispora* MnPs were significantly up-regulated relative to glucose medium. Liquid chromatography/tandem MS (LC-MS/MS) analysis of culture filtrates identified peptides corresponding to three *C. subvermispora* MnP genes (Fig. 1). In identical media, none of the *P. chrysosporium* MnP genes were up-regulated, but significant accumulation of two LiP gene transcripts was observed relative to glucose (Fig. 1). No peroxidases were identified by LC-MS/MS analysis of *P. chrysosporium* culture filtrates.

**Multicopper Oxidases.** Nine multicopper (MCO)-encoding *C. subvermispora* genes may be relevant to lignin degradation. Multiple alignments emphasizing signature regions (26, 27) revealed the presence of seven laccases, in the strictest sense, one of which was previously known (28). This observation is in distinct contrast to the *P. chrysosporium* genome, which contains no laccases (12) (Fig. 2). Consistent with a role in lignocellulose modification, transcript levels corresponding to *C. subvermispora* laccase was significantly up-regulated (more than threefold; $P < 0.01$) in media containing ball-milled *P. grandidentata* wood (aspen) relative to glucose medium (Fig. 2).

In addition to the laccases, *C. subvermispora* MCO-encoding genes included a canonical ferroxidase (Fet3). Involved in high-affinity iron uptake, the Fet3 genes of *C. subvermispora* (Cesubv67172) and *Postia placenta* (Pospl129808) show significant up-regulation on aspen-containing medium, whereas the *P. chrysosporium* orthologue (Phchr26890) is sharply down-regulated under identical conditions (Fig. 2). This strongly suggests that iron homeostasis is achieved by different mechanisms in these fungi.

**Other Enzymes Potentially Involved in Extracellular Redox Processes.** Peroxide and free radical generation are considered key components of ligninolysis, and analysis of the *C. subvermispora* genome, transcriptome, and secretome revealed a diverse array of relevant proteins. These included four copper radical oxidases, cellobiose dehydrogenase, various other glucose–methanol–choline oxidoreductases, and several putative transporters. Possibly related to selectivity of ligninolysis, expression patterns exhibited by certain genes, e.g., methanol oxidase, differed significantly between *P. chrysosporium* and *C. subvermispora*. (*SI Appendix* and *SI Appendix*, Table S1, include detailed listings of all annotated genes, transcript levels, and LC-MS/MS identification of extracellular proteins.)

Of particular relevance to lignin degradation by MnP, we observed a significant expansion of the genes putatively involved in fatty acid metabolism (Table 2). Relative to the single gene in *P. chrysosporium* (encoding Phchr125220) the Δ-12 fatty acid desaturase gene family was particularly expanded (five paralogues) in *C. subvermispora*. The *P. chrysosporium* and *C. subvermispora*

**Table 1. Steady-state kinetic constants of three peroxidases from *C. subvermispora* genome vs. *P. chrysosporium* LiP and *P. eryngii* VP**

| Constant | *C. subvermispora* 99382 ("VP") | 118677 (LiP) | 117436 (MnP) | *P. chrysosporium* Y00262 (LiPH8) | *P. eryngii* AF007244 (VPL) |
|---|---|---|---|---|---|
| $Mn^{2+}$ | | | | | |
| $K_m$, μM | ND [b] | ND | 58.5 ± 8.5 | ND | 181 ± 10 |
| $k_{cat}$, $s^{-1}$ | 0 | 0 | 331 ± 20 | 0 | 275 ± 4 |
| $k_{cat}/K_m$, $mM^{-1}\cdot s^{-1}$ | 0 | 0 | 5,600 ± 500 | 0 | 1,520 ± 70 |
| VA | | | | | |
| $K_m$, μM | 3,120 ± 526 | 1,620 ± 290 | ND | 190 ± 17 | 4,130 ± 320 |
| $k_{cat}$, $s^{-1}$ | 8.6 ± 0.7 | 8.7 ± 0.6 | 0 | 17.5 ± 0.5 | 9.5 ± 0.2 |
| $k_{cat}/K_m$, $mM^{-1}\cdot s^{-1}$ | 2.8 ± 0.3 | 5.4 ± 0.7 | 0 | 92.0 ± 6.0 | 2.3 ± 0.1 |
| RB5 | | | | | |
| $K_m$, μM | 3.97 ± 0.65 | 4.48 ± 0.64 | ND | ND | 3.4 ± 0.3 |
| $k_{cat}$, $s^{-1}$ | 9.8 ± 0.9 | 7.3 ± 0.5 | 0 | 0 | 5.5 ± 0.3 |
| $k_{cat}/K_m$, $mM^{-1}\cdot s^{-1}$ | 2,460 ± 185 | 1,620 ± 138 | 0 | 0 | 1,310 ± 90 |

Reactions were at 25 °C in 0.1 M tartrate (pH 3 for VA, pH 3.5 for RB5, and pH 5 for $Mn^{2+}$). ND, not determined because of lack of activity. Means and 95% SEM are provided.

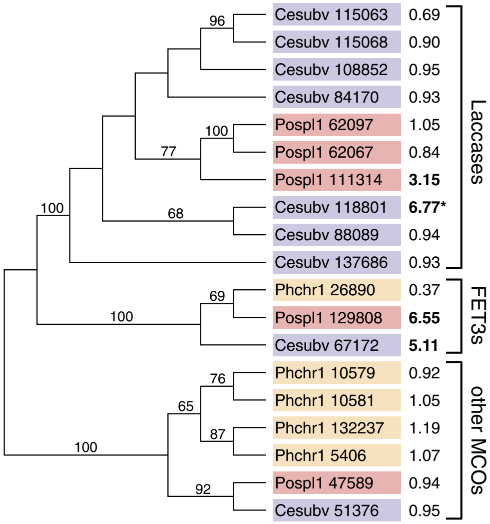


**Fig. 2.** Phylogenetic analysis of all MCO oxidases from *C. subvermispora*, *P. chrysosporium*, and the related polypore *P. placenta*. Analysis was performed by using RAxML with the WAG substitution matrix, γ-distributed rates among sites, a proportion of invariant sites and empirical amino acid frequencies (i.e., m = PROTGAMMAIWAGF). Shown is the maximum-likelihood tree found by using 1,000 heuristic searches, with bootstrap support shown for nodes with values greater than 50%. As in Fig. 1, transcript level ratios are adjacent to protein identification numbers. Complete *P. placenta* microarray data are available under Gene Expression Omnibus accession no. GSE12540 (33).

genes were previously designated *Pcfad2* and *Csfad2* (29, 30), respectively. Transcript levels of *P. chrysosporium Pcfad2* were significantly reduced (0.25-fold; $P < 0.01$) in media with aspen relative to glucose, whereas a *C. subvermispora* Δ-12 fatty acid desaturase (Cesubv124119) was up-regulated (2.9-fold; $P < 0.01$). With regard to Δ-9 fatty acid desaturases, only two *P. chrysosporium* genes were detected and, as in the case of Δ-12 fatty acid synthetases, both were down-regulated more than twofold ($P < 0.01$). Modest transcript accumulation (1.48-fold; $P = 0.03$) was observed for one of the four *C. subvermispora* Δ-9 fatty acid desaturases (Cesubv117066) in aspen wood media relative to glucose media. Increased numbers of MnP and lipid metabolism genes, viewed together with their expression patterns, are consistent with an important role for peroxyl radical attack on nonphenolic substructures of lignin.

**Carbohydrate Active Enzymes.** Overall, the number of GHs encoded by the *C. subvermispora* genome is slightly lower than that of other plant cell wall degrading basidiomycetes whose genomes have been sequenced (Dataset S1 and *SI Appendix*, Table S1). The number of GHs in *C. subvermispora* ($n$ = 171) is close to that in *P. chrysosporium* ($n$ = 177), and noticeably different in total number and in family distribution compared with the phylogenetically related brown rot fungus *P. placenta* ($n$ = 145; Fig. 3). Differences between *C. subvermispora* and *P. chrysosporium* are limited to a few families, but these distinctions might have consequences for degradation of plant cell wall polysaccharides. For example, *C. subvermispora* contained only three predicted proteins belonging to family GH7, an important group typically featuring "exo" cellobiohydrolases. In contrast, at least six GH7 protein models were identified in the *P. chrysosporium* genome. Family GH3, containing β-glucosidases involved in the hydrolysis of cellobiose, was represented by only six gene models in the *C. subvermispora* genome, unlike the 11 GH3 models found in *P. chrysosporium*. In addition, the *C. subvermispora* genome revealed only 16 cellulose binding modules (CBM1s), compared with 31 CBM1-containing protein models found in the *P. chrysosporium* genome.

In contrast to the oxidative systems, transcriptome and secretome analysis of GHs generally showed lower expression in *C. subvermispora* relative to *P. chrysosporium* (Table 3 and *SI Appendix*, Table S1). Transcripts corresponding to 30 *C. subvermispora* GH-encoding genes accumulated more than twofold ($P < 0.05$) in aspen wood- vs. glucose-containing media. In contrast, 52 *P. chrysosporium* GH-encoding genes were up-regulated (more than twofold; $P < 0.05$). MS unambiguously identified 60 and 121 proteins in filtrates from aspen wood media of *P. chrysosporium* and *C. subvermispora* cultures, respectively, among which 18 and three, respectively, corresponded to GHs.

Genes encoding likely cellulases showed only modest transcript levels in *C. subvermispora* (Table 3). *C. subvermispora* transcripts corresponding to single copies of a CBM1-containing cellobiohydrolase (GH7), a CBM1-containing endo-β-1,4-glucanase (GH5), and a GH12 endoglucanase, all canonical cellulases, were significantly up-regulated (more than twofold; $P < 0.01$) in aspen wood relative to glucose media. Under identical conditions, accumulating *P. chrysosporium* transcripts included four GH7 cellobiohydrolases, two GH5 endo-β-1,4-glucanases, and two GH12 endoglucanases (Table 3).

The foregoing analysis is limited to expression patterns of genes with putative function inferred from sequence comparisons. However, many of the predicted proteins that show no significant sequence similarity to known proteins could be important in selective ligninolysis. Specifically, we identified 139 "hypothetical" *C. subvermispora* proteins whose sequences show

**Table 2. Number, overall relatedness, and transcript levels of genes putatively involved in lipid metabolism**

| *C. subvermispora* | | | | | | *P. chrysosporium* | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Protein ID | Glc | BMA | B/G | *P* value | Comment | Protein ID | E-value | ID, % | Glc | BMA | B/G | *P* value |
| Δ-12 fatty acid desaturase (COG 3239) | | | | | | | | | | | | |
| 124119 | 11.01 | 12.54 | 2.90* | < 0.01 | — | 125220 | $1.00 \times 10^{-67}$ | 72 | 12.76 | 10.77 | 0.25* | <0.01 |
| 58880 | 10.36 | 10.29 | 0.96 | 0.729 | — | 125220 | $3.00 \times 10^{-70}$ | 72 | 12.76 | 10.77 | 0.25* | <0.01 |
| 109092 | 10.58 | 10.23 | 0.78 | 0.0149 | — | 125220 | $5.00 \times 10^{-59}$ | 72 | 12.76 | 10.77 | 0.25* | <0.01 |
| 155708 | 10.67 | 10.11 | 0.68 | < 0.01 | — | 125220 | $2.00 \times 10^{-77}$ | 72 | 12.76 | 10.77 | 0.25* | <0.01 |
| 112068 | 12.74 | 12.66 | 0.94 | 0.653 | *Csfad2* (29); *Pcfad2* (30) | 125220 | 0.00 | 72 | 12.76 | 10.77 | 0.25* | <0.01 |
| Δ-9 fatty acid desaturase (COG 1398) | | | | | | | | | | | | |
| 117066 | 11.78 | 12.35 | 1.48 | 0.0298 | *CsOle1* & *PcOle1* (29) | 128650 | 0.00 | 81 | 13.82 | 12.48 | 0.40* | <0.01 |
| 87875 | 8.93 | 8.94 | 1.01 | 0.88 | — | 121154 | $2.00 \times 10^{-68}$ | 33 | 13.49 | 12.38 | 0.46* | 0.017 |
| 117063 | 8.95 | 8.91 | 0.97 | 0.527 | 5′ needs editing | 121154 | $2.00 \times 10^{-62}$ | 33 | 13.49 | 12.38 | 0.46* | 0.017 |
| 121693 | 9.64 | 9.51 | 0.92 | 0.179 | — | 121154 | $1.00 \times 10^{-154}$ | 33 | 13.49 | 12.38 | 0.46* | 0.017 |

Normalized microarray data are presented as $\log_2$ signal strength average of fully replicated experiments. Significant accumulation (B/G ratio) of transcripts in BMA relative to glucose-grown (Glc) cultures was determined using the Moderated $t$ test and associated FDR. See Gene Expression Omnibus accession no. GSE14736 (33) for *P. chrysosporium* data. Both gene families are expanded in *C. subvermispora* relative to *P. chrysosporium*. BMA, ball-milled aspen; COG, clusters of orthologous groups; FDR, false detection rate.
*Significant ratio (<0.5-fold to >2-fold).

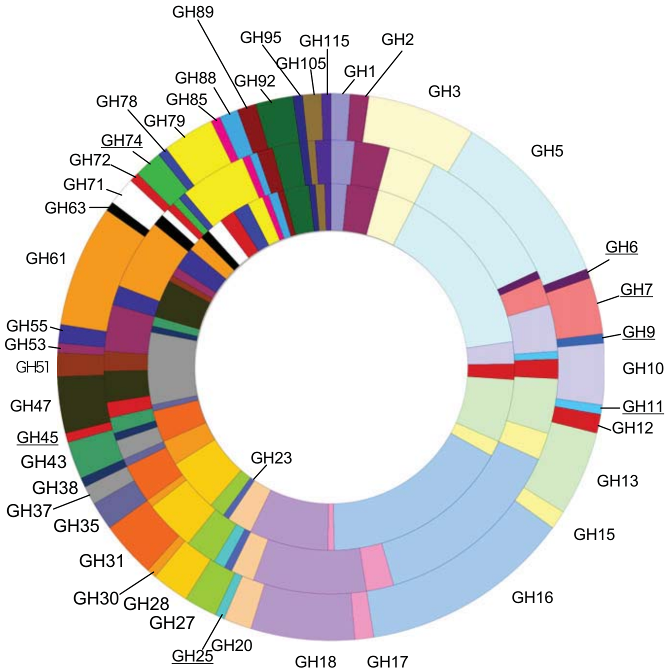


**Fig. 3.** Distribution of GHs in *P. placenta* (inner ring), *C. subvermispora* (middle ring), and *P. chrysosporium* (outer ring). Families absent from at least one species are underlined. Detailed listings of gene numbers within these and other species appear in Dataset S1, and expression patterns (transcript and protein) are presented in *SI Appendix*, Table S1.

no significant similarity to *P. chrysosporium* models but were otherwise highly expressed, i.e., transcript levels more than two SDs above the genome-wide mean ($n = 12084$, $X = 10.56$) or more than twofold transcript accumulation in aspen wood media vs. glucose or unambiguously identified via MS (at least two unique peptide sequences).

## Discussion

*C. subvermispora* and *P. chrysosporium* are both members of the order Polyporales, but they differ sharply in their ability to selectively degrade lignin. The genetics and physiology of *P. chrysosporium* have been intensively studied for decades. Largely because of its efficient degradation of plant cell walls, including the recalcitrant lignin, *P. chrysosporium* was selected as the first sequenced basidiomycete (12). In contrast, *C. subvermispora* has received less attention, although its selective lignin degradation is well known (2). Overall, our comparisons of *C. subvermispora* and *P. chrysosporium* gene repertoires, together with expression patterns on a complex lignocellulose substrate, suggest divergent strategies of plant cell wall degradation and provide clues about mechanisms of selective delignification.

Generally accepted as important components of lignin degradation systems, class II peroxidases were skewed toward expansion of the number of MnPs and accompanied by a putative LiP (Cesubv118677) and a VP (Cesubv99382). To confirm these predictions, both peroxidases were obtained by *E. coli* expression, and their steady-state kinetic constants for oxidation of selected peroxidase substrates were compared with those of a typical MnP from the *C. subvermispora* genome (Cesubv117436), a well characterized VP from *P. eryngii* (GenBank AF007244), and the well studied *P. chrysosporium* LiP isozyme H8 (all expressed in *E. coli*). Cesubv118677 and Cesubv99382 are able to directly oxidize VA and RB5, a unique characteristic of VP, exhibiting similar catalytic efficiency values to those observed for typical VPs. Moreover, both peroxidases are unable to oxidize $Mn^{2+}$, despite the presence in Cesubv99382 of a putative oxidation site for this cation. Thus, considering their sequences (Fig. 1 and *SI Appendix*) and catalytic activities (Table 1), these two peroxidases seem to represent an intermediate evolutionary state between LiP and VP.

In addition to the distinct repertoire of class II peroxidases, selective ligninolysis of *C. subvermispora* may be related, in part, to the expansion and coexpression of the genes putatively involved in lipid metabolism. Substantial evidence implicates MnP involvement (7, 8) in lignin degradation, but this enzyme cannot directly cleave the dominant nonphenolic structures within lignin. Nevertheless, several studies support mechanisms involving peroxidation of lipids (3). The expansion of *C. subvermispora* desaturase and MnP gene families, together with their high ex-

**Table 3. Expression of *C. subvermispora* and *P. chrysosporium* cellulases**

| | *C. subvermispora* | | | | | | | *P. chrysosporium* | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | LC-MS/MS (unique peptides)† | | Microarrays* | | | | | LC-MS/MS (unique peptides)† | | Microarrays* | | | |
| | | | | Signal ($\log_2$) | | | | | | | Signal ($\log_2$) | | | |
| Putative activity/family | ID no. | Glc | BMA | Glc | BMA | B/G ratio | *P* value | ID no. | Glu | BMA | Glu | BMA | B/G ratio | *P* value |
| CBH1/GH7 | 136606 | — | — | 11.0 | 12.6 | 3.02‡ | <0.01 | 126964 | — | — | 10.6 | 10.7 | 1.08 | 0.45 |
| CBH1/GH7 | 89943 | — | 1 | 8.84 | 8.96 | 1.09 | 0.09 | 137042 | — | — | 10.1 | 10.3 | 1.13 | 0.18 |
| CBH1/GH7 | 109983 | — | — | 9.09 | 9.03 | 0.96 | 0.32 | 127029 | — | 3‡ | 10.3 | 12.1 | 3.53‡ | <0.01 |
| CBH1/GH7 | — | — | — | — | — | — | — | 137372 | — | 5‡ | 9.6 | 12.8 | 9.18‡ | <0.01 |
| CBH1/GH7 | — | — | — | — | — | — | — | 129072 | — | — | 10.4 | 12.2 | 3.40‡ | <0.01 |
| CBH1/GH7 | — | — | — | — | — | — | — | 137216 | — | — | 10.2 | 14.5 | 19.6‡ | <0.01 |
| CBH2/GH6§ | 72777 | — | 2‡ | — | — | — | — | 133052 | — | 2‡ | 11.8 | 15.3 | 11.5‡ | <0.01 |
| EG/GH5 | 79557 | — | — | 10.2 | 14.0 | 13.9‡ | <0.01 | 6458 | — | — | 12.1 | 14.8 | 6.46‡ | <0.01 |
| EG/GH5 | 117046 | — | — | 9.8 | 10.8 | 1.99 | 0.02 | 4361 | — | 2‡ | 10.5 | 14.1 | 12.2‡ | <0.01 |
| EG/GH12 | 34428 | — | — | 8.95 | 10.9 | 3.81‡ | <0.01 | 8466 | — | 2‡ | 11.4 | 14.0 | 5.94‡ | <0.01 |
| EG/GH12 | 111819 | — | — | 9.75 | 10.0 | 1.20 | 0.07 | 7048 | — | 3‡ | 12.1 | 15.1 | 8.16‡ | <0.01 |

BMA, ball-milled aspen; FDR, false detection rate; Glc, glucose.

*As in Table 2, normalized microarray data are presented as $\log_2$ signal strength average of three fully replicated experiments. Significant accumulation (B/G ratio) of transcripts in BMA relative to glucose grown cultures was determined using the moderated *t* test and associated FDR.

†Number of unique peptides detected by LC-MS/MS after 5 d growth on BMA or glucose medium. Complete microarray and LC-MS/MS results are listed in *SI Appendix*, Table S1. For detailed *P. chrysosporium* microarray and LC-MS/MS data, see refs. 33 and 31, respectively.

‡Significant ratio and/or peptide score.

§Initial microarrays did not feature probes for the *C. subvermispora* gene encoding GH6 (protein model Cesubv72777), but multiple ESTs and the presence of detectable peptides show the gene is expressed, and likely at substantial levels.

pression levels relative to *P. chrysosporium* (Table 2 and Fig. 1), are consistent with a role in lignin degradation.

Overall numbers and family distributions of GH-encoding genes were similar between *C. subvermispora* and *P. chrysosporium* (Fig. 3), but subtle differences in number and expression were noted. Among the cellulases, cellobiohydrolases (*cel7s*) and endoglucanases (*cel5s* and *cel12s*) were particularly notable in their transcript and protein accumulation in *P. chrysosporium* cultures (Table 3). In contrast, expression of the *C. subvermispora* cellulolytic system was substantially lower than *P. chrysosporium*, whereas the converse was observed for enzymes important in extracellular oxidative systems (Figs. 1 and 2, Table 2, and *SI Appendix*, Table S1).

These observations provide functional models that may explain the shift toward selective ligninolysis by *C. subvermispora*. Definitive mechanisms remain uncertain, but our investigations identify a subset of potentially important genes, including those encoding hypothetical proteins. More detailed functional analysis is complicated by the insoluble nature of lignocellulose substrates and by the slow, asynchronous hyphal growth of lignin degrading fungi. Direct and persuasive proof of gene function would be aided by development of experimental tools such as gene disruption/suppression or isozyme-specific immunolocalization of secreted proteins.

## Methods

**Genome Sequencing, Assembly, and Annotation.** A whole genome shotgun approach was used to sequence *C. subvermispora* monokaryotic strain B (16) (US Department of Agriculture Forest Mycology Center, Madison, WI). Assembly and annotations are available through interactive visualization and analysis tools from the Joint Genome Institute genome portal (http://www.jgi.doe.gov/Ceriporiopsis) and at DNA Data Base in Japan/European Molecular Biology Laboratory/GenBank under project accession no. AEOV00000000. Details regarding the assembly, repetitive elements (Dataset S2), ESTs annotation, and specific gene sets are provided separately (*SI Appendix*, Figs. S1–S6).

**MS.** Soluble extracellular proteins were concentrated from *C. subvermispora* cultures containing ball-milled aspen as previously described for *P. chrysosporium* (31) This medium allows rapid growth on a lignocellulose substrate more relevant than glucose- or cellulose-containing media. However, the milling process pulverizes wood cell walls and the culture conditions may not replicate "natural" decay processes. Sample preparation and nano-LC-MS/MS analyses were performed as described in *SI Appendix*. Peptides were identified by using a Mascot search engine (Matrix Science) against protein sequences of 12,125 predicted gene models described earlier. Complete listings of carbohydrate active enzymes and oxidative enzymes, including peptide sequences and scores, are provided in *SI Appendix*, Table S1.

**Expression Microarrays.** NimbleGen arrays (Roche) were designed to assess expression of 12,084 genes during growth on ball-milled aspen (*P. grandidentata*) or on glucose as sole carbon sources. Methods are detailed in *SI Appendix*, and all data deposited under Gene Expression Omnibus accession no. GSE34636.

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# Correction

## MICROBIOLOGY

Correction for "Comparative genomics of *Ceriporiopsis subvermispora* and *Phanerochaete chrysosporium* provide insight into selective ligninolysis," by Elena Fernandez-Fueyo, Francisco J. Ruiz-Dueñas, Patricia Ferreira, Dimitrios Floudas, David S. Hibbett, Paulo Canessa, Luis F. Larrondo, Tim Y. James, Daniela Seelenfreund, Sergio Lobos, Rubén Polanco, Mario Tello, Yoichi Honda, Takahito Watanabe, Takashi Watanabe, Ryu Jae San, Christian P. Kubicek, Monika Schmoll, Jill Gaskell, Kenneth E. Hammel, Franz J. St. John, Amber Vanden Wymelenberg, Grzegorz Sabat, Sandra Splinter BonDurant, Khajamohiddin Syed, Jagjit S. Yadav, Harshavardhan Doddapaneni, Venkataramanan Subramanian, José L. Lavín, José A. Oguiza, Gumer Perez, Antonio G. Pisabarro, Lucia Ramirez, Francisco Santoyo, Emma Master, Pedro M. Coutinho, Bernard Henrissat, Vincent Lombard, Jon Karl Magnuson, Ursula Kües, Chiaki Hori, Kiyohiko Igarashi, Masahiro Samejima, Benjamin W. Held, Kerrie W. Barry, Kurt M. LaButti, Alla Lapidus, Erika A. Lindquist, Susan M. Lucas, Robert Riley, Asaf A. Salamov, Dirk Hoffmeister, Daniel Schwenk, Yitzhak Hadar, Oded Yarden, Ronald P. de Vries, Ad Wiebenga, Jan Stenlid, Daniel Eastwood, Igor V. Grigoriev, Randy M. Berka, Robert A. Blanchette, Phil Kersten, Angel T. Martinez, Rafael Vicuna, and Dan Cullen, which appeared in issue 14, April 3, 2012, of *Proc Natl Acad Sci USA* (109:5458–5463; first published March 20, 2012; 10.1073/pnas.1119912109).

The authors note that the author name Ryu Jae San should instead appear as Jae San Ryu. The corrected author line appears below. The online version has been corrected.

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CORRECTION