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Transcriptomic Responses of the Softwood-Degrading White-Rot Fungus *Phanerochaete carnosa* during Growth on Coniferous and Deciduous Wood▽†

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To identify enzymes that could be developed to reduce the recalcitrance of softwood resources, the transcriptomes of the softwood-degrading white-rot fungus *Phanerochaete carnosa* were evaluated after growth on lodgepole pine, white spruce, balsam fir, and sugar maple and compared to the transcriptome of *P. carnosa* after growth on liquid nutrient medium. One hundred fifty-two million paired-end reads were obtained, and 63% of these reads were mapped to 10,257 gene models from *P. carnosa*. Five-hundred thirty-three of these genes had transcripts that were at least four times more abundant during growth on at least one wood medium than on nutrient medium. The 30 transcripts that were on average over 100 times more abundant during growth on wood than on nutrient medium included 6 manganese peroxidases, 5 cellulases, 2 hemicellulases, a lignin peroxidase, glyoxal oxidase, and a P450 monooxygenase. Notably, among the genes encoding putative cellulases, one encoding a glycosyl hydrolase family 61 protein had the highest relative transcript abundance during growth on wood. Overall, transcripts predicted to encode lignin-degrading activities were more abundant than those predicted to encode carbohydrate-active enzymes. Transcripts predicted to encode three MnPs represented the most highly abundant transcripts in wood-grown cultivations compared to nutrient medium cultivations. Gene set enrichment analyses did not distinguish transcriptomes resulting from softwood and hardwood cultivations, suggesting that similar sets of enzyme activities are elicited by *P. carnosa* grown on different wood substrates, albeit to different expression levels.

Softwood, which is generated by gymnosperm plant species, is the predominant form of land plant biomass in the Northern hemisphere (8). The plentiful renewable supply of wood makes it an attractive feedstock for many industrial uses, including biofuel production (18). Softwood is also among the most recalcitrant lignocellulosic feedstocks, particularly to bioprocess technologies (42). The recalcitrance of softwood lignocellulose to bioprocess technologies has been attributed to its higher lignin content, smaller pore size, and fewer hemicellulose-derived acetyl groups (25). Despite this recalcitrance, various microorganisms have evolved the ability to transform softwood fiber, the best studied of which are the white-rot and brown-rot fungi of the phylum *Basidiomycota* (13). White-rot fungi are the only microorganisms known to effectively degrade all components of lignocellulose, while brown-rot fungi depolymerize wood polysaccharides and leave the lignin as a modified residue (41).

While the majority of white-rot fungi characterized to date effectively degrade hardwood, *Phanerochaete carnosa* is a white-rot fungus that was isolated almost exclusively from softwood (2). Previous analyses of proteins secreted by *P. carnosa* grown on spruce and cellulose identified peptides corresponding to enzymes involved in lignocellulose degradation, including cellulases, xylanases, glyoxal oxidases (GLOX), and peroxidases (19). Notably, many of the peptide sequences recovered in the proteomic analysis of *P. carnosa* matched conserved regions of multigene families, so the contributions of specific genes could not always be determined (19). The emergence of high-throughput methods for transcriptome analysis (38) opens the door for in-depth exploration of the contributions of specific genes to degradation of softwoods by *P. carnosa*.

The current study reports the first transcriptome analysis of the softwood-degrading white-rot fungus *P. carnosa*. This study also represents the first application of next-generation RNA-sequencing technologies (mRNA-Seq) to directly compare the transcriptomes of a wood-degrading basidiomycete grown on multiple wood samples, including balsam fir, lodgepole pine, white spruce, and sugar maple. By analyzing *P. carnosa* gene expression following growth on coniferous softwood (fir, pine, and spruce) and hardwood (maple) substrates and by comparing patterns of gene expression to previous analyses of the model *Phanerochaete chrysosporium*, we aimed to characterize the effect of lignocellulose composition on gene expression in *P. carnosa* and to predict key activities that could reduce the recalcitrance of softwood to bioprocess technologies.

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MATERIALS AND METHODS

Fungal strain and culture conditions. The naturally isolated homokaryotic *P. carnosa* strain HHB-10118-sp was obtained from the U.S. Department of Agriculture (USDA) Forest Products Laboratory (Madison, WI) and grown on solid or liquid medium. Wood cultivations were prepared by using a blender to grind balsam fir (*Abies balsamea*), lodgepole pine (*Pinus contorta*), white spruce (*Picea glauca*), or sugar maple (*Acer saccharum*) and then sifting air-dried samples through 3.35-mm^2- and 1.5-mm^2-pore-size sieves. Fiber that passed through the 3.35-mm^2 sieve but was retained by the 1.5-mm^2 sieve was recovered, and 4-g samples were transferred to 500-ml beakers containing 10 ml of B3 buffer (2 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.1 g $CaCl_2 \cdot 2H_2O$, 0.73 g 2,2-dimethylsuccinic acid, 0.5 mg thiamine-HCl, 0.2 g ammonium tartrate, and 10 ml mineral solution per liter in H_2O, pH 4.5) (16). The mineral solution contained 1.5 g nitrilotriacetate, 0.5 g $MnSO_4$, 1 g NaCl, 100 mg $FeSO_4$-$7H_2O$, 100 mg $CoSO_4$, 100 mg $ZnSO_4$, 10 mg $CuSO_4$-$5H_2O$, 10 mg $AlK(SO_4)_2$, 10 mg H_3BO_3, and 10 mg $NaMoO_4$ per liter H_2O. Thiamine-HCl, ammonium tartrate, and 2,2-dimethylsuccinic acid were added as filter-sterilized solutions, while all other media were steam sterilized for 20 to 30 min. Liquid cultivations contained 14 ml YMPG medium (2 g yeast extract, 10 g malt extract, 2 g peptone, 10 g glucose, 2 g KH_2PO_4, 1 g $MgSO_4 \cdot 7H_2O$, 1 g asparagine per liter in H_2O) in 500-ml beakers and were steam sterilized for 20 min.

Each culture medium was inoculated with an 11-mm circular agar plug taken from the growing edge of *P. carnosa* cultivated on solid YMPG (with agar). Cultivations were incubated under stationary conditions at 27°C until the diameter of the mycelial mat reached 4 cm (6 to 9 days), at which point the central 28 mm of growth was harvested. Cultivation of *P. carnosa* on fir, pine, spruce, and maple for 6 to 9 days was previously correlated with detectable biotransformation of each lignocellulosic substrate (unpublished data). Since cultivations were initiated using YMPG agar plugs, the transcriptomes of *P. carnosa* grown on fir, pine, spruce, and maple were compared to the transcriptome of *P. carnosa* grown on YMPG liquid medium. After cultivation, excess liquid was removed using Miracloth, and mycelia were flash frozen in liquid nitrogen and then stored at −80°C.

RNA extraction and sequencing. Total RNA was isolated from frozen samples using the RNeasy Plant Mini Kit (Qiagen Inc., Mississauga, ON, Canada) according to the manufacturer's protocol for plant tissues and filamentous fungi and including the optional on-column DNase digestion. Total RNA was sent to the Centre for the Analysis of Genome Evolution and Function (CAGEF, Toronto, ON, Canada) for sample preparation and sequencing. cDNA was synthesized and prepared for sequencing using the mRNA-Seq Sample Prep Kit (Illumina Inc., San Diego, CA). The samples were run in independent lanes, and paired-end sequences of 38 bp were obtained using the Illumina Genome Analyzer IIx.

Sequence annotation. Paired reads were converted to FASTQ format using an in-house PHP script and mapped to version 1.0 of the *P. carnosa* genome (http://genome.jgi-psf.org/Phaca1) (DOE-JGI, Walnut Creek, CA) using Novoalign (Novocraft) with default parameters, which filters out homopolymers and low-quality reads. Gene models were then predicted using the Maker genome annotation pipeline (3), whereby the Augustus gene prediction tool was used to analyze version 1.0 of the *P. carnosa* genome (31), and gene predictions were improved using Maker to integrate initial models with mRNA-Seq tag contigs. The BLAST algorithm was used to functionally annotate the resulting gene models based on their similarity to sequences of open reading frames predicted from the *P. chrysosporium* genome version 2.0 (36). Orthologs with reciprocal best hits were identified, and gene models predicted to encode proteins of interest to this study were reannotated manually. Transcript abundance was calculated based on the number of mRNA-Seq reads mapping to a given gene model and normalized to reads per million per kb of the predicted gene model. The corresponding heat map was created using an in-house PHP script, as was done previously (9).

GO Slim analysis. Individual genes were placed into GO Slim categories based on their automated annotations using the map2slim.pl tool available from the Gene Ontology Consortium (http://www.geneontology.org/GO.slims.shtml). Enzyme categories predicted to participate in lignocellulose transformation were added to the generic GO Slim for molecular function defined by the Gene Ontology Consortium. Each gene product was assigned to a single GO Slim category, and categories with fewer than 15 gene products were merged with the corresponding parent categories. To identify GO Slim categories that were significantly enriched on wood substrates compared to YMPG, an enrichment analysis was performed using Gene Set Enrichment Analysis 2.0 (32). The enrichment analysis applied a $\log_2$ ratio of classes as the metric for ranking genes and gene set randomization as the permutation type. The results were considered significant if the *P* value was less than 0.05 and the false-detection rate was less than 10% (32).

Phylogenetic analysis. Gene models predicted to encode manganese peroxidase (MnP) and lignin peroxidase (LiP) were aligned using ClustalW within Biology Workbench 3.2 (http://workbench.sdsc.edu) using the following parameters: gap open penalty = 15, gap extension penalty = 0.2, and delay divergent sequences = 30%. Bayesian trees were constructed using MrBayes 3.1 (14, 27) assuming the general time-reversible model for DNA sequence evolution, with gamma-distributed rate variation across sites. Phylogenetic trees were sampled every 100 generations until the average standard deviation of split frequencies was below 0.01, which occurred at 2,160,000 generations. The first 25% of trees were discarded as burn-in, and the remaining trees were used to calculate a 50% majority rule consensus tree rooted with a cytochrome *c* peroxidase (CCP) from *Saccharomyces cerevisiae*. CCP belongs to the class I peroxidases, while MnP and LiP belong to class II (22, 39).

Quantitative reverse transcription-PCR (qRT-PCR). Reverse transcription was performed using RevertAID H Minus Moloney murine leukemia virus (M-MuLV) reverse transcriptase (Fermentas Canada Inc., Burlington, ON, Canada), T_{25}VN primer (with 25 thymidine nucleotides, followed by an adenosine, cytosine, or guanosine [represented by "V"], and then any of the 4 nucleotides [represented by "N"]), and 30 ng total RNA in a 50-μl reaction volume. The resulting cDNA was diluted with 150 μl water, and 2 μl of each diluted sample was transferred to a reaction tube containing SYBR green JumpStart *Taq* ReadyMix (Sigma-Aldrich Canada Ltd., Oakville, ON, Canada) and 1 μM gene-specific primers (see Table S1 in the supplemental material) in a 25-μl volume. The reaction products were quantified based on plasmid standard curves using the DNA Engine Opticon 2 detection system (Bio-Rad Laboratories Canada Ltd., Mississauga, ON, Canada).

RESULTS

Transcriptome sequencing. mRNA-Seq was used to study the transcriptome of *P. carnosa* when grown on fir, pine, spruce, and maple wood samples and on YMPG liquid medium. A total of 152 million paired-end reads were obtained and filtered by the mapping program, and 63% of these reads were mapped to the 10,257 *P. carnosa* gene models predicted by Augustus and Maker. By mapping the mRNA-Seq data to *P. carnosa* transcript and genome sequences obtained by JGI (http://genome.jgi-psf.org/Phaca1), an additional 13% of reads were mapped to splice junctions or introns of predicted genes. The remaining reads are likely from untranslated regions (UTRs), intergenic regions, unpredicted genes, or unassembled genes. Notably, 9,154 of the models (88%) could be assigned a molecular function based on the homology of predicted protein sequences to proteins in other organisms and the presence of conserved functional domains within the predicted protein sequence. Where possible, transcript sequences collected in the current study are cross-referenced to GenBank accession numbers and JGI protein identifiers (IDs) to facilitate the retrieval of transcript sequences (see Fig. S1 in the supplemental material).

Of the 10,257 gene models with associated transcript reads, 533 were at least 4 times more abundant in *P. carnosa* grown on at least one wood substrate than in that grown on YMPG (see Fig. S1 in the supplemental material), 162 were at least 4 times more abundant during growth on all wood media than on YMPG, and 115 were at least 4 times less abundant during growth on all wood media than on YMPG. Notably, the 10,257 gene models are close in number to the 10,048 protein-coding genes predicted from the completed genome of *P. chrysosporium* (36). Given the phylogenetic similarity of *P. carnosa* and *P. chrysosporium*, this result suggests that the majority of *P. carnosa* genes were included in this study.

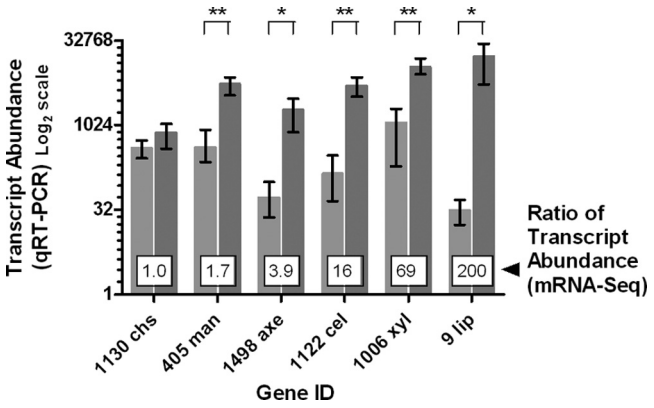

FIG. 1. Biological reproducibility of the transcript abundances determined by mRNA-Seq. Transcript abundance in *P. carnosa* during growth on fir (dark-gray bars) and YMPG (light-gray bars) was determined by qRT-PCR. Transcript ID numbers are indicated. *chs*, chitin synthase; *man*, mannanase; *axe*, acetyl xylan esterase; *cel*, cellulose binding; *xyl*, xylanase; *lip*, lignin peroxidase. The numbers on the bars are the ratios of transcript abundances from mRNA-Seq analysis of cultivations grown on fir and YMPG medium; single asterisks indicate significance at $P < 0.05$, double asterisks significance at $P < 0.005$ ($n = 4$). The error bars indicate the range of the data set. Reference data for each Gene ID are presented in Fig. S1 in the supplemental material.

Validation of mRNA-Seq patterns. To test the technical reproducibility of the mRNA-Seq experiments, four of the five samples were each sequenced in two lanes, and the numbers of reads mapping to each gene model were compared between the lanes. Read numbers per gene model were highly reproducible, with R^2 values greater than 0.99 for RNA from *P. carnosa* grown on YMPG, fir, spruce, and maple (see Fig. S2 in the supplemental material). RNA from the pine-grown culture was sequenced in one lane and was not tested for technical reproducibility.

We used real-time qRT-PCR to test the biological reproducibility of the RNA-Seq data for six transcript sequences in four replicate cultivations of *P. carnosa* grown on YMPG and ground fir (Fig. 1). These included five transcript sequences that were found by mRNA-Seq to be between 1.7 and 200 times more abundant in *P. carnosa* during growth on fir than during growth on YMPG and that are predicted to encode a mannanase (transcript 405), acetyl xylan esterase (transcript 1498), cellulose-binding protein (transcript 1122), xylanase (transcript 1006), and lignin peroxidase (transcript 9). A transcript predicted to encode chitin synthase (transcript 1130), which was found in similar amounts during growth on all tested substrates, was also quantified by qRT-PCR. Transcript sequences predicted by mRNA-Seq analyses to be more abundant in *P. carnosa* during growth on fir than during growth on YMPG were also significantly more abundant in replicate cultivations analyzed by qRT-PCR (Fig. 1). This analysis suggests that transcript counts at least 1.7 times higher in wood cultivations than in liquid cultures represent biologically relevant differences in transcript abundance.

Differentially regulated transcripts. To identify gene classifications that had higher transcript abundance in *P. carnosa* grown on all wood substrates than in those grown on YMPG, individual gene models were placed into GO Slim categories, and transcript abundance patterns were evaluated (Fig. 2). Fifty-five percent of gene annotations fit into specific GO Slim categories, and transcript sequences that clustered in GO Slim categories predicted to encode lignocellulose-degrading activity, including oxidoreductase activity, peroxidase activity, monooxygenase activity, hydrolase activity, and glycosyl hydrolase activity, were significantly enriched in *P. carnosa* grown on each of the wood substrates compared to YMPG (Fig. 2). In contrast, transcripts predicted to encode structural proteins had lower abundance in *P. carnosa* grown on each wood than on that grown on YMPG.

To predict specific catalytic activities that had the greatest difference in transcript abundance in *P. carnosa* grown on wood compared to YMPG, the 30 transcripts that were at least 100 times more abundant during growth on wood substrates (average values) than during growth on YMPG were evaluated in more detail (Table 1). Sixteen of these sequences (53%) were predicted to encode proteins involved in plant cell wall degradation, including 6 MnPs, 5 cellulases, 2 hemicellulases, a LiP, glyoxal oxidase, and a P450 monooxygenase. The three most highly abundant transcripts in wood-grown cultivations compared to YMPG-grown cultivations were predicted to encode MnP activity.

Cellulose and hemicellulose degradation. In fungi, cellulose degradation is mediated by at least three concerted cellulolytic activities: endoglucanases, cellobiohydrolases, and β-glucosidases. Endoglucanases hydrolyze internal glycosidic linkages, while cellobiohydrolases release cellobiose from either the reducing or nonreducing end of cellulose polymers. β-Glucosidase alleviates the inhibitory effect of cellobiose on endoglucanase activity by hydrolyzing the substrate to glucose (12, 40).

Among the wood-grown cultivations, transcripts predicted to encode each of these cellulolytic activities were generally most abundant in pine cultivations and lowest in fir cultivations (Fig. 3). Exceptions included a predicted GH 7 (transcript ID 144) and predicted GH61s (transcript IDs 606, 734, 3147, and 2705). Interestingly, these transcripts were most abundant in *P. carnosa* grown on maple. Overall, two gene models were predicted to encode GH7 cellobiohydrolases, and one was predicted to encode a GH6 cellobiohydrolase. Higher expression of GH7 than GH6 activity is also consistent with previous proteomic analyses of culture supernatants from *P. carnosa* grown on cellulosic substrates (19).

Transcripts encoding β(1,4)-endoglucanases from glycoside hydrolase (GH) families 5 and 12 were between 2 and over 300 times more abundant in *P. carnosa* cultivations grown on all wood substrates than in those grown on YMPG, while transcript sequences encoding a putative cellobiose dehydrogenase (transcript 493) were between 1.3 and 21 times more abundant in *P. carnosa* cultivations grown on all wood substrates than in those grown on YMPG. Notably, cellobiose dehydrogenase activity might be important for limiting the inhibitory effects of cellobiose and other oligosaccharide hydrolysis products. Similar to previous transcriptomic analysis of *P. chrysosporium* grown on red oak (28), the abundance of transcripts predicted to encode GH3 β-glucosidases in *P. carnosa* grown on wood substrates was comparatively low.

GH61 proteins have been shown to dramatically improve the impact of commercial cellulases, despite the apparent lack of ability to hydrolyze cellulosic substrates (11). In addition to typical cellulase activities, seven transcript sequences predicted

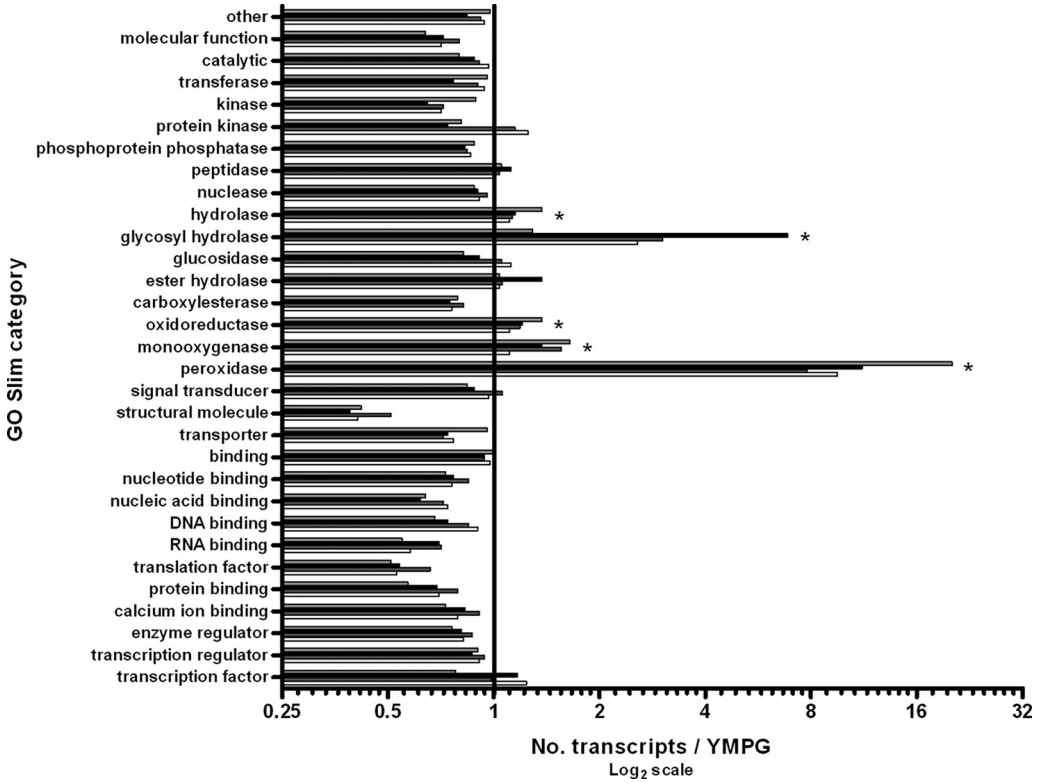

FIG. 2. Relative abundances of GO Slim classifications among *P. carnosa* transcripts isolated from each wood cultivation compared to cultivation on YMPG. The light-gray bars correspond to cultivation on fir, the black bars correspond to cultivation on pine, the dark-gray bars correspond to cultivation on spruce, and the white bars correspond to cultivation on maple. The asterisks indicate GO Slim categories that are significantly enriched in all wood cultivations ($P < 0.05$); the false-detection rate was less than 10%.

to encode GH61 proteins were between 8.8 and 740 times more abundant in *P. carnosa* cultivations grown on wood substrates than in those grown on YMPG. In fact, among transcripts encoding putative cellulases, a GH61 (transcript 1039) was the most highly abundant in *P. carnosa* grown on wood substrates compared to YMPG, based on the average transcript abundance for all wood cultivations (Table 1). Three additional transcript sequences were predicted to encode GH61 proteins, though their abundances were similar in wood and YMPG cultivations.

While cellulose is a linear polymer of repeating cellobiose subunits, hemicellulose composition and structure vary depending on the plant source, as well as the cell wall layer. For instance, while xyloglucan is the main hemicellulose present in primary cell walls of both hardwood and softwood, the corresponding secondary cell wall hemicelluloses differ. Glucuronoxylan comprises approximately 20 to 30% of the secondary cell wall polysaccharides in hardwood, while galactoglucomannan is the main hemicellulose in secondary cell walls of conifers (29). Minor hemicelluloses in hardwood and softwood fiber include glucomannan (2 to 5%) and glucuronoarabinoxylan (5 to 15%), respectively (29).

Similar to the expression of genes encoding cellulolytic activity, transcripts predicted to encode main-chain hemicellulases were most abundant in pine cultivations and lowest in fir cultivations compared with the other wood substrates (Fig. 3). Transcript sequences predicted to encode GH12 and GH74 endoglucanases (transcripts 5329 and 3295) that target primary cell wall polysaccharides were between 3.7 and 50 times more abundant in *P. carnosa* grown on wood substrates than in those grown on YMPG; four transcript sequences predicted to encode GH10 xylanases (transcripts 254, 1006, 2727, and 1086) were also more abundant in wood cultivations than on YMPG. In particular, transcript 1006 was the most abundant hemicellulase-encoding transcript from wood cultivations and was 900 times more abundant in pine cultivations than in YMPG cultures.

Similar to *P. chrysosporium*, two transcript sequences predicted to encode GH5 mannanases were detected (transcripts 405 and 8675) and were up to 59 times more abundant in wood cultivations than in YMPG cultures. Comparatively low abundance of transcripts encoding two putative GH2 β-mannosidases (transcripts 908 and 1389) was also observed in all cultivations; however, differential abundance between wood and YMPG cultivations was not observed.

In addition to hemicellulose-specific glycoside hydrolases, differential abundance of transcripts predicted to encode a glucuronoyl esterase from carbohydrate esterase (CE) family 15 (transcript 4531) and two CE1 acetyl xylan esterases (transcripts 1498 and 490) were up to 16 and 170 times more abundant in *P. carnosa* grown on wood substrates, respectively. Similar to predicted glycoside hydrolases, among the wood-grown cultivations, the abundance of transcripts predicted to encode hemicellulose-specific carbohydrate esterases was

TABLE 1. The 30 most abundant transcripts from *P. carnosa* during growth on wood relative to growth on YMPG

Gene ID[a]	Predicted activity	No. of reads/no. of reads from YMPG				
		Fir	Pine	Spruce	Maple	Avg
45	Manganese peroxidase	5,793	3,169	4,360	2,402	3,931
31	Manganese peroxidase	3,303	2,062	1,136	1,734	2,059
383 + 781	Manganese peroxidase	1,492	1,017	202	370	770
23	Serine-threonine rich	765	699	600	719	696
970	Phosphatidylethanolamine binding	1,468	331	207	290	574
119	Nonribosomal protein synthetase	549	407	455	416	457
42	Glyoxal oxidase	657	246	360	372	409
9	Lignin peroxidase	201	92	64	949	326
1006	Xylanase GH10	69	904	86	228	322
1168	Manganese peroxidase	653	236	108	254	313
1138	Manganese peroxidase	588	185	97	182	263
1039	Cellulose binding GH61	40	744	114	88	247
1512	Cellulose binding iron reductase	22	809	90	50	243
1428	NADPH dehydrogenase	284	247	171	249	238
314	Aspartic peptidase	416	218	119	169	230
9500	Dioxygenase	495	38	223	43	200
697	Manganese peroxidase	431	105	88	119	185
88	*O*-Methyltransferase	96	119	158	278	163
254	Xylanase GH10	31	333	119	119	151
2506	Aminotransferase	449	11	32	25	129
361	Cellobiohydrolase GH6	21	345	89	58	128
664	Endoglucanase GH5	10	333	116	40	125
144	Cellobiohydrolase GH7	46	12	185	249	123
732	P450 monooxygenase	98	113	167	110	122
2246	Cellulose binding GH61	12	179	170	121	121
6787	Dioxygenase	274	29	124	33	115
3683	Unknown	115	124	74	128	110
26	Serine-threonine rich	169	79	69	124	110
130	S53 protease	84	124	111	118	109
323	Cellobiohydrolase GH7	11	324	61	32	107

[a] Reference data for each Gene ID are presented in Fig. S1 in the supplemental material.

highest in pine cultivations, with the exception of transcript ID 490, which was most abundant in *P. carnosa* grown on spruce, followed by maple.

Given anticipated differences in the hemicellulose compositions of softwood and hardwood substrates, a gene set enrichment analysis was performed using transcript data from wood cultivations to identify genes with differential transcript abundance in *P. carnosa* grown on softwood substrates compared to maple cultivations. However, gene product categories that were at least four times more abundant in all softwood cultivations than in maple cultivations were not observed (data not shown), suggesting that similar sets of carbohydrate-active enzymes (CAZymes) are elicited by *P. carnosa* grown on different wood substrates, even though specific CAZymes are expressed to different levels.

Lignin degradation. Fungal degradation of lignin is promoted by the activities of four oxidative enzymes: LiP, MnP, versatile peroxidase (VP), and laccase. Similar to *P. chrysosporium* (21), genes encoding LiP and MnP, but not VP and laccase, were detected in the *P. carnosa* genome sequence. LiP and MnP are hemoproteins that require H_2O_2 to oxidize aromatic substrates and Mn^{2+}, respectively. In the case of MnP, Mn^{3+} is then stabilized by organic acids such as oxalate, forming chelates that oxidize phenolic lignin structures (10).

Gene models predicted to encode LiPs and MnPs were aligned to each other and to corresponding gene models from *P. chrysosporium* to ensure that only full-length gene models were reported. In cases where the models appeared to be partial sequences, their locations in the genome were determined and partial models were combined (e.g., transcripts 383 plus 781 and 9982 plus 9923). BLAST analysis of version 1.0 of the *P. carnosa* genome did not reveal additional *lip* and *mnp* sequences, suggesting that *P. carnosa* encodes seven MnPs (transcripts 31, 45, 383 plus 781, 1138, 1168, 1579, and 697), three LiPs (transcripts 9, 489, and 8106), and one LiP-like protein that does not contain the conserved ligninase domain (transcript 9982 plus 9923). These findings differ from the *P. chrysosporium* genome, which is predicted to encode five MnPs and 10 LiPs (21). A phylogenetic analysis of peroxidase genes revealed that the four *P. carnosa lip* and *lip*-like genes form a cluster, as do five of the seven *mnp* genes, suggesting that most LiP and MnP activities likely evolved through gene duplication after *P. chrysosporium* and *P. carnosa* speciation (see Fig. S3 in the supplemental material).

Transcript sequences from each of the seven *P. carnosa mnp* genes were 27 to 5,800 times more abundant in *P. carnosa* cultivations grown on each wood substrate than in YMPG cultivations (Fig. 3). The relative abundances of transcript sequences in *P. carnosa* grown on wood substrates compared to those grown on YMPG were highest for three *mnp* transcripts (45, 31, and 383 plus 781) (Table 1). While transcripts predicted to encode carbohydrate-active enzymes were generally most abundant in pine cultivations and least abundant in fir cultivations compared to those in other wood substrates, transcripts predicted to encode MnPs were most abundant in fir

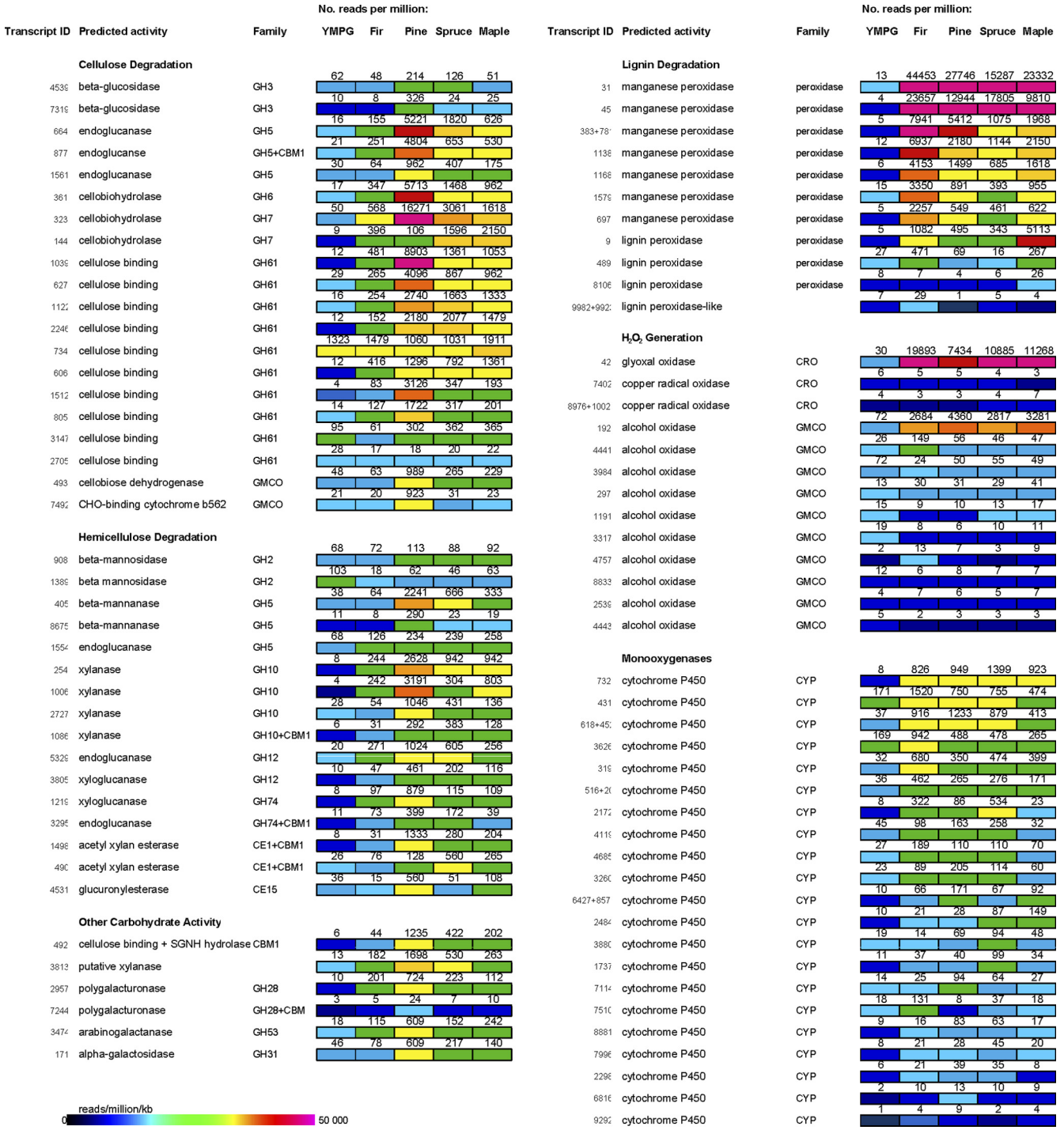

FIG. 3. Heat maps and abundances for selected transcripts expressed by *P. carnosa* during growth on wood and YMPG substrates. CBM, carbohydrate-binding module; CE, carbohydrate esterase; CRO, copper radical oxidase; CYP, cytochrome P450 monooxygenase; GH, glycosyl hydrolase; GMCO, GMC oxidoreductase.

cultivations and lowest in spruce cultivations, except transcript 45, which was lowest in maple cultivations (Fig. 3).

Of the three *lip* sequences, transcript 9 was between 64 and 950 times more abundant in wood cultivations than in YMPG cultivations, with the greatest abundance in the maple cultivation. Similarly, *lip* transcript 8106 was most abundant in *P. carnosa* grown on maple, where it was 3.5 times more abundant than in YMPG cultivations; however, this transcript was less abundant in the other wood cultivations than on YMPG. The *lip* transcript 489 was 2.6 to 18 times more abundant in *P. carnosa* grown on fir, pine, and maple than on that grown on YMPG, with the greatest abundance during growth on fir.

Transcript 9982 plus 9923, which is predicted to encode a LiP-like protein, was 4.5 times more abundant in *P. carnosa* grown on fir and was less abundant in the other wood cultures than on YMPG.

Several enzymes have been proposed to provide the H_2O_2 required for LiP and MnP activity, including GLOX, GLOX-related copper radical oxidases (CRO), and alcohol oxidase (AOX) (17, 37). Transcript 42, corresponding to a candidate *glox*, was the third most abundant transcript in *P. carnosa* grown on wood substrates in absolute terms (see Table S2 in the supplemental material), and it was 250 to 660 times more abundant in wood cultivations than on YMPG. Two related sequences that were also predicted to encode CRO enzymes (transcripts 7402 and 8976 plus 10028) were less than 1.9 times more abundant in *P. carnosa* grown on wood. Four predicted *aox* genes were up to 91 times more abundant during growth on wood (transcripts 192, 4441, 297, and 2539), while six others were generally less abundant during growth on wood (transcripts 3984, 1191, 3317, 4757, 8833, and 4443).

Monooxygenases. Various monooxygenases have been implicated in the degradation of small lignin fragments and other aromatic compounds. Accordingly, it is conceivable that monooxygenase activities could facilitate growth on softwood substrates by detoxifying lignin degradation products and the higher extractive content than is present in most hardwood species (23, 26). In *P. carnosa*, 322 transcript sequences were predicted to encode monooxygenase activity, and approximately 40% of these were identified as putative cytochrome P450 monooxygenases. Twenty-one of the sequences predicted to encode P450 monooxygenases were at least five times more abundant in one or more wood cultivations than on YMPG (Fig. 3). Notably, transcripts predicted to encode P450 monooxygenase activity likely represent a subset of all P450 genes in the *P. carnosa* genome. Accordingly, since *P. chrysosporium* carries approximately 150 full-length P450 genes (5), an intriguing possibility is that the complement of P450 genes of *P. carnosa* is expanded compared to that of *P. chrysosporium*. Detailed analysis of the *P. carnosa* genome is required to confirm this prediction.

DISCUSSION

To date, most analyses of softwood decay have focused on brown-rot fungi that initiate the degradation of wood polysaccharides using Fenton chemistry (15). These include a systematic analysis of the genome, transcriptome, and proteome of the lignocellulose-degrading brown-rot fungus *Postia placenta* (20, 35). These analyses identified oxidases predicted to generate extracellular Fe^{2+} and H_2O_2 and relatively few cellulolytic GHs or GHs with carbohydrate-binding modules compared to the white-rot fungi that have been characterized to date (20).

Similar to other white-rot fungi, the current analysis of *P. carnosa* identified glycoside hydrolases from families 2, 3, 5, 6, 7, 10, 12, 28, 31, 53, 61, and 74 that were more abundant in cultivations grown on softwood substrates than in those grown on YMPG. Notably, the number of transcript sequences predicted to encode GH61 proteins was higher than the number of transcript sequences predicted to encode cellulolytic activity.

Overall, the relative abundance of transcripts predicted to encode cellulase activity was higher than that of transcripts predicted to encode hemicellulase activity. Given the differences in hemicellulose compositions typically present in softwood and hardwood fiber, the profile of hemicellulase-encoding transcripts that were elicited by *P. carnosa* during growth on spruce, fir, and pine was expected to differ from the profile of those expressed during growth on maple. Although close analysis of transcript abundances summarized in Fig. 3 reveals higher abundance in pine cultivations of transcripts encoding cellulases, main-chain hemicellulases, and hemicellulose-specific carbohydrate esterases, a gene set enrichment analysis indicated that the overall distributions of transcripts predicted to encode CAZymes involved in cellulose and hemicellulose hydrolysis were similar in softwood and hardwood cultivations. It is possible that the grinding and steam sterilization of the wood samples increased the availability of cellulose and hemicellulose present in the lignocellulosic substrates and thereby reduced substrate differences. Alternatively, the expression of CAZymes in *P. carnosa* may be a concerted response to growth on lignocellulose, at least at the early growth stage investigated here. A time course study is now under way to determine whether differential expression of CAZymes in *P. carnosa* grown on fir, pine, spruce, and maple is more pronounced at later stages of cultivation.

Transcripts encoding enzymes involved in lignin degradation (peroxidases and H_2O_2-generating enzymes) were the most abundant gene products isolated from *P. carnosa* grown on wood substrates, both in absolute terms and in comparison to growth on YMPG (Table 1; see Table S2 in the supplemental material). In contrast, of the 80 most abundant transcripts expressed by *P. chrysosporium* grown on red oak, 22 corresponded to GHs or carbohydrate-binding modules (CBMs), and only 4 were predicted to encode lignin-degrading enzymes (28).

The ratio of *lip* to *mnp* genes and transcripts also appears to differ between *P. carnosa* and *P. chrysosporium*. The draft sequence of the *P. carnosa* genome predicts seven genes that encode MnP and only three genes that encode LiP; the abundance of transcripts corresponding to individual *mnp* genes was also generally higher than the number corresponding to individual *lip* genes in *P. carnosa* grown on wood substrates. In contrast, the *P. chrysosporium* genome predicts five *mnp* and 10 *lip* genes (21), and transcripts from individual *lip* genes appear to be more numerous than *mnp* transcripts in *P. chrysosporium* grown on wood and under low-nitrogen (ligninolytic) conditions (6, 28, 34).

Differences in modes of lignin degradation catalyzed by MnP and LiP could affect the efficiency of lignocellulose degradation by *P. carnosa* and *P. chrysosporium*. LiPs can directly oxidize a variety of aromatic substrates, whereas MnPs mediate lignin degradation through chelates of oxidized Mn^{3+} ions. Compared to LiP, the Mn^{3+} chelates have weaker oxidizing power and mainly act on phenolic structures. However, chelates of Mn^{3+} ions are likely more able to diffuse through plant cell walls than the larger lignin peroxidases (10). This property could facilitate the degradation of guaiacyl (G) lignin in softwood, which can form more dense structures than guaiacyl-syringyl (GS) lignin (4), perhaps due to its higher fraction of condensed biphenyl C-C bonds (30). In fact, differences between G and GS lignins have been shown to affect the rate of

degradation by *P. chrysosporium*, which depolymerizes natural and synthetic G lignins more slowly than natural and synthetic GS lignins (7, 24).

Our analysis of *P. carnosa* transcriptomes revealed a consistent genetic response to growth on both coniferous and deciduous substrates whereby enzymes involved in lignin degradation were the most highly expressed, followed by cellulase and then hemicellulase activities. This expression pattern is consistent with analyses of wood fiber isolated from *P. carnosa* cultivations, which predominantly reveal loss of lignin (Mahajan et al., submitted). Since the expression of ligninases and CAZymes is expected to change as lignocellulose is degraded (1, 33), future studies will evaluate temporal regulation of these transcripts to predict which are most critical to early and late stages of wood decay.

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