Significant Alteration of Gene Expression in Wood Decay Fungi

Postia placenta and Phanerochaete chrysosporium

by Plant Species

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Identification of specific genes and enzymes involved in conversion of lignocellulosics from an expanding number of potential feedstocks is of growing interest to bioenergy process development. The basidiomycetous wood decay fungi Phanerochaete chrysosporium and Postia placenta are promising in this regard because they are able to utilize a wide range of simple and complex carbon compounds. However, systematic comparative studies with different woody substrates have not been reported. To address this issue, we examined gene expression of these fungi colonizing aspen (Populus grandidentata) and pine (Pinus strobus). Transcript levels of genes encoding extracellular glycoside hydrolases, thought to be important for hydrolytic cleavage of hemicelluloses and cellulose, showed little difference for P. placenta colonizing pine versus aspen as the sole carbon source. However, 164 genes exhibited significant differences in transcript accumulation for these substrates. Among these, 15 cytochrome P450s were upregulated in pine relative to aspen. Of 72 P. placenta extracellular proteins identified unambiguously by mass spectrometry, 52 were detected while colonizing both substrates and 10 were identified in pine but not aspen cultures. Most of the 178 P. chrysosporium glycoside hydrolase genes showed similar transcript levels on both substrates, but 13 accumulated >2-fold higher levels on aspen than on pine. Of 118 confidently identified proteins, 31 were identified in both substrates and 57 were identified in pine but not aspen cultures. Thus, P. placenta and P. chrysosporium gene expression patterns are influenced substantially by wood species. Such adaptations to the carbon source may also reflect fundamental differences in the mechanisms by which these fungi attack plant cell walls.

Efficient and complete degradation of woody plant cell walls is generally ascribed to certain basidiomycetes collectively referred to as white rot fungi (14). Commonly associated with woody debris and forest litter, these fungi can depolymerize, degrade, and fully mineralize all cell wall polymers, including cellulose, hemicelluloses, and the normally rather recalcitrant polymer lignin. Such plant cell wall deconstruction requires complex extracellular oxidative and hydrolytic systems, and these have been studied extensively in the model white rot fungus Phanerochaete chrysosporium (34). Mechanistic aspects of the degradative processes remain uncertain, but the field has attracted interest because woody feedstocks are increasingly viewed as potential sources for high-value low-molecular-weight products (3).

Brown rot wood decay fungi, exemplified by Postia placenta, exhibit patterns of cell wall degradation distinct from those of white rot. In particular, these fungi rapidly depolymerize cellulose early in the decay process, but even after extensive decay, the lignin remains in situ as a modified polymeric residue (45, 65). The involvement of low-molecular-weight, diffusible oxidants, especially hydroxyl radicals, has long been suspected (10, 11). The generation of hydroxyl radicals by a nonenzymatic Fenton reaction (H₂O₂ + Fe²⁺ + H⁺ → H₂O + Fe³⁺ + ·OH) has been implicated repeatedly for brown rot (recent studies include references 9, 40, and 62) and, to a lesser extent, white rot (2).

Irrespective of strikingly different decay patterns, P. placenta and P. chrysosporium are phylogenetically related Polyporales fungi, both lying within the “Phlebia” clade (6, 20, 21). Recent comparative genome analyses (40, 57) are consistent with oxidative depolymerization of cellulose by P. placenta, including a substantial contraction in the number of genes potentially involved in hydrolytic attack on crystalline cellulose. For example, genes encoding extracellular exocellulohydrolases are absent. In contrast, the P. chrysosporium genome reveals numerous genes encoding cellulosomes and an array of high-oxida-
tion-potential lignin peroxidases. Viewed together with biochemical analyses, the preponderance of literature strongly supports a conventional hydrolytic mechanism of cellulose degradation by *P. chrysosporium*. Nevertheless, considerable uncertainty persists, especially with respect to genetic multiplicity, a prominent feature of the *P. chrysosporium* genome (41).

For example, at least 6 sequences are predicted to encode cellulbiohydrolase I (CBH), all of which are members of glycoside hydrolase (GH) family 7 (19; http://www.cazy.org/). The role(s) of these genes is poorly understood, but structural diversity within such families may reflect subtle functional differences (for an example, see reference 43) that permit adaptation to a changing substrate composition and/or other environmental conditions.

Substrate preferences among certain wood decay fungi are well known, with brown rot species often associated with gymnosperms and white rot fungi more typically isolated from angiosperms (14, 61). First reported by Holzbaur and Tien for lignin peroxidase (25), numerous studies involving defined media have demonstrated the substantial influence of substrate on gene expression by *P. chrysosporium* (reviewed in references 15 and 31) and *P. placenta* (40). Although differential transcriptional regulation and shifting secretome patterns have also been observed in comparing defined media to cultures containing a woody substrate (46, 47, 57), side-by-side quantitative comparisons are lacking. To address this issue, we show here that differences in the lignocellulosic substrate dramatically alter the transcript and secretome profiles of both *P. chrysosporium* and *P. placenta*.

**MATERIALS AND METHODS**

**Culture conditions and characterization.** RNA and protein were obtained from *P. chrysosporium* strain RP78 and *P. placenta* strain MAD-698-R (Forest Mycology Center, Forest Products Lab) grown in Highley's basal salt medium (23) containing 0.5% (wt/vol) ball-milled bigtooth aspen (*Populus grandidentata*) (BMA), ball-milled white pine (*Pinus strobus*) (BMP), or glucose as the sole carbon source. Each 2-liter Erlenmeyer flask contained 250 ml medium and was inoculated with approximately 10^7 *P. chrysosporium* spores or with *P. placenta* mycelia scraped from the surface of potato dextrose agar. *P. chrysosporium* and *P. placenta* cultures were incubated for 5 days on a rotary shaker (150 rpm) at 37°C and room temperature, respectively.

Consistent with previous analysis of *P. chrysosporium* grown for 5 days in medium containing ball-milled aspen, standard assays for lignin peroxidase (52), manganese peroxidase (46), glyoxal oxidase (32, 33), and cellulbiose dehydrogenase (4) showed no activity. None of these activities were detected in any *P. placenta* cultures, a predictable result given the absence of those genes. Carbohydrate compositions of inoculated and uninoculated media are listed in Table S3 in the supplemental material.

For RNA analysis, mycelia from triplicate cultures were collected by filtration through Miracloth (Calbiochem, EMD Biosciences, Gibbstown, NJ), squeeze dried, and snap-frozen in liquid nitrogen. Pellets were stored at −80°C until use. For mass spectroscopic analysis, culture filtrates were processed after 5 and 14 days of incubation.

**Expression microarrays.** *P. chrysosporium* and *P. placenta* Roche NimbleGen array designs are available under platforms GPL11022 and GPL31673, respectively, within the Gene Expression Omnibus (GEO) (http://www.ncbi.nlm.nih.gov/geo/index/).

Total RNA was purified from frozen mycelial pellets, converted to Cy3-labeled cDNA, hybridized to microarrays, and scanned as described previously (57). The 12 arrays used in these experiments were scanned on an Axon 4000B scanner ( Molecular Dynamics), and data were extracted using NimbleScan v2.4. Quantile normalization and robust multiarray averaging (RMA) (26) were applied to the raw data by using DNAStar ArrayStar v4 (Madison, WI). Expression levels were based on log2 signals, and significant differences in expression were determined using the moderated t test (48), with the false discovery rate (FDR) (5) threshold set at P values <0.05. The MIAME-compliant (8) microarray expression data were deposited in NCBI’s Gene Expression Omnibus. The newly acquired data can be viewed/downloaded together with previously deposited data, which include results from glucose-grown, microcrystalline cellulose-grown, and BMP-grown arrays (40, 56, 57).

Competitive reverse transcription-PCR (RT-PCR) was used to quantitatively transcription of *P. placenta* genes encoding an aryl alcohol dehydrogenase and three putative P450s. Gene-specific primers and ampiclon information for these genes are listed in Table S1 in the supplemental material. The quantitative RT-PCR methodology was used previously for confirmation of *P. chrysosporium* (56) and *P. placenta* (57) microarrays.

**Mass spectrometry.** Soluble extracellular protein was precipitated from culture filtrates by direct addition of solid trichloroacetic acid (TCA) to 10% (wt/vol), and trypsin-generated peptides were analyzed by nano-liquid chromatography–tandem mass spectrometry (nano-LC-MS/MS), using an Agilent 1100 nanoflow system (Agilent, Palo Alto, CA) connected to a hybrid linear ion trap-Orbitrap mass spectrometer (LTQ-Orbitrap; Thermo Fisher Scientific, San Jose, CA) equipped with a nanoelectrospray ion source as described previously (57). Using protein databases for *P. placenta* (http://jgi.doe.gov/Postia; nonredundant haploid set) and *P. chrysosporium* (http://jgi.doe.gov/whterot; BestModels_2_1), the MS/MS spectra were analyzed using an in-house Mascot search engine (version 2.2.07; Matrix Science, London, United Kingdom). Mascot searches were done with a fragment ion mass tolerance of 0.6 Da, a parent ion tolerance of 15 ppm, and methionine oxidation as a variable modification. Scaffold (version Scaffold_3_00_6; Proteome Software Inc., Portland, OR) was used to validate MS/MS-based peptide and protein identifications. Protein identifications were accepted if they contained at least 2 uniquely identified peptides and if protein probabilities exceeded 95%, as determined by the Protein Prophet algorithm (4). To access detailed information, *P. chrysosporium* and *P. placenta* protein model identification numbers are preceded by “Pch” or “Ppl,” respectively, and the corresponding data can be accessed directly via their respective JGI genome portals. The protein pages include information from the Gene Ontology (GO) database for each InterPro domain. 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 within the SwissProt database. All other proteins were designated “hypothetical.”

**Microarray data accession numbers.** The MIAME-compliant (8) microarray expression data were deposited in NCBI’s Gene Expression Omnibus and are accessible through GEO accession number GSE29659.

**RESULTS**

On media containing glucose, BMA, or BMP, we identified peptides corresponding to 356 *P. chrysosporium* genes (Fig. 1, upper right panel; see Table S2 in the supplemental material). Focusing our analyses on the woody substrates, 31 proteins were identified in both *P. chrysosporium* and *P. placenta*, and all were previously detected in BMA (57). These included well-characterized glycoside hydrolases, such as a family 6 (GH6) cellulbiohydrolase II (51), two family 7 GH1s (43, 54, 55), two family 12 and one family 5 endo-1,4-β-glucanase (18, 53), a family 28 polygalacturonase and a farnogalacturonase, a family 55 1,3-β-glucosidase (27), a family 16 likely laminarinase (30), a family 51 putative arabinofuranosidase, and a family 35 putative β-galactosidase. A total of 30 proteins were identified in BMA but not in BMP (Fig. 1, upper right panel). With two exceptions, all had been reported earlier (57). The two exceptions were a putative mitochondrial ATP carrier protein (Pchr343565) and a serine protease (Pchr3855). The former was also present in glucose medium and was likely the result of hyphal autolysis, but the latter was detected exclusively in BMA and featured a clear secretion signal. The proportion of proteins of likely intracellular origin was especially high in glucose medium and after 14 days in wood cultures, when starvation-induced lysis is expected (see Table S2).

Fifty-seven *P. chrysosporium* proteins were confidently de-
tected in BMP but not in BMA. Among these, only two proteins, both of unknown function (Pchr8221 and Pchr134789), had been reported in an earlier BMA analysis (57). We could not confidently assign these to BMA medium here because only a single peptide was detected for each.

Carbohydrate-active enzyme (CAZy)-encoding genes represented 33% (20 of 61 genes) and 45% (40 of 88 genes) of the total \textit{P. chrysosporium} protein genes identified in BMA and BMP, respectively, but the number and distribution of medium-specific proteins better illustrate the influence of substrate (Fig. 1, lower bar graph). Of 30 \textit{P. chrysosporium} genes confidently identified in BMA but not in BMP, only three were predicted to encode CAZys. Possibly reflecting the relative abundance of \textit{O}-acetylgalactoglucomannans in softwoods, \textit{P. chrysosporium} proteins found exclusively in BMP included two family GH74 proteins (28), a GH92 \textit{1,2-\textalpha;}-mannosidase, two GH27 \textalpha;-galactosidases (17), and three GH10 endo-1,4-\textbeta;-xylanases (13). Five GH61 proteins, a grouping of considerable recent interest (16), were also observed only in BMP. Twenty-nine of the glycoside hydrolase proteins identified after 5 days of growth were also observed after 14 days (see Table S2 in the supplemental material). Nine new GHs were identified in these older cultures, all in BMP (GH27 protein Pchr4422, GH28 protein Pchr4449, GH31 protein 125462, GH43 proteins Pchr333 and Pchr133070, GH7 protein Pchr137216, GH78 protein Pchr122292, GH92 protein Pchr3431, and the possible GH74 protein Pchr28013).

When a mutant strain of \textit{P. chrysosporium} was cultured on the softwood species \textit{Pinus nigra} (black pine), eight CAZys were detected (46), five of which were also found in our BMP cultures (GH3 protein Pchr129849, GH5 protein Pchr5115, GH74 protein Pchr134556, and GH17 proteins Pchr137372 and Pchr127029). In contrast to the results with black pine, we did not detect peptides matching GH88 protein Pchr840, GH37 protein Pchr140267, or GH92 protein Pchr3431. (The latter protein was detected in 14-day BMP medium.) Recent LC-MS/MS and transcriptome analyses of \textit{Phanerochaete carnosa}-colonized \textit{Picea glauca} (white spruce) support the importance of common extracellular CAZys (37, 38), but it is unclear whether these comparisons are meaningful given the substantial differences in our experimental design.

\textit{P. chrysosporium} oxidoreductases detected in BMP included the copper radical oxidase CRO2 (59), cellobiose dehydrogenase (36), and an FAD-dependent oxidoreductase of unknown function, whereas BMA cultures contained a glucose oxidase-like GMC oxidoreductase, a catalase, and an alcohol oxidase (see Table S2 in the supplemental material). The latter protein (Pchr126879) is 89% identical to the methanol oxidase of the brown rot fungus \textit{Gloeophyllum trabeum} (12). In black pine, oxidoreductase identification was limited to a single CRO2 peptide (46). Based on peptide matches to the \textit{P. chrysosporium} database, five putative oxidoreductases were detected in \textit{P. carnosa}-colonized spruce, but none corresponded to those observed here in BMP (38).

A total of 413 \textit{P. placenta} proteins were identified, of which a total of 71 were identified in either BMA or BMP, and 51 of these were present in both BMA and BMP (Fig. 1; see Table S2 in the supplemental material). With one exception, a puta-
Comparative phosphodiesterase (Ppl127047), all proteins detected in BMA were observed previously (57). Manual inspection suggests that the gene model corresponding to Ppl127047 is inaccurate, possibly containing 2 or more genes. Twenty-eight of the proteins common to the two media corresponded to glycoside hydrolase-encoding genes, and all had homologous sequences in *P. chrysosporium* (see Table S2). Among these common enzymes was a putative GH5 endo-1,4-β-glucanase (Ppl115648). The Ppl115648 protein lacks a cellulose binding domain, but the catalytic domain is similar to those of known endoglucanases. In addition to BMP and BMA, the protein has been detected in medium containing microcrystalline cellulose (Pchr139777). The Pchr139777 protein contains a highly conserved domain of unknown function (DUF1237) and was detected only in BMP for both *P. chrysosporium* and *P. placenta*. Twenty-nine of the glycoside hydrolase proteins of unknown function (hypothetical) were confined to BMA and/or BMP cultures (Table 1), and among these, 6 had no clear homolog in *P. placenta*. These hypothetical genes included 47 hypothetical proteins in BMA and in BMP. Peptides corresponding to GH18 proteins (Ppl107968 and Ppl119925) and a GH28 protein (Ppl105117) were detected in BMA but not in BMP after 14 days, whereas GH2 (Ppl114395) and GH5 (Ppl121713) enzymes were observed only in BMP.

Microarray analyses revealed large numbers of differentially regulated genes in glucose- versus BMA- or BMP-grown cultures. Transcript levels in BMA cultures were largely consistent with those in previous reports (57). Two hundred thirty-six *P. chrysosporium* genes accumulated >2-fold (*P < 0.05*) higher levels in BMP than in glucose, while transcripts of 136 genes were more abundant in glucose than in BMP. Fewer *P. chrysosporium* genes were upregulated in BMP than in glucose (27), but 174 were upregulated in glucose relative to BMP.

### Table 1. *P. chrysosporium* genes encoding transcripts with >2-fold accumulation and detectable proteins in BMA and/or BMP cultures

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**a** Microarray data are presented as average log signal strengths for three fully replicated experiments. Significant accumulation (A/P ratio) of transcripts in ball-milled aspen (A) relative to ball-milled pine (P) cultures was determined using the moderated *t* test (48) and associated FDR values (5) (*P* values). Data are ranked according to A/P ratios. The number of unique peptides was detected by LC-MS/MS after 5 days of growth on glucose (G), BMA (A), or BMP (P) medium. Numbers in brackets indicate the number of peptides assigned to an allelic variant.

**b** The *P. placenta* genome contains distantly related sequences within these families, but their similarity lies below our E value threshold (<10^-17).
TABLE 2. *P. placenta* genes encoding transcripts with >2-fold accumulation and detectable extracellular proteins\(^a\)

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\(^a\) Calculations are as described in the footnotes to Table 1. To identify *P. chrysosporium* homologs, the 12,438 *P. placenta* protein models represented on microarrays were aligned with all 10,048 v2 *P. chrysosporium* protein models by using Timelogic (Active Motif, Carlsbad, CA) hardware accelerated double-affinity Smith-Waterman alignments as previously described (57).

\(^b\) Gene models lie adjacent on scaffold 67 and are predicted to encode identical proteins. Transcripts differed only slightly, suggesting a possible assembly error.

\(^c\) Complete MS/MS results are listed in Table S2 in the supplemental material. Only proteins with 2 or more peptides in BMP and/or BMA are listed.

encoding polyphenol oxidase (Ppl114245), amine oxidase (Ppl98543), peroxidase (Ppl111839), a laccase (Ppl46931), alcohol dehydrogenase (Ppl55493), two P450s (Ppl97939 and Ppl128850), and a copper radical oxidase, CRO5 (Ppl56702). Peptides corresponding to the *P. placenta* cro5 gene were identified in BMA and BMP.

*P. placenta* genes upregulated >2-fold in BMP relative to BMA were particularly rich in genes encoding oxidoreductases, including 15 cytochrome P450s, and transcript levels of 10 of these P450s were upregulated >4-fold (Fig. 2). All but 2 of the 85 BMP-upregulated genes, hypothetical proteins Ppl121538 and Ppl106710, were matched to a *P. chrysosporium* homolog (see Table S2 in the supplemental material).

**DISCUSSION**

Our results show that gene expression profiles of *P. chrysosporium* and *P. placenta* are significantly influenced by the wood substrate. Analyses of transcript levels in all three pairwise comparisons (BMA-BMP, BMP-glucose, and BMA-BMA) showed combined totals of 378 and 513 regulated genes in *P. chrysosporium* and *P. placenta*, respectively. More specifically, 240, 201, and 47 *P. chrysosporium* genes showed differential accumulation in BMA-glucose, BMP-glucose, and BMA-BMP comparisons, respectively. For *P. placenta*, the same comparisons showed 250, 372, and 162 regulated genes, respectively. In this connection, it should be noted that unless otherwise specified (e.g., Fig. 2), a threshold of >2-fold transcript accumulation (*P < 0.05*) was imposed.

Biological and technical variation was low under the conditions employed (GEO accession number GSE29659), and as in earlier studies (56–58), qRT-PCR results affirmed the relative transcript abundances (Fig. 3). However, it should be emphasized that we were examining steady-state transcript levels, with no information regarding stability/turnover. Furthermore, responses may be indirect, and given the current limitations in automated gene annotation, we have surely underestimated the number of regulated genes. Beyond this, time course experiments, which are prohibitively

**FIG. 2.** Heat map showing hierarchical clustering of *P. placenta* genes with >4-fold (*P < 0.05*) transcript accumulation in BMP relative to BMA (first 19 genes) and in BMA relative to BMP (last 15 genes). The scale above the map shows log₂-based signals.
investigations of the of proteins in lignocellulose degradation. Affirming previous
differentially regulated or not, surely support an important role
especially those associated with high transcript levels, whether
interpreted cautiously.

absence of detectable extracellular peptides should be inter-
extracellular Fenton chemistry via iron reduction. In short, the
kDa (Ppl128976) features a secretion signal, and previous stud-
transcript signals were observed in both BMA (log2
ing to
weight may be overlooked. For example, peptides correspond-
ble and stable proteins. Important proteins with high turnover
shotgun approach reported here favors identification of solu-
and/or differential regulation. However, the nonquantitative
mechanisms (16), significant (P < 0.01) transcript accumulation (>4-
fold) and peptides were detected for GH61 proteins Pchr41650
(Table 2) and Pchr121193 (Table 3). Peptides corresponding to
GH61 proteins Pchr41123, Pchr4691, and Pchr122129 were
also detected, but their transcript accumulation relative to that
in glucose medium was modest, at 1.05-fold, 1.81-fold, and
1.27-fold, respectively.

Cellobiose dehydrogenase (CDH), an enzyme implicated in
Fenton chemistry (reviewed in reference 2) and cellulose depolymerization (39), was highly expressed in BMP (log, signal,
13.97; 16 unique peptides) (Table 3). In this connection, we
also identified peptides corresponding to a putative aldose
1-epimerase (ALE1) in BMP but not in BMA (Table 2). Al-
though peptides were absent (ALE1) or unconvincing (CDH)
in BMA, transcript accumulation was significantly higher than
that in BMP. The coordinate expression of ALE1 and CDH
was previously noted in P. chrysosporium cultures containing
microcrystalline cellulose as the sole carbon source (58).
Possibly, these enzymes are physiologically connected through
the generation of the β-anomer of cellobiose, the preferred sub-
strate of CDH (22).

In contrast to the case for P. chrysosporium, significant (>2-
fold) accumulation of glycoside hydrolase transcripts was not
observed in BMA-grown P. placenta (Table 1), although the
putative GH28 enzymes polygalacturonase (Ppl111730) and
rhamnogalacturonase (Ppl58192) were upregulated in BMP
(Table 1). However, high transcript levels and extracellular
peptides were observed in many instances (Table 3; see Table
S2 in the supplemental material). Among these, a potential
GH5 endoglucanase (Ppl115648), albeit one without a cellu-
lose binding domain, was highly expressed in P. placenta
cultured in both BMA and BMP. This observation lends support
to the possible involvement of an endo-acting 1,4-β-glucanase,
but it should be mentioned that no other likely cellulases were
expressed until day 14, when a second putative 1,4-β-glucanase
(Ppl117690) appeared. The P. placenta genome contains two
predicted GH61 genes, and both showed only modest tran-
script levels (log2 signal averages ranging from 8.86 to 9.21)
and no peptides detected (see Table S2).

Although few P. placenta glycoside hydrolases exhibited sig-
ificant (>2-fold) differential regulation on the lignocellulosic
substrates, many oxidoreductase-encoding genes were substan-
tially regulated. Transcripts corresponding to the copper radical
oxidase gene cro5 were more abundant in BMA than in
BMP, and peptides were detected in BMA and BMP (Table 1;Fig. 2). The predicted protein (Ppl56703) features 4 N-termi-
al repeats of a highly conserved domain (WSC; IPR013994)
possibly involved in carbohydrate binding. Also accumulating
in BMA relative to BMP were transcripts corresponding to
polyphenol oxidase, a peroxidase, laccase, and a putative fla-
in-containing oxidoreductase.

Most surprising was the accumulation of oxidoreductase
transcripts in BMP relative to those in BMA (Fig. 2 and 3). These
included 15 genes encoding cytochrome P450s, a func-

expensive at current microarray costs, would likely reveal
additional genes involved in early colonization and during
advanced decay.

Protein identifications supported an important role for many
genes, particularly those associated with high transcript levels
and/or differential regulation. However, the nonquantitative
shotgun approach reported here favors identification of solu-
able and stable proteins. Important proteins with high turnover
rates or those bound to the substrate or of low molecular
weight may be overlooked. For example, peptides correspon-
ding to P. placenta gpl1 were not detected, even though very high
transcript signals were observed in both BMA (log2 = 14.78)
and BMP (log2 = 14.90). The predicted mature protein of 21.5
kDa (Ppl128976) features a secretion signal, and previous stud-
ies of P. chrysosporium (50) support a role for this protein in
extracellular Fenton chemistry via iron reduction. In short, the
absence of detectable extracellular peptides should be inter-
preted cautiously.

On the other hand, unambiguous protein identifications,
especially those associated with high transcript levels, whether
differentially regulated or not, surely support an important role
of proteins in lignocellulose degradation. Affirming previous
investigations of the P. chrysosporium cellulolytic system,
elevated transcript levels and proteins were observed for
genes encoding CBH1s (CEL7C [Pchr127029] and CEL7D
[Pchr137372]) (see Table S2 in the supplemental material),
CBH2 (CEL6 [Pchr133052]) (Table 3), and endoglucanases
(CEL5A [Pchr6458] and CEL12A [Pchr8466]) (Table 1). Pos-
sibly enhancing cellulolytic activity by nonhydrolytic mecha-
nisms (16), significant (P < 0.01) transcript accumulation (>4-
fold) and peptides were detected for GH61 proteins Pchr41650
(Table 2) and Pchr121193 (Table 3). Peptides corresponding to
GH61 proteins Pchr41123, Pchr4691, and Pchr122129 were
also detected, but their transcript accumulation relative to that
in glucose medium was modest, at 1.05-fold, 1.81-fold, and
1.27-fold, respectively.

Ce...
Highly expressed *P. placenta* and *P. chrysosporium* genes encoding proteins detected in culture filtrates by LC-MS/MS.

<table>
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* Listing of genes with transcript signals >2 standard deviations (SD) above the genomewide average and with at least 2 unique peptides identified. For *P. placenta*, the BMA log2 value was 10.95, with an SD of 1.599, and the BMP value was 10.96, with an SD of 1.606. For *P. chrysosporium*, the BMA value was 11.55, with an SD of 1.417, and the BMP value was 11.549, with an SD of 1.391. Models with obvious inaccuracies and in need of manual editing are shown in bold. Asterisks indicate differentially regulated genes (>2-fold transcript accumulation; P < 0.05). All abbreviations are as in Tables 1 and 2.
many interesting hypothetical proteins. Some of these are more precisely termed “proteins of unknown function,” including the 8 differentially regulated \( P. \) \( \text{placenta} \) gene products identified with LC-MS/MS as extracellular proteins and bearing no apparent homologs in \( P. \) \( \text{chrysosporium} \) Table 2). Based on transcript levels and protein identification, these and other genes seem worthy targets for future investigation.

ACKNOWLEDGMENTS

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