Comparative analysis of white rot and brown rot fungi: genomes, transcriptomes and secretomes

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Lignocellulose in vascular plant cell walls is one of the largest sinks for fixed global carbon and is receiving increased attention as a potential biofuels feedstock. Relatively few organisms can efficiently convert the recalcitrant polymer blend in lignocellulose to monomeric components. The main exceptions are certain basidiomycetes, which attack wood through two main decay types called white-rot and brown-rot. Wood-decaying basidiomycetes are essential contributors to carbon cycling in forest soils, and brown-rot fungi are additionally important because they are a major cause of failure in wooden structures.

White-rot fungi degrade all components of plant cell walls, including cellulose, hemicellulose and lignin. Although they cannot grow on lignin alone, they have the unique ability to completely degrade a large proportion to CO_2 and H_2O. This biodegradative strategy exposes the structural polysaccharides of plant cell walls, thus making them susceptible to hydrolysis by cellulases and hemicellulases. Brown-rot fungi such as *Postia placenta* employ a different approach. Early in the decay process, they rapidly depolymerize cellulose but without concomitant weight loss. As decay progresses, brown-rot fungi modify lignin extensively, but the products remain *in situ* as a polymeric residue. Given the incomplete ligninolysis that occurs during brown-rot, it remains unclear how these fungi gain access to plant cell wall polysaccharides. It seems probable that the two decay types share at least some mechanisms, because molecular phylogeny, morphological considerations, and substrate preference suggest that brown-rot fungi have repeatedly evolved from white-rot fungi (*1*). *P. placenta* is closely related to the model white-rot fungus, *Phanerochaete chrysosporium*, and recent comparison of their genomes (*2*) indicate that the derivation of brown-rot is characterized largely by the contraction or loss of multiple gene families that are thought to be important in typical white-rot, such as cellulases, lignin peroxidases (LiPs), manganese peroxidases (MnPs), copper radical oxidases (CROs), cellobiose dehydrogenase (CDH), and pyranose-2-oxidase (POX). This general pattern of simplification is consistent with the view (*3*) that brown-rot fungi, having evolved novel mechanisms for initiating cellulose depolymerization, have cast off much of the energetically costly lignocellulose-degrading apparatus that is retained in white-rot fungi, such as *P. chrysosporium*.

To further our understanding of lignocellulose degradation, broad computational comparisons of the *P. placenta* and *P. chrysosporium* genomes have been undertaken in conjunction with high throughput examination of transcript profiles and secreted proteins. More specifically, full genome NimbleGen expression arrays and liquid chromatography-tandem mass spectrometry (LC-MS/MS) are being used to characterize transcriptomes and secretomes of these fungi when cultivated in defined media, in media containing ball milled wood as sole carbon source, and in blocks of colonized wood.

Analysis of the *P. placenta* genome has elucidated a repertoire of genes and expression patterns distinct from those of all known cellulose-degrading microbes, including *P. chrysosporium*. The *P. placenta* genome completely lacks cellulose-binding domains and

the number of glycoside hydrolases is relatively low owing in part to the paucity of cellulases. No exocellobiohydrolases and only two potential β-1,4 endoglucanase genes have been identified. Nevertheless, several glycoside hydrolase-encoding genes, including a putative endoglucanase, are highly expressed when *P. placenta* was grown under cellulose induction.

Many investigations of white-rot and brown-rot mechanisms support the participation of low molecular weight oxidants. Hydroxyl radical, generated via the Fenton reaction ($H_2O_2 + Fe^{2+} + H^+ \Rightarrow H_2O + Fe^{3+} + \cdot OH$), has been repeatedly implicated as a diffusible oxidant in brown rot, and to a lesser extent, in white rot. As reviewed (*4*), three somewhat overlapping mechanisms of oxidative degradation have been advanced. One view emphasizes the importance of CDH. In the case of *P. placenta*, genome analysis has convincingly shown that CDH is absent (*2*). Another view invokes the role of low molecular weight glycopeptides that catalyze extracellular iron reduction. Initially identified in *P. chrysosporium* (*5*), potential orthologs of these glycopeptide-encoding genes were identified in the *P. placenta* genome and in one case, increased transcript levels have been observed in cellulose-containing media. Accordingly, a role for these glycoproteins in a *P. placenta* Fenton system is possible. The third mechanism involves extracellular quinone redox cycling (*6*). Evidence supporting this system includes cellulose induction of genes encoding quinone reductase, quinate transporter, phenylalanine ammonia lyase and laccase. However, the importance of hydroquinone-driven Fenton chemistry in *P. placenta* remains unclear because this fungus secretes high levels of oxalate (*7*), and Fe^{3+}-oxalate chelates are poorly reducible by hydroquinones (*8*).

Transcript levels of Fet3 and Frt1, central components of iron acquisition systems, are substantially increased in cellulose-grown cultures of *P. placenta*, but not in identically grown *P. chrysosporium* cultures (*2, 9*). While cellulose itself may sequester Fe^{3+} (*10*), the generation of Fe^{3+}–oxalate and potentially other redox active iron-chelates might also contribute to lower the effective concentration of bioavailable iron that is accessible to the organism. Thus, cellulolytic conditions might switch on the high-affinity iron uptake system to ensure proper levels of intracellular iron in *P. placenta*.

Also compatible with Fenton mechanisms, elevated transcripts and proteins corresponding to structurally divergent oxidases (e.g. copper radical oxidases, glucose-1-oxidases and methanol oxidases) and putative iron reductases have been identified principally in *P. placenta* cultures. Viewed together with the significant number of hemicellulases secreted in cellulose medium, wood decay by *P. placenta* likely involves attack by both oxidative and hydrolytic mechanisms.

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