

Distinct growth patterns and time-dependent secretome alterations by two taxonomically divergent brown rot fungi

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Project Goals: The overarching goal, within which this study's aims fit, is to discover which genes are differentially up-regulated across the mycelia of brown rot wood-degrading fungi *in planta*, particularly at the leading edge of wood decomposition. These unique fungi accomplish what we have difficulty achieving – energy from plant biomass. To do this, brown rot fungi apparently couple an oxidative pretreatment step with enzymatic saccharification in discrete space, via partitioning of reactions. We have previously shown evidence that these are governed by differential expression, but with genes putative, transport out of hyphae unclear, and the secretome poorly studied, historically, a comprehensive approach is needed that can also limit ('winnow') data sets from powerful global analytical tools to focus on the genes and pathways that matter. To address this, we are comparing global expression profiles among mycelial regions and matching what we see to what they do, in terms of changing wood physiochemistry. To map a 'connectome' in a wood-fungal interaction, specifically, we must also match gene expression patterns with the extracellular secretome and with physiochemical wood modifications.

Specific to this project, the **Objectives** were 1) to focus efforts on the secretome of brown rot fungi *in planta* in order to match gene expression patterns generated in whole-transcriptome efforts and 2) to expand our work on *Postia placenta* to include fungi from other distinct clades. Brown rot fungi have a polyphyletic history, and we make a significant assumption when extrapolating from *one* brown rot fungus to *all* brown rot fungi.

Abstract:

Brown rot fungi are wood-degrading fungi that employ both oxidative and hydrolytic mechanisms to degrade wood. Hydroxyl radicals that facilitate the oxidative component are powerful non-selective oxidants and are incompatible in discrete space with hydrolytic enzymes. Differential gene expression has been implicated in *Postia placenta* to segregate these reactions, but it is unclear if this two-step mechanism varies in other brown rot fungi with different traits and life history strategies, and that occupy different niches in nature. We used proteomics to analyze a progression of wood decay on thin wafers, using brown rot fungi with significant taxonomic and niche distance - *Serpula lacrymans* (Boletales; 'dry rot' lumber decay) and *Gloeophyllum trabeum* (order Gloeophyllales; slash, downed wood). Both fungi produced greater oxidoreductase diversity upon wood colonization and greater glycoside hydrolase activity later, consistent with a two-step mechanism. The two fungi invested very differently, however, in terms of growth (infrastructure) versus protein secretion (resource capture), with ergosterol/extracted protein ratio increased 7x with *S. lacrymans* than with *G. trabeum*. In line with their native substrate associations, hemicellulase specific activities were dominated by mannanase in *S. lacrymans* and by xylanase in *G. trabeum*. Consistent with previous observations, *S. lacrymans* did not produce GH 6 cellobiohydrolases (CBH) in this study, despite belonging to the order Boletales which is distinguished among brown rot fungi by having CBH genes. This work suggests that distantly

related brown rot fungi employ staggered mechanisms to degrade wood, but that the underlying strategies vary among taxa.

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