

### 143. Spatially co-localizing the incompatible oxidative and enzymatic steps during fungal brown rot wood degradation - Early Career Program

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**Project Goals:** Wood-degrading fungal genomes are increasingly sequenced and annotated, including brown rot functional types starting with *Postia placenta* in 2009. Wood-degrading fungi metabolize wood either by removing lignin to access carbohydrates, or they extract carbohydrates without extracting significant amounts of lignin. The brown rot fungi evolved more recently and at least seven different times from ancestral white rot lineages, suggesting an advantage in efficiency. As the first annotated genome representative of ‘typical’ brown rot fungi, *P. placenta* is capable of an oxidative hydroxyl radical pretreatment that occurs concurrently with enzymatic saccharification of woody carbohydrates. This consolidation of otherwise incompatible reactions is fundamentally interesting and has great implication on the potential to consolidate harsh pretreatments with saccharification in a single processing step. Therefore, our research goals are as follows:

- 1) physically sample wood degraded by the brown rot fungus *P. placenta* in order to map coincident pretreatment and saccharification reactions and to correlate relevant lignocellulose chemistry,
- 2) image pH and porosity at the fungus-plant interface and layer this data with images showing cellulase ingress, and
- 3) map, along the active hyphal front, the co-occurring expression of iron reductases associated with pretreatment and of cellulases used in saccharification.

**Abstract:** Enzymatic bioconversion of lignocellulose plant tissues generally requires an initial pretreatment step, followed by saccharification and then fermentation or other downstream processing approaches. Consolidated bioprocessing (CBP) of lignocellulose combines enzymatic sugar release (saccharification) with fermentation, but pretreatments typically remain separate and costly. In nature, lignocellulose-degrading brown rot fungi consolidate pretreatment and saccharification, likely using spatial gradients to partition these incompatible reactions. Our goal is to characterize how this is achieved, in order to better understand the fungus and to potentially apply this approach in a mimicked consolidated approach.

The goal of this research is characterizing this relevant biological system, with objectives (stated above) to 1) physically sample wood degraded by the brown rot fungus *Postia placenta* to map reactions spatially and to correlate with cell wall modifications, 2) produce images of the environmental variables (pH and porosity) affecting cellulase ingress over time during brown rot, and map, along the active hyphal front, the co-occurring expression of iron reductases associated with pretreatment and of cellulase involved in saccharification. These are spatially-focused goals.

Therefore, my respective approaches involve either small-scale, spatially resolved characterization (Obj. 1), or appropriately resolved microscopy (Obj. 2 & 3).

To date, we have completed Objective 1 and published the findings in a single, large paper in *International Biodeterioration and Biodegradation* in 2013. Results show a spatially-segregated ‘zone of interest’ near the hyphal front, where depolymerization measured by alkali solubility of residues occurs ahead of both lignin modifications and active cellulase. These results include caveats on detection limits, etc., but suggest that Objectives 2 and 3, in progress, will be highly valuable. It also has shifted the hypotheses away from the assumed two-step staggering of reactions to instead assume that lignin may be modified by non-oxidative reactions and that cellulases may not be pivotal as the second step. At present, we have transformed *Piccia pastoris* to produce the brown rot cellulase PpCel5B for immunolabel, and will soon have imaged its ingress into wood cell walls at the resolution possible with the transmission electron microscope (TEM). This has been coupled with the Objective 3 effort to use fluorescence in situ hybridization (FISH) to co-localize expression of those genes assumed pivotal to brown rot, overlaying the depolymerization front we located in Objective 1. The result will be a full picture of temporal progression of brown rot, using a spatial gradient developed in wafers to do so.

### **References**

Schilling, J.S., Duncan, S.M., Presley, G.N., Filley, T.R., Jurgens, J.A., and Blanchette, R.A. (2013) Colocalizing incipient reactions in wood degraded by the brown rot fungus *Postia placenta*. *International Biodeterioration and Biodegradation* 83: 56-62.

*Funding statement: This work is supported by the Early Career Program and within the Office of Biological and Environmental Research in the DOE Office of Science.*