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

Robert A. Blanchette,1 Shona M. Duncan,1 Timothy Filley,2 Joel Jurgens,1 Justin Kaffenberger1, Jon Menke1* (menk0001@umn.edu), Gerald N. Presley, Jiwei Zhang, and Jonathan S. Schilling1

1University of Minnesota, Saint Paul; 2Purdue University, West Lafayette, IN

http://schillinglab.cfans.umn.edu

Project Goals: Wood-degrading fungal genomes are increasingly sequenced and annotated, including brown rot functional types, initiated with Postia placenta in 2009. Wood-degrading fungi metabolize wood either by removing lignin to access carbohydrates (white rot), or they extract carbohydrates using a mechanism to circumvent lignin, largely (brown rot). 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 have been 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) Use microscopy to image the fungus-plant interface along a continuum, and layer data with information about structural carbohydrate degradation and other wood modifications.

3) Map, along the active hyphal front, the co-occurring expression of oxidative genes associated with pretreatment and of glycosyl hydrolases (GHs) 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 spatially the co-occurrence of relevant reactions in this biological system of high relevance for bioenergy objectives. We are doing this with a novel directional growth strategy on wood wafers, using P. placenta as our model system and a variety of physiochemical and molecular tools to overlay spatial patterns in place of a 'noisier' temporal sequence from whole wood blocks. This project is nearly complete, with one key publication close to submission. From the physical sampling efforts, we learned that there is an area of depolymerization apparently beyond those areas of lignin demethylation and glycosyl hydrolase activity, occurring at the hyphal front colonizing wood. We have matched this observation to gene expression patterns, as well, using fluorescence in situ hybridization (FISH) and confocal microscopy but without the signal strength or the chemical sensitivity with wood physiochemical analyses to connect potential with outcomes. Instead, our final efforts have coupled qPCR with the coarse sampling information generated in Objective 1 to colocalize expression of

target genes. These results are showing conclusively three novel results. 1) This fungus (P. placenta), assumed in many literature reports to have constitutive expression of cellulases indeed does not, showing inducible patterns that lag behind the hyphal front. 2) Most of the genes we tested that are putatively associated with an oxidative brown rot 'pretreatment' are, in fact, being expressed ahead of cellulases with highest transcript counts at the hyphal front and repression at the induction point for cellulases. 3) These patterns are matched by carbon release induction/repression patterns as the wood is disassembled. This collectively is in line with the theory of brown rot as a two-step pretreatment/saccharification mechanism, consolidated over small spatial distances in wood. This offers great promise for consolidating bioprocessing steps and for understanding a vital carbon cycling pathway via wood decomposition, but other aspects such as putative gene functions and intra vs. extracellular fate of proteins remain unknown, particularly given the likelihood that hyphal fronts normally confront other colonizers in nature. Addressing these gaps are critical next steps in the fundamental side of this work, and this 'wafer' system of directional growth is proving highly valuable in segregating and targeting key reactions for deeper omics analyses.

References

1. 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.
2. International Biodeterioration and Biodegradation 83: 56-62.

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