Applying the biology of brown rot fungi to consolidated bioprocessing - Early Career Program

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Project Goals: *Postia placenta* is a wood-degrading brown rot fungus with a sequenced and annotated genome[1]. Like other 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 3) 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).

Small-scale physical sample analysis includes traditional wet chemical characterization, coupled both with spin-trap adduct recovery of hydroxyl radicals produced by the fungus and with C13-labeled tetramethyl ammonium hydroxide thermochemolysis for specific brown rot lignin modifications. For microscopy, I am utilizing fluorescence lifetime imaging (FLIM) with confocal detection for pH measurements, cryo-transmission electron microscopy (TEM) with electron tomography for porosity

measures, a complementary scanning transmission x-ray approach for porosity, and traditional TEM with immunolabeling to track cellulase ingress. I am also planning to co-localize chitinous fungal biomass, imaged using a traditional WGA-FITC dye, with fluorescence in-situ hybridization to measure mRNA transcribed from iron reductase and endoglucanase DNA sequences, made possible by the recent JGI-funded annotation of the *P. placenta* sequence. Overall, this research will help resolve how brown rot fungi consolidate oxidative pretreatments with enzyme-based saccharification, so that we might better understand and exploit natural synergies between bioconversion steps that we currently approach as separate, distinct steps.

References

1. Martinez et al. (40+ authors) (2009) Genome, transcriptome, and secretome analysis of wood decay fungus *Postia placenta* supports unique mechanisms of lignocellulose conversion. *PNAS* 106:1954-1959.