Spatial Connectomics to Identify Agents Relevant to Lignocellulose Deconstruction in Fungi

Jiwei Zhang[1], Gerald N. Presley[1], Kevin Silverstein[1], Melania Figueroa[1], Katarina Sweeney[1], Kenneth E. Hammel[2], Christopher J. Hunt[2], Ellen A. Panisko[3], and **Jonathan S. Schilling**[1]

[1]University of Minnesota, Saint Paul, [2]U.S. Forest Products Laboratory (FPL); [3]Pacific Northwest National Laboratory

URL: http://schillinglab.cfans.umn.edu

Project Goals: Our goal 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.

Objective 1 – **Zone localization:** Use the wood wafer design to resolve a depolymerization zone in *P. placenta* near the mycelial leading edge, and optimize RNA extraction for thin-sectioning.

Objective 2 - Fungal connectomics: Co-localize gene expression with the secretome and with relevant physiochemical modifications made to the wood, e.g. hemicellulose loss, porosity changes.

Objective 3 - Clade comparisons: Compare key zones among brown rot clades, in context of white rot same-clade ancestors, to target universal 'brown rot' genes and candidates for bioprocessing.

Abstract:
Some fungi are uniquely able to deconstruct lignocellulose, and their mechanisms have potential biofuels applications. A key hindrance to harnessing these mechanisms has been their spatial complexity. Our past work has shown that differentiated networks of hyphae that penetrate wood are not metabolically uniform, with critical reactions occurring near the hyphal front. Standard analyses of these fungi from artificial media or from colonized wood ground en masse fail to distinguish expression of key gene products in localized regions along growing hyphae.

Our focus for this research is specifically on brown rot fungi, a more recently evolved decay fungal group (relative to white rot) that circumvents the lignin barrier to extract sugars from lignocellulose. The genetic basis for how this capacity evolved away from white rot multiple times remains unknown, despite the modern options to align the compare brown rot and white rot fungal genomes. Our new collaboration aims to focus omics techniques to map and integrate expression over networks of wood-degrading fungal hyphae *in planta*. A similar approach, 'connectomics,' has been used to map the human nervous system, and its application here is timely. First, wood-degrading fungal genomes are an emerging resource, particularly brown rot functional types.

Second, we recently optimized a thin-section wood set-up that can finely resolve reaction zones along an advancing mycelium. Within these zones, we can employ deep omics approaches without the typical noise of whole-sample homogenization. By co-localizing gene expression, secretions, and wood modifications, we can prioritize the genes most useful for application.

Our goal is to discover which genes are differentially up-regulated across the mycelia of wood-degrading fungi *in planta*, particularly at the leading edge of wood decomposition. To do this, we need to compare global expression profiles among mycelial regions. 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. Given this potential for substrate-fungus feedback, we will cross-check genes using separate clade representatives for brown rot fungi alongside their white rot ancestors, harnessing the JGI MycoCosm portal and several key resources and expertise at the USDA Forest Products Laboratory and the Pacific Northwest National Laboratory.

References:

Presley, G.N., Zhang, J., Schilling, J.S.* (online first) A genomics-informed study of oxalate and cellulase regulation by brown rot wood-degrading fungi. Fungal Genetics & Biology doi.org/10.1016/j.fgb.2016.08.004.

Zhang, J., Presley, G.N., Hammel, K.E., Menke, J.R., Hu, D., Ryu, J.S., Orr, G., Schilling, J.S. *(2016) Localizing gene regulation reveals a staggered wood decay mechanism for the brown rot fungus *Postia placenta*. Proceedings of the National Academy of Sciences 113: 10968-10973.

Kaffenberger, J.T. and Schilling, J.S. (online first) Comparing lignocellulose physiochemistry after decomposition by brown rot fungi with distinct evolutionary origins. Environmental Microbiology doi:10.1111/1462-2920.12615.

Schilling, J.S., Kaffenberger, J.T., Liew, F.J., Song, Z. (2015) Signature wood modifications that reveal decomposer community history. PLoS ONE 10(3): e0120679. doi:10.1371/journal.pone.0120679.

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.

Schilling, J.S., Ai, J., Blanchette, R.A., Duncan, S.M., Filley, T.R., and Tschirner, U.W. (2012) Lignocellulose modifications by brown rot fungi and their effects, as pretreatments, on cellulolysis. Bioresource Technology 116: 147-154.

Funding statement

This work was supported by the U.S. Department of Energy Office of Science (Early Career Grant DE-SC0004012 from the Office of Biological and Ecological Research (BER) to J.S.S.; and BER Grant DE-SC0012742). Confocal microscopy is funded by user Facility grant #48607 at the Environmental Molecular Sciences Laboratory of Pacific Northwest National Laboratory (to J.S.S.).