Title of Project: RECOVERY ACT - Consolidating Biomass Pretreatment with Saccharification by Resolving the Spatial Control Mechanisms of Fungi

Institution: University of Minnesota

Date Received: 5/1/2010

Principal Investigator: Jonathan S. Schilling

Project Results:

Summary
This project is complete, with our goals met and with five publications in print, including one in 2016 in *PNAS*. Nineteen presentations resulted from this work, along with numerous invited talks by the PI, Schilling. To reiterate our goal, we were trying to better elucidate the mechanisms that a group of wood-degrading fungi (brown rot type) use to segregate extracellular oxygen radical-forming reactions from enzymes produced by the same fungus, in this case the JGI-sequenced *Postia placenta*. *P. placenta* was the first and only brown rot genome available when this grant was written. The brown rot 'two-step' approach is an accepted theory that has not been validated as a system. Harnessing or mimicking this approach in an industrial context could allow consolidation of harsh pretreatments of plant biomass with those enzymatic reactions required for releasing monomeric sugars. The specific objectives in this project were motivated to explore these co-occurring reactions and the physiological conditions that allow these reactions to be partitioned away from each other and from living fungal hyphae. Porosity and pH were the two key elements theorized to segregate the reactions at a submicron scale; therefore, our approach was to use highly-resolved spatial analyses to determine both the pore size in plant cells before and during brown rot and the pH differential that has been theorized to exist between the fungal cell wall and the inside of the secondary cell wall of the plant, in this case wood.

What we found over the course of the Early Career research was that much of the traditional paradigm for brown rot was based on a constitutive cellulase production pathway that we have shown to be incorrect. To do this, our most successful design over this research project was in a directional growth wood 'wafer' set-up. In short, we used space as a surrogate for time to produce a temporal sequence of wood decay as *P. placenta* invades wood. This first revealed a window of interest at the leading edge of the hyphal front (Schilling et al. 2013, *IBB*) where we confirmed early wood modifications without detectable cellulase activity, despite the long-held theory that cellulase production was constitutive. Second, we used transcriptomics (first via qPCR, later by RNAseq) to verify that cellulase transcripts in this fungus were being delayed (downregulated) while oxidoreductases were upregulated within a 48-hr ephemeral oxidative window (Zhang et al. 2016, *PNAS*). This 'two-step' mechanism was verified in the secretome of *P. placenta* and for other brown rot fungi (Presley et al. 2017, *FGB*; Presley and Schilling 2017, *AEM*). Finally, we verified the inducing mechanism, the release of cellobiose, not only inducing cellulases but also repressing oxidoreductases (Zhang and Schilling 2017, *FGB*). This high-quality set of papers tells a very clear story about a two-step oxidative-hydrolytic mechanism in *P. placenta*, controlled by the release of a single disaccharide, cellobiose. Rather than an inducible cellulase system, we have for the first time been able to capture this ephemeral pretreatment ahead of saccharification and show and inducible system, and we are currently writing a manuscript for Nature Communications where we have used functional genomics to verify this across brown rot fungal clades as a clear distinction in mechanisms from white rot fungal ancestors.

This apparent 'upgrade' in biomass deconstruction has been our focus and was the applied basis for funding from DOE. This is for several key reasons, each of which an area where we have delivered critical information during this grant research. First, a consolidation of harsh oxidative reactions alongside cellulase hydrolysis would be 'consolidated bioprocessing (CBP)' that we have not yet been able to achieve in industry. This consolidation would save a very expensive pretreatment step during commercial biomass

processing, and here, the fungi are clearly consolidating reactions using space to do so rather than time. Second, the genes involved in the unique oxidative step have never before been isolated. This allowed us to narrow the gene targets for discovery from thousands to less than 600 in the *PNAS* paper, and now to less than 40 by using functional genomics among clades for the *Nature Communications* effort. This will target gene discovery efforts and help identify the unifying features that make brown rot unique – both are enormous contributions to our field and to these applied interests. Third, we have identified a single sugar, cellobiose, that controls they key oxidative-hydrolytic transition during the 'two-step.' This is a clever mechanism for the fungus, and a novel finding that is already trending on the *FGB* website. More than the mechanism, however, is that this single control pathway offers a simpler option to do so in a bioreactor for industrial biomass processing.

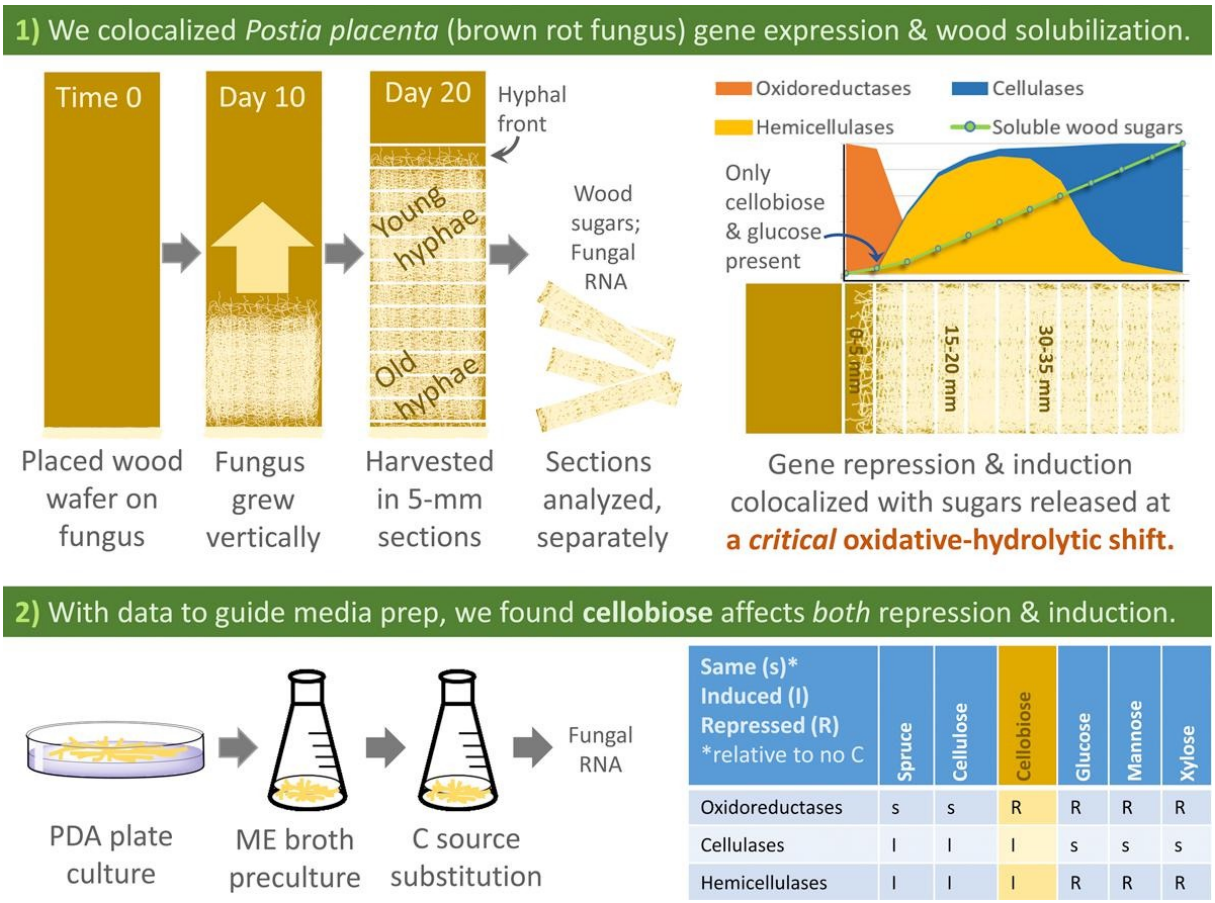
In conclusion, we have delivered on our promises for this grant and have published several key papers for the field and toward industrial application. Our particular success has been enabled by a 5-yr grant cycle and an ability to link objectives in a way to probe this mechanism deeply and follow questions raised by previous objectives. In this way, this Early Career grant has been able to accomplish the deliverables promised as well as adapt its objectives to the questions in a unified way to yield a very significant contribution to the science of biomass deconstruction.

Research

Objective 1 (Complete)

This first objective was to use what we considered a 'coarse' physical sampling (1-mm intervals) of wood as it was invaded from one direction by our test fungus, *P. placenta*. This was the 'wafer' design described above, and it has remained a very useful set-up for our experiments. This required extensive optimization of the set-up to slow growth and accentuate gradients. We accomplished this by using both gravity and a cross-grain orientation of a wood type (spruce) already low in diffusibility. This also required a highly laborious approach to sectioning and then sub-sampling wood, similar to slicing a loaf of bread and then cutting slices into several sub-slices. Many wafers were sectioned 70 times, and then sub-sectioned to create 210 total sub-samples for processing. We took all opportunities to streamline sample processing for certain techniques such as tetramethylammonium hydroxide (TMAH) thermochemolysis to measure lignin modifications, but this was undeniably a laborious effort with eight authors on the final manuscript now in print at *International Biodeterioriation and Biodegradation*. In this case, it was a necessary effort to target the coarse scale of reaction partitioning hypothesized, bracketing a zone of interest and offering a wood-based microcosm set-up to reproduce and resample this zone.

The specific methods for Objective 1 have been outlined in prior updates (Figure 1, below, is new and from a graphical abstract in the Zhang and Schilling 2017 *FGB* paper). In brief, the approach was to have *P. placenta* colonize a wood substrate cut to impede colonization and accentuate gradients, then measure endoglucanase co-localized with lignin modifications and carbohydrate depolymerization, both using characteristic changes induced by brown rot fungi. Key results are shown as Figures 2-4 (following pages), indicating the zone of depolymerization preceding both lignin modifications and the endoglucanase, suggesting co-localization in discrete space.



Same (s)* Induced (I) Repressed (R) *relative to no C	Spruce	Cellulose	Cellobiose	Glucose	Mannose	Xylose
Oxidoreductases	s	s	R	R	R	R
Cellulases	I	I	I	s	s	s
Hemicellulases	I	I	I	R	R	R

Figure 1 – Colonization of wood wafers by *Postia placenta*, and sectioning for sampling and co-localizing aspects of the brown rot mechanism. In this graphical abstract from Zhang and Schilling (2017) *FGB*, the wafer design is shown upper right, along with an analysis in upper left of wood sugars released in various zones along the wafers. These are shown matched to qPCR transcript data upper right. Then, C source culturing trials validated an induction/repression role for cellobiose. This general design was used for much of the published work as our space-for-time surrogate design.

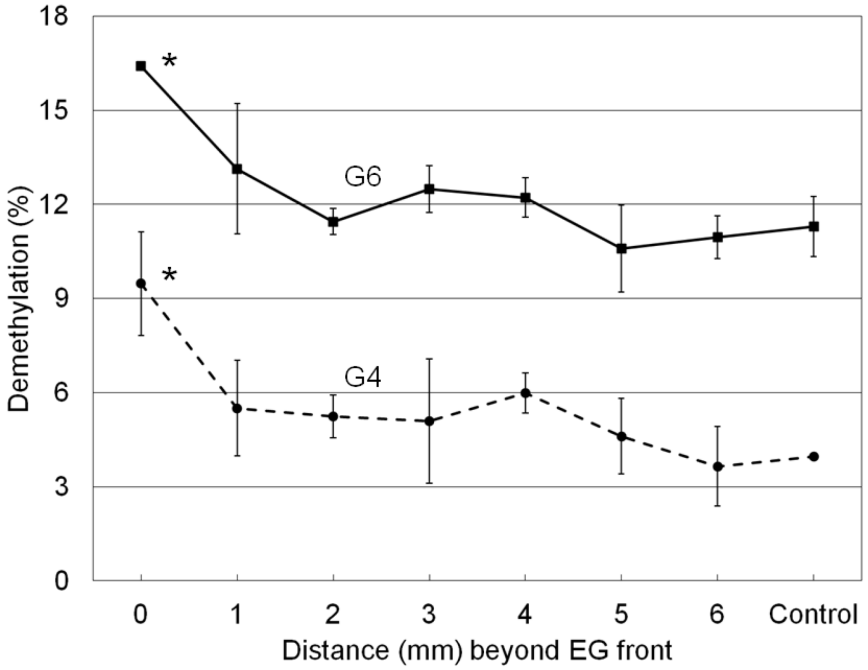


Figure 2 – The Schilling et al. (2013, *IBB*) results that indicated an early change in wood without the presence of active cellulases (*P. placenta* does not have exo-acting cellulases). Demethylation at G6 and G4 positions on lignin residues is theorized due to oxidative Fenton chemistry during brown rot 'pretreatments,' but here, significantly higher demethylation 'front' is coincident with, not ahead of, the 'front' of endoglucanase (EG).

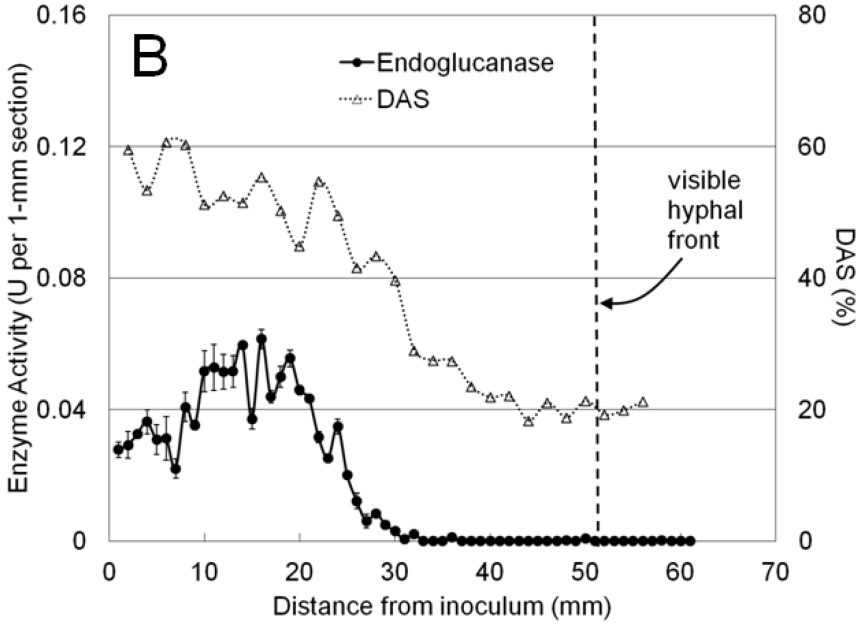


Figure 3 – The EG front, shown in Figure 2 coincident with lignin demethylation, lags behind visible hyphal front by approximately 2 cm, as well as behind the advance of hyphae imaged with chitin-specific dye using laser-scanning confocal microscopy (not shown). Depolymerization, as dilute alkali solubility (DAS), however, appears to increase ahead of the EG front.

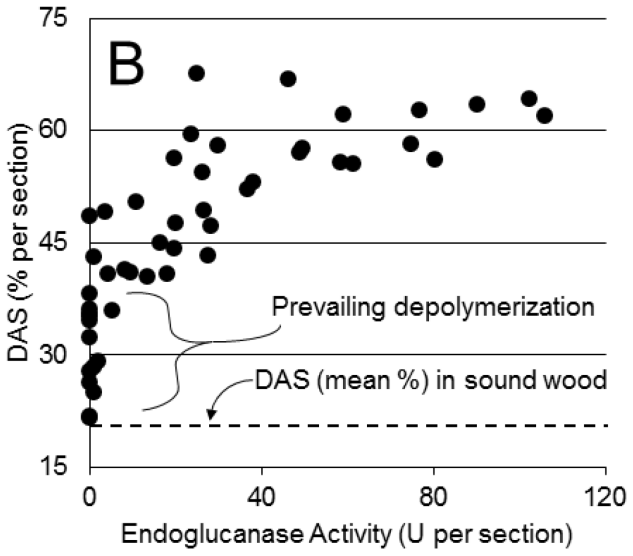


Figure 4 – Regressing the proxy for depolymerization against the EG progress (again, coincident with lignin demethylation), the zone of prevailing depolymerization is clearly > 1 cm, as measured at these detection limits and with the tools, as proposed.

The conclusion from this work is that if controlled partitioning of oxidative Fenton chemistry away from the cellulase is occurring, it is not at 'large distances away from hyphae, as is often written without citation. This work, however, seems to suggest two possibilities that are both ripe for follow-up: 1) the consequences in lignin modifications are not tied to the oxidative reactions responsible for depolymerizing holocellulose, and/or 2) the agent of depolymerization is not simple Fenton chemistry, as theorized. There are several reasons to assume #2 may be correct, and one of the most powerful aspects of the progress in Objective 2 is that in addition to providing a functionally-relevant measure of porosity, as proposed, if the enzyme is not within the cell wall (as we hypothesize) it may not be the critical second step in the two-step reaction. The *P. placenta* secretome, lacking cellobiohydrolases (CBHs), could be a result of discarding genes that are no longer necessary – perhaps EG will be the next to disappear, rather than being something unusually efficient on its own. This would suggest a one-step reaction, not a two-step reaction, and demand a highly spatially-targeted oxidative reaction in order to be energetically efficient. Non-localized Fenton reaction within a wood cell does not fit this description.

Objective 2 (Complete)
The second objective in the work involved co-localization of aspects of the brown rot mechanism that control *where* oxidative and enzymatic reactions occur. This work had two main components of the approach, one to localize porosity and the other pH (a functional differential from pH 1.5-5.5).

First, for porosity, we used several approaches to attempt to track the progress of wood 'loosening' and a key endoglucanase in the *P. placenta* simplified cellulase arsenal, Cel5b. To track the cellulase, rather than purifying low-levels of Cel5b from the P. placenta secretome, we have transformed *Pichia pastoris* to produce it for us, to ease purification and increase titers toward producing immunlabel tags for transmission electron microscopy (TEM), *in planta*.

The recombinant *P. placenta* endoglucanase Cel5B (rPpCel5B) was expressed in *P. pastoris* using the EasySelect Pichia expression kit (Invitrogen, Carlsbad, CA) as described in the manufacturer's protocols. Expression and purification of rPpCel5B was performed by the Biotechnology Resource Center at the University of Minnesota in St. Paul. Prior to production scale expression, transgenic *P. pastoris* strains capable of expressing rPpCel5B were identified via PCR with a target amplicon of PpCel5B cDNA. A transgenic strain containing the integrated PpCel5B cDNA sequence (*PiprPpCel5B)* was induced to produce and secrete the recombinant protein. After induction, *PiprPpCel5B* culture supernatant was

screened to determine the presence an active enzyme capable of cleaving AZO-CMC. After production-scale expression of the recombinant protein, *PiprPpCel5B* culture supernatant was concentrated by repeated ultrafiltration through a poly-ethersulfone 10-kD-cutoff membrane and rPpCel5B was further purified via DEAE-Sepharose CL-6B anion exchange chromatography. Fractions containing proteins with the approximate mass of glycosylated rPpCel5B (~50 kD) and the ability to cleave AZO-CMC were pooled, concentrated, and dialyzed against sodium citrate buffer (20 mM, pH 5.0). Dialyzed material was enzymatically deglycosylated (E-DEGLY; Sigma, St. Louis, MI) to confirm the presence of a protein with the theoretical mass of non-glycosylated PpCel5B (~34 kD).

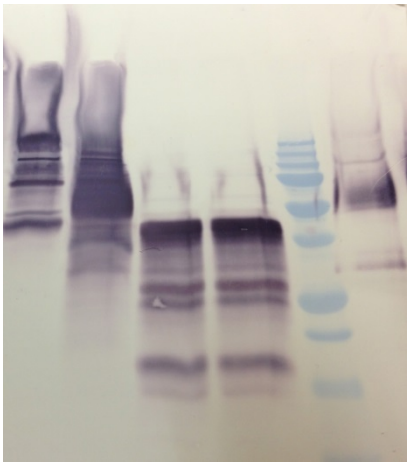


Figure 5 - Western blotting of proteins secreted by *PiprPpCel5B* in culture and wild type *P. placenta* in rotted birch. Lane 1: *PiprPpCel5B* culture supernatant under non-inducing conditions. Lane 2: *PiprPpCel5B* supernatant 72 hours after induction with methanol. Lanes 3 and 4: protein extracts from birch obtained 34 days after inoculation with *P. placenta*. Lane 5: molecular weight markers (Top to bottom: 250 kD; 150 kD; 100 kD; 75 kD; 50 kD; 37 kD; 25 kD; 20 kD; 15 kD); Lane 6: partially purified rPpCel5B.

Partially purified concentrated rPpCel5B protein in sodium citrate buffer (Figure 5, lane 9) was used for immunization of New Zealand White Rabbits to obtain antisera. Antisera production services were contracted through Pacific Immunology (Ramona, CA). Antisera obtained from rabbits seven weeks after immunization detected the presence of a protein with an approximate mass of the rPpCel5B target protein in extracts obtained from Birch rotted by *P. placenta* via Western blotting (Figure 5, lane 3 and 4). Results suggest antibodies in post-immunization antisera are capable of detecting PpCel5B expressed by *P. placenta* in rotted wood and wild type and recombinant proteins are both glycosylated (Figure 5, lane 4 and 6) Experiments are in progress to confirm that antibodies contained in the antisera have specific affinity to the PpCel5B expressed by *P. placenta* in rotting wood. These experiments will also determine the degree to which, if any, antibodies produced by the rabbit hybridize to proteins secreted by wild type *P. pastoris* in culture that share epitopes with proteins secreted by in *P. placenta* rotted wood.

Once antisera specificity was determined, antisera was used in immuno-electron microscopy (TEM, specifically) to assess red pine rotted by *P. placenta*. This assessment established very low presence of PpCel5B within the wood cell walls of gamma irradiated red pine, which we may be incorporating in our *Nature Communications* paper but within the supplemental material. This TEM work was challenging, and with an ongoing and new user facility grant at PNNL, we transferred some of the antibody there to continue doing this work going forward. This lack of EG within the wood cell wall supports previous work from the 1980s, that the enzyme is too large to enter the wood cell wall. However, these microscopy efforts using polyclonal secondary antibodies are supportive more than they are conclusive, so this approach of using supplemental figures to support data generated from chemical analyses is logical.

Second, for the pH measurements, we have used a bromophenol blue dye most recently to attempt hyperspectral small-scale imaging using a powerful light microscopy system in Biodale, on campus. Brown rot fungi are theorized to maintain a pH differential between the fungal hyphae and deeper within the

secondary cell walls of wood. This ranges from pH 5.5 within wood to <2 near hyphae, as determined previously using a patch clamp technique. This shift from blue to yellow is obvious, but also very universal, not patterned. This does not work to prove or disprove a pH differential between fungal hyphae in wood cells and the wood itself, however, because of the lack of resolution. This interface may be submicron, and wood cells in our systems average 30 microns total diameter, with the lumen around 7 microns diameter or larger in many cases. Getting pH, a dynamic environmental variable, with minimal disruption to a fungus growing *in planta* and in a multi-phase environment with areas of low porosity is challenging. In the coming year, we will continue to approach the pH measurements with imaging tools, with the help of Lawrence Berkeley National Laboratory (LBNL), as planned. We met with Seema Singh in San Francisco in December 2013, to discuss imaging options, and this collaboration offers some options for increasing resolution.

Third, to assess wood porosity changes over the course of brown rot, *Postia placenta* (MAD698R) was grown on a wood wafer vertically placed on a surface of the fungal lawn, as in the other studies. The fungus grew directionally on the wood piece, from bottom to top, which allowed us to dissect sections of wood pieces infected by the fungus at different growth/wood-decaying stages. Four sections were harvested from each wafer: non-infected zone; 0-5mm zone from the fungal growth front; 15-20mm from the fungal growth front; and 30-35mm from the fungal growth front. We have been harvesting 10 for the solute exclusion porosity analyses. The data have been acquired, but we are waiting to include these alongside proteomics data that were being generated separately as part of a newer DOE BER grant that includes proteomics efforts at PNNL.

In these porosity data is a trend of increasing pore spaces for any size of PEG molecules when decay is advanced. Individual values of each group (PEG molecule and zone) have been analyzed by t-test to evaluate statistical significance in their pore space differences from the non-infected zone. The data suggested that more heavily decayed wood such as the 30-35mm zone increased the pore space for all the tested PEG molecules, but not by a large margin. It was also suggested that such increased pore space was more pronounced for smaller molecules such as PEG400K and Ethylene glycol (EG), and significant difference was seen in the 15-20mm zone in addition to the 30-35mm zone. These porosity measures have become increasingly interesting, as the RNAseq efforts (below) have revealed that among the oxidative genes upregulated, three non-oxidative CAZy family genes putatively involved in cell wall swelling (a pectinase and two expansins) are also highly upregulated at the front. Porosity has long been debated in brown rot literature, with an increase being necessary for cellulase ingress but previous coarse porosity studies suggesting little change.

Objective 3 (Complete)
To further explore the molecular mechanisms behind the partitioning of saccharification and brown rot pretreatment (e.g., oxidation), we led an effort to detect the transcripts of genes associated with these processes. At first, this was designed for microscopy and in situ hybridization efforts, but the success of the wafer set-up convinced us to first try qPCR in these sections to attempt to reveal an early zone of differential expression. This successfully isolated a 'zone of upregulation' for the lignocellulose-oxidation genes, showing us a pattern among 16 genes of differential expression that had never been shown before and that supported a two-step oxidative-hydrolytic brown rot mechanism. This was submitted to *PNAS*, with supportive reviews save for an interest in RNAseq whole transcriptome data rather than qPCR. We therefore adapted the study for RNAseq, showed a similar pattern, but with the whole transcriptome to show for it. This was published in *PNAS* because of this consistency as well as its novelty. The key figures are shown in order, below, along with an additional figure for our Nature Communications submission as a venn diagram, outlining our use of functional genomics to narrow the gene targets going forward.

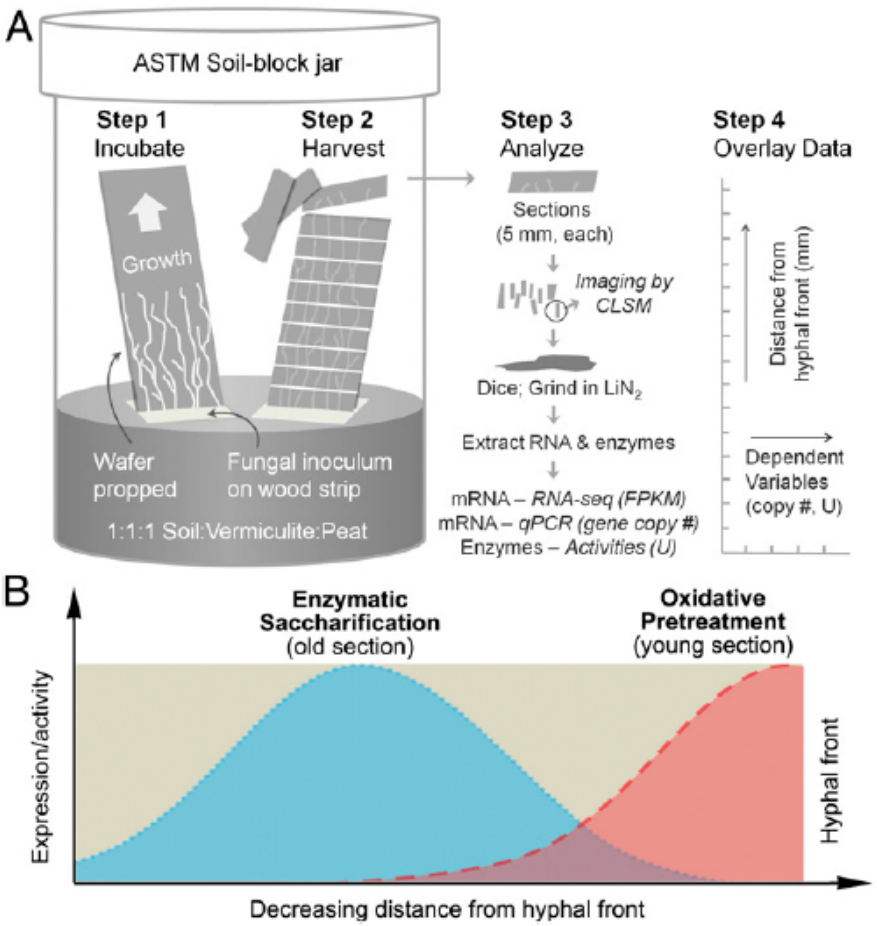


Figure 6 - Schematic of wafer setup and staggered mechanism hypothesis. (A) *P. placenta* was cultured in modified American Society for Testing and Materials (ASTM) soil-block microcosms, with wood wafers propped to force fungi to grow vertically. This setup generated a spatiotemporal gradient along the advancing mycelium, delineated by the visible advancing hyphal front and reconstructed from sections. LIN2, liquid nitrogen. (B) Data from RNA-seq, quantitative PCR (qPCR), confocal laser scanning microscopy (CLSM), and enzymatic activity assays were layered (i.e., stacked) in specific zones relative to the hyphal front and used to test the hypothesis that brown rot fungi stagger the expression of genes and extracellular enzymes during wood decay.

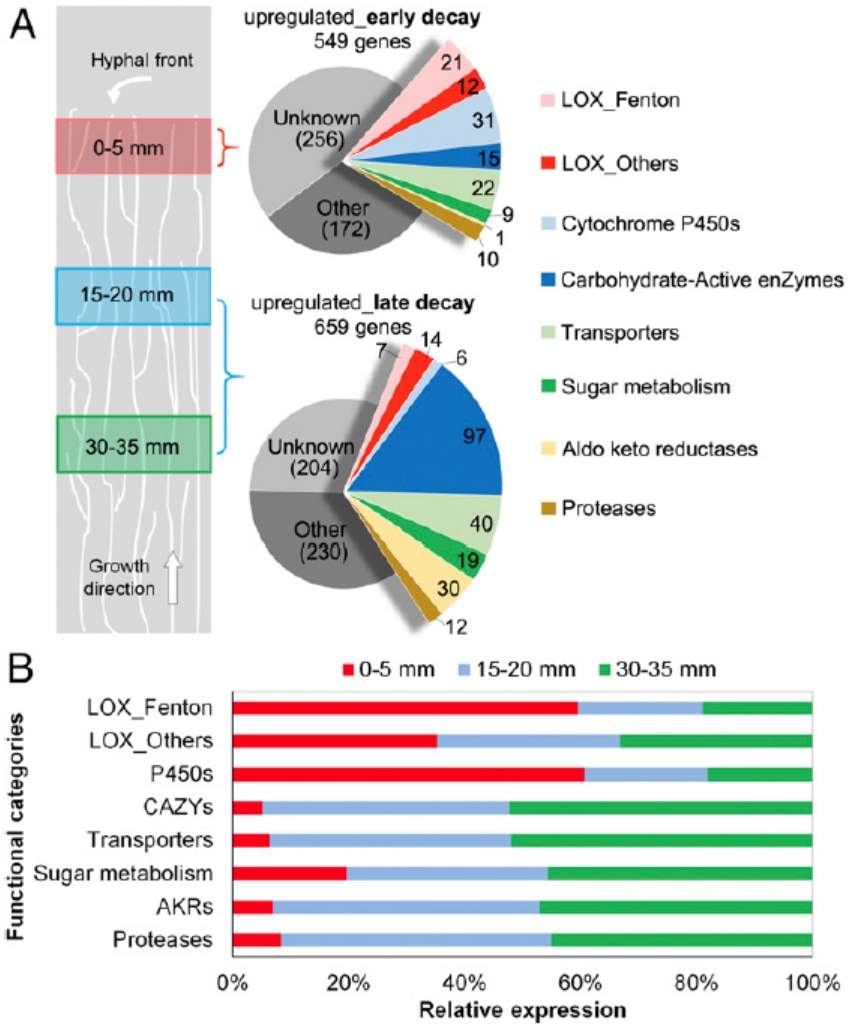


Figure 7 - Gene transcription patterns along the advancing hyphal front in aspen wafers as measured by RNA-seq. (A) Distribution of DEGs. Relative to sections at 15–20 mm and 30–35 mm, which represent late decay, 549 genes were up-regulated in sections at 0–5 mm, which represent early decay. Conversely, 659 genes were up-regulated during late decay. Data were obtained from three bioreplicates at each position. (B) Relative expression levels in different gene categories. The relative FPKM levels of DEGs in each category were compared among the sections of the three lengths. For DEGs, the fold change was >4 (FDR < 0.05) and the FPKM was >5. The LOX_Fenton category includes enzymes that may support extracellular Fenton chemistry (e.g., via extracellular H2O2 generation, iron reduction and homeostasis, hydroquinone biosynthesis, quinone redox cycling). LOX_Others includes genes that may be involved in lignin/aromatic compound modification, oxalate metabolism, polysaccharide monooxygenation, and protection from reactive oxygen species (9, 57). CAZy genes were assigned according to the CAZy database (www.cazy.org) (58). The assignment of other gene categories is provided in Dataset S3. AKR, aldo keto reductase.

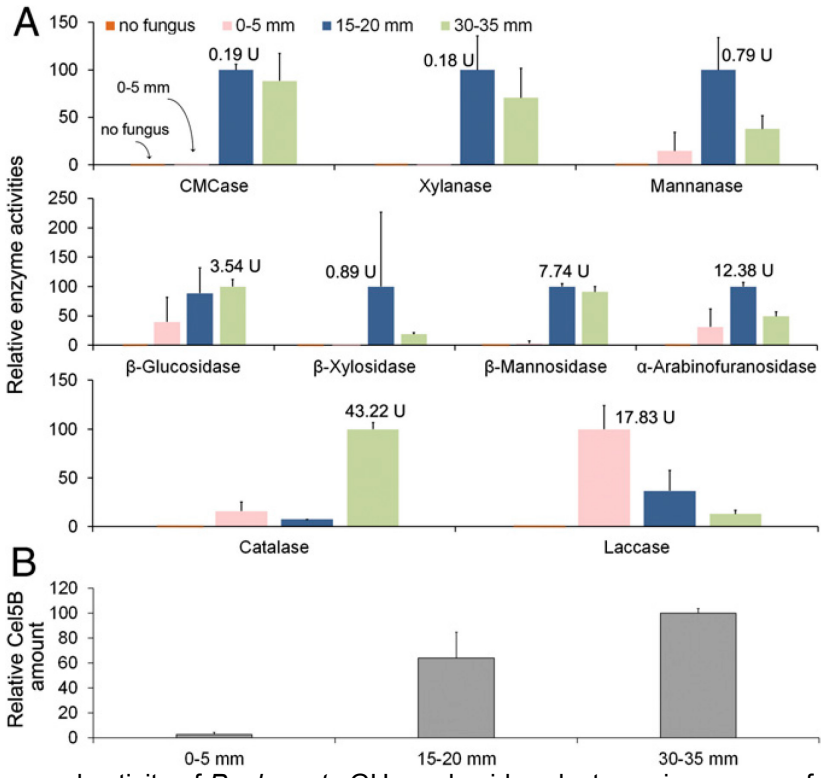


Figure 8 - Presence and activity of *P. placenta* GHs and oxidoreductases in aspen wafers. (A) Relative activities of relevant water-extracted enzymes with likely lignocellulolytic roles (SI Materials and Methods). Activities were measured as units per milligram of total fungal protein and were normalized for each enzyme by setting the highest activity (shown beside the column) at 100. (B) Relative amounts of secreted endoglucanase Cel5B (protein ID 103675/117690) via Western blotting. Error bars represent the SD of two bioreplicates, with 12 wafers' sections pooled for each replicate.

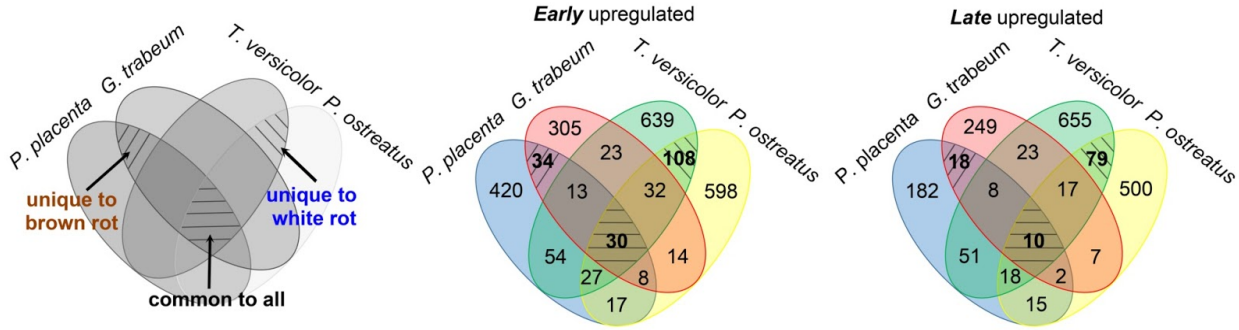


Figure 9 – The 549 upregulated genes targeted in the *PNAS* work (Figure 7a), was reduced to 34 ("unique to brown rot") using functional genomics among four fungi, an effort poised for submission to *Nature Communications* that will include the DOE Early Career grant acknowledgement due to the *P. placenta* data being integrated. Unique and common differentially-expressed genes (DEGs) as a function of fungus and wood decay stage. The stage-dependent genes, i.e., early- or late-upregulated genes (*4-fold change with FDR < 0.05, see SI for DEG definitions*) are shown among brown rot fungi (*P. placenta, G. trabeum*) and white rot fungi (*T. versicolor, P. ostreatus*) after assigning the same MCL_ID to orthologous genes in all tested fungi (*see Dataset 1 for OrthoMCL groups*).

Conclusions: These three completed Objectives have yielded significant publication and presentation products, as well as having been leveraged by the PI Schilling toward user facility grants and another, larger DOE BER grant with an 'omics' focus on wood-degrading fungal mechanisms. More than the quantity of

the research, however, is its quality. Brown rot fungal mechanisms have been elucidated by work done either in artificial media (not representative) or in solid wood as whole blocks (noisy). These have, by design, overlooked the ephemeral (48 hours, in our system) oxidative pretreatment step unique to brown rot. Our design has isolated that step, spatially, and within that zone we have concluded three very important, novel aspects of the brown rot mechanism that overturn long-standing dogma: 1) Brown rot cellulases are induced after a very short oxidative step at the hyphal front, upending the traditional model of brown rot cellulases as a constitutive system and a distinguishing feature from white rot. 2) Brown rot deploy an oxidative pretreatment that relies on some familiar genes (e.g. iron reductases) but not apparently others that have long been theorized as critical to brown rot pretreatments (e.g. alcohol oxidases). 3) The brown rot 'two-step' is common among multiple brown rot clades, representing a convergently evolved approach to deconstruct wood – this transition is mediated by cellobiose released from wood. In revealing these three key aspects of brown rot, we have pushed the field forward as well as narrowed the targets for gene discovery within this biological system primed to be harnessed, commercially.

Products Delivered:

Peer-reviewed Publications

Zhang, J., Schilling, J.S.* (2017) Role of carbon source in the shift from oxidative to hydrolytic wood decomposition by *Postia placenta.* Fungal Genetics & Biology 106:1-8.

Presley, G.N., Zhang, J., Schilling, J.S.* (2017) 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.

Presley, G., Schilling, J.S.* (2017) Distinct growth and secretome strategies in two taxonomically-distinct brown rot fungi. Applied & Environmental Microbiology 83:7 11 e02987-16

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.

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.

Posters and Presentations

Zhang, J, JS Schilling, J M Figueroa, K Sweeney, G. Presley, KE Hammel, C Hunt, H Suzuki, E Panisko, G Orr, D Hu. Spatial connectomics to identify agents relevant to lignocellulose deconstruction in fungi. Department of Energy, Genomic Sciences Program annual PI meeting, Tysons Corner, VA March 7, 2016.

Presley, G.N., Zhang, J., Schilling, J.S. Colocalization of wood modifications and secretome composition during the colonization of wood by *Postia placenta* and *Gloeophyllum trabeum*. 28th Fungal Genetics Conference, Asilomar, CA, March 19, 2015.

Zhang, J., Presley, G.N., Schilling, J.S. Brown Rot Fungus *Postia placenta* and its Differential Expression as it Degrades Wood. 28th Fungal Genetics Conference, Asilomar, CA, March 19, 2015.

Schilling, J.S., Figueroa, M., Hammel, K.E., Hunt, C.J., Panisko, E.A., Presley, G.N., Zhang, J. (2015) Spatial connectomics to identify agents relevant to lignocellulose deconstruction in fungi. DOE Genomic Sciences BER program grantee meeting, Tyson's Corner, VA, February 24, 2015.

Schilling, J.S., Blanchette, R.A., Duncan, S.M., Filley, T., Jurgens, J., Presley, G. Mimicking fungal biomass decomposition, assuming a biphasic mechanism. DOE Genomic Sciences BER program grantee meeting, Tyson's Corner, VA, February 23, 2015.

Schilling, J.S., Presley, G., Menke, J., Zhang, J. (2014) Tracking footprints of wood-degrading fungi that do the two-step. Mycological Society of America (MSA), East Lansing, MI. June 8-11, 2014.

Presley, G., Schilling, J.S. (2014) Probing the *Postia placenta* secretome in early stages of wood decay. Mycological Society of America (MSA), East Lansing, MI. June 8-11, 2014.

Zhang, J., Presley, G., Song, Z., Oliver, P., Schilling, J.S. (2014) Partitioning transcriptions of the oxidative enzymes and cellulase reveals the spatially control mechanisms of fungi during wood degradation. Mycological Society of America (MSA), East Lansing, MI. June 8-11, 2014.

Presley, G.N., Schilling, J.S. Correlation of expression patterns in wood-degrading brown rot fungi to wood modifications: Using space as a gauge for time. AIChE annual meeting, San Francisco, CA, November 4, 2013.

Schilling, J.S., Blanchette, R.A., Duncan, S.M., Filley, T., Jurgens, J., Presley, G. Mimicking fungal biomass decomposition, assuming a biphasic mechanism. DOE Genomic Sciences BER program grantee meeting, Bethesda, MD, February 26, 2013.

Menke, J., Ryu, J., Presley, G., Duncan, S., Jurgens, J., Blanchette, R., Filley, T., Hammel, K., Schilling, J. Spatial assessment of oxidative and enzymatic reactions in brown rotted wood. The American Phytopathological Society (APS) and Mycological Society of America (MSA) Joint Annual Meeting, Austin, TX, August 12, 2013.

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