# Gene Regulatory Networks Enabling Fungi to Selectively Extract Sugars from Lignocellulose

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**Project Goals:** Fungi dominate the biological decomposition of wood and other lignocellulosic plant tissues in nature. These saprotrophs offer us a proven model for making energy, sustainably, from biomass. They also offer those with commercial interests a range of pathways for unlocking sugars embedded in lignin. Their strategies range from 'white rot' mechanisms that remove lignin to gain access to polysaccharides to 'brown rot' mechanisms that selectively extract sugars, leaving most lignin behind. This metabolic diversity could be harnessed, industrially, but research has generally been focused more toward white rot delignification pathways. White rot fungi can unsheath polysaccharides by selectively removing lignin, a capacity that historically attracted interest for the potential to extract intact fibers for papermaking. Modern bioenergy schemes, however, do not aspire for intact fibers - instead, the goal is to depolymerize polysaccharides to release fermentable sugars (saccharification), saving lignin as a co-product, if possible. This is a better fit for the carbohydrate-selective pathways of brown rot fungi, but our grasp of fungal brown rot metabolism lags behind what we know about white rot.

Our collaborative project is aligned to address these gaps, with the **goal** of producing an integrated regulatory model for brown rot. Our proposed objectives insure stand-alone advances, but will also synergize to push ideas forward in a systems context.

**Objective 1** is to identify fungal gene regulation patterns that distinguish brown rot fungi from fungi with other decay modes (e.g., white rot). We plan to compare fungi among relevant lineages but with varied carbohydrate-selectivities. We will culture these strains on solid wood wafers, spatially mapping gene expression and then overlaying fungal/wood metabolite patterns to enable temporally-resolved functional genomics. These maps can isolate patterns unique to brown rot and can target characterization.

**Objective 2** focuses on characterization, starting with a short list generated in an earlier transcriptomics study, and progressively adding objective 1 gene targets. We plan to use routine single-/multi-cellular *in vitro* transformation pipelines, but will complement this with efforts to develop a brown rot transformation system, enabling *in vivo* manipulations (e.g., Crispr-Cas9).

**Objective 3** is to use metabolomics to map metabolite-expression feedback over time, providing networks of gene regulation. This approach promises to advance our understanding of this unique brown rot strategy, beyond current ROS-centric models toward a systems view.

**Abstract:**

Certain filamentous fungi are uniquely able to deconstruct lignocellulose, and their poorly understood mechanisms have potential biofuels applications. A key hindrance to harnessing these fungal 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 omics analyses of these fungi from artificial media or from colonized wood ground en masse fail to distinguish expression of key gene products occurring 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*. Wood-degrading fungal genomes are an emerging resource, particularly brown rot functional types. 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, as well as understanding the underlying regulation of a globally important decomposition mechanism – brown rot.

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 where reactive oxygen species (ROS) are deployed. To do this, we need to compare global expression profiles among mycelial regions. To map this wood-fungal interaction, we must 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 JGI and the Pacific Northwest National Laboratory.

**References:**

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