White-Rot Fungi Utilize Lignin-Derived Compounds as a Carbon Source

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Project Goals: This study aims to investigate the hypothesis that white-rot fungi can simultaneously depolymerize lignin extracellularly and catabolize depolymerization products intracellularly as carbon and energy sources. Understanding this potential biological activity and identifying the most promising fungal strains for lignin turnover and catabolism will justify future investment in genetic tool development to enable metabolic engineering in white-rot fungi for lignin bioconversion to bioproducts.

Lignin is currently an undervalued aromatic polymer in lignocellulosic biorefineries, mainly due to its heterogeneity and recalcitrance, but it is a key substrate to enable a sustainable plant-based bioeconomy. White-rot fungi (WRF) are recognized as the most efficient lignin-degrading organisms in Nature and are thus potential biocatalysts for lignin bioconversion. However, while lignin *depolymerization* by WRF has been studied for decades and is well accepted, the ability of WRF to *catabolize* lignin remains virtually unknown.

To ascertain if WRF simultaneously depolymerize lignin and utilize lignin-derived compounds, we are employing in *silico*, in *vivo,* and in *vitro* approaches based on hypothesis-driven, systems-biology studies. Recent ^{13}C-labeling analyses with two WRF that exhibit different lignocellulose degradation patterns have revealed that these organisms can indeed funnel carbon from lignin degradation products to central metabolism. Considering the lack of information on aromatic catabolic pathways in WRF, we have initiated pathway and enzyme discovery via in *silico* analyses. Specifically, we are conducting homology searches in two selected fungal genomes using as *template* enzymes already characterized in bacteria and yeast. Based on these results, we have hypothesized a pathway that WRF most likely use for the conversion of 4-hydroxybenzoic acid, an abundant aromatic compound found ester-linked to lignin in poplar. Multi-omic analyses (transcriptomics, proteomics, and metabolomics) based on differential substrate have also facilitated enzyme down-selection and confirmed metabolites from the hypothesized pathway. Lastly, we have initiated enzyme validation via *in vitro* studies to verify the function of the down-selected enzymes (e.g. decarboxylases, hydroxylases, and dioxygenases). Overall, the knowledge gained through this work will serve as a foundation to employ WRF in lignin bioconversion to bioproducts and provide deeper insight into global carbon cycling.

This research is supported by the U.S. Department of Energy (DOE), Office of Biological and Environmental Research (BER) under the Early Career Award Program. A portion of the research has been performed using EMSL (grid.436923.9), a DOE Office of Science User Facility sponsored by the Office of Biological and Environmental Research. A portion of the work has been also conducted by the U.S. DOE Joint Genome Institute, a DOE Office of Science User Facility, supported by the DOE Office of Science under Contract No. DE-AC02-05CH11231. Part of this work was also supported by a Laboratory Directed Research and Development project at the National Renewable Energy Laboratory.