Elucidation of aromatic catabolic pathways in white-rot fungi

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This project aims to investigate the hypothesis that white-rot fungi can simultaneously depolymerize lignin extracellularly and catabolize depolymerization products intracellularly as carbon and energy sources. Evaluating this hypothesis will provide deeper understanding of the role of white-rot fungi in facilitating carbon sequestration in Nature. Additionally, identifying the most promising fungal strains for lignin turnover and catabolism will catalyze future efforts in genetic tool development to enable metabolic engineering in white-rot fungi for lignin bioconversion to bioproducts.

Lignin is the second most abundant plant-based biopolymer on Earth and represents up to 40% of the energy density of lignocellulosic biomass. Even though lignin is a massive natural carbon and energy reservoir, only a small group of basidiomycete fungi, namely white-rot fungi (WRF), have evolved the ability to efficiently depolymerize and mineralize lignin to CO_2 and H_2O. Considerable research efforts have been undertaken to understand how WRF depolymerize lignin but the biochemical reactions that convert lignin into CO_2 have been largely neglected. In fact, it is unclear if WRF intracellularly catabolize lignin-derived aromatic compounds to utilize them as a carbon and energy source, or rather if lignin is depolymerized and mineralized extracellularly merely to facilitate access to cellulose and hemicellulose for use as a primary carbon source.

To date, we have employed ^{13}C-isotope labeling, systems biology approaches, and *in vitro* enzyme assays to definitively demonstrate that two WRF, *Trametes versicolor* and *Gelatoporia (Ceriporiopsis) subvermispora*, funnel carbon from lignin-derived aromatic compounds into central carbon metabolism via intracellular catabolic pathways [1]. Specifically, ^{13}C-isotopic labeling approaches showed that these WRF utilize poplar-derived aromatic compounds (e.g. 4-hydroxybenzoic acid (4-HBA)) as a carbon source. *In silico* genome analysis led us to hypothesize a complete catabolic pathway for 4-HBA and identify multiple homologous sequences for enzymes with putative oxidative decarboxylase, hydroxylase, and ring-opening dioxygenase activities, which are among the main biochemical reactions acting on aromatic compounds. Spatial and differential proteomic and metabolomic analyses supported the proposed catabolic pathways and showed alternative catabolic steps in *T. versicolor* that were not present in *G. subvermispora*.

Based on the *in silico*, proteomics, and transcriptomics results, we down-selected enzymes for further *in vitro* characterization, and we have assigned a function to six fungal enzymes (including oxidative decarboxylases, hydroxylases, and ring-opening dioxygenases). Interestingly, even though we selected homologous enzyme pairs from both WRF with similar -omics trends, in a few cases only one of the studied fungi showed activity for the proposed substrate. Based on all the observations from this study [1], we hypothesized that 4-HBA preferentially undergoes oxidative decarboxylation to hydroquinone and subsequent hydroxylation to 1,2,4-benzenetriol in *G. subvermispora* before ring cleavage, whereas 4-HBA would preferentially undergo hydroxylation to protocatechuate and further oxidative decarboxylation to 1,2,4-benzenetriol in *T. versicolor*. Examining additional decarboxylases and hydroxylases as well as enzymes from other protein families that can perform the same or similar oxidative reactions, such as cytochromes P450 with aromatic hydroxylation activity, will also be key for elucidating enzyme preferences for specific substrates. Overall, this work forms the foundation of a new research area based on lignin catabolism by WRF, which could be further exploited to convert the undervalued biopolymer lignin into value-added compounds.

Publication

[1] Carlos del Cerro, Erika Erickson, Tao Dong, Allison R. Wong, Elizabeth K. Eder, Samuel O. Purvine, Hugh D. Mitchell, Karl K. Weitz, Lye Meng Markillie, Meagan C. Burnet, David W. Hoyt, Rosalie K. Chu, Jan-Fang Cheng, Kelsey J. Ramirez, Rui Katahira, Wei Xiong, Michael E. Himmel, Venkataramanan Subramanian, Jeffrey G. Linger, and Davinia Salvachúa. Intracellular pathways for lignin catabolism in white-rot fungi. ***PNAS***, in press. **2021**.

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