Lignin Valorization through Biological Funneling

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Project Goals: The BioEnergy Science Center (BESC) focuses on fundamental understanding and elimination of biomass recalcitrance. BESC's approach to improve accessibility to the sugars within biomass involves (1) improved plant cell walls for rapid deconstruction and (2) multi-talented microbes for converting plant biomass into biofuels in a single step [consolidated bioprocessing (CBP)]. Biomass research works with two potential bioenergy crops (switchgrass and *Populus)* to develop improved varieties and to understand cell wall biosynthesis pathways. We test large numbers of natural variants and generate specific modified plants samples. BESC's research in deconstruction and conversion targets CBP manipulating thermophilic anaerobes and their cellulolytic enzymes for improved conversion, yields, and titer. Enabling technologies in biomass characterization, 'omics, and modeling are used to understand chemical and structural changes within biomass and to provide insights into mechanisms.

Lignin structure and composition vary significantly across plant species. Regardless of the feedstock type, in many modern biorefinery designs, lignin is typically not utilized for fuels and chemicals production, but instead is slated to be burned for process heat because its inherent heterogeneity and recalcitrance make it difficult to selectively valorize.[1] Indeed, despite many decades of lignin depolymerization research, most catalytic strategies to break down lignin yield a highly heterogeneous slate of aromatic compounds.

In nature, some microbes have evolved catabolic pathways that enable the utilization of lignin-derived aromatic molecules as carbon and energy sources. Aromatic catabolism typically occurs via upper pathways that act as a "biological funnel" to convert heterogeneous lignin-derived substrates to central intermediates, such as protocatechuate or catechol. These compounds subsequently undergo ring cleavage and are further converted, often via the β-ketoadipate pathway, to central carbon metabolism. Recently, we employed a natural aromatic-catabolizing organism, *Pseudomonas putida* KT2440, to demonstrate that these metabolic pathways can be harnessed and engineered to convert both aromatic model compounds and heterogeneous, lignin-enriched streams into value-added compounds.[2-5] To make this concept of biological funneling a reality will require systems-level understanding of the catabolic pathways used by microbes to convert lignin to monomers and then convert these monomers to value-added compounds.[6]

Here, we will present several insights into lignin depolymerization and aromatic catabolism by *P. putida* KT2440 and other aromatic-catabolic microbes. From a time-resolved proteomics experiment in a real-world, lignin-rich substrate with an analysis of both the extracellular and intracellular fractions, we identified multiple enzymes that are exclusively expressed and secreted in the substrate that are known to cleave bonds in lignin. Additionally, from both proteomics and transcriptomics, we have identified multiple enzymes of interest for localization studies to better understand the flow of aromatic carbon from lignin to central carbon metabolism in the context of cellular structure. To date, we have validated a GFP-based labeling approach with a known aromatic catabolic enzyme. A localization study is ongoing from the extracellular enzymes through the upper aromatic catabolic pathways and the β -ketoadipate pathway.

Additionally, we are examining the ability of *P. putida* to catabolize new types of lignin substrates. Specifically, caffeoyl alcohol has been recently shown to be a naturally occurring polymer in seed coats of several plant species.[7-8] These monomers form homogeneous, linear ether-linked lignin (dubbed C-lignin), which represents a promising new avenue for engineering homogeneous plant lignin for more effective valorization approaches. Using a C-lignin extract, we have demonstrated that *P. putida* can depolymerize and catabolize C-lignin-rich substrates, demonstrating the ability to potentially use microbial strategies to valorize these novel lignins. Overall, this work demonstrates that the use of microbial aromatic catabolism coupled to advances in lignin chemistry *in planta* may one day enable an approach to valorize lignin by overcoming its inherent heterogeneity to produce fuels, chemicals, and materials.

References

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