

Investigating the spatial organization of aromatic catabolism in *P. putida* KT2440

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Project Goals: The Center for Bioenergy Innovation (CBI) vision is to accelerate domestication of bioenergy-relevant, non-model plants and microbes to enable high-impact innovations at multiple points in the bioenergy supply chain. CBI addresses strategic barriers to the current bioeconomy in the areas of 1) high-yielding, robust feedstocks, 2) lower capital and processing costs via consolidated bioprocessing (CBP) to specialty biofuels, and 3) methods to create valuable byproducts from the lignin. CBI will identify and utilize key plant genes for growth, composition and sustainability phenotypes as a means of achieving lower feedstock costs, focusing on poplar and switchgrass. We will convert these feedstocks to specialty biofuels (C4 alcohols, C6 esters and hydrocarbons) using CBP at high rates, titers and yield in combination with cotreatment, pretreatment or catalytic upgrading. CBI will maximize product value by *in planta* modifications and biological funneling of lignin to value-added chemicals.

The valorization of lignin—an abundant, recalcitrant, and heterogeneous polymer in plant cell walls—is critical to enable the bioeconomy. Biological funneling of lignin-derived aromatic compound mixtures to single value-added products via engineered *Pseudomonas putida* KT2440 has emerged as a means to overcome heterogeneity. However, the spatial organization of lignin catabolism remains unclear, yet has implications for metabolic engineering strategies as well as our collective knowledge of nutrient acquisition by soil bacteria. Here, we first explore spatiotemporal dynamics of the *P. putida* exoproteome during cultivation on lignin-rich media. We observe that many enzymes with known and putative roles in aromatic catabolism are selectively packaged into outer membrane vesicles (OMVs) from early to late stationary phase, corresponding to the shift from bioavailable carbon to oligomeric lignin as a carbon source. Functional assays demonstrate that enzymes contained in the OMVs are active and catabolize aromatic compounds, which supports OMV-mediated extracellular breakdown of lignin-derived aromatics as a strategy for nutrient acquisition by soil bacteria. Second, we present the discovery that two undescribed proteins in *P. putida* are important to the uptake of *p*-coumarate and ferulate, which are abundant hydroxycinnamic acids in plant cell walls. Together, these works improve our understanding of the spatial distribution of lignin-derived aromatic catabolism and seek to support improved efficiency of microbial lignin conversion.

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