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Project Title.

Systems Metabolic Engineering of *Pseudomonas putida* for the Bioproduction of C6 Chemicals from Lignin-derived Aromatics

Abstract.

Pseudomonas putida KT2440 (KT2440) is a gram-negative, non-platform bacterium with robust metabolism of aromatic compounds and tractable genetic traits. This project focuses on the engineering of KT2440 strain for the bioproduction of C6 chemicals from aromatics that can be derived from lignin degradation. As a largely untapped aromatic carbon source, lignin plays a critical role in the economic utilization of lignocellulosic biomass. In the preliminary study, the PI's lab has developed a proof-of-concept KT2440 strain that reached 17.4% of the theoretical yield (titer at 2.5 g/L) for the direct biosynthesis of C6 diacid, i.e., adipic acid, from lignin-derived aromatics. In this project, the team aims to optimize the adipate production and further use it as a central hub to expand the product portfolio to include additional industrially important C6 chemicals, i.e., 6-hydroxycaproate (6-HCA), 1,6-hexanediol (1,6-HDO), 6-aminocaproate (6-ACA), and 1,6-hexanediamine (1,6-ACA).

The overall engineering goal is supported by specific research objectives for the KT2440 host, including to broaden its carbon utilization capability to S lignin compounds, to enable its application in microaerobic/anaerobic processes, and to install/optimize new biosynthetic pathways. The project goal will be achieved through an integrated approach of systems metabolic engineering that is enabled by synthetic and systems biology tools built in the PI's lab.

The team expects fundamental knowledge gained about metabolism and regulatory networks in KT2440 to have broad impacts on future engineering and industrial applications of the strain. Furthermore, in light of recent progress in degradations of polyethylene terephthalate (PET) plastics, the resulting aromatic stream can be fed to strains engineered in this project for the bioproduction of C6 chemicals, which extends applications of this research.