Conversion of lignin-derived aromatic compounds into lipids by engineered *Rhodococcus opacus* strains

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Project Goals: The goal of this project is to interrogate the metabolic networks and genetic regulation that control the utilization of and tolerance to thermochemically depolymerized lignin, focusing on phenolics, in *R. opacus*.

Lignocellulosic biomass is a renewable feedstock that can be converted into biofuels and high-value chemicals using microorganisms. However, pretreatment of lignocellulosic biomass often releases toxic compounds that inhibit microbial growth. In addition, lignin has been an untapped carbon source due to its recalcitrance and toxic monomeric units (e.g., aromatic compounds). Our work focuses on developing *Rhodococcus opacus* as a new chassis for conversion of aromatic compounds into triacylglycerols (TAGs), biodiesel precursors (1). *R. opacus* is a promising host due to its high tolerance to and utilization of aromatics as a sole carbon source. Importantly, *R. opacus* can accumulate TAGs up to ~80% of cell dry weight under nitrogen-limiting conditions. To enhance its innate aromatic-degrading capacity, we applied adaptive evolution, a growth-based strain selection method, by sequentially sub-culturing cells in diverse combinations of lignin-derived aromatic compounds as sole carbon sources. Our adapted strains demonstrated higher growth rates and higher lipid accumulation compared to the wild type strain. Whole genome sequencing, RNA-seq, and ^{13}C-fingerprinting analysis have identified possible aromatic tolerance and utilization mechanisms such as degradation pathway upregulation. We will present progress towards development of *R. opacus* as a microbial cell factory.

(1) WD Hollinshead, WR Henson, M Abernathy, TS Moon and YJ Tang. Rapid Metabolic Analysis of *Rhodococcus opacus* PD630 via parallel ^{13}C-Metabolite Fingerprinting, *Biotechnol. Bioeng.* 113, 91-100 (2016)

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