Aromatic tolerance and utilization in adapted *Rhodococcus opacus* strains for lignin bioconversion

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Project Goals: We aim to characterize and improve *Rhodococcus opacus* PD630 as a chassis for conversion of lignocellulosic biomass, specifically thermochemically depolymerized lignin (i.e., aromatics), to valuable products. This approach can add value to a previously under-utilized feedstock (lignin) and improve titers and productivities in lignocellulosic biorefineries.

Lignocellulosic biomass is a renewable feedstock that can be converted to valuable products. During biomass depolymerization, growth inhibitors can be generated which limit product yields, titers, and productivities, and the non-sugar component (i.e., lignin) is not used during fermentation. To increase tolerance to growth inhibitors and incorporate utilization of lignin for production of valuable products, we have explored tolerance to and utilization of lignin-derived aromatics by *Rhodococcus opacus* PD630, a bacterial strain that can accumulate triacylglycerols (biodiesel precursor) up to ~80% of its cell dry weight in nitrogen-limited culture conditions. Specifically, we performed adaptive evolution on lignin model compounds to improve the native aromatic tolerance of *R. opacus* and characterized adapted strains by multi-omics approaches. *R. opacus* strains adapted for growth on single and multiple lignin model compounds demonstrated improved growth and lipid accumulation compared to the wild-type strain. In addition, mixtures of adapted strains demonstrated improved growth on depolymerized lignin compared to the wild-type strain. Whole genome sequencing, RNA-Sequencing, ^{13}C-fingerprinting, and metabolomic approaches have identified possible mechanisms for improved aromatic tolerance and utilization such as upregulation of degradation pathways and transporters. We will present our progress towards the development of *R. opacus* as a microbial cell factory.

References

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