

Elucidating Aromatic Utilization Mechanisms in Engineered *Rhodococcus opacus* Strains for Lignin Valorization

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Project Goals: We aim to combine adaptive evolution, gene deletion analysis, and multi-omics approaches to identify aromatic tolerance and utilization mechanisms in the promising biofuel production strain *Rhodococcus opacus* PD630 (*R. opacus*). Our systems biology approach provides insights into the catabolic potential of *R. opacus* as a chassis for the conversion of lignocellulose, specifically thermochemically depolymerized lignin (i.e., aromatics), into valuable products.

R. opacus is naturally tolerant to aromatic compounds found in lignin-derived mixtures. We have demonstrated the potential of *R. opacus* for increased survivability in high concentrations of aromatics through adaptive evolution. Through genomic and functional characterization of wild type and adapted strains, pathways for aromatic degradation and funneling into central metabolism have been elucidated. Expression profiles have only been generated for select carbon sources, however, limiting our understanding of aromatic utilization and tolerance [1, 2].

To increase our knowledge of aromatic utilization and tolerance, we grew wild type *R. opacus* PD630 and mutant strains in minimal media supplemented with model lignin breakdown products at a total aromatic concentration permissive to WT growth. Additionally, we grew the mutant strains at higher concentrations of the relevant aromatics to examine the transcriptional changes which supported the increased-tolerance phenotype. Additionally, ¹³C metabolic flux analysis and targeted metabolomics were completed for WT/mutants growth on aromatics to rigorously measure and compare how aromatic substrates were consumed [3, 4].

We have been performing multi-omics analyses and gene deletion experiments to determine mechanisms of aromatic tolerance and utilization. Specifically, we have utilized transcriptomics, machine learning-based transcript-to-flux prediction models, and recently developed synthetic biology tools to elucidate the intriguing mechanisms of aromatic utilization [5-9]. This study will deepen our understanding of aromatic tolerance and utilization mechanisms in diverse *R. opacus* mutants by expanding the list of aromatic compound mixtures [10-12]. In

addition, this work will enable us to provide a genome-scale model of *R. opacus* to facilitate the development of the promising biofuel production organism.

Publications (+ Corresponding author)

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