Elucidating aromatic tolerance and utilization in adaptively evolved *Rhodococcus opacus* strains for lignin valorization

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Project Goals: We aim to combine adaptive evolution and multiple–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.

The oleaginous microbe *R. opacus* is naturally tolerant to aromatic compounds commonly found in lignin-derived mixtures. We have demonstrated the potential of *R. opacus* for increased survivability in higher concentrations of aromatic compounds 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 – which were not permissive to WT growth – 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 phenol to rigorously measure and compare how aromatic substrates were consumed [3, 4].

We found similar transcriptional profiles in the established beta-ketoadipate pathway and aromatic gene clusters for mutant and wild type strains grown on the same carbon source. Instead of wild type and mutant strains differing in aromatic transcription profiles, the carbon source seems to drive differences in expression. An amino acid labeling experiment performed with phenol supported this finding by showing only minor differences between WT and mutant strains, suggesting that enhanced aromatic tolerance in adapted strains might be the product of not only

increased rates of aromatic utilization but also other unknown factors. Further work is currently underway to characterize and define unknown mechanisms of aromatic tolerance and utilization through analysis of differentially expressed transcripts, use of machine learning-based transcript-to-flux prediction models, and recently developed synthetic biology tools [5-8]. 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 in which these strains are fully characterized.

Publications (+ Corresponding author)

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5. DM DeLorenzo, WR Henson and TS Moon[+]. Development of Chemical and Metabolite Sensors for *Rhodococcus opacus* PD630. *ACS Synth. Biol.* 6, 1973–1978 (2017)

6. DM DeLorenzo, AG Rottinghaus, WR Henson and TS Moon[+]. Molecular toolkit for gene expression control and genome modification in *Rhodococcus opacus* PD630. *ACS Synth. Biol.* 7, 727–738 (2018)

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