Multi-omic analysis of adapted *Rhodococcus opacus* strains for the characterization of aromatic metabolism

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Project Goals: We aim to combine adaptive evolution and multi-omic approaches to identify aromatic tolerance mechanisms in *Rhodococcus opacus*. We aim to utilize identified genes and pathways to engineer an optimized *R. opacus* strain for the conversion of lignocellulose into valuable products.

Depolymerization of lignin results in a mixture of aromatic compounds that inhibit microbial conversion of lignocellulose into value-added fuels and chemicals. *R. opacus* is a promising strain for biofuel production due to its inherent tolerance for aromatic compounds. However, mechanisms for its aromatic tolerance and utilization remain undercharacterized. *R. opacus* has also been underutilized due to a lack of reliable engineering tools. To identify degradation pathways and tolerance mechanisms for aromatic mixtures in *R. opacus*, we performed adaptive evolution on 32 combinations of lignin model compounds, and used multi-omic approaches, including genomics, transcriptomics, targeted metabolomics, and lipidomics. Additionally, we developed a toolbox for reliable gene expression control and genome modification in *R. opacus*, and applied the developed tools to confirm our findings from these multi-omic analyses.

The key findings are as follows. First, adapted strains showed improved growth compared to wild-type on model lignin compound mixtures (up to 1900% growth improvement). Second, whole genome sequencing identified genes involved in redox reactions that were mutated in four distinct phenolic adaptation experiments with no mutations in two control adaption experiments. One of the mutated enzymes, superoxide dismutase, showed decreased activity in PVHG6, the adapted strain with the most improved growth, suggesting that redox reactions are important for aromatic tolerance and utilization. Third, using transcriptomics, we identified multiple aromatic transporters, five aromatic funneling pathways, and two degradation routes of the β-ketoadipate pathway for five aromatic compounds. Fourth, synthetic biology tools, including a reliable system for genome modification and the first reported CRISPR interference (CRISPRi) system for *Rhodococcus*, have been developed and applied to confirm the identified degradation pathways for lignin model compounds. Fifth, we found that the alteration of the mycolic acid and phospholipid membrane composition is likely to be a strategy of *R. opacus* for phenol tolerance. Together, our results provide insights into mechanisms for aromatic tolerance and utilization, facilitating the further development of *R. opacus* as an improved chassis for renewable bioproduction.

Publications
1. WR Henson, T Campbell, D DeLorenzo, Y Gao, B Berla, SJ Kim, M Foston, TS Moon and G Dantas. Multi-omic elucidation of aromatic catabolism in adaptively evolved *Rhodococcus opacus*. *Metab. Eng.* 49, 69–83 (2018)

2. WR Henson, F Hsu, G Dantas, TS Moon and M Foston. Lipid metabolism of phenol tolerant *Rhodococcus opacus* strains for lignin bioconversion. *Biotechnol Biofuels*. 11:339 (2018)

3. DM DeLorenzo and TS Moon. Selection of stable reference genes for RT-qPCR in *Rhodococcus opacus* PD630. *Sci. Rep.* 8:6019 (2018)

4. 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)