

Characterizing the transcriptomes of *Rhodococcus opacus* strains adapted to model lignin breakdown products

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Project Goals: Using *R. opacus* strains adapted to model lignin breakdown products, we are identifying the genetic components and RNA expression changes which impart aromatic tolerance.

The oleaginous microbe *R. opacus* is naturally tolerant to aromatic compounds found in lignin-derived compound mixtures. *R. opacus* has been selected as a potential biological chassis for lignin conversion to lipids, and its mutants with improved aromatic tolerance have been generated by adaptive evolution. We have previously characterized the genetic alterations to *R. opacus* after aromatic adaptation. However, the differential expression profiles of mutant clones have only been described for individual compounds or one mixture of these compounds, limiting our understanding of aromatic tolerance and utilization in *R. opacus* [1, 2].

In this work, we used a multipartite study design to comprehensively characterize these mutants. We completed growth experiments, comparing wild-type *R. opacus* PD630 (WT) and the mutant strains in minimal media at a total aromatic concentration permissive to WT growth. For each compound and each compound mixture, we also measured the growth rate of adapted mutant strains grown at a high concentration not permissive to WT growth. Additionally, ¹³C metabolite profiling and lipidomics have been performed along with transcriptomics. Importantly, we are now contextualizing the increased growth and compound utilization by comparing the transcriptomic profiles of the adapted strains grown on aromatic compounds at the permissive or nonpermissive concentrations. 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

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. A Yoneda, WR Henson, NK Goldner, KJ Park, KJ Forsberg, SJ Kim, MW Pesesky, M Foston, G Dantas and TS Moon. Comparative transcriptomics elucidates adaptive phenol tolerance and utilization in lipid-accumulating *Rhodococcus opacus* PD630. *Nucleic Acids Res.* 44, 2240–2254 (2016)

This work is funded by the U.S. Department of Energy (DOE), Office of Biological and Environmental Research, Biological System Sciences Division, award # DE-SC0018324.