

## **Multi-omics analysis of *Rhodococcus opacus* strains evolved for optimized lignocellulose bioconversion**

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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. 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.**

Lignin-derived aromatics inhibit microbial conversion of lignocellulose-based sugars to fuels and chemicals, and microbial lignin valorization strategies require consumption of complex aromatic mixtures. *R. opacus*, a promising biofuel production strain, can tolerate and consume individual lignin-derived aromatics, but degradation pathways and tolerance mechanisms for aromatic mixtures are not well characterized. To address this knowledge gap, we performed adaptive evolution using lignin model compound mixtures and characterized adapted strains using multiple omics approaches. Adapted strains showed up to 1900% growth improvement compared to the wild-type strain in model lignin compound mixtures. We identified multiple genes involved in redox reactions that were affected by mutations in four distinct adaptation experiments. Both the wild-type and adapted strain PVHG6 co-consumed multiple lignin model compounds, and transcriptomics showed distinct funneling degradation pathways upregulated in response to each aromatic compound. Together, our results provide new insights into the complex metabolism of aromatic compound mixtures by *R. opacus* and suggest that tuning redox metabolism can enable improved catabolism of aromatic compounds.

### **Publications**

1. WD Hollinshead, WR Henson, M Abernathy, TS Moon and YJ Tang. Rapid Metabolic Analysis of *Rhodococcus opacus* PD630 via parallel <sup>13</sup>C-Metabolite Fingerprinting, *Biotechnol. Bioeng.* 113, 91-100 (2016)
2. Yoneda A, Henson WR, Goldner NK, Park KJ, Forsberg KJ, Kim SJ, Pesesky MW, Foston M, Dantas G, Moon TS. Comparative transcriptomics elucidates adaptive phenol tolerance and utilization in lipid-accumulating *Rhodococcus opacus* PD630, *Nucleic Acids Res.* 18, 2240-2254 (2016)

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