A Concerted Systems Biology Analysis of Aromatic Metabolism in

*Rhodococcus opacus* PD630

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Project Goals:

1. Use $^{13}$C-MFA to reveal R. opacus’ phenol metabolism
2. Connect flux data with transcription profiling and metabolite analysis to show phenol metabolism regulations.
3. Determine whether R. opacus phenol utilization is hindered by other aromatic and non-aromatic substrates.
4. Test adaptively evolved strains to determine how their central metabolic network has changed.

*Rhodococcus opacus* PD630 metabolizes aromatic substrates and naturally produces branched-chain lipids, which are advantageous traits for lignin valorization. To provide insights into its lignocellulose hydrolysate utilization, we performed $^{13}$C pathway tracing, transcriptional profiling, biomass composition analysis, and metabolite profiling in conjunction with $^{13}$C-metabolic flux analysis (MFA) of phenol metabolism. We found that 1) phenol is metabolized through the ortho branch of the β-ketoacip late pathway; 2) phenol-fed cultures have high TCA cycle fluxes with overflow succinate secretion; 3) NADPH is generated mainly via NADPH-dependent isocitrate dehydrogenase; 4) Active cataplerotic fluxes increase plasticity in the TCA cycle; and 5) gluconeogenesis occurs partially through the reversed Entner–Doudoroff pathway (EDP). We also found that phenol-fed *R. opacus* PD630 generally has lower sugar phosphate concentrations (e.g., fructose 1,6-bisphosphatase < 5%) compared to metabolite pools in glucose-fed *Escherichia coli* (set as 100%), while pool sizes of its TCA metabolites (malate, succinate, and α-ketoglutarate) are higher than those in *E. coli*. In addition, glucose catabolite repression is absent in *R. opacus*, but phenol utilization can be hindered by the presence of other aromatic substrates (e.g., benzoate). Three adaptively-evolved strains display different growth rates when fed with phenol as a sole carbon source, but they demonstrate a conserved central flux network.

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