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–ketoadipate 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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