Characterizing growth and metabolism of *Rhodococcus opacus* PD630 on real lignin breakdown products

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Project Goals: The goal of this project is to determine the mechanisms by which *Rhodococcus opacus* PD630 is able to convert depolymerized lignin to lipid-based biofuels. Our previous work has examined how *R. opacus* metabolizes several aromatic model compounds, but it does not extend to how this bacterium is able to tolerate and utilize depolymerized lignin as a sole carbon source. The specific aim of this work is to tailor the depolymerization reaction conditions and catalysts to generate lignin breakdown products suited to the metabolism of *R. opacus*.

R. opacus is a soil bacterium that is naturally able to tolerate and utilize aromatic substrates as sole carbon sources, as well as accumulate high titers of lipids [1, 2]. To date, published studies have focused on how *R. opacus* is able to tolerate and consume model aromatic compounds (phenol, benzoate, etc.), and have provided many novel insights into this species' aromatic and lipid metabolism [3-6]. However, there is a gap in knowledge about how *R. opacus* consumes real-world lignin breakdown products (LBP), as well as how the specific thermocatalytic process conditions and catalysts used to produce LBP affect *R. opacus* metabolism. Our team has employed diverse lignin chemical conversion processes, cell cultivation, GC-MS analysis of LBP consumptions, ^{13}C-based dynamic labeling of intracellular metabolites, and metabolite pool size quantifications, which offer rich insights into LBP metabolism in *R. opacus*.

First, we characterized LBP composition and its effect on *R. opacus* growth. We found that the LBP composition is highly dependent on the biomass source (e.g., switchgrass or poplar), reaction conditions (e.g., solvent, temperature, and residence time), and the catalyst (e.g., palladium or nickel on activated carbon). Our palladium catalyst produced more aromatic compounds than the nickel catalyst did. *R. opacus* was able to consume LBP generated with either catalyst as the sole carbon source, although cultures reached higher cell density when grown using LBP from the palladium catalyst.

Second, we developed a convenient method for gas chromatography–mass spectrometry to analyze and compare the compounds present in LBP cultures. This method can identify which compound *R. opacus* is able to consume. By GC-MS analyses of culture samples with different LBP compositions, we found that the major fractions of LBP consumed in all *R. opacus* culture samples were a mixture of ketone, furfural, and phenolic compounds.

Third, we measured and compared the intracellular concentrations of ~20 central carbon metabolites when *R. opacus* was grown with glucose, benzoate, phenol, and three defined aromatic mixtures. We found that metabolic concentrations were highly variable based on the carbon substrates present. This finding shows that *R. opacus* has a flexible metabolism, and demonstrates how changes in LBP composition can affect central metabolism.

Fourth, we measured intracellular metabolite concentration from cells growing with LBP, and have carried out isotopic pulse-trace experiments with LBP. LBP-fed cells had higher intracellular sugar phosphate concentrations than glucose-fed *E. coli* did, and their intracellular TCA cycle metabolite concentrations were ~50% of those of *E. coli*, suggesting that *R. opacus* is able to co-consume the sugar and aromatic fractions of LBP. Isotopic pulse-trace experiments have indicated that there is a significant lag phase (~3 hours) for the uptake of LBP after medium switching from phenol to LBP, likely because the cells need lag phase to sense the LBP and to produce the appropriate transporters and degradation enzymes.

Future work will focus on optimizing the thermocatalytic reaction parameters to produce LBP more compatible with the metabolic capabilities of *R. opacus*. Additionally, transcriptomic analysis will be performed to establish how accurately growth on the model compounds simulates growth on real LBP. The findings from this work improve our understanding of *R. opacus*' metabolism for future lignin valorization, and they will inform development of a machine learning-based predictive model.

Publications (+ Corresponding author)

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