Systems metabolic engineering of *Novosphingobium aromaticivorans* for lignin valorization

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Project Goals: To engineer a non-model bacterium, *Novosphingobium aromaticivorans*, for valorization of depolymerized lignin to value-added bioproducts. The project involves (1) discovery and optimization of pathways for assimilation of lignin-derived aromatic compounds, (2) engineering conversion pathways that match the stoichiometry of aromatic catabolism, and (3) development of genome-scale mapping techniques to identify new engineering targets in non-model bacteria.

Lignin is one of the abundant renewable materials found in nature. This heterogeneous aromatic polymer is composed of a variety of guaiacyl (G), syringyl (S), and *p*-hydroxyphenyl (H) monomers that are connected by diverse chemical linkages. Lignin valorization would improve biofuel economics, potentially through bacterial conversion of thermochemically depolymerized lignin into valuable bioproducts. *Novosphingobium aromaticivorans* F199 is an Alphaproteobacterium capable of degrading G, S, and H monomers and, due to its genetic tractability, is an emerging model organism for conversion of lignin-derived aromatic compounds. However, F199 cannot natively catabolize every component of depolymerized lignin, which limits conversion yields.

We are identifying new aromatic degradation pathways to increase the catabolic potential of *N. aromaticivorans* F199, using a combination of barcoded transposon insertion sequencing, proteomics, and *in vitro* biochemistry. In several cases, *N. aromaticivorans* F199 is known to assimilate an aromatic compound but the pathway requires additional characterization, as we demonstrated with the aromatic monomer syringate.[1] Additionally, F199 can catabolize previously undescribed compounds, such as the β-1 linked dimer 1,2-diguaiacylpropane-1,3-diol (DGPD). We have recently identified a novel enzyme, LsdE, required for DGPD catabolism.[2] However, there are multiple aromatic compounds for which *N. aromaticivorans* F199 lacks the necessary catabolic pathway. We have previously isolated additional Sphingomonads that metabolize several of these compounds and are currently investigating the relevant pathways for transfer to F199. Of note, one of these isolates is remarkably similar at the genetic level to *Sphingobium* sp. SYK-6, a well characterized model for bacterial lignin degradation.

To further enable the genetic exploration of lignin catabolic pathways and to integrate these into novel hosts, we are developing new genetic tools for *N. aromaticivorans* F199. A broad host range plasmid that replicates in the strain has been identified. To better understand the effect of host genetic variation on pathway function, we are also adapting a newly-developed technique of bacterial quantitative trait locus (QTL) mapping. We have acquired and sequenced additional strains of *N. aromaticivorans* and demonstrated intraspecific recombination using genome shuffling. By combining novel pathway discovery with this developing genetic toolset, we can engineer *N. aromaticivorans* F199 to more efficiently valorize lignin.

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