Systems metabolic engineering of *Novosphingobium aromaticivorans* for lignin valorization

Marco N. Allemann (allemannmn@ornl.gov),[1] Christopher C. Azubuike (azubuikecc@ornl.gov),[1] Gerald N. Presley,[1] Leah H. Burdick,[1] Richard J. Giannone,[1] David C. Garcia,[1] and **Joshua K. Michener**[1]

[1]Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, TN

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 *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) 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.[1]

We are identifying new aromatic degradation pathways to increase the catabolic potential of F199, using a combination of barcoded transposon insertion sequencing, proteomics, and *in vitro* biochemistry. We demonstrated this approach with the aromatic monomer syringate,[2] the β-1 linked dimer 1,2-diguaiacylpropane-1,3-diol (DGPD),[3] and, more recently, the monomer guaiacol. However, there are multiple aromatic compounds for which F199 lacks the necessary catabolic pathway. We have previously isolated additional Sphingomonads that metabolize several of these compounds and are currently identifying and characterizing the relevant pathways for transfer to F199. These new strains include a *Novosphingobium* isolate that can assimilate the β-β linked dimer pinoresinol and a *Sphingobium* isolate that is remarkably similar at the genetic level to *Sphingobium* sp. SYK-6 and can likewise assimilate the 5-5 linked dimer dehydrodivanillic acid (DDVA).

In addition to introducing new pathways, we are also optimizing native assimilation pathways in F199 to efficiently channel more lignin-derived carbon into central metabolism intermediates. Simultaneously, we are converting the resulting intermediates into value-added products, such as building blocks for bio-derived polymers. Finally, to better understand the effect of host genetic variation on pathway function, we are adapting a novel technique, bacterial quantitative trait locus (QTL) mapping, to F199. We have demonstrated intraspecific recombination between strain of *N. aromaticivorans* and are currently studying and optimizing this process. By combining novel pathway discovery, heterologous expression, and optimization, we are engineering *N. aromaticivorans* F199 to be more efficient at valorizing lignin-derived compounds.

This work was supported by the U.S. Department of Energy Office of Science, through the Office of Biological and Environmental Research (BER) Early Career Research Program. Preliminary data was collected through the BioEnergy Science Center and Center for Bioenergy Innovation, Bioenergy Research Centers funded by BER.

1. Azubuike, C. C., Allemann, M. N. & Michener, J. K. Microbial assimilation of lignin-derived aromatic compounds and conversion to value-added products. *Curr. Opin. Microbiol.* **65**, 64–72 (2022).

2. Cecil, J. H., Garcia, D. C., Giannone, R. J. & Michener, J. K. Rapid, Parallel Identification of Catabolism Pathways of Lignin-Derived Aromatic Compounds in Novosphingobium aromaticivorans. *Appl. Environ. Microbiol.* **84**, AEM.01185-18 (2018).

3. Presley, G. N. *et al.* Pathway discovery and engineering for cleavage of a β-1 lignin-derived biaryl compound. *Metab. Eng.* **65**, 1–10 (2021).