Redundancy in Aromaric O-demethylation and Ring Opening Reactions in *Novosphingobium aromaticivorans* and their Impact in Biological Conversion of Lignin

Jose M. Perez[1,2,3]* (perez8@wisc.edu), Wayne S. Kontur,[2,3] Carson Gehl,[2,3,4] Derek M. Gille,[2,3] Yanjun Ma,[2,3] Alyssa V. Niles,[2,3] German Umana,[2,3] Timothy J. Donohue,[2,3,5] and **Daniel R. Noguera[2,3]**

[1]Department of Civil and Environmental Engineering, University of Wisconsin-Madison, Madison, WI; [2]DOE Great Lakes Bioenergy Research Center, Madison, WI; [3]Wisconsin Energy Institute, University of Wisconsin-Madison, Madison, WI; [4]Department of Biomedical Engineering, University of Wisconsin-Madison, Madison, WI; [5]Department of Bacteriology, University of Wisconsin-Madison, Madison, WI, USA

Website: https://www.glbrc.org/

Project Goals: The overall project aims to valorize the lignin fraction of plant biomass via chemical fractionation and depolymerization followed by conversion of the resulting mixtures of aromatic compounds into single valuable chemicals by genetically engineered bacteria. The goal of this study was to identify the key O-demethylases and ring-opening dioxygenases involved in the degradation of plant-derived aromatic compounds in *Novosphingobium aromaticivorans*

One of the major components of plant biomass is lignin, an heterogenous and recalcitrant aromatic heteropolymer. One strategy to make value from lignin is to use chemical techniques to deconstruct it into mixtures of phenolic compounds and to funnel these mixtures into a single product using engineered bacteria. *Novosphingobium aromaticivorans* DSM12444 can naturally degrade multiple lignin-derived phenolic compounds and it has been previously engineered to produce 2-pyrone-4,6-dicarboxylic acid (PDC) from a variety of compounds that are naturally catabolized via 3-methoxygallic (3-MGA) acid or protocatechuic acid (PCA). Two critical reactions involved in aromatic compounds catabolism by *N. aromaticivorans* that can affect their conversion into PDC are O-demethylation and oxidative aromatic ring opening.

In this work, we investigated enzymes predicted to be responsible for O-demethylation of syringic acid, vanillic acid, and 3-MGA, and enzymes predicted to be responsible for the ring opening of 3-MGA, gallic acid, and PCA. Our results show that DesA is involved in syringic acid and vanillic acid O-demethylation, whereas LigM is involved in vanillic acid and 3-MGA O-demethylation. We also found evidence of a potential new O-demethylase involved in the O-demethylation of 3-MGA into gallic acid. In addition, our results support that LigAB is the main enzyme responsible for the aromatic ring opening of 3-MGA, gallic acid, and PCA. However, we also found a previously uncharacterized aromatic ring opening dioxygenase, LigAB2, that has high activity with gallic acid and plays a minor role in the degradation of 3-MGA and PCA.

The data obtained in this study revealed a previously uncharacterized route for aromatic compound degradation in *N. aromaticivorans* that involves O-demethylation of 3-MGA into gallic acid followed by aromatic ring opening. We predict that in wild-type *N. aromaticivorans* the carbon flux from syringic acid is channeled ~85% via aromatic ring opening of 3-MGA and ~15% via its O-demethylation to gallic acid. Finally, by inactivating O-demethylation of 3-MGA in the originally constructed PDC-producing strain of *N. aromaticivorans*, the resulting strain (PDC2) increases the PDC yield from syringic acid to nearly stoichiometric.

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