Title: Probing Lignin Deconstruction and Catabolism in Soil *Pseudomonas* species

Authors: Rebecca A. Wilkes,[1] Allison Werner,[2] Neha P. Kamat,[1] Gregg Beckham,[2] and **Ludmilla Aristilde[1]** (ludmilla.aristilde@northwestern.edu),

Institutions: [1]Northwestern University, Evanston, IL; [2]National Renewable Energy Laboratory, Golden, Co

Project Goals: The overarching research goals are to elucidate the reaction network responsible for the metabolic functionalities of the outer membrane vesicles (OMVs) in soil *Pseudomonas* species with demonstrated accelerated lignin catabolism and subsequently evaluate the metabolic relationship of OMVs in fueling lignin-derived carbon fluxes towards intracellular biosynthetic pathways.

Abstract Text: The first objective of this project is to elucidate lignin deconstruction and carbon assimilation flux using whole-cell cultures. The working hypothesis is that the species optimized for coupled OMV-mediated lignin deconstruction and intracellular assimilation of lignin derivative will incorporate lignin carbons into cellular metabolites, whereas the species with only OMV-mediated lignin deconstruction will accumulate lignin derivatives in the spent media. In preliminary experiments, cells of different *Pseudomonas* species (*P. putida* KT2440, *P. putida* S12, and *P. protegens* Pf-5) are grown in nutrient solutions containing different lignin structures [lignosulfonate (LS) and lignin alkali (LA)] supplemented without or with glucose (a sugar) or acetate (a short-chain carboxylic acid). In addition to monitoring cell growth and carbon consumption from the lignin compounds, we are monitoring changes in the lignin structure. Thus far, we have used Fourier-transform infrared spectroscopy (FTIR) to track the vibrational peak (1585 cm^{-1}) associated with the lignin aromatic skeleton, which disappeared in the LS structure after two-week exposure to all three species. These results imply the breakdown of the LS aromatic structure. We performed total organic carbon analysis to determine the extent to which LS-derived carbon was consumed. We plan to apply nuclear magnetic resonance spectroscopy and high-resolution liquid chromatography-mass spectrometry analysis to identify modification in the LS structure and specific LS-derived breakdown products, respectively. We are also developing mixed isotope tracer experiments to capture the intracellular assimilation of lignin breakdown products.

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