Plant-Microbe Interfaces: Systematic discovery and prediction of novel LuxI-type quorum sensing signals in members of the *Populus* microbiome

Amy L. Schaefer[1]* (amyschae@uw.edu), Omar Demerdash,[2] Dale A. Pelletier,[2] **Caroline S. Harwood**,[1] **E. Peter Greenberg**,[1] and **Mitchel J. Doktycz**[2]

[1]Department of Microbiology, University of Washington, Seattle; [2]Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, TN

http://PMI.ornl.gov

Project Goals: The goal of the Plant-Microbe Interfaces (PMI) SFA is to characterize and interpret the physical, molecular, and chemical interfaces between plants and microbes and determine their functional roles in biological and environmental systems. *Populus* and its associated microbial community serve as the experimental system for understanding the dynamic exchange of energy, information, and materials across this interface and its expression as functional properties at diverse spatial and temporal scales. To achieve this goal, we focus on 1) defining the bidirectional progression of molecular and cellular events involved in selecting and maintaining specific, mutualistic *Populus*-microbe interfaces, 2) defining the chemical environment and molecular signals that influence community structure and function, and 3) understanding the dynamic relationship and extrinsic stressors that shape microbiome composition and affect host performance.

Acyl-homoserine lactone (acyl-HSL) quorum sensing (QS) has received considerable interest as a possible target for controlling microbial activities and as a model for communication system involved in coordinating activities of groups of bacteria. Recent advances have shown there are two related families of acyl-homoserine lactone quorum-sensing signal synthesis enzymes (LuxI-type enzymes), which differ in whether they acquire the organic acid reaction substrate as an acyl-acyl carrier protein (ACP) intermediate or an acyl-coenzyme A (CoA) intermediate. The acyl-CoA-utilizing enzymes are of particular interest because one has served as a model to understand the reaction mechanism of acyl-homoserine lactone synthases, and because there might be a great diversity in signals synthesized by this family of enzymes. Using a bioinformatics approach, we found the CoA class of LuxI homologs is common in genomes of α-Proteobacteria isolated from *Populus* tree roots. To systematically study acyl-HSL diversity among these isolates, we developed an experimental pipeline that includes the following steps: i) determination of the potential CoA substrate inventory encoded in a given genome; ii) utilization of a radiolabel ^{14}C-methionine protocol to detect acyl-HSLs synthesized in the presence of potential CoA substrates; iii) AiiA lactonase treatment of the ^{14}C-product to confirm it is an acyl-HSL compound; and iv) purification and structural identification of the acyl-HSL using high-resolution mass spectrometry. There is also an opportunity to leverage these CoA-type LuxI enzymes for computational based structural analysis and molecular docking experiments designed to predict the substrates for these enzymes. Because the potential CoA-substrates are 'simple' (relative to the ACP-linked substrates), we can create a tailored library of potential CoA substrates to dock with a predicted protein model of a given LuxI-type synthase. The experimental protocol will validate the computational results and aid in prioritizing signaling

partners for further evaluation. Taken together, these approaches should be useful in expanding the range of acyl-HSL signal diversity among plant-associated bacteria.

The Plant Microbe Interfaces Scientific Focus Area is sponsored by the Genomic Science Program, U.S Department of Energy, Office of Science, Biological and Environmental Research.