Plant-Microbe Interfaces: Determining the rate and consequences of horizontal gene transfer in the rhizosphere by simulating lateral spread of salicylate catabolism genes

Stephan Christel[1], Joshua K. Michener[1]* (michenerjk@ornl.gov), Paul E. Abraham,[1] Alyssa A. Carrell,[1] Sara S. Jawdy,[1] Tao Yao,[1] Julie E. Chavez,[1] Leah Burdick,[1] Dawn M. Klingeman,[1] Timothy J. Tschaplinski,[1] Jin-Gui Chen,[1] Wellington Muchero,[1] Melissa A. Cregger,[1] and **Mitchel J. Doktycz**[1]

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

http://pmiweb.ornl.gov/

Project Goals: The goal of the 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.

Plants affect the composition of the rhizosphere microbiome through the secretion of biomolecules. For example, *Populus* trees produce high levels of salicylic acid conjugates that are thought to play a role in structuring its microbiome, inhibiting the growth of some microbes, while serving as a carbon source to others. However, rhizosphere microbes are continually evolving to maximize their own fitness, perhaps changing the growth effects of compounds like salicylates. Horizontal gene transfer (HGT) is one of the main drivers of prokaryotic diversity and is hypothesized to play a significant role in how microbes compete in the rhizosphere and evade host controls. In turn, mutations in the plant host can change the abundance and composition of secreted small molecules. The functional consequences of these dynamic evolutionary interactions are key to understanding and predicting the stability of plant-microbe interactions in the rhizosphere.

To assess the potential consequences of successful HGT events in the *Populus* rhizosphere, *Pseudomonas* isolates from *Populus* were engineered to express a pathway to metabolize salicyl alcohol. These modified strains successfully used salicyl alcohol and salicylic acid as the sole carbon and energy source. Global proteomic measurements showed minimal disruption to the native physiology after pathway acquisition, suggesting few barriers to pathway transfer in the rhizosphere. To test the effects on abundance and localization in the rhizosphere, genomically barcoded populations of wild-type and engineered strains from one isolate, *Pseudomonas* sp. GM17, were inoculated onto otherwise sterile plant roots. In the absence of synergistic strains and on roots of unaltered *P. trichocarpa* BESC819, DNA barcode sequencing revealed no discernable effect of acquiring the salicyl alcohol catabolic pathway.

However, concentrations of salicyl alcohol and salicylic acid are low in native *Populus* roots since these aromatic compounds are generally secreted as glycosylated salicylates. Therefore, we tested whether the fitness effects of salicyl alcohol catabolism depend on epistatic interactions with the host or with other members of the rhizosphere microbiome. Experiments were conducted with *Rahnella* sp. OV744, a species that increases availability of salicyl alcohol by deglycosylating salicin, and with a genetically-modified *Populus* variant overexpressing XBAT35. The roots of the XBAT35 line contain high concentrations of salicylic acid-related conjugates, including a 5- to 15-fold increase in tremuloidin and a 3- to 7-fold increase in salicin. We expect that the effect of catabolic pathway acquisition will be increased when its substrate is more prevalent. These experiments will aid in understanding how plant-microbe-community interactions modulate microbial composition, localization, and ultimately function.

Oak Ridge National Laboratory is managed by UT-Battelle, LLC for the U.S. Department of Energy under contract no. DE-AC05-00OR22725. 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 under FWP ERKP730.