

Plant-Microbe Interfaces: Measuring rates and consequences of horizontal gene transfer in the *Populus* rhizosphere

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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.

Microbes have larger population sizes and shorter generation times than trees. As a result, the plant-microbe interaction is constantly being challenged by microbial mutants with new phenotypes. We are investigating how these mutually-beneficial interactions are maintained despite evolutionary challenges. In particular, horizontal gene transfer (HGT) can rapidly move genes and pathways between physically-adjacent microbes. When host-microbe recognition is mediated by specific molecular signals, those interactions are vulnerable to hijacking by unrelated microbes that acquire the necessary pathway through HGT.

We are developing sequencing-based techniques for tracking HGT in microbial communities, using next-generation capture-C to associate plasmids and hosts in a function-independent fashion. This approach is capable of differentiating strains with variable plasmid occupancy, though further optimization is ongoing to decrease the fractional detection limit.

In addition, we are identifying gene clusters in sequenced rhizosphere isolates and transferring them into naïve hosts. Focusing initially on gene clusters involved in degradation of salicylates, plant defense compounds common in *Populus*, we are genetically modifying non-degrading strains to introduce new aromatic degradation pathways. These modified strains will then be tested for colonization efficiency to determine whether pathway acquisition affects root colonization.

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