Plant-Microbe Interfaces: Simplified community approach to investigate multiple levels of selection in a host-microbiome relationship

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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.

The long-lived woody perennial *Populus* harbors a diverse consortium of microbial associates. To gain insight into the complex *Populus* host and microbial interactions, we developed a synthetic community system that employs subsets of bacteria isolated from *Populus*. Selection of bacterial communities is controlled by multiple interacting factors including environment, host genetics, and bacterial genetics. To explore some of these factors, we constructed two types of synthetic communities, (1) a highly diverse 150-member community to examine the interaction of host genetics and environment, and (2) two communities (*Variovorax* and *Rhizobium*) of closely related bacterial strains to examine how bacterial genetics determine colonization of host organ specificity. These communities were used to inoculate double autoclaved soil containing host plants. For the 150-community we used *Populus trichocarpa, Populus tremula x alba,* and *Arabidopsis thaliana*, to test the effect of host species and exposed them to three environmental perturbations (control ambient temperature, warming, and cold). We used 16S rRNA sequencing to determine which strains colonized the hosts and how the environment affects community structure. We found strong selection of the bacterial community based on host genus (*Populus* and *Arabidopsis*, 28.4%), host species (10.8%), and environmental perturbations (8.9%). *Arabidopsis* samples were mainly colonized by a *Pantoea* species, while in *Populus* a *Rhodanobacter* and a *Mycobacterium* species were the main constituents of the community. To examine what genetic traits lead to colonization on the bacterial side, we inoculated *Populus trichocarpa* and *Populus deltoides* plants with a community of *Rhizobium* strains and *Variovorax* strains. We used metagenomic sequencing to determine which strains colonized either the

rhizosphere (region around the roots) or the endosphere (interior region of the roots). We then used a comparative genomics approach to determine what genetic traits could lead to colonization of a specific compartment. We found 4 strains of *Variovorax* (out of 28), 32 strains of *Rhizobium* (out of 82) enriched in the endosphere, 9 strains of *Variovorax,* and 32 strains of *Rhizobium* enriched in the rhizosphere. In general genes involved in fatty acid biosynthesis, lipid transport and metabolism, and amino acid transport were enriched in the rhizosphere regardless of bacterial species. These experiments show the utility of synthetic communities to answer questions on multiple environmental scales.

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