Plant-Microbe Interfaces: Cultivating a representative *Populus* microbiome?

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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.

Environmental samples of *Populus* tissues harbor a diverse microbiome. Many of the functional attributes of *Populus* are a result of both its genetic potential and its microbiome. The integral role of the microbial community in plant performance is now widely recognized, and increasingly, the constituents of plant microbiomes are being defined. While phylogenetic surveys have revealed who is present in the *Populus* microbiome, and show that this composition can depend on, and respond to, environmental conditions and stresses, the challenge shifts to determining why particular microbes are selected and how they collectively function in concert with their host.

To dissect and understand the complexity of natural plant and microbial communities, we are developing experimental plant-microbial community systems amenable to laboratory conditions. We targeted the isolation of representative bacterial strains from environmental samples of *Populus* roots using a direct plating approach. This resulted in 3205 unique bacterial isolates from 4 phyla, 9 classes, 16 orders, 46 families and 118 genera. All isolates have been identified with 16S rRNA sequencing, with a portion (14%) having genome sequences. The greatest number of isolates are from 5 genera; *Pseudomonas* (18.2%), *Bacillus* (13.7%), *Rhizobium* (13.4%) *Streptomyces* (11.8%) and *Variovorax* (5.9%). The majority of the isolates were cultured from the root endosphere (50%) followed by rhizosphere (37.3%) and unsterilized root tissues (12.7%) of *Populus*. The representativeness of bacterial isolates has been assessed by comparison of 16S sequences to 16S amplicon sequences from environmental surveys. Many of the bacterial strains represent relatively abundant genotypes present within the *Populus* rhizosphere and endosphere. While this culture collection represents a large fraction of the abundant phyla present within the *Populus* rhizosphere, efforts are underway to isolate additional phyla. Additionally, with the initiation of a JGI-CSP, genome sequencing has been carried out on representatives from all 4 phyla and 9 classes, encompassing 12 orders, 34 families and 84 genera. These isolates and genome sequences have facilitated comparative genomic analyses and experimental plant-microbial community approaches to understand the dynamics of microbiome organization. Reference microbiomes comprised of low diversity microbial communities representing abundant taxa from

environmental samples were created and tested for their ability to reproducibly colonize *Populus*. We found that colonization is reproducible across replicates and host plants, despite isolation from different host genotypes. Community member abundances in general are similar to single strain inoculation indicating potential for unique niche site colonization. Communities increased root growth compared to non-inoculated host plants. Further, these studies are beginning to uncover characteristics of host-microbe selectivity and identify molecular mechanisms underlying community assembly and function. This work allows us to determine how individual microbes contribute in a community and enables a mechanistic understanding of how plant and microbial genetics lead to complex community phenotypes.

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