7. Plant Microbe Interfaces: Defining the functional diversity of the *Populus* root microbiome

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Project Goals: The goal of the PMI SFA is to understand the genome-dependent molecular and cellular events involved in establishing and maintaining beneficial interactions between plants and microbes. *Populus* and its associated microbial community serves as the experimental system for understanding how these molecular events manifest themselves within the spatially, structurally, and temporally complex scales of natural systems. To achieve this goal, we focus on 1) characterizing host and environmental drivers fro diversity and function in the *Populus* microbiome, 2) utilizing microbial model system studies to elucidate *Populus*-microbial interactions at the molecular level and dissecting the signals and pathways responsible for initiating and maintaining microbial relationships and 3) develop metabolic and genomic modeling of these interactions to aid in interpreting the molecular mechanisms shaping the *Populus*-microbial interface.

The beneficial association between plants and microbes exemplifies a complex, multi- organism system that is shaped by the participating organisms and the environmental forces acting upon it. Studying the integral plant–microbe system in native, perennial environments provides a great opportunity for discovering plant–microbial system functions relevant to DOE missions related to bioenergy and carbon-cycle research and understanding of ecosystem processes. Therefore, *Populus* and its associated microbial community are being studied as part of our Plant-Microbe Interfaces project (http://pmi.ornl.gov). *Populus* trees are host to a variety of microorganisms within their endosphere and rhizosphere that can influence host phenotypes. Our goal is to understand the phylogenetic and functional diversity within the *Populus* microbiome and to elucidate the metabolic and molecular mechanisms responsible for shaping the *Populus*-microbial interface. To begin to untangle this complex ecosystem, we have applied cultivation dependent and cultivation independent techniques to capture and characterize the *Populus* root microbiome. Utilizing direct plating methods we have isolated and begun to characterize a large collection of *Populus* rhizosphere and endosphere bacterial strains. Through a JGI-CSP project, we are currently sequencing the genomes of many of these isolates and applying comparative genomics to identify functions important for the formation of mutualistic relationships with the host. However, our isolate collection may not be representative of the *Populus* microbiome. Population distributions from rRNA gene-based approaches on *Populus* suggest that the high-GC Gram-positives, *Planctomycetales*, *TM7*, *Crenarchaea*, and *Acidobacteria*, may be underrepresented in culture-based efforts. Further, because of the low microbial biomass relative to plant biomass, metagenomes have been difficult to obtain. Finally, certain endophytic groups have been difficult to isolate and culture in laboratory settings. To address these issues, a method of enriching live endophytes from *Populus* root homogenates for isolation and metagenomic investigation is being developed. Endophytic bacterial communities were enriched using differential and density gradient centrifugation. Total DNA was extracted from enriched and unenriched samples, and the endophytic bacterial community composition was determined by 16S rDNA sequencing, using the MiSeq platform. Our enrichment protocol reduced

the number of contaminating chloroplast DNA reads by approximately 10 fold. The enrichment also significantly increased the reads of Actinobacteria, Planctomycetia, and Alpha- and Gamma-proteobacteria classes. Live bacterial enrichments were also inoculated to agar plates for isolation and flow sorting for single-cell genomics. The ability to perform single-cell multi-omic analyses will allow for in-depth characterization of rare endophytic bacteria residing within the natural root system of *Populus.*

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