

Plant-Microbe Interfaces: Model communities of *Populus* bacterial isolates to study mechanisms of microbiome function

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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 for 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 function of the plant-microbiome system is a result of complex interactions among microbiome members, host plant and the environment. Direct experimentation on the microbiome is difficult due to the inability to culture the microbiome in the laboratory. Our approach is to test hypotheses generated from natural system studies within model communities constructed from cultivable and genome-sequenced members of *Populus trichocarpa* genotypes, fungi and bacteria isolates. Our bacterial isolate collection consists of >2700 bacterial strains, of which ~250 have been sequenced to date through a JGI CSP. Using genomic content, strain functional data, and microbiome community data as a guide, we select communities of organisms to colonize germ-free *Populus* cuttings in constructed community experiments.

In studies of microbiome response to adverse plant growth conditions, we observe a common set of bacteria that respond to plant stress. To study the contribution of individuals to this complex phenotype, we developed two separate 10-member bacterial communities representing >90% of the estimated functional diversity present within natural microbiome metagenomes (by KEGG annotations). The 10 strains for each community represent abundant and diverse orders identified in previous studies of natural *Populus* microbiomes, including α -, β - and γ - *Proteobacteria*, *Bacilli*, and *Actinobacteria*. Both communities increased root growth when inoculated on axenic host plants. Both communities were dominated by specific genera within beta- and gamma-proteobacteria, despite isolation from different host species. We observe positive and negative

correlations between community members in replicate samples, an example of emergent behavior that would not be observed in one-on-one studies or general level sequencing studies. When subjected to carbon limitation stress (shading), we observe a host transcriptional response indicative of pathogen defense, suggesting that host carbon dynamics may influence microbiome structuring. This system provides a framework by which complex natural host-microbe interactions can be studied within a more simplified constructed community.

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