Plant-Microbe Interfaces: Constructed communities of *Populus* and bacterial isolates to study microbiome function

Collin Timm[1], Alyssa Carrell[1], Sara Jawdy[1], Lee Gunter[1], Zamin Yang[1], Jayde Aufrecht[1,] Shen Lu[1], Nancy Engle[1], **Timothy Tschaplinski**[1], **Dale Pelletier**[1*](pelletierda@ornl.gov), **Dave Weston**[1], Gerald A Tuskan[1], Mitchel Doktycz[1]

[1]Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, TN

http://PMI.ornl.gov

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.

Functions of a plant-microbiome system are the result of complex interactions between microbiome members and the host plant. Direct experimentation on the microbiome is difficult due to the inability to culture the microbiome in the laboratory. Our solution is to study constructed communities designed to represent the microbiome, which consists of diverse, cultivable members isolated from natural systems. Our isolate collection consists of >2700 bacterial strains, of which ~200 have been genome-sequenced to date through a JGI CSP. Using genomic content, strain functional data, and microbiome community data as a guide, we selected a community of diverse microbial isolates to colonize axenic *Populus* cuttings in microbiome replacement experiments.

Our first goal was to identify the contribution of individual members to host phenotype in a community environment. A *Pseudomonas* representative from our isolate collection was chosen based on several factors including 1) its ability to produce the plant hormone indole-3-acetic acid; 2) the ability to grow using plant metabolites sucrose and 3-hydroxybenzoate as sole carbon sources, and 3) the enhancement of root hair production in *Arabidopsis*. The second bacterial isolate, from the *Burkholderia* genera, has predicted enzymes for growing on multiple carbohydrate sources and it colonized *Populus* cuttings at a high density (10^8 CFU/g root). When inoculated on *Populus* cuttings, these strains increased root growth relative to uninoculated controls. The enhanced root growth in the dual-inoculated samples can be explained by the

combination of the two individual effects. Transcriptome and metabolome data showed responses that were unique to individual bacterial treatments, and the expression of genes and metabolites in the mixed conditions was consistent with the combination of the effects of the individual strains.

In ongoing work we have begun studying two large communities, each consisting of 10 genome-sequenced bacterial isolates from *P. deltoides* or *P. trichocarpa,* respectively. The 10 strains from each constructed community represent abundant and diverse orders of natural *Populus* microbiomes identified in previous studies, including α-, β- and γ- *Proteobacteria*, *Bacilli,* and *Actinobacteria.* Both constructed communities increased root growth when inoculated on axenic host plants and were dominated by *Burkholderia* and *Pantoea* genera, despite isolation from different hosts and different genomic content. Finally, we observed 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 genera level sequencing studies. These promising results provide a foundation for future constructed community studies.

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