Plant-Microbe Interfaces: Whole genome sequence analysis of bacterial strains isolated from the *Populus* microbiome

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Project Goal: 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 an initial 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 will focus on 1) characterizing the natural variation in *Populus* microbial communities within complex environments, 2) elucidating *Populus*-microbial interactions at the molecular level and dissecting the signals and pathways responsible for initiating and maintaining microbial relationships, and 3) performing metabolic and genomic modeling of these interactions to aid in interpreting the molecular mechanisms shaping the *Populus*-microbial interface.

Populus trees are host to a variety of microorganisms within their endosphere and rhizosphere that can have positive effects on the host. 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 isolated bacterial strains native *Populus deltoides* roots collected in central Tennessee and North Carolina. A diverse array of bacterial strains (>1000) comprising some 7 class and 89 genera of bacteria were isolated from the rhizosphere (529) and root endosphere (558). The isolates comprise Actinobacteria (15%), Bacilli (17%), Flavobacteria (6%), Sphingobacteria (3%), and a- (22%) b- (15%) and g- (22%) proteobacteria. In order to explore potential metabolic and physiological diversity present within *Populus* microbiome isolates, we performed whole genome sequencing on a subset of 43 bacterial isolates. Isolates where chosen for sequencing based on a variety of factors including abundance in native ecosystems, ability to colonize plants, both microbial and plant phenotyping and physiological properties. Sequence data was generated by Illumina HiSeq2000 paired-end sequencing of 500 bp insert libraries. Sequence reads were assembled using Velvet and annotated by the ORNL automated genome annotation pipeline. The initial metabolic analyses of the sequenced strains targeted a comparison of KEGG orthologous groups. Metabolic activities of the organisms were predicted using the same KEGG annotation pipeline and then analyzed using network tools to identify clusters of KEGG orthologous groups that are specific for each isolate or group of isolates. The analysis revealed metabolic diversity of the isolates. Metabolic reconstructions

generated for isolates provided further insight into the nature of metabolic interactions of the isolates with the plant host.

The Plant Microbe Interfaces Scientific Focus Area is sponsored by the Genomic Science Program, U.S. Department of Energy, Office of Science, Biological and Environmental Research