Plant-Microbe Interfaces: Network integration uncovers gene-targets involved in plant-microbial interactions

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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.

The interactions between plants and their constituent microbiomes are complex. Thus, it is important to delineate potential host factors that may shape the composition of the microbiome to bring us one step closer to understanding this complex set of interactions. Here, we investigate the relationship between microbial relative abundance and host genomic variation within *Populus trichocarpa* for both leaf and xylem tissues. Leveraging a kmer-based pipeline to extract putative taxa from bulk total-RNA samples, we identified hundreds of taxa. We focused on four leaf and four xylem genera-level taxa that overlap with the PMI culture collection. We observed *Acinetobacter*, *Bradyrhizobium*, *Enterobacter*, and *Pantoea* in xylem tissue; and *Bacillus*, *Chryseobacterium*, *Paenibacillus*, and *Streptomyces* in leaf tissue. The relative abundance of each of these taxa were then used as phenotypes in a genome wide association study (GWAS). Significant single nucleotide polymorphism associations are annotated with nearby genes. Utilizing novel network integration approaches that leverage extant knowledge from multiple 'omic data sources, called random walk with restart filter (RWR-filter) and random walk with restart lines of evidence (RWR-LOE), we refine the GWAS gene set to obtain a set of high confidence genes with biological context for each respective phenotype. We used gene annotation and network topological context around the respective genes to create a set of conceptual models that provide hypotheses to explore for future CRISPR/Cas9 experiments. In xylem, by integrating the annotations of the gene sets for each taxa to identify associations present in two or more phenotypes, we find a putative signal transduction cascade. In particular, we find associations to members of various transcription factor families, genes involved in signaling, transport, cell-

division, cell-wall cellulose synthesis, the abscisic acid phytohormone pathway, and post-translational modification involving ubiquitin protein degradation. In addition, our analysis allows for investigation of the host-factors associated with individual phenotypes. For the *Chryseobacterium* found in leaf tissue, for example, we find associations to genes involved in the auxin and salicylic-acid phytohormone pathways, abiotic stress, flavonoid biosynthesis, nitrogen metabolism, and protein degradation. Together our approach allows for a thorough investigation of the host-microbial interactions, and thereby, identifies target host genes that may play an important role in shaping the microbial community.

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