Plant-Microbe Interfaces: Transient expression assays, stable transgenics, and a genome editing system for studying *Populus*-microbe interactions

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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) developing metabolic and genomic modeling of these interactions to aid in interpreting the molecular mechanisms shaping the *Populus*-microbial interface.

The symbiosis between perennial plants and microbial associates has critical implications for diverse phenomena including global carbon, water and nutrient cycling as well as biomass production on marginal croplands. As such, characterizing the molecular genetics underlying such interactions holds tremendous potential in engineering biological systems for enhanced carbon sequestration and sustainable biomass production.

Over the last six years of PMI SFA research, a number of genetic loci regulating the interactions between *Populus* and soil microbes have been identified, mainly through genome-wide association studies (GWAS) and quantitative trait locus (QTL) mapping. While these natural variants and QTL pedigrees continue to serve as valuable resources for evaluating host genotype influences on microbial community composition, diversity and function, genetic materials with a more defined background (i.e., only with alternation in expression of a single gene) can provide key insights to pinpoint the function of these genetic regulators at the molecular and biochemical levels. Previously, we have established a *Populus* mesophyll protoplast transient expression system which has been used for the studies of protein subcellular localization, protein-protein interaction, protein degradation and gene activation and repression. Recently, this system has also been successfully applied to chromatin immunoprecipitation (ChIP) and ChIP-sequencing (ChIP-seq).

We are also developing a number of additional systems including the generation of transgenic lines in *Populus deltoides* and *Populus trichocarpa* backgrounds. Transformation efficiency is determined by both genetic and non-genetic factors. Both *Populus* species and within-species genotypes vary greatly at the gene, allelic and nucleotide levels. Because the genotype-specific variation is large, there is no universal transformation protocol suitable for all *Populus* genotypes

at this time. The 1084 *P. trichocarpa* GWAS population provides a rich source of raw materials for identifying superior genotypes for efficient transformation based on callus induction efficiency. From this population, we have identified a number of genotypes that are transformable. Non-genetic factors including tissue type, callus-induction medium composition, shoot induction medium composition, *Agrobacterium* strain, culture conditions and vector type will be further optimized for improving transformation efficiency. In addition to traditional tissue culture and an *agrobacterium*-based transformation method, we will apply the CRISPR/Cas9 genome-editing system to specifically disrupt the function of selected target genes in *Populus*. Our initial test will be on the disruption of a gene encoding a lectin receptor-like kinase that has been shown to play a key role in *Populus-Laccaria bicolor* interactions.

Because the time required to generate stably transformed *Populus* plants and to propagate plant materials is lengthy (~9 to 15 months), we are also establishing a hairy root transformation system using *Agrobacterium rhizogenes* in *Populus*. This system can be used to rapidly assess gene expression and function, and can also be used as a screening method to select genes for the generation of stable *Populus* transgenic lines.

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.