5. Plant-Microbe Interfaces: Tripartite plant-fungal-bacterial 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 fro 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 molecular events leading to recognition and colonization of a host plant by beneficial microorganisms are poorly understood. Our ongoing research is aimed at identifying and isolating microbes associated with natural *Populus* ecosystems in order to determine molecular, genetic and cellular events involved in recognition and establishment of beneficial microbial interactions with *Populus*. Several research investigations aimed at enhancing our understanding of plant-microbe interactions are underway, focused on elucidating the genetic and molecular mechanisms of the interactions of the host plant and the bacterial network associated with fungal partners within natural *Populus* ecosystems.

The mycorrhizal symbiosis is the most widespread plant-microbe association that supports forest growth and sustainability. Based on the *Populus-Laccaria* ectomycorrhizal model, this project is focused on identifying specific host-derived genetic determinants by characterizing a core set of genes regulating *Populus-Laccaria* interactions. To this end, we have combined quantitative trait loci analyses (QTL) of *Laccaria* colonization phenotypes in a *P. trichocarpa* x *P. deltoides* F_1 pedigree, transcripts level expression analyses under the same colonized condition, high- throughput genotyping and whole genome resequencing to characterize sequences in the target genomic regions. We have identified a species-specific whole-gene deletion in *P. deltoides*, which co-segregated with colonization efficiency. This gene was absent in 60 *P. deltoides* genotypes from diverse geographical origins but was highly conserved in 673 *P. trichocarpa* genotypes evaluated. Variants resulting in up to 2X more colonization by the fungal symbiont have been identified in *Populus*. Transgenic lines in *Arabidopsis* and in *Populus* have been constructed to investigate the functionality of a couple of target genes.

Plants have developed a complex defensive response system to protect themselves against invasion by detrimental organisms, often mediated by plant hormones. Invading organisms, in turn, have developed various methods to circumvent the plant's defenses or control plant cell function to their benefit. Pathogens attempt to manipulate the plant response by producing effectors that target different components of the JA and ET signaling pathways in such a fashion that colonization is favored. Similarly mutualistic fungi affect plant hormone signaling cascades to achieve colonization although knowledge of the mechanisms behind most of these differences is in its infancy. In this work, we demonstrate that MiSSP7, an effector protein produced by *L. bicolor*, targets plant-encoded JAZ proteins, in particular PtJAZ6, and interacts with it in the nucleus of the plant. Through this interaction, MiSSP7 is able to block the activity of MeJA and promote the proliferation of both bacteria and *L. bicolor* in plant tissues. This effect is likely due to the ability of MiSSP7 to stabilize the JAZ protein and reduce the jasmonic acid induced degradation of the JAZ protein. Thus, the jasmonic acid responsiveness of the host plant would be affected by the microbial environment to foster symbiotic interactions.

In complex soil ecosystems, fungi are surrounded by diverse microbial communities, which modulate the mycorrhizal symbiosis. These include the so-called mycorrhiza helper bacteria (MHB), which are thought to assist mycorrhiza formation and symbiosis. Moreover, some mycorrhizal and root-associated fungi possess bacterial endosymbionts. Because very little is known about the role of these helper and endosymbiotic bacteria in *Populus*-fungi interactions, this project is aimed at dissecting the signaling mechanisms underlying *Populus*–fungal–bacterial interactions. To this end, we have sequenced genomes of bacterial endosymbionts and helper bacteria, constructed mutant libraries and have begun mutant phenotype screening. We have cleared the endosymbiont from several fungal strains and observed fitness costs to the host under specific conditions. We demonstrate that some helper bacterial strains influence *Populus–L. bicolor* colonization and some mutants are affected in their beneficial effect. This study provides new insights into the mechanism of multi-partite interaction between *Populus* and its complex microbial communities.

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