Plant-Microbe Interfaces: Emerging research directions

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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.

The molecular events that lead to recognition and colonization of a host plant by beneficial bacteria or fungi are poorly understood. Ongoing research in this project is aimed at identifying molecular, genetic and cellular events in *Populus* involved in recognition and establishment of beneficial microbial interactions, and at identifying and isolating microbes associated with natural *Populus* ecosystems. Results from these ongoing studies have led to initiation of new research directions to enhance our understanding of plant-microbe interactions. New efforts are focused on elucidating the molecular mechanisms of microbial biofilm formation on plant roots, the role of helper bacteria in promoting beneficial plant-fungal interactions, and on development of a *Populus* protoplast transient expression system for the molecular and biochemical characterization of regulatory proteins.

The mutualistic association between plant roots and microbes form an environment that is ideal for biofilm formation, including sufficient moisture and nutrients, which are supplied by the plant host. This project is focused on identifying the specific conditions in the plant rhizosphere that trigger biofilm formation in selected microbes. To this end, we have tested the ability of selected microbes to form biofilms under various growth conditions using a well-described biofilm formation assay. Data from multiple *Pseudomonas* strains indicate differential responses to additions in the growth media. For example, sucrose, a common constituent of plant root exudates, appears to induce biofilm formation in GM49. Likewise, the presence of high levels of phosphate induces biofilm formation in GM60 and GM67. These and other phenotypes will form the basis for selecting microbes on which to focus in-depth studies to elucidate molecular pathways involved in root colonization.

The soil is probably one of the most complex ecosystems in which plant–fungal–bacterial interactions operate. Mycorrhizal fungi are surrounded by complex microbial communities, which modulate the mycorrhizal symbiosis that impacts biomass production, defense against

pathogens, and tree nutrition. Among this diverse microbial community, the so-called mycorrhiza helper bacteria (MHB) are thought to assist mycorrhiza formation and symbiosis. Since very little is known about the role of MHB in *Populus*-fungi interactions, this project is aimed at dissecting the signaling mechanisms underlying *Populus*–fungal–bacterial interactions. To this end, we have analyzed the morphological changes induced in *Laccaria bicolor* by several bacterial strains isolated from the *Populus* rhizosphere, and performed *Populus–L. bicolor*–bacteria co-cultures under controlled greenhouse conditions. We demonstrate that some bacterial strains influence *Populus–L. bicolor* colonization and have a clear beneficial effect. This study provides new insights into the mechanism of interaction between *Populus* and its complex microbial communities.

Transcriptomcis, proteomics and metabolomics have uncovered a suite of genes involved in *Populus*-microbe interactions. However, it remains unclear how these components interact with each other and are coordinated in the same signaling cascades to regulate some specific processes of plant-microbe interactions. Molecular and biochemical characterization of these genes is essential for elucidating the molecular mechanism underlying plant-microbe interactions. We have established a protocol for efficient isolation of protoplasts from *Populus* leaf mesophyll cells. Subsequently, we have established a protoplast transient expression system using *Populus* protoplasts. We demonstrated that *Populus* protoplasts respond to biotic and abiotic stimuli in a similar manner as that in intact plants. Furthermore, by using a series of fluorescent fusion proteins, we have established subcellular localization of various organelle markers. The *Populus* protoplast transient expression system will be used to study protein-protein interactions, protein-DNA interactions, post-translational modifications, protein degradation, and artificial microRNA-mediated gene silencing. Furthermore, together with gene reporters, this system will be used to examine the specific role of candidate genes in regulating plant-microbe interactions. The *Populus* protoplast transient expression system represents a much-needed system for research in the post-omics era.

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