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

In this project, we are focused on understanding Populus–microbe interactions at the molecular level to dissect the signals and pathways important for initiating and maintaining symbiotic relationships with Populus. Our goal is to elucidate molecular, spatial, and temporal dynamics involved in Populus-microbe interactions using systems biology approaches and directed analytical methodologies. We are interested in how bacteria selectively respond to and become associated with Populus; and how microbially induced molecular and cellular events impact plant growth, health, and fitness. Ultimately, these data will be used to construct model plant- microbial communities to better understand the underlying rules to community assembly and the functional contributions that result from arrangements of multiple organisms.

Our current research is focused on dissecting the signaling pathways involved in plant-bacterial interactions using select Populus-derived bacterial isolates that were chosen based on phenotypic screens and genome inventory data. One area of focus in these isolates is cyclic-di-GMP signaling, which often controls exopolysaccharide (EPS) production, biofilm formation, motility, and other colonization factors. In the robust root colonizer Pantoea sp. YR343, we have employed transposon mutagenesis using a strain that overexpresses the diguanylate cyclase orf2884 in order to identify gene products that function in response to high levels of cyclic-di-GMP production. We have identified at least 60 different gene products, several of which are predicted to have a role in exopolysaccharide (EPS) production and transport, as well as several transcription factors. RNAseq analyses of the c-di-GMP overexpressing strain identified many more candidate genes whose expression is controlled by c-di-GMP levels. By combining these datasets, we have identified a subset of genes on which to focus our initial colonization studies. We are currently working to characterize the functions of these gene products in biofilm formation, EPS production, motility and plant colonization. These mutants provide a set of tools for developing a broader understanding of the molecular mechanisms involved in root colonization by bacteria.

A genomic analysis of Populus isolates belonging to the Proteobacteria, revealed that many possess a newly discovered, but not very well explored plant–bacterial signaling system called the OryR system. Originally discovered in the rice pathogen Xanthomonas oryzae, OryR, which is a transcription regulator, responds to an unknown signal in rice plant extracts to control virulence. We found that an OryR
homologue, now called PipR, in the Populus endophyte, Pseudomonas sp. GM79 responds to Populus leaf macerates to activate gene expression. The genomic region surrounding the GM79 plant-responsive pipR gene includes two genes annotated as peptidases and also genes coding for a putative ABC-type peptide transporter. Pseudomonas sp. GM79 strains that we constructed with mutations in the putative peptidases showed increased responses to Populus macerates. A strain with a mutation in a gene coding for the putative ABC-type peptides transporter did not respond to Populus leaf macerates. We hypothesize that the plant signal(s) enters the bacterial cells by active transport and that the peptidases affect the activity of the signal. We have partially purified the signal and the purified material can be partially inactivated by one of the peptidases. We believe that a better understanding of these OryR-type plant signal receptors and their plant signals is of general importance as they occur in dozens of bacterial species that are associated with economically important plants. Our analysis of the PipR system in a Populus-associated strain opens up the door to studies of a specific Populus-bacterial interaction that is previously unexplored.

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