Plant Microbes Interfaces: Probing the molecular mechanisms of plant-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 of 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.

An analysis of 154 genome sequences of Proteobacteria isolated from root tissues of the *Populus deltoides* revealed that many (n=32) of them encode a transcription factor that is closely related to a protein named OryR from the rice pathogen *Xanthomonas oryzae*. OryR detects an unknown plant compound and when bound by the plant ligand, activates transcription of genes involved in virulence. We have been investigating an OryR homolog, which we call PipR, in the *Populu*s non-pathogenic endophyte *Pseudomonas* sp. GM79. Our recently published work showed that GM79 PipR activates expression of the downstream peptidase gene, *pipA*, in response to plant leaf macerates, peptide-rich hydrolysates (peptone), and high concentrations of a specific tripeptide (ser-his-ser). Recent work has focused on identifying compounds that can activate *pipA* in a PipR-dependent manner at concentrations that might be present in plants. Mutant analyses of genes flanking *pipR* suggest that the PipR signal(s) enter the bacterial cells by active transport via an ABC-type transporter. The periplasmic binding protein component of the ABC-type transporter binds the plant and peptone signal(s) tightly, and we used this as a tool to purify and characterize the signal(s) from peptone. We identified several D-isomer peptides that can activate the PipR system, some at physiologically relevant (low mM) concentrations. The finding that PipR is most responsive to D-form peptides suggests similar compounds could be the active signal in *Populus* macerates. Plants are known to produce a variety of peptides that are involved in signaling for the regulation of growth and development and in defense against pathogen infection. Our findings raise the

possibility that plant-produced peptides may also be important for establishing beneficial plant-microbe interactions. We have also identified PipR homologs in other members of the *Populus* microbiome and have surveyed them for PipR activity. We believe that a better understanding of these PipR-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. As a complementary project, we are interested in understanding the role of secondary metabolites in the PMI microbiome and have identified a variety of potentially interesting gene clusters using antiSMASH analyses.