Plant-Microbe 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 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

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We have shown that quorum-sensing (QS) genes are prevalent in Proteobacteria isolated from roots of the Eastern cottonwood tree, *Populus deltoides*. Many of these isolates encode an orphan LuxR homolog, which is closely related to OryR from the rice pathogen *Xanthomonas oryzae*. OryR does not respond to acyl-homoserine lactone (AHL) QS signals, instead it detects an unknown plant compound. We discovered an OryR homolog, named PipR, in the endophyte *Pseudomonas* sp. GM79. We examined the genomic region surrounding *pipR* and found genes annotated as a peptide transporter and peptidases. We purified the peptidases and found they are most active against compounds with terminal proline and alanine residues. A reporter responsive to the GM79 PipR homolog was used to show that, similar to *X. oryzae* OryR, its activity increased in the presence of plant leaf macerates, but it was not influenced by AHLs. Because of the abundance of flanking peptide metabolism genes, we hypothesized the PipR signal may be peptide-like in nature. We found that protein hydrolysates (peptone) activated the reporter in a PipR-dependent manner and a specific tripeptide showed moderate inducer activity. Strains with peptidase gene mutations showed increased responses to plant leaf macerate, peptone and the tripeptide signal(s), relative to the wildtype. A mutation in the putative ABC-type peptide transporter locus blocked the response to plant leaf macerate, peptone, and the tripeptide signal(s). We hypothesize that the plant/peptone/tripeptide signal(s) enters the bacterial cells by active transport and that the peptidases affect the signal, likely by enzymatic degradation, in a negative feedback loop. We have also determined that the periplasmic binding protein component of the ABC-type transporter binds the plant and peptone signal(s) tightly and we are using this as a tool to purify and characterize the signal(s). Our analysis of the PipR system in a *Populus*-associated strain opens the door to studies of a specific *Populus*-bacterial interaction that is unexplored. 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.

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