Plant-Microbe Interfaces: Identification of genes controlled by a plant-responsive transcription factor in the *Populus* endophyte *Pseudomonas* GM79

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Project Goals: The goal of the PMI SFA is to characterize and interpret the physical, molecular, and chemical interfaces between plants and microbes and determine their functional roles in biological and environmental systems. *Populus* and its associated microbial community serve as the experimental system for understanding the dynamic exchange of energy, information, and materials across this interface and its expression as functional properties at diverse spatial and temporal scales. To achieve this goal, we focus on 1) defining the bidirectional progression of molecular and cellular events involved in selecting and maintaining specific, mutualistic *Populus*-microbe interfaces, 2) defining the chemical environment and molecular signals that influence community structure and function, and 3) understanding the dynamic relationship and extrinsic stressors that shape microbiome composition and affect host performance.

A number of plant-associated proteobacteria, including members of the *Populus* microbiome, have LuxR-family transcription factors that we refer to as PipR subfamily members. PipR proteins play roles in interactions between bacteria and their plant hosts, and some are important for bacterial virulence in plants. We identified a plant-derived ethanolamine derivative, *N*-(2-hydroxyethyl)-2-(2-hydroxyethylamino) acetamide (HEHEAA), as a potent effector of PipR-mediated gene regulation in the *Populus* root endophyte *Pseudomonas* GM79. To better understand which genes are controlled by PipR in GM79, we performed RNAseq transcriptomic analyses and identified a regulon of approximately 40 genes. The positively regulated genes (n=13) included those encoding N-terminal peptidases, a HEHEAA signal transporter, and a two-component regulator, which is inactive under laboratory growth conditions. Surprisingly, the majority of PipR-controlled genes exhibited lower gene expression in the presence of HEHEAA. The PipR-HEHEAA repressed genes included those involved in hydrogen cyanide production and anaerobic respiration on nitrate. Additional experimentation confirmed PipR-HEHEAA regulation of cyanide production and nitrate respiration. We were surprised to find that the influence of PipR-HEHEAA on cyanide production and nitrate respiration was mediated solely through the activity of the PipR-controlled peptidase, PipA. We are attempting to understand the mechanism of this regulatory pathway through proteomics experiments.

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