Plant-Microbe Interfaces: Structural basis for a bacterial Pip system plant effector recognition protein

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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 of plants. We identified an ethanolamine derivative, *N*-(2-hydroxyethyl)-2-(2-hydroxyethylamino) acetamide (HEHEAA), as a potent effector of PipR-mediated gene regulation in the plant endophyte *Pseudomonas* GM79. HEHEAA-dependent PipR activity requires an ATP-binding cassette-type active transport system, and the periplasmic substrate-binding protein (SBP) of that system binds HEHEAA. To begin to understand the molecular basis of PipR system responses to plant factors, we crystallized a HEHEAA-responsive SBP in the free- and HEHEAA-bound forms. The SBP, which is similar to peptide-binding SBPs, was in a closed conformation. A narrow cavity at the interface of its two lobes is wide enough to bind HEHEAA, but cannot accommodate peptides with side chains. The polar atoms of HEHEAA are recognized by hydrogen-bonding interactions, and additional SBP residues contribute to the binding site. This binding mode was confirmed by a structure-based mutational analysis. We also show that a closely related SBP from the plant pathogen *Pseudomonas syringae* pv *tomato* DC3000 does not recognize

HEHEAA. However, a single amino acid substitution in the presumed effector-binding pocket of the *P. syringae* SBP converted it to an HEHEAA-binding protein. The *P. syringae* PipR depends on a plant effector for activity and our findings imply that different PipR-associated SBPs bind different effectors.

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