Plant-Microbe Interfaces: Identification of gene products involved in plant colonization by *Pantoea* sp. YR343 using a plant-responsive diguanylate cyclase

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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.

Populus deltoides (poplar) hosts a diverse microbiome that influences its growth and productivity. The ability of plant growth promoting bacteria to exert beneficial effects on plant hosts is mediated through chemical and physical associations with plant tissues. *Pantoea* sp. YR343, a gamma-proteobacterium isolated from the rhizosphere of *P. deltoides*, forms robust biofilms along the root surfaces of *Populus* and possesses plant growth-promoting characteristics, such as phytohormone production and phosphate solubilization. The mechanisms governing biofilm formation along plant roots by bacteria, including *Pantoea* sp. YR343, are not fully understood and many genes involved in this process have yet to be discovered. Because the signaling molecule cyclic di-GMP plays an important role in biofilm formation, we employed a strategy for identifying putative colonization factors by modulating c-di-GMP expression in *Pantoea* sp. YR343. To this end, we identified three diguanylate cyclases, enzymes that synthesize c-di-GMP, that are expressed during colonization of plant roots. Overexpression of one of these diguanylate cyclases (encoded by PMI39_02884) significantly impacted exopolysaccharide production, motility, and biofilm formation. This overexpression strain was utilized for a genetic screen to identify genes that respond to high levels of c-di-GMP. Several genes were identified, including a UDP-galactose lipid carrier transferase (PMI39_01848) and a capsule polysaccharide transporter (PMI39_03059), which are predicted to function in EPS production. Transposon mutants affecting these genes were further characterized for their ability to colonize plant roots.

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