Plant Microbe Interfaces: Quorum sensing systems are prevalent in the *Populus* microbiome

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Project Goal: 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 an initial 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 will focus on 1) characterizing the natural variation in *Populus* microbial communities within complex environments, 2) elucidating *Populus*-microbial interactions at the molecular level and dissecting the signals and pathways responsible for initiating and maintaining microbial relationships, and 3) performing metabolic and genomic modeling of these interactions to aid in interpreting the molecular mechanisms shaping the *Populus*-microbial interface.

As part of the ORNL Plant-Microbe Interfaces Science Focus area, we are characterizing the natural diversity of microbial associates of *Populus* and elucidating the molecular mechanisms by which these organisms interact. We sampled a population of *P. deltoides* as it occurs along the Caney Fork River in Tennessee in 2009. Analysis of 16s rRNA sequences indicates the *Populus* bacterial communities are dominated by Acidobacteria, Alphaproteobacteria, and Gammaproteobacteria and the Proteobacteria are the predominant group isolated from *Populus* endophyte and rhizosphere samples (Gottel et al, Appl Environ. 77:5934). Many Proteobacteria use acyl-homoserine lactone (AHL) signals for cell density-dependent gene regulation, in a process known as quorum sensing and response. LuxI-type proteins synthesize small, diffusible AHL signals that function with LuxR-type signal receptors to control gene expression. Most known AHLs possess a fatty acyl side chain, derived from fatty acid biosynthesis, of varying side chain length and substitution. Recently we discovered novel AHL-type signals (*p*-coumaroyl-HSL, cinnamoyl-HSL, and isovaleryl-HSL) made by several soil- and plant-associated Alphaproteobacteria. This suggests that there may be additional novel HSL-type signals made by bacteria.

We initially screened ~120 Proteobacteria isolated from *P. deltoides* for AHL production and found >80% Alphaproteobacteria and >20% of the Gammaproteobacteria isolates to be positive. This suggested AHL signaling is prevalent in *Populus* microbial communities. When we examined the entire genome sequence of 40 of the Proteobacterial isolates, AHL signaling genes were even more prevalent than we predicted from AHL screening results as at least one *luxI*- or *luxR*-type gene was present in 10/10, 3/6, and 24/24 of the sequenced Alphaproteobacteria, Betaproteobacteria, and Gammaproteobacteria genomes, respectively. Interestingly, sequence analysis of some

of the *luxI*-type genes suggests the potential for novel AHL-type signals. Sequence analysis of the *luxR*-type genes suggests some receptors proteins may be responsive to an unknown plant-derived compound, as has reently been reporter for bacteria associated with certain crop plants (Subramoni et al, Appl Environ. 77:4579).

AHL quorum sensing often controls the production of “public goods” such as antimicrobials and exoezymes, as well as aggregation factors and conjugal transfer processes. In order to define the AHL regulon of a particular bacterium, mutants in either the *luxI*- or *luxR*-type genes are often constructed and analyzed relative to wild-type. However, not all AHL-producing bacteria are genetically tractable. To examine AHL-regulons in bacteria without constructing AHL-mutants we have demonstrated that purified AiiA lactonase, an enzyme that hydrolyzes the HSL ring of AHL signals, can be added to bacterial cultures to inhibit AHL-regulated phenotypes and gene expression. Using this enzyme we are defining the AHL-regulons of *Populus*-associated bacteria, by using RNAseq (Hirakawa et al, J Bacteriol. 193:2598).

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