8. Plant-Microbe Interfaces: Probing the Molecular Mechanisms of Plant-Microbe Interactions

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Project Goals: We are focused on understanding *Populus*–microbe interactions at the molecular level to dissect the signals and pathways important for initiating and maintaining symbiotic relationships with *Populus*. Our goal is to elucidate the molecular, spatial, and temporal dynamics involved in *Populus*-microbe interactions using systems biology approaches and directed analytical methodologies. We are interested in how bacteria selectively respond and become associated with *Populus*; and how microbially induced molecular and cellular events impact plant growth, health, and fitness. Ultimately, these data will be used to construct model plant-microbial communities to better understand the underlying rules to community assembly and the functional contributions that result from arrangements of multiple organisms.

Our current research is focused on dissecting the signaling pathways involved in plant-microbe interactions using select Populus-derived isolates that were chosen based on phenotypic screens and genomic inventory data. One area of focus in these isolates is cyclic-di-GMP signaling, which often controls exopolysaccharide production, motility, and other colonization factors. In the robust root colonizer *Pantoea* YR343, we have employed promoter libraries and live imaging to identify three c-di-GMP signaling genes, *orf2884, orf3006,* and *orf3134,* which are highly expressed during *Populus* root colonization but not in laboratory culture. These genes encode diguanylate cylases, which are enzymes that synthesize c-di-GMP, that were more highly induced by growth on *Populus* and/or wheat roots. We have engineered strains to overproduce c- di-GMP in *Pantoea* YR343 and the *Pseudomonas* strains GM17 (an inhibitor of *Laccaria* fungal growth), GM41 (a helper of *Laccaria* fungal growth), and GM30 (an inducer of plant root proliferation). Ongoing RNAseq analyses of the c-di-GMP-overexpressing strains aim to elucidate putative c-di-GMP-controlled exopolysaccharide genes believed to play a role during root colonization. A second signaling system of interest is that of acyl-homoserine lactone signal quorum sensing (QS). Our recent work has shown the genes encoding QS signal synthases (*luxI* genes) and QS signal receptors (*luxR* genes) are prevalent in members of the *Populus* microbiome. We also observed many examples of a recently described subfamily of orphan *luxR*-genes encoded in the genomes of *Rhizobium* and *Pseudomonas* strains isolated from *Populus*. This LuxR subfamily is unusual in that it is believed to respond to an unknown plant- derived signal, not a bacterially produced acyl-homoserine lactone signal. We have created reporter fusions in order to follow LuxR activity in a *Populus* isolate, *Pseudomonas* GM79, and found that plant macerate is required for LuxR activity. These reporter fusions now enable experiments aimed at the elucidation of the plant compounds that serve as a LuxR ligand.

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