

CRISPRi-Mediated Analysis of Biofilm Formation in Plant Growth Promoting *Pseudomonas fluorescens*

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Project Goals: Rhizobacterial communities provide benefits to plants in a variety of ways. To decipher specific molecular mechanisms by which rhizobacteria interact and benefit to plants, we study model rhizosphere communities in laboratory experimental systems. These simpler systems can be interrogated using a variety of approaches, including genetics.

Rhizobacteria of the *Pseudomonas fluorescens* group exhibit beneficial activities on multiple plants including *Populus* trees. *P. fluorescens* was characterized as a mycorrhizal helper bacteria (MHB) that promotes growth of the ectomycorrhizal fungus *Laccaria bicolor* at *Populus* roots. *P. fluorescens* also exhibits strain-specific promotion of aspen seedling growth in a laboratory experimental system [1]. The mechanisms underlying these beneficial interactions between roots and microbes still remain poorly characterized. To gain deeper insight into the rhizobacterial mechanisms, we investigated the spatial and temporal dynamics of the colonization by *P. fluorescens* of mycorrhizal and non-mycorrhizal roots of aspen seedlings [2]. Seedlings were grown in vertical plates in the laboratory, inoculated with a fluorescently labeled *Pseudomonas* strain, and root colonization was monitored over a period of five weeks. We observed an unsuspected diversity of bacterial assemblages at seedling roots that changed over time and were strongly affected by root mycorrhization with *Laccaria bicolor*. Biofilm assemblies on mycorrhizal and non-mycorrhizal roots were distinct, thick biofilms exhibiting internal channel-like structures were observed on non-mycorrhizal root surfaces whereas a layer of bacterial cells stacked along their long axis were found embedded within a gel-like substance at mycorrhizal roots. In the binary system, *P. fluorescens* SBW25 formed dense biofilms on aspen roots after 5 weeks and produced significant PGP phenotypes, suggesting that biofilms are associated with PGP activities [2].

In bacteria, the secondary signaling molecule cyclic diguanosine monophosphate (c-di-GMP) is a central regulator of bacterial transition from motile to biofilm life-styles [3]. We hypothesized that c-di-GMP is connecting rhizosphere signals to specific changes in cellular functions that trigger biofilm formation at roots. C-di-GMP is synthesized by enzymes called diguanylate cyclases (DGCs), degraded by phosphodiesterases (PDEs), and bound by effector proteins that regulate specific cellular functions. Among the 55 c-di-GMP-associated proteins encoded by *P. fluorescens* SBW25, six genes that were transcriptionally responsive to the presence of roots in our data sets and/or previously reported as environmentally induced genes [4], were selected for genetic interrogation. The 6 selected genes were down regulated using the CRISPR interference system (CRISPRi) for expression knockdown and phenotypes related to biofilm formation,

motility, and resistance to reactive oxygen species (ROS) were scored. Despite the high functional redundancy of the c-di-GMP regulatory network in *P. fluorescens* [3], we found that each knocked down gene exhibited measurable phenotypes relevant for interaction with plant roots (i.e., biofilm formation, ROS stress). These results support the notion that root-responsive c-di-GMP-associated proteins are important for the regulation of root-associated phenotypes. The corresponding genes will be our primary targets for future knockout experiments to study the molecular mechanisms that associate biofilm formation, patterns of root colonization, and plant growth promotion.

References

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