Plant-Microbe Interfaces: Developing a synthetic community system to test preferential allocation to nitrogen-fixing bacteria

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Project Goals: The goal of the PMI SFA is to 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 the 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 focus on 1) characterizing host and environmental drivers for diversity and function in the *Populus* microbiome, 2) utilizing microbial model system studies to elucidate *Populus*-microbial interactions at the molecular level and dissecting the signals and pathways responsible for initiating and maintaining microbial relationships and 3) develop metabolic and genomic modeling of these interactions to aid in interpreting the molecular mechanisms shaping the *Populus*-microbial interface.

Plant-microbiome function results from complex interactions among microbial members, host plant genetics/physiology and surrounding environmental conditions. Once the plant – microbiome is established (after initial colonization events), a key question is whether the host plant can actively discriminate among mutualists by 'rewarding' beneficial members through preferential allocation of carbon. To begin to address this question, we developed and characterized reference microbiomes consisting of 10 bacterial strains representing abundant and functionally diverse orders that were isolated and genome-sequenced, from natural *Populus* microbiota. These include potential diazotrophic strains. Subsequently we investigated the ability of five diazotrophs to colonize and function in a *P. trichocarpa* host. After three weeks of co-culture conditions, our results showed strain-specific preferences for plant organs and tissues as indicated by CFUs and qPCR analyses. *Rahnella* sp. OV588 was determined to be a robust colonizer of *Populus* tissues and a *nifH* deletion mutant was generated for that strain for further functional characterization. Using a germ-free magenta box system with calcined clay substrate, *P. trichocarpa* genotype 819 was either uninoculated or inoculated with wild-type OV588 or a *nifH* deletion mutant. All experimental combinations were provided with either Hoagland's complete nutrient medium (with N) and without N. In the no N condition, plants cultured with OV588 showed a 48% increase in total plant dry weight relative to uninoculated plants or plants with the *nifH* mutant strain. Furthermore, acetylene reduction assays of whole *Populus* plants

showed ~5-fold increase in ethylene production when colonized by wild-type OV588 compared to uninoculated or *nifH* mutant-inoculated plants. There were no significant differences in ethylene production, total dry weight or chlorophyll concentration when N was included in the growth medium regardless of the bacterial inoculum. Experiments using $^{15}N_2$ gas in the plant growth chamber are underway. Our work here suggests that N_2 is being fixed by *Rahnella* sp. OV588, which contributes to enhanced plant growth under N-limiting conditions. Future studies will use a dual label of $^{13}CO_2$ and $^{15}N_2$ within a split root system to address questions of preferential allocation within a community context.