

Plant-Microbe Interfaces: Comparative genomics and functional characterization of *Populus*-associated endophytes

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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.

Populus trees host a diverse community of microbes found closely associated with the plant roots (rhizosphere) and inside plant tissues (endosphere). These complex communities are shaped by the functional diversity expressed by their members as well as the environmental forces acting on them. Previous studies have shown that the microbial community found inside the plant is less diverse than the host-associated rhizosphere community, consistent with the idea that the endosphere represents a unique environmental niche. The mechanisms by which some microbes are able to bypass plant defenses and survive within plant tissues, however, are not well understood. To better understand what makes an endophyte an endophyte, we are using a combination of comparative genomics, microbial physiology, and molecular genetics to characterize and identify genetically tractable model endophytes to study the molecular mechanisms that promote an endophytic lifestyle. Starting with a collection of 30 genome sequenced endophytes isolated from *Populus*, we are characterizing plant-associated microbial behaviors including phytohormone production, siderophore production, motility, biofilm formation, and plant colonization. In addition, we are determining whether these isolates are genetically tractable by constructing fluorescent strains through plasmid transformation or chromosomal integration. This collection also includes thirteen *Variovorax* isolates, twelve of which were isolated from the endosphere and one from the rhizosphere of *Populus*. Genomic comparisons indicate that these isolates are diverse within the *Variovorax* genus, despite being isolated from a common host. We show that the rhizosphere-isolated strain *Variovorax* sp.

YR216 is the most distantly-related of the *Populus*-associated isolates. Unlike the endophyte isolates, *Variovorax* sp. YR216 lacks ACC deaminase activity and does not efficiently colonize root tissue. By combining comparative genomic analyses with molecular genetics, these studies will provide insights into the microbial pathways and signaling events involved in plant host recognition and survival.

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