Plant-Microbe Interfaces: Characterization of natural products from the Populus microbiome

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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 serve 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.

The Populus root microbiome is an incredibly diverse community, comprising organisms from across plant, animal, oomycete, fungal, viral, archaeal and bacterial taxa. Bacteria from the soil are known to harbor many gene clusters encoding complex natural products that can act as signaling molecules, antibiotics, and antifungals. We set out to characterize the natural product potential of bacteria from a plant root community in order to understand its biosynthetic diversity as well as begin to determine keystone members and associated molecules that regulate community structure and plant health. The model plant system used was Populus, the first fully genome-sequenced tree species having an already well-characterized root metagenome.

We first considered metagenomic samples collected from the roots of P. deltoides and examined the overall bacterial diversity and natural product variety, comparing to other plant and human microbiomes to show the predicted species and natural product richness. The diversity of bacteria in the plant microbiome is greater than the well-studied human gut microbiome, and the organisms within this community have greater biosynthetic potential as well. We next utilized the fully sequenced genomes of over 400 bacterial isolates, representing the four major bacterial phyla in the metagenome, connecting molecules to genomes and surveying the overall natural product potential. While some species harbor greater numbers of clusters, especially Actinobacteria of the genus Streptomyces, we found over 10 clusters per organism on average, with over 4000 predicted clusters. Comparison to known natural product gene clusters revealed that only 1% of clusters produced an already-characterized secondary metabolite, revealing the great potential to discover compounds with novel structures and possibly novel activities.
About 15% of the predicted clusters could not be connected to known natural products classes, revealing the potential to discover structurally novel metabolites. Of the remaining clusters, many grouped within classes known to produce molecules with antibiotic or antifungal properties. Ribosomally synthesized and post-translationally modified peptide natural products were both prevalent in the collection and divergent from previously characterized molecules. These natural products, which are peptides that have been modified by additional enzymes, were the most abundant class of natural product identified, being more common than even nonribosomal peptide and polyketide clusters. Lactones and siderophores, molecules known to be important for quorum sensing and iron acquisition, respectively, were prevalent in the genomes and suggest a high level of communication as well as pressure to compete for resources. These molecules are involved not just in microbe-microbe interactions, but have consequences in signaling to the plant to enable colonization and nutrient exchange.

While the diversity and richness of natural product gene clusters within the genome-sequenced fraction of the Populus microbiome reveals an additional layer of complexity in the community, the presence of a gene cluster does not necessarily mean that the compound will be produced. Thus, we set out to determine if genome-sequenced Streptomyces isolates were capable of producing compounds with antifungal and antibiotic activity under laboratory conditions. Streptomyces sp. OK461 and Streptomyces sp. OK006 each contain an identical lasso peptide gene cluster with known Gram-positive antibacterial activity. Both isolates inhibited the growth of Gram-positive isolates, and methanolic extracts containing the lasso peptide replicate this activity. Thus, complex molecules such as the lasso peptide are involved in interspecies signaling and communication, shaping community structure and therefore influencing the overall health and growth of the host plant.

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