Plant-Microbe Interfaces: Accessing the hidden diversity of the poplar microbiota through targeted metagenomics and cultivation.

Mircea Podar* (podarm@ornl.gov), Joel Turner, Leah Burdick, Dawn Klingeman, Zamin Yang, Dale Pelletier and **Mitchel Doktycz**

Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, TN

http://PMI.ornl.gov

Project Goals: The goal of the Plant-Microbe Interfaces (PMI) SFA is to characterize and interpret the physical, molecular, and chemical interfaces between plants and microbes and determine their functional roles in biological and environmental systems. Populus and its associated microbial community serve as the experimental system for understanding the dynamic exchange of energy, information, and materials across this interface and its expression as functional properties at diverse spatial and temporal scales. To achieve this goal, we focus on 1) defining the bidirectional progression of molecular and cellular events involved in selecting and maintaining specific, mutualistic Populus-microbe interfaces, 2) defining the chemical environment and molecular signals that influence community structure and function, and 3) understanding the dynamic relationship and extrinsic stressors that shape microbiome composition and affect host performance.

The bulk of the microbial taxonomic diversity from soils and the *Populus* rhizosphere has remained uncultured. Currently, we only have representatives of 4 of the 21 phyla with abundance of >0.1% of the rhizosphere community. Competition and inhibition could restrict the growth of some organisms in the presence of others. It is also likely that many of the uncultured bacterial taxa require chemical compounds synthesized either by the plant or by other members of the community. A comprehensive understanding of the role of microbes in *Populus'* physiology requires expanding the range of bacterial taxa used in the assembly of controlled communities used for laboratory mechanistic studies. For yet-unculturable microbes, obtaining genomic information will aid inferring their physiological potential and dependencies, which in turn could lead to cultivation.

We developed flow cytometric fractionation of the complex *Populus* microbiome, based on various cellular characteristics including size and morphology, DNA content and cell wall constituents. Following sorting of populations containing 1000-5000 cells and genomic amplification by multiple displacement, we obtained mini-metagenomes enriched in low abundance microbial constituents of *Populus* rhizosphere, including Thaumarchaeota and candidate bacterial phyla OD1, TM6 and TM7. Metagenomic sequencing resulted in draft genomes that are currently being used in comparative genomic analyses with relatives from other microbiomes.

We also applied single cell isolation and high throughput cultivation using flow cytometry cell sorting. We were successful in isolating in pure culture a member of Acidobacteria (*Terriglobus* sp.) and one of Verrucomicrobia, (*Roseimicrobium* sp), the first representatives of those phyla from the poplar rhizosphere. We also isolated novel representatives of Actinobacteria, Proteobacteria and Bacteroidetes. Those organisms are currently being characterized physiologically and at genome level. Ongoing effort is also directed towards isolation of candidate bacterial phyla representatives.

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