

## **Plant-Microbe Interfaces: Simplified community approach to investigate the dynamic host-microbiome relationship**

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**Project Goals: The goal of the 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 long lived woody perennial *Populus* harbors a diverse consortium of microbial associates. To gain insight into complex *Populus* host and microbial interactions, we isolated over 3,200 bacteria and developed a synthetic community system that employs subsets of these microbes. The isolate collection contains representatives from many of the dominant and abundant community members found in *Populus* field studies. However, much of the microbial taxonomic diversity from the *Populus* rhizosphere has not been cultured. We have representatives of 6 of the 21 phyla with abundance of >0.1% of the community. Thus, we have ongoing efforts to cultivate and characterize *Populus* rhizosphere bacteria that are under-represented or absent from our existing collection using single cell sorting on plant-derived culture systems (Podar *et al.*, 2019). Our prior studies utilized reduced communities (< 10 members) to evaluate questions relating to the functional genetics underlying plant – microbe interactions. Now, we have created a synthetic community approach that allows for the design and evaluation of large complex communities (>150 members) to determine how host plant genetics, nutrient and environmental interactions shape community interactions and function. To create the synthetic community, all 16S rRNA sequences from the sequenced bacterial strains within our culture collection were extracted, trimmed to the V4-V6 region, and aligned. Using our DISCO-microbe software (Carper *et al.*, 2020), we have designed a community consisting of 150 members that spans 4 phyla, 9 classes, 12 orders, 32 families and 77 genera. These 150 members have been inoculated into double autoclaved soil containing *P. trichocarpa* plants and exposed to differing environmental conditions for growth over 3 weeks: control (C), warm temperatures (W), cold temperature (CT), low nitrogen (LN) and warm temperature and low nitrogen (WLN). Overall, inoculated plants were smaller than the uninoculated controls suggesting an initial negative effect from the microbial load. The negative effects between inoculated and un-inoculated were statistically significant for number of leaves, change in stem height and leaf area, although this effect depended on the environmental condition.

The W and WLN conditions had the greatest effect phenotypically on the stem height and leaf area. Initial sequencing of the bacterial community of C- and CT-conditioned plants identified 95 out of 150 bacterial members present in host tissues. Tissue type (rhizosphere, root, stem and leaves) was the main factor in structuring the community in both the weighted (45.8%,  $p=0.0003$ ) and unweighted (34.0%,  $p=0.0001$ ) UniFrac metrics. The environmental condition also played a role in structuring community with more variation explained from the unweighted (20.3%,  $p=0.0001$ ) than weighted (9.3%,  $p=0.0035$ ) UniFrac metrics. This suggests that community differences between the environmental conditions is primarily from changes in low abundance community members. The two most abundant members in both environmental conditions were *Rhodanobacter* and *Paraburkholderia* strains. Within the CT leaves, a *Pantoea* strain was the most abundant microbe. Additionally, several strains were found across both treatments and all tissues sampled, including *Rahnella aquatilis*, a strain with demonstrated nitrogen fixing ability. Further investigations are ongoing to better understand how *Populus* structures its microbiome in response to genetic and environmental change.

## References

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