Plant-Microbe Interfaces: Metaproteomics reveals shifts in microbial activity and regulation in artificially assembling communities as they respond to environmental variation

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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.

Populus interacts with numerous microbes that have been shown to enhance its health, growth, and development. While PMI researchers have observed distinct microbiome compositions that associate with the *Populus* rhizosphere and root endosphere, little is known about the dynamic molecular mechanisms that influence microbial community assembly across distinct environments. As environmental conditions fluctuate (e.g., nutrients, pH), individual microbes will alter their cellular behavior and this will ultimately impact how they interact with other community members. Unfortunately, studying microbe-microbe interactions and behaviors in a natural setting is challenging, because of the complexity of natural ecosystems and the limited control over environmental factors. A strategy to approach this major challenge relies on addressing community complexity in a progressive manner, by first building a detailed understanding of relatively simple subsets of the community and then achieving high predictive power through combining different building blocks (e.g., hosts, community members, and environmental factors). Emergent properties observed in simpler communities can then be re-evaluated as more complex systems are studied and, when a particular property becomes less relevant, then higher-order interactions can be identified.

In this study, ten taxonomically diverse bacterial members of the *Populus deltoides* rhizosphere were co-cultured either in a complex (R2A) or minimal glucose (MOPs+glucose) medium. Metaproteomics were utilized to understand community assembly, structure, adaptation, and

functionality. Microbial structure was assessed, and results showed that microbial relative abundance stabilizes after few passages and the community is dominated by few microbial strains. In general, we observed two different stable microbial community compositions between the two media types tested. Proteome-wide analyses revealed that *Pseudomonas* was the dominant member in both media, and expressed proteins involved in the synthesis of antibiotics, toxins, and siderophores. Pairwise-microbe interaction data reinforced these antagonistic properties of *Pseudomonas* by showing a presence of zone inhibition for most members of the community.

As members of the community are challenged with unfavorable conditions created either intentionally by other members of the community (e.g., production of antibiotics from *Pseudomonas*) or unintentionally through the collective activity of community metabolism (e.g., limited nutrients, lower oxygen levels, etc.), microbial persistence requires the expression of necessary coping mechanisms. The proteome-wide analysis of *Pantoea* and *Bacillus,* which maintained relatively high protein biomass across the rich media passages, revealed two distinct behavioral changes used to cope with the changing environments. In brief, *Pantoea* was observed to effectively adapt to the changes in the communities of R2A media by increasing the abundance of proteins related to antibiotic resistance and motility as well as shifting metabolism from aerobic to anaerobic processes. *Bacillus*, on the other hand, sporulated as an adaptive response to nutritional deprivation and environmental stress.

Overall, metaproteomics provided an accurate assessment of community assembly across two distinct growth media and improved our understanding of the molecular mechanisms associated with dynamic microbe-microbe interactions and behaviors across changing communities and environments. For this 10-member artificial community, *Pseudomonas* acted as a detrimental member of the community through the production of proteins responsible for the synthesis of antibiotics, toxins, and siderophores. In response to unfavorable conditions, other members maintained relatively high abundances by adapting or becoming dormant. Moving forward, these findings will be leveraged to predict the behavior of more complex systems.

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