**Plant-Microbe Interfaces: Protein post-translational modifications in a 10-member microbial community from the *Populus* rhizosphere offer critical insights into how microbes adapt to changing environments**

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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.**

Bacterial members within microbial communities that associate with the *Populus* rhizosphere adapt to their environmental conditions not only by modulating the abundance of the proteins they produce, but also by regulating the functions of these molecules. Post-translational modifications (PTMs) of proteins are one of the most important, yet understudied mechanisms that microbes use to rapidly activate, alter or suppress protein functions. Optimized bioinformatic pipelines for metaproteomics exploiting *de novo* peptide sequencing now allow researchers to broadly characterize PTMs in proteins extracted from bacterial isolates and communities. Here, we used this metaproteomics capability to interrogate the PTM landscape from a simplified artificial community consisting of ten predominant bacterial members in *Populus deltoides* rhizosphere co-cultured in complex and minimal glucose media.[1] The advantage of this synthetic community is its low complexity, high controllability, and reproducibility in a laboratory setting from which molecular insights that are closely representative of how bacteria behave in their natural environments can be obtained.

Briefly, equal volumes of *Bacillus* sp. Bc15, *Caulobacter* sp. AP07, *Duganella* sp. CF402, *Pantoea* sp. YR343, *Paraburkholderia* sp. BT03, *Pseudomonas* sp. GM17, *Rhizobium* sp. CF142, *Sphingobium* sp. AP49, *Streptomyces* sp. YR139, and *Variovorax* sp. CF313 with the same normalized cell densities were mixed in either a complex medium or minimal medium supplemented with 0.2% glucose, harvested after 48-hrs, then transferred into fresh media by

diluting 1:10 for a total of 15 passages in biological triplicates. From these 15 passages, a metaproteomics workflow was applied to study PTMs.

Our results showed that approximately 18% of proteins identified for all organisms at both rich and minimal media conditions had PTMs with biological significance. The most frequently occurring types of PTMs were methylations, dehydrations, and oxidations/hydroxylations. Such modifications were part of proteins impacting prominent cellular processes, including translation, ribosomal structure and biogenesis, energy production and conversion, protein turnover and chaperon functions, as well as amino acid metabolism and transport. Protein-centric analyses highlighted two types of PTM regulation: static modifications that are essential for proper protein function; and dynamic, reversible modifications that alter the functional or structural state of proteins. Two representative examples of these types of PTM-based regulations were a static β-methylthiolation modification localized on a universally conserved aspartic acid residue in bacterial ribosomal proteins S12; and a dynamic lysine methylation only present in the sequences of elongation factor thermo unstable proteins for some bacterial members being impacted by nutrient depravation.

Overall, this study demonstrates that high-resolution mass spectrometry not only affords the ability to broadly characterize PTMs in a biological system, but that it also provides a level of sensitivity capable of revealing regulatory mechanisms influencing the activity of single proteins.

**References/Publications**

1 Brown, S. D. *et al.* Twenty-one genome sequences from Pseudomonas species and 19 genome sequences from diverse bacteria isolated from the rhizosphere and endosphere of Populus deltoides. *J Bacteriol* **194**, 5991-5993, doi:10.1128/JB.01243-12 (2012).

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