

## **Plant-Microbe Interfaces: Development of an experimental approach to achieve spatially resolved plant root-associated metaproteomics using an agar-plate system**

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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 plant-microbe interface is a region where microbes respond to and interact with their plant hosts. Plants secrete root exudates that shape the microbiome, yet it is poorly understood how microbiome functions change with respect to space and time across plant roots. Methodologies that facilitate spatio-temporal metaproteomic studies of root-associated microbiomes are yet to be realized. To address these challenges, we developed a novel extraction method that facilitates spatial characterization of microbiomes with bottom-up proteomics using a plant-agar culture system under controlled laboratory conditions. Spatially defined agar ‘plugs’ of interest near or distant from the plant roots were excised and subsequently processed using a novel peptide extraction method that uses magnetic microparticle beads for agar removal from the samples allowing for metaproteomic measurements. As a proof-of-principle, a previously studied 10-member bacterial community constructed from a *Populus* root system was grown in an agar-plate with a 3-week-old *P. trichocarpa* plant. High-resolution metaproteomic measurements that are used to infer both bacterial community composition and function were obtained across two time points (24-h and 48-h) for three distinct agar plugs collected at the root base, root tip, and a region distant from the root. The spatial resolution of these measurements provide evidence that the root-associated microbiome structure and proteome expression changes across the plant root interface. Interrogation of the individual bacterial proteomes revealed functional profiles related to their behavioral associations with the plant root, in which chemotaxis and augmented metabolism likely supported predominance of the most abundant member. Obtaining spatio-temporally resolved microbial communities along different regions of the plant root was enabled using this novel peptide extraction method, which is a critical step towards understanding how plants select for or

against specific microbes. More broadly, we anticipate this novel extraction approach will benefit other soft-agar plate studies that study nutrient exchange among microbes.

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