## 3. Plant Microbe Interfaces: Proteomic Characterization of Endophyte and Rhizosphere Microorganisms and their Impacts on Plants

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**Project Goal: The goal of the PMI SFA is to understand the genome-dependent molecular and cellular events involved in establishing and maintaining beneficial interactions between plants and microbes. *Populus* and its associated microbial community serves as the experimental system for understanding how these molecular events manifest themselves within the spatially, structurally, and temporally complex scales of natural systems. To achieve this goal, we focus on 1) characterizing host and environmental drivers for diversity and function in the *Populus* microbiome, 2) utilizing microbial model system studies to elucidate *Populus*-microbial interactions at the molecular level and dissecting the signals and pathways responsible for initiating and maintaining microbial relationships and 3) develop metabolic and genomic modeling of these interactions to aid in interpreting the molecular mechanisms shaping the *Populus*-microbial interface.**

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Protein expression patterns, and changes in those patterns, provide information on metabolic and other strategies employed by bacteria, fungi, and plants that participate in natural communities such as the root microbiomes of *Populus* trees. Ongoing projects in the PMI SFA are studying the proteome responses of these various organisms to treatments ranging from application of hormones to interactions with other members of the *Populus* microbiome community.

*Populus* proteome response to a constructed soil microbial community. We have acquired leaf proteome data from *Populus* specimens inoculated with a 3-member bacterial community (see poster by Weston et al.), as well as from axenic control *Populus* specimens. The goal of this study is to determine whether observed phenotype differences are accompanied by changes in the *Populus* proteome. Preliminary analysis reveals that slightly less than half of the approximately 4400 detected protein groups are common to all samples, with ongoing work aimed at identifying proteins of altered abundance in the inoculated plants.

Proteomes of mycorrhizal roots in *Populus*. We have performed proteomics characterization of *Populus* roots that exhibit varying degrees of colonization by the ectomycorrhizal fungus *Laccaria bicolor*. Identification of both fungal and plant proteins will provide biological insights into their interactions. To determine figures of merit for proteins that are potentially important in the colonization process, but may be difficult to measure because of their small size or transient expression, we have shown that one such protein (MiSSP7) is amenable to detection by our LC- MS-MS protocol as a pure standard, but may be masked by matrix effects when present in a full proteome.

Effects of plant hormones on protein expression. As a n ew class of plant hormones, strigolactones (SLs) act as a key inhibitor of shoot branching, stimulate seed germination of root parasitic plants, and promote hyphal branching and root colonization of symbiotic arbuscular mycorrhizal fungi. They also regulate many other aspects of plant growth and development. We have perfomed quantitative proteomics using an isobaric chemical labeling reagent, iTRAQ, to identify the proteome regulated by SLs in *Arabidopsis* seedlings. We found that in addition to regulating the abundance of proteins implicated in SL pathways, SLs also regulate the expression of a number of proteins that have not been previously assigned to SL pathways. Furthermore, we observed a drastic difference between the SL-regulated transcriptome and the SL-regulated proteome. These findings provide a new tool to investigate the molecular mechanism of action of SLs.

Proteomes of *Populus* endophyte and rhizosphere bacterial isolates. The increasing availability of genome sequences for PMI bacterial isolates (see poster by Pelletier et al.) has enabled proteomics investigations of selected members of this growing collection. Changes in protein expression resulting from an applied treatment (e.g., environmental stress, an applied chemical, or presence of another organism) can reveal metabolic pathways involved in the response to the treatment. Examples of studies of PMI isolates include responses of *Pseudomonas* sp. strain GM41 to tryptophan, and of *Pantoea* sp. strain YR343 to *Populus* root exudate. It will be interesting to consider detected proteomes across bacterial strains in light of comparative genomics studies being performed elsewhere in the PMI (see poster by Ussery et al.) To facilitate these measurements, we are refining our LC-MS-MS protocols to achieve higher throughput.

Future studies. We are planning metaproteomics measurements on natural rhizospheric communities of *Populus*, and integration with metagenomics analyses (See poster by Schadt *et al.*).

Current and new datasets are being made available across the PMI project using the Proteomics Workflow implemented in the PMI Knowledgebase (see poster by Ussery *et al*.).

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