205. Plant Microbe Interfaces: Proteome Characterization of Plant-Bacterial and Fungal- Bacterial Interactions

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Project Goals: 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 fro 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.

We are studying protein expression patterns, and changes in those patterns, in experiments involving bacteria and fungi isolated from the root microbiomes of Populus trees.

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. Proteome data were obtained for leaves 1, 2, and 3 weeks post- inoculation. Approximately 2000 protein groups were identified for each individual measurement, summing to >5600 protein groups across the entire experiment, of which nearly 1700 exceed a minimum abundance criterion for quantitative comparison. The 20 most abundant proteins observed for each treatment account for 16 to 18% of the total protein abundance, and are dominated by ribosomal proteins, photosystem I and II proteins, peptidyl-prolyl cis-trans isomerases, and RuBisCo. Not surprisingly, the leaf proteomes showed only background-level signals from proteins of the inoculated bacterial strains. Despite clear growth phenotype differences between inoculated and uninoculated plants at the 3 week time point, as well as marked differences in metabolite profiles from a similar inoculation experiment, we observed only a few proteins that differed in abundance between the inoculated and control plants. However, the numbers of differentially abundant proteins increased with time. The fraction of observed proteins in several KOG functional categories (RNA processing and modification; energy production and conversion; translation, ribosomal structure and biogenesis; amino acid transport and metabolism; carbohydrate transport and metabolism; posttranslational modification, protein turnover, chaperone; and cytoskeleton) is higher than the corresponding fraction in the full predicted Populus proteome, while the observed fraction in the signal transduction mechanisms KOG is less than the full predicted proteome. The results indicate that observed growth phenotype and metabolic profiles do not strongly correlate with changes in protein abundance, suggesting future studies such as the roles of post-translational modification in regulation of enzyme

activity and deeper proteome measurements to interrogate low- abundance proteins such as transcription factors.

Effect of helper bacteria on the proteome of Laccaria bicolor. A Pseudomonas strain (GM41) isolated within the PMI project from the Populus microbiome has previously been shown to enhance the growth and root colonization by the mycorrhizal fungus L. bicolor. In initial proteome measurements of co-cultures of the fungus with this Mycorrhizal Helper Bacterial isolate (MHB; Labbé et al., 2014), we have identified both bacterial and fungal proteins that are present only in the co-culture, but not in cultures of either organism grown alone. We are examining these proteins for evidence that will identify mechanisms underlying the MHB phenomenon.

Proteome of Mortierella elongata and a bacterial endosymbiont. Mortierella elongata is a commonly isolated soil fungus that plays an important role in forest soil ecosystem processes by affecting plant growth, carbon sequestration, and nitrogen cycling. This fast-growing fungus hosts a bacterial endosymbiont, Candidatus Glomeribacter sp. We have applied mass spectrometry-based quantitative proteomics to investigate changes in protein abundance, as well as post-translational modifications. This analysis reveals that the abundances of over 400 fungal proteins significantly changed in response to the reduced abundance of endosymbiont in normal growth medium, providing functional insights into fungal host-endosymbiont interaction.

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