Plant Microbe Interfaces: Proteomics at the Plant-Microbe Interface

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Project Goal: 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 an initial 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 will focus on 1) characterizing the natural variation in *Populus* microbial communities within complex environments, 2) elucidating *Populus*-microbial interactions at the molecular level and dissecting the signals and pathways responsible for initiating and maintaining microbial relationships, and 3) performing metabolic and genomic modeling of these interactions to aid in interpreting the molecular mechanisms shaping the *Populus*-microbial interface.

The proteomes and metaproteomes of plants, bacteria, and fungi offer insights into the mechanisms that these organisms employ at plant-microbe interfaces. We have initiated proteomics studies of several organisms involved in these interfaces, with an emphasis on those involving *Populus* trees, to identify processes in which proteins are important for establishment and maintenance of the interface.

-Proteomics of *Laccaria bicolor* mycelia We have performed "shotgun" proteomics of mycelia from the ectomycorrhizal fungus *Laccaria bicolor* grown under a variety of conditions, including different fungal strains, media compositions, cold and heat stresses, and proximity to roots of *Populus* plants. The average number of *L. bicolor* proteins identified per sample was ~1200. Approximately 440 proteins were detected in at least one replicate of each condition, establishing a "core" proteome for *L. bicolor*.

-Analysis of small proteins encoded by small genes in bacterial and plant species To complement work pioneered by Xiaohan Yang to identify small-protein-encoding genes (see poster by Li et al.), we are developing proteomics methods to target small proteins from plant tissues. After evaluating several methods for enriching small proteins in an *E. coli* model system, we applied the most promising technique (in-gel digestion of low molecular weight regions from 1D SDS-PAGE separations) to studying small proteins in *Arabidopsis* roots and shoots. Of a total of > 5000 proteins identified from unfractionated extracts and small protein fractions, a few tens of proteins were identified exclusively in gel fractions corresponding to molecular masses below 20 kilodaltons. Evidence for expression of these proteins supports

improved annotation of the corresponding small genes, and provides candidates for further studies of biological function.

-Proteomics studies of roots from field-sampled mature *Populus* trees The ability to study the root proteome of plants is an important first step towards studying plant-microbe interactions in the rhizosphere. Extraction of proteins from plants is technically challenging, and further complications arise when the samples are obtained from mature tree roots. We have identified an approach that reliably yields proteins from roots of naturally occurring *Populus* trees, sampled during PMI collection trips to the Yadkin River in North Carolina. We typically identify >1000 proteins from each root sample, despite heterogeneity in morphology, differences in location, soil type, etc.; from 4 individual trees, we have evidence for ~2600 proteins to date (with some redundancies due to gene duplications, etc.). A "core" proteome that is common to all these root samples contains several hundred proteins. Analyses of functional categories and subcellular locations are ongoing.

-Comparison of label-free, metabolic labeling, and isobaric chemical labeling for quantitative proteomics Using a model soil bacterial species, *Pseudomonas putida* F1, we have compared figures of merit such as depth of proteome coverage, quantification accuracy, precision, and reproducibility for several quantitative proteomics methods using a high-performance hybrid mass spectrometer, the LTQ Orbitrap Velos. Each approach has particular merits, and the final choice of approach depends on the requirements of the experiment at hand. Our results indicate that isobaric chemical labeling has the highest quantification precision, label-free quantification provides the largest number of protein identifications, and metabolic labeling is intermediate in both measures.

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