An overview of EMSL's Mass Spectrometry Capability

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This presentation will include a brief overview of mass spectrometry capability at the Environmental Molecular Sciences Laboratory (EMSL), a U.S. Department of Energy (DOE) national scientific user facility located at Pacific Northwest National Laboratory, with selected application highlights. EMSL provides integrated experimental and computational resources for discovery and technological innovation in the environmental molecular sciences to support the needs of DOE and the nation.

As an illustrative example, I will describe our collaborative endeavor to characterize the soybean root hair system proteome and gain novel insight into the means by which rhizobia infect legume roots to establish a nitrogen-fixing symbiosis. Our ultimate goal is to utilize soybean root hairs to explore, at a systems level, the biology of a single, differentiated plant cell type.

Root hairs, single tubular cells formed from the differentiation of epidermal cells (trichoblasts) on primary and secondary roots, are involved in water and nutrient uptake and represent the colonization site of leguminous roots by rhizobia, soil bacteria that establish a nitrogen fixing symbiosis. Their ability to enter into a nitrogen fixing symbiosis with soil bacteria (i.e., rhizobia), and thus to be grown without the need for expensive nitrogen inputs, is one of the key, unique properties of legumes (e.g., soybean). Among other advantages, biological nitrogen fixation provides for a very favorable input-to-output ratio for biodiesel production from soybean. The availability of the genome sequence (www.phytozome.net/soybean) now enables detailed functional genomic analysis of soybean.

Soybean root and root hair samples were isolated at various times (0-72h) after inoculation with *B. japonicum* and frozen in liquid nitrogen. Mock-inoculated samples were also generated. A total of 78 samples were analyzed (including 3 biological replicates). The AMT tag database was constructed based on RPLC-MS/MS results from SCX fractions generated using 6 pools of the above samples. Subsequently, we performed comparative proteomic analysis of the time series of the infection using label-free approach (RPLC-FTMS). Similar samples, at the same time points, were also analyzed for gene expression (by microarrays and HTP sequencing) and for metabolites (via GS/LC-MS/MS), and corresponding phosphopeptide enriched samples have been analyzed using the 8-plex-iTRAQ strategy.

Approximately 7,000 proteins from soybean and *B. japonicum* were detected in root hairs. The proteins identified are involved not only in basic cell metabolism, but also in functions more specific to the single root hair cell, including water and nutrient uptake, vesicle trafficking, hormone and secondary metabolism. For example, consistent with the role of the root hair in nutrient uptake, a variety of transport proteins were identified. A variety of plant hormones are known to modulate legume nodulation. Therefore, it was of interest to find biosynthetic enzymes for virtually all of the known plant phytohormones in the soybean root hair proteome. Statistical analysis identified more than 1,000 proteins significantly regulated in root hairs in response to the symbiotic bacteria. The proteomic data are currently being integrated with phosphoproteomic, metabolomic and transcriptomic data to provide a systems level view of the root hair response to *B. japonicum* infection.