Plant-Microbe Interfaces: Developments in integrated omics to link microbial metabolism to community structure/function in plant/microbial systems

Robert Hettich,[1] (hettichrl@ornl.gov) Sanjeev Dahal,[2] Dale Pelletier,[2] Greg Hurst,[1] Nancy Engle,[2] Timothy Tschaplinski,[2] and **Mitchel Doktycz**[2]

[1]Chemical Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN; [2]Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, TN

http://PMI.ornl.gov

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 serve 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.

The availability of new mass spectrometric platforms for higher performance and enhanced measurement speeds can be combined with optimization of new sample preparation protocols to dramatically enhance the impact of omics measurements for plant-microbe research. For example, a traditional deep proteome measurement (LC/LC-MS/MS) might require more than 20 hours of MS measurement time. Thus, there is a strong need for development of methods to dramatically increase measurement throughput while maintaining measurement depth. To this end, we have installed and demonstrated a new proteome measurement scheme based on a high-performance LC-MS/MS instrument (ThermoFisher Q-Exactive Orbitrap mass spectrometer). This instrument provides measurement speeds of up to 12 Hz, enhanced dynamic range measurement, mass accuracies in the low ppm range, and mass resolutions exceeding 100,000 (FWHM). This provides the ability to execute multi-dimensional LC/LC-MS/MS experiments in a higher throughput fashion (up to 5-6 complete runs per day), with equal or superior mass accuracy and measurement depth. By utilizing autosamplers for automated sample injection campaigns, we have been able to dramatically improve the precision and reproducibility of proteome measurement, which greatly enhances differential proteome measurements. These advances increase proteome measurement throughput by 6-10X overall, thereby opening new avenues for the larger sample campaigns expected for both microbial and plant studies. To extend the range of experimental information possible for metabolomic studies, we have undertaken implementation of LC-MS measurement capabilities for a range of semi-volatile and non-volatile metabolites to complement our existing GC-MS capabilities. To this end, we have implemented a high performance reverse phase LC-MS/MS approach (based on Q-Exactive Orbitrap MS) for bacterial and plant metabolomic studies.

This instrument is configured with an autosampler/high performance LC system to permit fairly large-scale metabolomic measurement campaigns with high mass accuracy, deep dynamic range, and tandem mass spectrometry fragmentation. These advances should help transform the capacity for executing detailed "integrated omics characterization campaigns," such as the study discussed below.

We have applied these new tasks and procedures to understand the microbial metabolism of higher order salicylates. One of the defining characteristics of *Populus* is the production of these secondary metabolites, which are involved in host defense and signaling mechanisms. Two interesting bacterial strains, *Rahnella* sp. OV744 and *Pseudomonas* sp. GM16, are involved in the degradation of simple phenolic compounds such as salicin and salicyl alcohol respectively through uncharacterized mechanisms. We hypothesize that these two strains can utilize pathways to cooperatively grow on salicin. We acquired both metabolomic and proteomic data for mono- and co-cultures of *Rahnella* sp. OV744 and *Pseudomonas* sp. GM16. The hypothesis tested with this experiment was that in a co-culture of salicin-containing medium, OV744 would metabolize salicin to salicyl alcohol, which would then be used as a carbon source by GM16. Culture experiments were designed to test this hypothesis, as well as to potentially provide information on the underlying enzymes and pathways employed by the two species in metabolizing salicylates. When monitoring a microbial co-culture in salicin using qPCR strain specific primers, growth of both OV744 and GM16 was observed, suggesting a cross-feeding of salicyl alcohol leading to growth of both strains. By using various quantitative and multi-omics (proteomics and metabolomics) approaches, we demonstrated that the individual pathways for salicin and salicyl alcohol metabolism are present in OV744 and GM16, respectively. Furthermore, we establish that in salicin co-culture, these two strains utilize both of these pathways to cross-feed salicyl alcohol. The research presents one of the potential mechanisms for microbial transformation of salicin and potentially other more complex HOS, and demonstrates the intricate interactions that occur within the plant microbiome.

The Plant Microbe Interfaces Scientific Focus Area is sponsored by the Genomic Science Program, U.S Department of Energy, Office of Science, Biological and Environmental Research.