Multi-Omics Analysis of a Mycorrhizal System

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Project Goals: The goal of the Environmental Sensing and Response SFA is to decipher the molecular dialog between rhizobacteria, plant roots, and ECM fungi that leads to the establishment of symbiotic interactions and beneficial effects for the plant. The mechanisms for environmental sensing and response between rhizosphere community members span multiple types of biological interactions that can be revealed by using an integration of metabolomics, proteomics, and transcriptomics data through computational analysis. Analysis of multi-omics data proposes specific molecular mechanisms of mycorrhizal interactions that can be directly validated through biological experiments.

In terrestrial ecosystems, plants are never solitary entities. Rather, they exist as complex meta-organisms, comprised of plant hosts and communities of interacting soil microorganisms, which include mutualistic fungi and bacteria. Symbiosis between soil bacteria, mycorrhizal fungi, and tree species leads to coordinated resource exchange and enhanced productivity and resiliency in forest ecosystems. These symbiotic associations provide multiple benefits to the host tree, especially under conditions of limiting nutrient availability. In return, the rhizosphere microorganisms acquire photosynthetically-derived carbon from the plant in form of sugars and organic acids. There are significant knowledge gaps in our understanding of how rhizosphere communities establish and maintain symbiotic interactions that are beneficial to the plant host. We address these knowledge gaps using a laboratory model of mycorrhizal symbiosis between *P. tremuloides* (aspen) and the ectomycorrhizal fungi *Laccaria bicolor* (Laccaria) and *Paxillus involutus* (Paxillus), multiomics analysis, and machine learning approaches. Aspen seedlings were grown in sand pots, alone or in mycorrhizal association with either Laccaria or Paxillus. Root transcriptomic, community metabolomic, and root membrane proteomic data were collected, through collaborations with JGI and EMSL. Data was collected for each of the 'omics data types and integrated into a system-scale model of mycorrhizal interactions.

Transcriptomics: In an analysis of aspen root transcriptomic data, about 60% of short reads have multiple possible alignments to *P. trichocarpa* genes (JGI genome, v3) demonstrating the value of statistical approaches like 'Bowstrap' for community transcriptomics. 1376 significantly differentially expressed genes (6%) in response to mycorrhizal interaction were detected by ANOVA.

Metabolic Models: Transcriptomics can be used to predict metabolism using PRMT [1]. 1235 predicted metabolites are present in the metabolic model, of which 55 (4%) are differentially metabolized in response to mycorrhizal interaction.

Proteomics: Application of statistical modeling tool 'BowStrap' [2] yielded significant improvements in our ability to detect significant protein expression from EMSL proteomics data. A total of 5646 proteins were detected as significantly expressed. BowStrap-predicted

protein expression level was substantially different from those predicted by standard alignment method using unique reads only (r = 0.69). There were 1776 observations where BowStrap detected significant protein expressions missed using unique reads alignments, including 358 proteins uniquely detected by BowStrap, and 536 proteins (22% of proteome) detected to be differentially expressed in the membrane fraction as a function of mycorrhizal interaction.

Metabolomics: There were a total of 165 metabolites detected in aspen root, 88 of which (53%) were identified as 'unknown'. Eleven metabolites (7%) were identified as significantly different by ANOVA as a function of mycorrhizal interactions.

Linking Omics Data: The combination of omics data has the potential to generate a system-scale representation of biological interactions [3,4]. Integrated datasets can reveal novel observations that no single omics dataset could uncover. Predicted links between metabolic models and unknown compounds from metabolic data, informed by correlations between metabolomics and proteomic data, potentially identifies 'unknown' metabolites. Linking proteomics data with transcriptomics data identifies potential post-translational regulatory mechanisms that are undetectable by either transcriptomic or proteomic data alone.

While any single omics dataset is valuable, combining multi-omics data into models provides new insights no single omics data can offer. By linking transcriptomics, proteomics, and metabolomics in a single model, it is possible to predict specific interaction mechanism-related biological phenomenon, (e.g., biosynthesis of signaling molecules, post-transcriptional modifications of mRNA, post-translational modification of proteins, and linking metabolic models to observed metabolomics) that illuminate the molecular mechanisms of rhizosphere community interaction and seedling phenotype. Crucially, this data integration helps prioritize protein targets for biological experimental validation.

References

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Funding statement. This contribution originates from the "Environment Sensing and Response" Scientific Focus Area (SFA) program at Argonne National Laboratory.