Integrated Pan-omics Measurements for Systems Level Characterization of Biological Systems


Beyond Genome Potential - Applications of Pan-Omics Approaches for Microbial Communities

**Project Goals**

Achieving a predictive systems level understanding of plants, microbes and microbial communities requires an integrative experimental approach to ultimately enable solutions to energy, environment, and climate challenges. We are applying advanced mass spectrometry (MS)-based capabilities for comprehensive molecular characterizations (proteomics – including post-translational modifications – metabolomics, lipidomics, and glycomics) of biological systems. Evaluation of a common solvent-based extraction protocol indicates that it can be used to isolate proteins, metabolites, and lipids from the same sample for subsequent omics analyses. Additional advancements include targeted proteomics methods (activity-based protein profiling and selected reaction monitoring) and elucidation of protein proteoforms through integrated top-down, bottom-up, and post-translational modifications measurements. This pan-omics approach allows new biological insights by providing a holistic view of complex phenotypic relationships between environmentally important microorganisms and higher organisms, as well as metabolic activities within microbial communities.

An integrated pan-omics strategy

- **Sample extraction and processing**
  - Extraction procedures for: Communities (soils, microbial mat, fungal gardens, water column, etc.), Plants (root hairs, woody tissue), Extracted components: Proteins (sub-cellular fractions, activity-based enrichments, post-translational modifications and intact protein analysis), Metabolites, Lipids, Glycosids
- **Quantitative approaches**
  - Integrated peak intensities, labeling strategies, and spectrum counts

Technology developments to enable current and future pan-omics measurements

- Higher throughput and more sensitive measurements from smaller samples
- Improved electrospray-mass spectrometry interfaces
- Instrument modifications to enable better accumulation of ions
- Improved instrument data acquisition speeds
- Informatics approaches that improve quantification and omics coverage (e.g. at a given FDR)

Pan-omic Sample Preparation to Increase Biological Insights and Reduce Variability

Illustration of pan-omic sample processing procedure to allow for extraction of proteins, metabolites, and lipids from a single sample. This procedure provides coverage comparable to separate procedures for each measurement type, and reduces both sample matrix and sampling-related variables.

**Spatially structured cellulose degradation in symbiotic microbial-fungal gardens**

Collaborators: Frank Aylward & Cameron Currie, University of Wisconsin-Madison

Spatial classification of the L. gongylodorus protist and fungal garden based on changes in protein abundances. We speculate that based on the pH resistance and nature of cellulose/cellulose degradation that Prevotella has a short-term advantage during the pulse. The balance quickly recovers following the return to stable conditions. Utilizing metabolomics and 16s rRNA gene complementary insights, providing a basis for hypothesis generation and testing.

**Functional resilience of a rumen community during repeated pH perturbations**

Collaborators: Amy Boaro & Birgitta Ahhring, Washington State University

pH perturbation had a negative impact on Fibrobacter and a positive impact on Prevotella based on changes in protein abundances. We speculate that based on the pH resistance and nature of cellulose/cellulose degradation that Prevotella has a short-term advantage during the pulse. The balance quickly recovers following the return to stable conditions. Utilizing metabolomics and 16s rRNA gene complementary insights, providing a basis for hypothesis generation and testing.

**Spatially structured fungal garden**

Collaborators: Frank Aylward & Cameron Currie, University of Wisconsin-Madison

Meta-proteomics and meta-metabolomics results suggest that L. gongylodorus transitions from using root exudates in the top stratrum to producing it in the bottom

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