Development of Integrated "Omics" Approach for Assessing Microbial Cycling of Carbon in Prairie Soil Using a Model Soil Bacterium: *Arthrobacter chlorophenolicus*

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Project goals : Soils of the U.S. Great Prairie store more than 30% of the terrestrial organic carbon in the continental U.S. This area is expected to experience dramatic changes in precipitation patterns, with either longer drought periods and more extreme precipitation events, as a consequence of climate change. However, the impact of a changing climate on the stored carbon pools in these soils is currently not known. This project aims to evaluate the impact of altered rainfall patterns on the carbon cycling dynamics of the soil microbiota in Kansas native prairie soil using a combination of omics approaches.

To develop protocols for RNA and protein extraction from soil, we spiked the soil with a soil bacterium that has been genome sequenced, *gfp* (green fluorescent protein)-tagged *Arthrobacter chlorophenolicus*. The *gfp* gene was used as an internal standard for accurate quantification. The model strain was inoculated into sterile and non-sterile Kansas prairie soil, amended or not with general (acetate) and specific (chlorophenol) carbon substrates.

Total RNA was extracted from the samples and is currently being sequenced using the Illumina platform to obtain metatranscriptomes. Target genes of interest were quantified by quantitative PCR and RT-QPCR. Total proteins were also extracted from the same samples to obtain metaproteomes. Metaproteomics proved particularly challenging due to the high humic acid content in the Kansas prairie soil. Therefore, we tested and optimized methods for extraction of proteins from the soil for subsequent measurement by shotgun metaproteomics via 2d-LC-MS/MS on an LTQ Velos mass spectrometer.

The first RNA-based results confirmed that the *gfp* transcript could be detected under most conditions and was thus a good estimator of *A. chlorophenolicus* abundance and activity. The initial metaproteome data indicated that several of the enzymes involved in acetate and chlorophenol degradation pathways were expressed in soil. In addition, several proteins involved in response to stress (thioredoxin, chaperonin, cold-shock proteins, etc.) were expressed. We also detected high levels of a flagellin protein in the soil amended with chlorophenol. Comparison of *A. chlorophenolicus* protein yields from sterile and non-sterile soil showed the impact of high background soil diversity on complicating the proteomic results.

These methods are now being applied to the samples acquired from the rainfall manipulation plots at the Konza Prairie Long-Term Ecological Research station. At JGI we have sequenced over 150 Gb of DNA from Kansas soil. To analyze the data using omics we have developed a new comprehensive functional database. We also focused on improving the sequence annotation by screening sequences belonging to particular KO (KEGG Orthology) families. We used this information to build a HMM (Hidden Markov Model). The database was structured into "maudules", which were chosen to represent specific functions with ecological context (such as denitrification, methanogenesis, etc.). This validation approach

will enable us to directly explore omics data for key functions of interest, and to integrate the different omics analyses to improve our understanding of carbon cycling processes in the prairie.

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