42. The Study of Microbial Communities using 'Metaomic' Approaches at Naturally Reducing Subsurface Sites

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Project Goals: Diverse microbial communities exist in subsurface environments that possess significant metabolic potential to effect global carbon, nitrogen and metal cycles including the transformation of radionuclides. The objectives of this ongoing project are: 1) to apply systems-level biology through the application of 'metaomics' approaches (collective analyses of whole microbial community DNA, RNA and protein) to the study of microbial environmental processes and their relationship to carbon, nitrogen and metals including the influence of microbial communities on uranium contaminant mobility in subsurface settings undergoing natural attenuation, 2) improve methodologies for data generation using metaomics technologies and the analysis and interpretation of that data and 3) use the data generated from these studies towards microbial community-scale metabolic modeling (see also "Genome-scale Reconstruction of Metabolic Networks from Microbial Communities at Sites Undergoing Natural Attenuation of Uranium").

To meet the goals of this project, two subsurface sites from the Department of Energy (DOE) Rifle Integrated Field Research Challenge Site (RIFRC) are being interrogated using a suite of metaomic approaches. The first site consists of sediments from the Winchester 2007 gallery, 'JB" well locations and was chosen due to the occurrence of natural attenuation of uranium (uranium reduction in the absence of biostimulation or other remedial interventions) which has critical implications towards the design and implementation of remedial strategies for uranium removal from groundwater. Although biostimulation experiments via the addition of acetate to groundwater have not taken place here, uranium reduction has been verified by absorption spectroscopy. Monitored natural attenuation from a practical standpoint is an important and likely necessary, complement to any bioremediation strategy that might be employed in such settings due to the size and scope of the remediation required. The second and more recent sites of study within this project have been collected from Colorado River floodplain sediments representing recent sediment depositions. Overbank deposits in the floodplain have become enriched in C, Fe and S minerals. Aggradation processes have led to the subsequent burial of these enriched sediments creating "hotspots" of biogeochemical activity which serve as analogs to the buried naturally reduced sediments at the JB sites.

From the 'JB' sediments (including the JB01-05' at 4m depth) metagenomic (DNA) and metatranscriptomic (RNA) sequence has been generated using the Illumina HiSeq and MiSeq platforms While uranium reduction has been confirmed at these sites, complete immobilization of uranium has not been noted and results from this analysis provides insights into this finding. Taxonomic profiles generated from both assemblies and high quality alignments (>60bp quality trimmed read length at >80% composite identity) to the NCBI NT database revealed that for both the metagenomic and metatranscriptomic data sets, the most abundant species based on best matches (~28% DNA, ~14% RNA) are to relatives of the facultative anaerobic chemolithotroph, *Thiobacillus denitrificans* which is capable of coupling the oxidation of inorganic sulfur compounds to the reduction of oxidized nitrogen compounds. While there is interest in the use of *T. denitrificans* for use in the removal of nitrate and sulfide in environmental settings, this organism has also been shown

to be capable of the oxidization of U(IV) to soluble U(VI) in the presence of nitrate potentially accounting for the lack of complete uranium immobilization at this site. Evidence for the presence of metal reducing bacterial relatives (although not necessarily demonstrated to reduce uranium) such as *Rhodoferax ferrireducens*, were determined. Intriguingly, evidence for other biological mechanisms that could serve as potential contributors to uranium immobilization were also determined including homologs to the genes responsible for the synthesis of delftibactin, a secondary metabolite responsible for the biomineralization of gold as part of a protective mechanism against gold toxicity.

The relatively high abundances of *T. denitrificans* and *R. ferrireducens* in the JB data has served as motivation to develop a single combined genome-scale model based on a constraint-based metabolic reconstruction of the two organisms to gain an understanding of organism interaction. While a manually curated metabolic model for *R. ferrireducens* was already available, only an automatically generated model (through Model SEED) was available for *T. denitrificans* which has required additional manual curation to facilitate its use in this study. In order to match the biomass objective functions of the two organisms, the *T. denitrificans* model was augmented to share the same reaction stoichiometry as *R. ferrireducens* for DNA, RNA, Protein, phospholipids and lipopolysaccharides synthesis and missing metabolic capabilities such as sulfur oxidation reactions associated with known SOX genes have been added. Once both of the individual models were capable of producing flux through their respective biomass reactions, the stoichiometry matrices underlying each organism's model was combined. To generate a combined biomass reaction function, the objective function was chosed to be a weighted combination of *R. ferrireducens* and *T. denitrificans* equations producing 21 combined models based on the spectrum of biomass stoichiometry reweightings. Preliminary flux balance analysis and flux variability analysis are underway as well as additional work to include modifying the bounds of intracellular reaction fluxes based on metatranscriptome data.

The recently acquired floodplain samples represent an important opportunity to contrast microbial community diversity and function especially the coupling of carbon, nutrient and metal cycles with the results obtained from the JB sites. To that end, biological and technical replicates of metaomic data are currently being produced. To improve the contiguity of metagenomic assemblies (which subsequently form a framework for metatranscriptomic and metaproteomic analysis and computational modeling) in addition to the use of the Illumina sequencing platform, the use of PacBio sequencing is being investigated. PacBio sequencing has the ability to produce significantly longer read lengths (3kb or longer) when compared to Illumina platforms (<300bp) although with reduced accuracy (~90%). Therefore, new approaches for hybrid assembly including steps which incorporate mapping Illumina assemblies to PacBio reads in an iterative fashion are being tested. These approaches are broadly applicable and can be applied to other environments dominated by microbially mediated elemental cycling processes.

This project is supported by the Office of Biological and Environmental Research in the DOE Office of Science.