Genome-scale Metabolic Modeling of Microbial Communities at Sites Undergoing Natural and Stimulated Attenuation of Uranium

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Project Goals: The interactions between microorganisms in subsurface microbial communities are highly connected and complex, playing important roles in the cycling of carbon, nitrogen, and metals. As part of a larger research project using ‘metaomics’ approaches to study these processes (see also “The use of ‘Metaomic’ and Modeling Approaches at Subsurface Sites to Study Carbon, Nutrient and Metal Cycling in Microbial Communities”) the objectives of this work are to: 1) use data from ‘metaomic’ approaches to reconstruct metabolic networks of microbial communities undergoing natural attenuation of uranium, 2) provide insights into the potential mechanisms that lead to numerical dominance of specific bacterial taxa under different environmental conditions, 3) study the interaction and collaboration among members of the microbial community and 4) integrate gene expression data characterizing metabolic activity into genome-scale models to describe microbial activity in sub-optimal states.

Reductive immobilization of soluble U(VI) to the insoluble U(IV) is an efficient remediation strategy of subsurface groundwater contaminated with uranium. While U(VI) can be reduced to U(IV) by stimulation of indigenous bacteria with amendments of organic carbon, there are zones of natural attenuation of U(VI) at the Rifle Integrated Field Research Challenge (RIFRC) site, where U(VI) can be both reduced and remobilized in the absence of biostimulation. Elucidation of the potential mechanisms of microbial community structure and metabolism under these conditions of natural attenuation can facilitate more efficient remediation design and management strategies.

In order to meet the goals of this ongoing project, our earlier efforts investigated the microbial community structure at a site previously identified as undergoing natural attenuation of uranium using metagenomic data. Due to a similarity in genome content between subsurface microbial community members assigned to the same taxonomic class at our study site, we pursued a pan-genome-scale approach to subsequently analyze metabolic potential at the class-level. A statistical analysis of the functional profiles from the JB site indicated that within the numerically dominant taxonomic classes (α-, β-, γ- and δ-proteobacteria, and Actinobacteria) there is an abundance of enzymes related to CO2 fixation (e.g. Rubisco in the α- β-, and γ- proteobacteria, and PEPCase in the Actinobacteria). In contrast, in acetate amended sites within the numerically dominant δ- proteobacteria, there is a high abundance of enzymes related to N2 fixation (e.g. Nitrogenase). Collectively, these results reveal different community structures and metabolic functions mediating C and N cycling under contrasting environmental conditions.

Using metagenomic and reference genome datasets, pan-genome-scale metabolic networks were reconstructed for α-, β-, γ- and δ-proteobacteria, and Actinobacteria, respectively. The models were optimized and gaps filled to ensure that they are capable of growth in geochemical conditions similar to that of the RIFRC site. These class-level models were then integrated into a Dynamic Multi-species Metabolic Modeling (DMMM) framework for investigating the interaction and collaboration among community members. The model analysis indicates that β-proteobacteria like Thiobacillus denitrificans may dominate the community at the JB site due to its ability to use inorganic electron donors for energy and fix CO2 as its major carbon source. Through electron transport with cytochrome bc1 complexes and NADH-Q oxidoreductase, a tight coupling between Fe(II) oxidation and NO3 reduction can be established to support use of CO2 as the main source of carbon. Similarly, reduced inorganic sulfur...
compounds may be oxidized to sulfate by ferricytochrome c with reduction of NO3 as a terminal electron acceptor and fixation of CO2 as the major carbon source.

Interaction and collaboration of microbial community members were quantitatively estimated through the DMMM approach. While competitive interactions mainly occur in the community for electron donors and acceptors, and carbon sources, the simulations indicate that there are potential syntrophic interactions between β-proteobacteria (e.g. T. denitrificans) and Actinobacteria (e.g. Streptomyces). Actinobacteria may, for example, use the products of sulfur oxidation (e.g., sulfate) from β-proteobacteria as the final electron acceptor for CO2 fixation under anoxic conditions. Although these class-level models were able to capture potential syntrophic interactions between classes, further complexity exists at the species level, leading to important metabolic differences between class members. To facilitate species-level modeling efforts, we have currently focused on developing and validating models for both Anaeromyxobacter dehalogenans and T. denitrificans. Both models were developed by combining the Model SEED web resource with a novel algorithm for resolving bioenergetic inconsistencies. Furthermore, both models were curated to ensure consistency with available experimental data for growth on a variety of carbon sources and electron acceptors. We were then able to investigate the microbial community dynamics with species-level resolution using genome scale models of Geobacter sulfurreducens, G. metallireducens, A. dehalogenans, Rhodoferrax ferrireducens, and T. denitrificans, integrated using the DMMM approach.

Subsurface microorganisms may grow within either optimal or sub-optimal states depending on ever changing environmental conditions. Hence, application of a flux balance analysis (FBA) that seeks to maximize or minimize an objective function may not be always appropriate for describing and predicting microbial activities in the subsurface. Therefore, we are currently developing methods to integrate metatranscriptomic (gene expression) data into the genome-scale models, thereby elucidating functional mechanisms of the community indicative of, and relevant to, sub-optimal states. These combined models will be incorporated into the DMMM framework to improve the predictive capability of the genome-scale models.

This project is supported by the Office of Biological and Environmental Research in the DOE Office of Science DESC0006837.