Uranium Biomineralization by Natural Microbial Phosphatase Activities in the Subsurface

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The project goal is to examine the role of phosphohydrolases in naturally occurring subsurface bacteria for the purpose of promoting the immobilization of uranium through the formation of insoluble uranium phosphate minerals. Our prior work focused on pure culture and soil column studies that utilized contaminated soils from the DOE Oak Ridge Field Research Center (ORFRC), demonstrating that microbial phosphatase activity liberated sufficient concentrations of inorganic phosphate (PO_4^{3-}) to promote uranium-phosphate mineral formation under oxic and anoxic conditions at different pH values (pH 5.5 and 7). Current objectives are to: (1) examine the diversity of the microbial communities present in Area 2 soil slurry incubations and examine treatments [glycerol-2-phosphate (G2P) or glycerol-3-phosphate (G3P) amendments at pH 5.5 or pH 6.8] conditions that promote intracellular polyphosphate formation, (2) examine the biochemistry of multiple acid phosphatases identified in the completed genomes of the ORFRC *Rahnella* strain Y9602 and reference strain *Rahnella aquatilis* ATCC 33071, and (3) examine the application of positron emission tomography (PET) to track subsurface bacteria in soil column studies. In collaboration with DOE LBNL investigators, we have examined microbial community dynamics of ORFRC Area 2 subsurface microbial populations responding to G2P and G3P amendments with the PhyloChip microarray. Within 36 days, treatments with G3P and G2P yielded 9.1 mM and 4.7 mM PO_4^{3-}, respectively. Treatments at pH 6.8 enriched members of the phyla *Crenarchaeota*, *Euryarchaeota*, *Bacteroidetes*, *Flavobacteria*, *Sphingobacteria*,and *Proteobacteria* while treatments at pH 5.5 enriched in the phyla *Crenarchaeota*, *Euryarchaeota*, and *Proteobacteria*. Electron microscopy of soil slurry treatments indicates that G2P (pH 5.5) enhanced intracellular polyphosphate formation. Our two *Rahnella* genome sequencing projects (*Rahnella* sp. Y9602 and *Rahnella aquatilis* ATCC 33071) led by JGI have been completed. Comparative genomic studies between the two strains are underway to examine possible influences the contaminated ORFRC had on the Y9602 strain. Five candidate low-molecular weight acid phosphatases have been cloned and biochemical analysis of substrate range(s) are underway and will be used examine alternative phosphate substrates as well as allow for real-time gene expression analysis of cultured and mixed community phosphate-solubilizing studies. Our current collaboration with DOE BNL researchers has examined the use PET imaging as a method to visualize subsurface microbial processes. Our recently published studies demonstrated that the phosphate solublizing *Rahnella* sp. Y9602 labeled with 2-deoxy-2-(^{18}F)fluoro-D-glucose could be visualized in real-time after being introduced to soil columns. These initial studies demonstrate metabolically active subsurface microorganisms could be visualized under varying soil column conditions relevant to the ORFRC. The combined multiphasic approach of pure culture and soil slurry studies coupled to genome-enabled studies will provide a greater understanding of microbial community dynamics involved in phosphate-mediated U(VI) sequestration and biomineralization.