Project #1024775

Title: Promoting uranium immobilization by the activities of microbial phophatases

Principal Investigator: Sobecky, Patricia A.

Organization: Georgia Tech Research Corporation

Results To Date: Patricia Sobecky and Martial Taillefert - Progress Report

The following is a summary of progress in our project “Promoting uranium immobilization by the activities of microbial phosphatases” during the second year of the project.

(1). Assignment of microbial phosphatases to molecular classes. One objective of this project is to determine the relationship of phosphatase activity to metal resistance in subsurface strains and possible contributions of horizontal gene transfer (HGT) to the dissemination of nonspecific acid phosphatase genes. Non-specific acid phosphohydrolases are a broad group of secreted microbial phosphatases that function in acidic-to-neutral pH ranges and utilize a wide range of organophosphate substrates. To address this objective we have designed a collection of PCR primer sets based on known microbial acid phosphatase sequences. Genomic DNA is extracted from subsurface FRC isolates and amplicons of the expected sizes are sequenced and searched for conserved signature motifs. During this reporting period we have successfully designed and tested a suite of PCR primers for gram-positive and gram-negative groups of the following phosphatase classes: (i) Class A; (ii) Class B; and (iii) Class C (gram negative). We have obtained specific PCR products for each of the classes using the primers we have designed using control strains as well as with subsurface isolates.

We have recently shown (Martinez et al. 2006) the broad and remarkable extent of horizontally transferred PIB-type ATPase genes encoding efflux proteins that provide a mechanism for heavy metal resistance among metal resistant FRC bacteria from contaminated subsurface soils. Based on these findings, we have hypothesized that the occurrence of genes encoding bacterial acid phosphatases may also exhibit evidence of HGT-mediated dissemination among microbial communities in the contaminated subsurface. Ongoing studies and analyses of phosphatase sequences amplified from subsurface isolate will determine if HGT has contributed to the dissemination of phosphatases that are providing a beneficial phenotype to subsurface microorganisms. We are also in the process of designing additional PCR primers that will yield larger amplicons for additional HGT analyses as well as cloning phosphatase genes from subsurface strains that we are currently characterizing for U biomineralization activities.

(2). Characterization of phosphatases using whole cells and cell extracts. During this report period we have also been examining the phosphatase activities at different pHs as pH is likely to significantly influence enzyme activity. In addition, pH is an important parameter of the contaminated subsurface environment at the FRC and can limit and/or prevent microbial activity. The pH-activity profiles of whole cells show activity at pH 4-7. The enzyme activity occurs at more acidic pH, with the

maximal activity occurring at pH 5.5. Ongoing experiments will determine metal dependency and temperature effects on enzyme activity.

In addition, we have initiated studies to determine the cellular location and substrate range of acid phosphatase(s) (data not shown) in several of the phosphate-liberating FRC subsurface isolates (i.e., Rahnella and Bacillus) and Arthrobacter strains. Arthrobacter strains are included as we are testing whether or not these isolates may be accumulating (rather than liberating) phosphate via storage as polyphosphate. This premise is particularly intriguing and if verified represents another potential mechanism for U immobilization by subsurface microorganisms. In the third year of the project studies will broaden the range of organophosphorus compounds to be tested and peptide sequencing of acid phosphatases will be conducted following their isolation from denaturing SDS-PAGE.

(3). Accumulation of inorganic phosphate and removal of U in acidic groundwater: A series of experiments have been conducted during this report period with a number of "phosphate-liberating" strains including the gram-negative Rahnella sp. strain Y9-602, isolated from FRC Area 3, Bacillus sp. strain Y9-2 and Arthrobacter sp. strain X34. The strains are incubated in synthetic (aerobic) groundwater, designed to replicate FRC conditions, and amended with 10 mM glycerol-3-phosphate (G3P) and 200 uM U(VI) after 36 hr of incubation. The objectives of these experiments are to determine (i) the rate of phosphate production from G3P via microbial phosphatase activity, (ii) if sufficient phosphate is produced to precipitate U phosphate, and (iii) the effects of pH on the rate of phosphate production and U precipitation. The rate of phosphate production by strain Y9-602 in the absence of U is linear and dependent on pH. At pH 5 the rate of phosphate production is 19.1 uM/hr and increases to 33.8 uM/hr at pH 7. When cells are incubated in the presence of U, phosphate production and viable cell counts (not shown) reach a steady state immediately after U addition, but rebound later. The rebound in both phosphate production and viable cell counts is dependent on how fast U precipitates which, in turn, is dependent on pH. At low pH 4.5 only ~ 8% of uranium is precipitated as U phosphate and there is no observable rebound in phosphate production or viable cell counts. It is not until > 85% of U is removed from solution that phosphate production and viable cell counts begin to rebound as demonstrated at 72 hours at pH 5.5 and within 48 hours at pH 7. At pH 7, both U phosphate and U hydroxide must be precipitated as the complete removal of uranium is observed in both control and live samples. At 96 h, > 90% of U precipitated from all solutions greater than pH 4.5. In the present experiments, pH and phosphatase activity are major determining factors for U precipitation.

A direct comparison of phosphate liberation activity and U removal between the three subsurface strains indicated that the phosphate liberated by the Rahnella sp. and Bacillus sp. remove >85% of U from solution. The Arthrobacter strain did not appear to liberate a sufficient amount of phosphate to promote the precipitation of U from solution. Experiments are underway to determine if the Arthrobacter strains are sequestering phosphate rather than releasing it under the experimental conditions being tested.

We have contacted a beamline scientist, Dr. Samuel Webb, at the Stanford Synchrotron Research Laboratory (SSRL) to determine the chemical composition of the uranium precipitate formed during the incubations. We will conduct a few preliminary XAS and XRD analyses shortly and write a beamtime proposal this Summer. These analyses should confirm the oxidation state of uranium and determine whether the precipitate formed during the incubations is a uranium

phosphate or hydroxide compound.

Lastly, this summer we will also begin to determine the speciation of uranium in the dissolved phase using capillary zone electrophoresis (CZE) to study the effect of the speciation on the biomineralization of U(VI). To our knowledge, the techniques developed have only been applied to measurements of UO22+ using different chromophores [1-3]. A method will be developed to separate and detect small uranyl-organic complexes that are likely to affect the mechanisms of uranium biomineralization. The method will be based on the separation of the organic compounds, such as G3P or organic acids, that are complexed by uranium using conventional electrolytes. The organic compound will be detected by absorbance in the UV range, and each peak identified will be collected by a fraction collector for uranium analysis by ICP-MS. We hope to be able to use this tool to identify the exact mechanism of uranium biomineralization in the presence of the organophosphorus compound. Literature Cited: 1). Evans, L. and G.E. Collins. 2001. Separation of uranium(VI) and transition metal ions with 4-(2-thiazolylazo)resorcinol by capillary electrophoresis. Journal of Chromatography 1:127-133. 2). Liu, B., L. Liu and J. Cheng. 1998. Separation of thorium, uranium and rare-earth elements with 2-[(2-arsenophenyl)-azo]-1,8-dihydroxy-7-[(2,4,6-tribromophenyl)azo]-naphthalene-3,6-disulfonic acid by capillary electrophoresis. Analytical Chimica Acta 358:157-162. 3). Oztekin, N. and F.B. Erim. 2000. Separation and direct UV detection of lanthanides complexed with cupferron by capillary electrophoresis. Journal of Chromatography (1-2):263-268.

Deliverables: Publications and presentations:

Martinez, R.J., Wang, Y., Raimondo, M.A., Coombs, J.M., Barkay, T., and Sobecky, P.A. 2006. Horizontal Gene Transfer of PIB-type ATPases among Bacteria Isolated from Radionuclide and Metal Contaminated Subsurface Sediments. Applied and Environmental Microbiology 72: 3111-3118.

Martinez, R.J., Beasley, M, Wilson, J.J., Taillefert, M., and Sobecky, P.A. Promoting Uranium Immobilization by the Activities of Microbial Phosphatases. American Society for Microbiology General Meeting Abstract (2006). Poster presentation.

Sobecky, P.A., Martinez, R.J., Beasley, M, and Taillefert, M. Promoting Uranium Immobilization by the Activities of Microbial Phosphatases. NABIR Annual PI Meeting Abstract (2006). Oral presentation.

Beasley, M.J., Martinez, R.J., Sobecky, P.A. and Taillefert, M. Uranium biomineralization as a result of bacterial phosphatase activity. American Chemical Society General Meeting Abstract (2006). Oral presentation.

In preparation: Beazley, M. J., Martinez, R. J., Sobecky, P. A., and Taillefert, M. Uranium biomineralization as a result of bacterial phosphatase activity: Insights from a bacterial isolate from a contaminated subsurface.