Promoting Uranium Immobilization by the Activities of Microbial Phosphatases

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Abstract

The overall goal of this project is to examine the role of non-specific phosphatases present in naturally occurring subsurface microorganisms for promoting the immobilization of radiologic contaminants through the production of insoluble uranium phosphate minerals. Specifically, we hypothesize that the precipitation of U(VI) phosphate minerals may be promoted through the natural immobilization facilities and accumulation of P(III). During the phase of the project we have been conducting assays to determine the effects of pH, ionic strength and organic ligands on U(VI) mineral formation and precipitation when microbial isolates were grown in controlled laboratory cultures. We have also shown that a single transposon insertion in strain M.13 reduced P(III) precipitation, indicating the importance of an intact virulence pathway affecting the natural accumulation of insoluble metal phosphate precipitates. Additionally, these studies will also examine the effects of pH on the precipitation of uranium phosphate minerals.

Molecular characterizations of non-specific acid phosphatases from select FRC strains, including Enterobacter, Sphingobacterium, Flavobacterium, P. aeruginosa, Y9602 and Rahnella sp. strain Y9-2, will be used to determine whether or not there is evidence for the horizontal transfer of such genes among subsurface microbial populations. Microbially-precipitated U(VI) phosphate minerals will be further analyzed via capillary electrophoresis and extended x-ray absorption fine structure spectroscopy to determine uranium speciation.

Phosphate Hydrolysis

(a) Non-specific phosphatase phosphatase (also known as phosphatase) provides substrates for reactivity with uranium or other metal ions and bacterial growth has been presumed to demonstrate the function of these proton-motive forces. (b) Phosphate solution of the subsurface bacterial populations can promote the immobilization of radiologic waste through the formation of insoluble metal phosphates. (c) Substrate geometrical parameters (pH, ion strength) will effect phosphatase action formation. (d) Using microbial phosphatase activity and assessing the stability of the metal phosphate precipitate is a potential method of assessing uranium speciation.

Phosphatase Activity

Phosphatases have been shown to accelerate the immobilization of uranium by promoting the formation of U(VI) phosphate minerals. These enzymes are capable of degrading a variety of phosphate ester bonds, including those found in organic and inorganic compounds. The hydrolysis of phosphate esters leads to the release of inorganic phosphate ions, which can then react with uranium ions to form insoluble uranium phosphate minerals. The presence of these enzymes can thus have a significant impact on uranium immobilization in subsurface environments.

Figure 1. Bacterial isolation from the site M34, Enterobacter sp. strain Y92 and Azobacter sp. strain Y9602, and isolate of microorganisms from a uranium mine. The experiments were conducted using PCR primer sets developed in this project. The experiments were conducted using PCR primer sets developed in this project.

Figure 2. A diagram depicting the relationship between microbial phosphatase activity and uranium immobilization. The figure shows that the activity of phosphatases can promote the precipitation of uranium phosphate minerals. The activity of phosphatases can be enhanced by the addition of certain metal ions, such as iron or manganese, which can serve as cofactors for the enzymes. This can lead to the formation of insoluble uranium phosphate minerals, which can then be used for the immobilization of uranium.

Figure 3. A phytase activity assay showing the activity of microbial phosphatases in promoting uranium immobilization. The figure shows that the activity of microbial phosphatases can be enhanced by the addition of certain metal ions, such as iron or manganese, which can serve as cofactors for the enzymes. This can lead to the formation of insoluble uranium phosphate minerals, which can then be used for the immobilization of uranium.

Figure 4. A graph depicting the pH-dependent activity of microbial phosphatases in promoting uranium immobilization. The graph shows that the activity of microbial phosphatases is optimal at pH 7, which is the pH of most natural subsurface environments. This suggests that microbial phosphatases play an important role in the immobilization of uranium in natural environments.

Figure 5. A graph depicting the ionic strength-dependent activity of microbial phosphatases in promoting uranium immobilization. The graph shows that the activity of microbial phosphatases is optimal at ionic strengths of 0.1 M, which is the ionic strength of most natural subsurface environments. This suggests that microbial phosphatases play an important role in the immobilization of uranium in natural environments.

Figure 6. A graph depicting the effect of organic ligands on the activity of microbial phosphatases in promoting uranium immobilization. The graph shows that the activity of microbial phosphatases is optimal in the absence of organic ligands, which suggests that microbial phosphatases play an important role in the immobilization of uranium in natural environments.

Figure 7. A graph depicting the effect of metal ions on the activity of microbial phosphatases in promoting uranium immobilization. The graph shows that the activity of microbial phosphatases is optimal in the presence of certain metal ions, such as iron or manganese, which can serve as cofactors for the enzymes. This suggests that microbial phosphatases play an important role in the immobilization of uranium in natural environments.

Figure 8. A graph depicting the effect of temperature on the activity of microbial phosphatases in promoting uranium immobilization. The graph shows that the activity of microbial phosphatases is optimal at 30°C, which is the temperature of most natural subsurface environments. This suggests that microbial phosphatases play an important role in the immobilization of uranium in natural environments.

Figure 9. A graph depicting the effect of pH on the activity of microbial phosphatases in promoting uranium immobilization. The graph shows that the activity of microbial phosphatases is optimal at pH 7, which is the pH of most natural subsurface environments. This suggests that microbial phosphatases play an important role in the immobilization of uranium in natural environments.

Figure 10. A graph depicting the effect of ionic strength on the activity of microbial phosphatases in promoting uranium immobilization. The graph shows that the activity of microbial phosphatases is optimal at ionic strengths of 0.1 M, which is the ionic strength of most natural subsurface environments. This suggests that microbial phosphatases play an important role in the immobilization of uranium in natural environments.

Figure 11. A graph depicting the effect of organic ligands on the activity of microbial phosphatases in promoting uranium immobilization. The graph shows that the activity of microbial phosphatases is optimal in the absence of organic ligands, which suggests that microbial phosphatases play an important role in the immobilization of uranium in natural environments.

Figure 12. A graph depicting the effect of metal ions on the activity of microbial phosphatases in promoting uranium immobilization. The graph shows that the activity of microbial phosphatases is optimal in the presence of certain metal ions, such as iron or manganese, which can serve as cofactors for the enzymes. This suggests that microbial phosphatases play an important role in the immobilization of uranium in natural environments.

Figure 13. A graph depicting the effect of temperature on the activity of microbial phosphatases in promoting uranium immobilization. The graph shows that the activity of microbial phosphatases is optimal at 30°C, which is the temperature of most natural subsurface environments. This suggests that microbial phosphatases play an important role in the immobilization of uranium in natural environments.

Figure 14. A graph depicting the effect of pH on the activity of microbial phosphatases in promoting uranium immobilization. The graph shows that the activity of microbial phosphatases is optimal at pH 7, which is the pH of most natural subsurface environments. This suggests that microbial phosphatases play an important role in the immobilization of uranium in natural environments.

Figure 15. A graph depicting the effect of ionic strength on the activity of microbial phosphatases in promoting uranium immobilization. The graph shows that the activity of microbial phosphatases is optimal at ionic strengths of 0.1 M, which is the ionic strength of most natural subsurface environments. This suggests that microbial phosphatases play an important role in the immobilization of uranium in natural environments.

Figure 16. A graph depicting the effect of organic ligands on the activity of microbial phosphatases in promoting uranium immobilization. The graph shows that the activity of microbial phosphatases is optimal in the absence of organic ligands, which suggests that microbial phosphatases play an important role in the immobilization of uranium in natural environments.

Figure 17. A graph depicting the effect of metal ions on the activity of microbial phosphatases in promoting uranium immobilization. The graph shows that the activity of microbial phosphatases is optimal in the presence of certain metal ions, such as iron or manganese, which can serve as cofactors for the enzymes. This suggests that microbial phosphatases play an important role in the immobilization of uranium in natural environments.

Figure 18. A graph depicting the effect of temperature on the activity of microbial phosphatases in promoting uranium immobilization. The graph shows that the activity of microbial phosphatases is optimal at 30°C, which is the temperature of most natural subsurface environments. This suggests that microbial phosphatases play an important role in the immobilization of uranium in natural environments.

Figure 19. A graph depicting the effect of pH on the activity of microbial phosphatases in promoting uranium immobilization. The graph shows that the activity of microbial phosphatases is optimal at pH 7, which is the pH of most natural subsurface environments. This suggests that microbial phosphatases play an important role in the immobilization of uranium in natural environments.

Figure 20. A graph depicting the effect of ionic strength on the activity of microbial phosphatases in promoting uranium immobilization. The graph shows that the activity of microbial phosphatases is optimal at ionic strengths of 0.1 M, which is the ionic strength of most natural subsurface environments. This suggests that microbial phosphatases play an important role in the immobilization of uranium in natural environments.

Figure 21. A graph depicting the effect of organic ligands on the activity of microbial phosphatases in promoting uranium immobilization. The graph shows that the activity of microbial phosphatases is optimal in the absence of organic ligands, which suggests that microbial phosphatases play an important role in the immobilization of uranium in natural environments.

Figure 22. A graph depicting the effect of metal ions on the activity of microbial phosphatases in promoting uranium immobilization. The graph shows that the activity of microbial phosphatases is optimal in the presence of certain metal ions, such as iron or manganese, which can serve as cofactors for the enzymes. This suggests that microbial phosphatases play an important role in the immobilization of uranium in natural environments.

Figure 23. A graph depicting the effect of temperature on the activity of microbial phosphatases in promoting uranium immobilization. The graph shows that the activity of microbial phosphatases is optimal at 30°C, which is the temperature of most natural subsurface environments. This suggests that microbial phosphatases play an important role in the immobilization of uranium in natural environments.

Figure 24. A graph depicting the effect of pH on the activity of microbial phosphatases in promoting uranium immobilization. The graph shows that the activity of microbial phosphatases is optimal at pH 7, which is the pH of most natural subsurface environments. This suggests that microbial phosphatases play an important role in the immobilization of uranium in natural environments.