Promoting Uranium Immobilization by the Activities of Microbial Phosphatases

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The goal of this project is to examine the role of nonspecific phosphohydrolases in naturally occurring subsurface microorganisms for the purpose of promoting the immobilization of radionuclides through the production of phosphate precipitates. This project will focus on uranium (U), a radionuclide that poses significant risk to human health and the environment. Nonspecific acid phosphohydrolases are a broad group of secreted microbial phosphatases that function in acidic-to-neutral pH ranges and utilize a wide variety of organophosphoester substrates. We hypothesize that subsurface microorganisms that exhibit (acid) phosphatase activity and are resistant to heavy metals have the potential to immobilize U via a biomineralization process. Biomineralization, defined as the immobilization of an element by nonredox microbial precipitation, could prove to be a feasible alternative or complementing remediation approach to U bioreduction and adsorption processes. The primary objective is to demonstrate that the intrinsic phosphatase activities of indigenous subsurface microbes result in the release/accumulation of sufficient PO_4^{3-} to cause the formation and precipitation of low solubility U-phosphate minerals in oxygenated groundwater and soil. We will examine three critical hypotheses: (1) acid phosphatases of subsurface microbes provide resistance to heavy metals, and this phosphatase-mediated resistance trait has been disseminated in subsurface populations by lateral gene transfer; (2) phosphatase activity of these subsurface microbes will promote U immobilization by formation of insoluble U-phosphate precipitates; and (3) subsurface geochemical parameters (pH, nitrate) will affect phosphate mineral formation by altering microbial phosphatase activity and/or affecting the stability of the metal phosphate precipitates. A three-step experimental approach is being conducted to examine the relationship between the occurrence of microbial phosphatases and metal resistance among the DOE Field Research Center (FRC) strains we are currently studying, along with the contribution of lateral gene transfer in the dissemination of these beneficial phosphatase genes. Our preliminary testing of a subset of isolates cultured from contaminated FRC soils and background (reference) soils indicate a higher percentage of FRC isolates exhibiting phosphatase phenotypes (i.e., in particular those we surmise to be PO_4^{3-}-irrepressible) when compared to isolates from the background reference site. A high percentage of strains exhibiting such putatively PO_4^{3-}-irrepressible phosphatase phenotypes was also shown to be resistant to lead as well as cadmium. We have designed PCR primer sets for amplification of classes A, B, and C acid phosphatases from the FRC subsurface isolates.