Viral infection of subsurface microorganisms and metal/radionuclide transport

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Microbially mediated metabolisms have been identified as a significant factor either directly or indirectly impacting the fate and transport of heavy metal/radionuclide contaminants. To date the role that viruses play influencing microbial mortality and the resulting community structure under varying redox conditions in subsurface sedimentary environments remains poorly understood. The objective of this project is to investigate viral infection of subsurface bacteria and the formation of contaminant-bearing viral particles. This objective will be approached by examining the following working hypotheses: (*i*) subsurface microorganisms are susceptible to viral infections by the indigenous subsurface viral community, and (*ii*) viral surfaces will adsorb heavy metals and radionuclides. In an effort to initially assess the significance of viral infection in subsurface microbial communities, the production of viral like particles in response to biostimulation of the microbial community was investigated by inoculating low nutrient culture medium containing acetate and nitrate with uranium and nitrate containing shallow subsurface sediment (25% mass/vol). No significant production of viral like particles was observed when acetate or nitrate was omitted from culture medium. Acetate was oxidized coupled to the reduction of nitrate to nitrite resulting in the increase in bacterial abundance and an initial increase in Viral Like Particles (VLP's) (Virus to Bacteria ration ca. 480 to 2,400 over the course of the study). During nitrate reduction to nitrite, VLP abundance increased from 1.1×10^5 to 4.6×10^7 VLP mL^{-1} (ca. increase of 430x). The production of 9mM nitrite resulted in a lag in nitrite reduction and a subsequent decrease in bacterial abundance. This also corresponded to a decrease in VLP abundance (4.9×10^6 VLP mL^{-1}). Interestingly when nitrite reduction resumed, VLP abundance again increased to 2.6×10^7 VLP mL^{-1} while bacterial abundance continued to decrease. In an effort to identify the viruses infecting indigenous bacteria, bacterial isolates were obtained. One nitrate reducing isolate, Alda10, was selected for further study. Analysis of the 16S rRNA gene sequence revealed the closest related organism in pure culture was *Pseudomonas fredricksbergensis* with 99%similarity. Mitomycin C induction of Alda10 resulted in the production of VLP's (1.9×10^6 VLPmL^{-1}). Transmission electron microscopy of the VLPs revealed particles similar to filamentous phage. Together these results suggest a lysogenic infection of *Pseudomonas* sp. Alda10. The production of VLP's in groundwater has implications for metal and radionuclide transport. Initial experiments indicate that metals will adsorb to the surface of *Escherichia coli* phage T4. It is therefore necessary to establish the potential relationship(s) between viruses, subsurface microbial communities, and contaminant metals/radionuclides to provide sufficient scientific understanding such that DOE sites would be able to incorporate coupled physical, chemical and biological processes into decision making for environmental remediation and long-term stewardship by establishing viral-microbial relationships and the subsequent fate and transport of heavy metals and radionuclides.