The kinetics and mechanism of selenate reduction by *Enterobacter cloacea*

N. YEE[1], D.Y. KOBAYASHI[2] AND J. MA[1]

[1]Department of Earth and Environmental Sciences, Rutgers, The State University of New Jersey, USA (nyee@andromeda.rutgers.edu, jincaima@pegasus.rutgers.edu)
[2]Department of Plant Biology & Pathology, Rutgers, The State University of New Jersey, USA (kobayashi@aesop.rutgers.edu)

The concentration and distribution of selenium species in near surface geologic and aquatic environments is strongly affected by microbial processes. Under aerobic and microaerophilic conditions, a wide variety of phylogentically distinct bacteria species have been shown to reduce Se(VI) and Se(IV) to form sparingly soluble elemental selenium. In order to quantify the geochemical impact of these microorganisms, accurate models must be developed to predict when these organisms will be active, and how rapidly they will reduce selenate and selenite. In this study, we quantified the kinetics of the selenate reduction reaction by soil microorganism *Enterobacter cloacae* and we identified the genes that control the selenate reduction process.

The kinetics of selenate reduction by *E. cloacae* were examined using batch experiments as a function of pH, temperature, and cell density. Electron microscopy and X-ray diffraction were employed to determine the morphology and crystallinity of the reduction products. Finally, we used transposon mutagenesis to produce mutants that have loss the ability to reduce selenate, and we characterized some of these mutants.

A rate law based on the Michaelis-Menten equation was developed to describe the selenate reduction kinetics over a range of pH conditions, temperatures, and cell densities. The reduction reaction formed nanoparticulate elemental selenium granules that were both excreted into solution and attached to the cell surface. Mutants constructed from transposon mutagenesis were unable to reduce selenate to Se^0 but were still able to reduce selenite, demonstrating that the selenate and selenite reductases are distinct enzymes. The link between the genetics and geochemistry of microbial selenium oxyanion reduction will be discussed.

New insights into the molecular mechanism of microbial metal respiration

THOMAS J. DICHRISTINA

School of Biology, Georgia Tech, Atlanta, GA 30332, USA

Metal-reducing members of the genus *Shewanella* respire anaerobically on a wide variety of compounds as terminal electron acceptor including oxidized iron [Fe(III)], manganese [Mn(IV)], uranium [U(VI)] and technetium [Tc(VII)]. The molecular mechanism of bacterial metal respiration, however, is poorly understood. A genetic complementation strategy has been followed to identify the *Shewanella* genes and proteins required for anaerobic respiration on Fe(III), Mn(IV), U(VI) and Tc(VII). Four rapid screening techniques have been developed to identify respiratory mutants deficient in anaerobic respiration on each metal. Mutant subclasses (designated Fer, Mnr, Urr and Tcr, respectively) have been identified by the inability to respire anaerobically on a specific terminal electron acceptor, while retaining the ability to respire on all other electron acceptors tested. Wild-type DNA fragments containing metal reduction-specific genes have been isolated by genetic complementation with a wild-type gene clone bank, and the metal reduction-specific genes have been identified by nucleotide sequence analysis. The metal reduction-specific genes provide new insight into the molecular mechanism of bacterial metal respiration.