The Molecular Basis for Selenate Reduction in *Citrobacter freundii*

JOANNE THEISEN[1] AND NATHAN YEE[1,*]

[1]Department of Environmental Sciences, Rutgers University, New Brunswick, NJ, USA
(*correspondence: nyee@envsci.rutgers.edu)

Introduction: The redox cycling of selenium in aquatic environments is strongly affected by microbial activity. *Citrobacter freudii* is a facultative anaerobic bacterium found in freshwater that is known to reduce selenate. The mechanisms involved in Se(VI) reduction by *C. freundii* are currently poorly understood. In this study, we conducted selenate reduction experiments and sequenced the genome of *C. freundii* to investigate the molecular basis of selenate reduction.

Materials and Methods: Experiments were conducted to monitor selenate reduction by *C. freundii* during growth on citrate as the carbon source. The concentrations of selenate, dissolved oxygen and protein biomass were monitored over time. Selenium reduction products were analyzed using X-ray absorption spectroscopy. DNA was extracted from *C. freundii* and PCR primers were designed to amplify the functional selenate reductase gene *ynfE*. To sequence the genome of *C. freundii,* a paired end library was constructed using an Illumina Nextera kit, and sequencing was performed using an Illumina Genome Analyzer IIX. Sequence assembly was performed using CLC Genomics Workbench 5.1. Gene annotation and protein sequence analysis was performed based on NCBI database and BLAST.

Results and Discussion: The results indicate that *C. freundii* catalyzes the reduction of selenate to elemental selenium in the absence of oxygen. We detected the functional selenate reductase gene *ynfE,* which is predicted to encode for a molybdenum-binding Tat-secreted protein. A FNR binding site was located immediately upstream of the *ynfE* gene suggesting the expression of the selenate reductase occurs under anaerobic conditions and is regulated by oxygen-sensing transcription factors. The genome analysis revealed the complete selenate reductase operon *ynfEGHdmsD,* showing high sequence identity to selenate reductases found in related gammaproteobacteria. Genes for molybdate uptake, molybdopterin guanine dinucleotide biosynthesis, twin arginine translocation, and FNR regulation were also identified in the genome sequence. Based on the experimental results and genome sequence, we developed a molecular model to describe anaerobic selenate reduction in *C. freundii*.