Comparative Physiology and Biochemistry of Metal-Reducing Organisms from Acidic and Neutral pH Environments

D.E. Cummings[1], M.E. Swenson[2], T.L. Tyler[2], and T.S. Magnuson (PI)[2]

[1]Point Loma Nazarene University, San Diego, CA; [2]Idaho State University, Pocatello, ID

Comparative biochemical and molecular approaches have revealed interesting similarities and differences between neutrophilic and extremophilic mineral transforming bacteria. New methods have been developed to investigate the biochemistry of electron transport to metals and minerals, and new findings challenge the paradigm of electron transfer mechanisms to metal and mineral electron acceptors. Acidophilic dissimilatory iron-reducing bacteria (DIRB) have not been extensively studied, in contrast to their neutrophilic (i.e., *Geobacter*) counterparts. They are important organisms in metal and mineral transformation in acidic environments, and could represent bioremediative agents in metal- and radionuclide-contaminated low pH systems. Our NABIR-funded work is focused on the physiology and biochemistry of a representative acidophilic iron reducer, *Acidiphilium cryptum* JF-5. Preliminary data gathered thus far show *A. cryptum* to be an excellent system for these types of studies. Physiology studies show that *A. cryptum* effectively reduces both Fe(III) and Cr(VI), similar to many neutrophilic DIRB. Analytical and preparative methods for isolating and characterizing the extracellular polymeric substance (EPS) of *Acidiphilium* and *Geobacter* have been developed. Preliminary biochemical analysis of *Acidiphilium* shows the presence of *c*-type cytochromes in cell surface/EPS extracts, including two high-mass (42 and 48 kDa) outer-membrane-associated cytochromes *c*. These cytochromes have been purified by ion exchange and gel filtration techniques, and spectroelectrochemical analysis suggests that these proteins are capable of Cr(VI) and Fe(III) reduction in vitro. Neutrophilic DIRB also contain a suite of *c*-type cytochromes, but in much greater overall abundance. Microscopy studies of *A. cryptum* using lectin probes reveal biofilm structures, and attachment to mineral surfaces, even at low pH. Neutrophilic DIRB have similar biofilm structures when grown with solid-phase electron acceptors. The results indicate that *A. cryptum* may influence the fate of not only Fe, but also Cr in low-pH environments. It thus appears that acidophilic DIRB share certain features and abilities with their neutrophilic counterparts, but also have other traits that allow for this type of metabolism in extreme environments. Future studies will include genome-enabled studies of *A. cryptum* (facilitated by the DOE Joint Genome Institute), analysis of protein-mineral interactions using *A. cryptum c*-type cytochromes, resolution of select redox proteins and protein complexes of *Acidiphilium* by multidimensional PAGE techniques, and further biochemical characterization of redox proteins present in both *Acidiphilium* and in neutrophilic DIRB.