Identification of Metal Reductases and Determination of their Relative Abundance in Subsurface Sedimentary Systems Using Proteomic Analysis

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Heavy metal and radionuclide contamination at U.S. Department of Energy (DOE) sites nationwide constitutes a major environmental problem. Of particular interest are U and Tc, as well as Fe and Mn, due to their potential direct and indirect effects on contaminant biogeochemical behavior. For the past decade bacteria that use metals as terminal electron acceptors have been isolated and identified. These bacteria include members of three major anaerobic groups: the denitrifying, sulfate-reducing, and Fe(III)-reducing bacteria. The electron transfer pathways within these bacteria are still not well understood. Moreover, this lack of information substantially impedes efforts to increase in situ bioremediation efficiency. Hence, identification of metal reductases and determination of their similarity among these bacterial groups are essential for understanding these mechanisms and assessing bioremediative potential at DOE sites.

We have used cell fractionation techniques to resolve subcellular protein fractions and quantify the purity of proteins within each enriched fraction. Additionally, we have applied classical biochemical separations of fractions to enrich for specific proteins responsible for metal reduction activity. The application of advanced proteomics techniques allows for the identification of all the proteins in the enriched fractions eliminating the need for purifying each protein to homogeneity. We are using orthogonal purification approaches in both series and parallel to create fractions containing different complements of proteins. As each fraction exhibits the metal reduction activity, the proteins common to all the fractions are the most likely targets for further study by molecular biological techniques.