Characterization of Metal-Reducing Communities and Isolates from Uranium-Contaminated Groundwater and Sediments

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The elucidation of how metal-reducing bacteria interact in a given community and how the community responds to stress and perturbations can help infer the interplay between stress pathways and gene networks that help optimize ecosystem function and stability. A goal of VIMSS is to characterize the responses of bacterial populations at multiple levels of resolution in order to understand biochemical capacity at DOE waste sites. Within this context, bacteria with desired functions (e.g., heavy metal reduction) have been isolated from the Oak Ridge Field Research Center (ORFRC). The ORFRC is located within the Y-12 Security Complex near Oak Ridge, TN in the Bear Creek Valley, and the site includes 243-acres of a previously disturbed contaminated area. The subsurface at the FRC contains one of the highest concentration plumes of mobile uranium located in the United States, and contains various levels of nitrate, heavy metal, and organic contamination (http://www.esd.ornl.gov/orifrc/).

Recently, biostimulation with ethanol was used to detoxify the contaminated groundwater. The experiment successfully reduced nitrate and uranium levels to a safe-to-humans level during the span of the trial. During the biostimulation, d-*Proteobacteria* were detected to predominate the subsurface groundwater, and sequences indicative of the genera *Desulfovibrio*, *Geobacter*, and *Anaeromyxobacter* were observed. Two isolates were achieved, *Anaeromyxobacter* fw109-5 and *Desulfovibrio* FW1012B. The *Anaeromyxobacter* genome has been sequenced at JGI, and the *Desulfovibrio* genome is underway.

Desulfovibrio FW1012B was isolated from well FW101-2B in the bio-stimulation zone during U(VI) reduction at FRC. The isolate can reduce sulfate and utilize pyruvate, fumarate, maleate, lactate, and 1,2-propanediol. Cr(VI) is reduced, and nitrate is reduced without growth. The isolate can utilize triethylphosphate, metaphosphate, and trimetaphosphate as a phosphorus source. The closest cultivated relative is *Desulfovibrio*

carbinoliphilus based upon the SSU rDNA gene sequence, and the closest relative with a completely sequenced genome is *Desulfovibrio fructosovorans*. (that was already said above)

Anaeromyxobacter fw109-5 is a mesophilic, iron-reducing bacterium that was isolated from groundwater that had a pH of 6.1 and contained approximately 1.4 mM nitrate and 0.9 μM hexavalent uranium. *Anaeromyxobacter* species are high G+C d-*Proteobacteria* related to the genus *Myxococcus*. Based upon SSU rRNA gene sequences, the closest cultivable relative is *Anaeromyxobacter dehalogenans* 2CP-C with 96.5% sequence identity. The strain fw109-5 grows in the pH range of 4.0 to 9.0, but optimal growth is observed from pH 7.0 to 8.0. To date, known electron donors include acetate, lactate, ethanol, and pyruvate, and electron acceptors include nitrate and iron(III) but not AQDS. Yeast extract and peptone do not support growth, and the organism requires low substrate concentrations for growth (i.e., oligotrophic conditions). Optimal growth occurs under anaerobic conditions, and microaerophilic conditions can be tolerated. The *Anaeromyxobacter* fw109-5 genome is approximatley 5.3 Mb in size with 4,336 candidate protein-coding genes. The slow-growing bacterium is predicted to have two SSU *rrn* genes (16S), and almost 30% of the predicted ORFs are classified as conserved hypothetical proteins. A large percentage of estimated ORFs are predicted to be part of a signal transduction pathway with enrichment in serine/theronine kinase putative proteins. In comparison, fw109-5 had similar numbers of putative two-component and one-component signal transduction proteins as other sulfate- and metal-reducing d-*Proteobacteria*, but fewer compared to *Myxococcus xanthus*. In addition, preliminary data suggest social behavior and sporulation. The genome is predicted to encode a full glycolytic and tricarboxylic acid cycle as well as a pyruvate dehydrogenase complex. Approximately 105 putative proteins are predicted to contain heme-binding sites, with almost half being multi-heme proteins.