Anaerobic Microbial Metal Redox Cycling by Members of the Beta Proteobacteria

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As a continuation of our NABIR studies investigating the immobilization of uranium onto biogenically produced iron minerals resulting from anaerobic nitrate-dependant Fe(II) oxidation, we recently isolated a novel *Dechloromonas* sp. strain LT-1 and a closely related organism, *Propionivibrio limicola* strain CR. Both of these organisms were isolated as dissimilatory perchlorate-reducing bacteria and were obtained from enrichments initiated with groundwater and subsurface sediments, respectively. Analysis of the complete 16S rDNA sequence indicated that strain LT-1 was 96% similar to *Dechloromonas* sp. CL24 in the beta subclass of the Proteobacteria. In contrast to all other tested *Dechloromonas* species, strain LT-1 did not oxidize Fe(II) with nitrate as the sole electron acceptor. However, strain LT-1 was capable of coupling growth to the reduction of Mn(IV) with acetate as the sole carbon and energy source. No growth occurred in the absence of manganese. [^{14}C]-acetate was oxidized to $^{14}CO_2$ during this metabolism. Further screening of other *Dechloromonas* species, including *Dechloromonas* strain RCB, the only described organism capable of anaerobic benzene oxidation, indicated that manganese reduction was a common trait in this genus. In addition to acetate, strain RCB could even oxidize [^{14}C]-benzene to $^{14}CO_2$, with Mn(IV) as the sole electron acceptor. Strain RCB is currently being sequenced by the Joint Genome Institute at Walnut Creek, CA. Three, eight, and forty kB libraries have been generated in puc18, p21, and Fosmid vectors, producing sequence reads representing greater than 10X coverage of this approximately 4.2 Mb genome. Gap closure and resolution of misassemblies are currently being conducted via resequencing and primer walking of the clonal libraries. An oligonucleotide library representing the entire genome has been constructed and is currently being used in microarray analyses.

In contrast to strain LT-1, 16S rRNA sequence analysis indicated that strain CR was 97.2% similar to *Propionivibrio limicola.* Phenotypic characterization revealed that in contrast to all previously described perchlorate-reducing bacteria, strain CR was capable of reducing Fe(III)-NTA coupled to the oxidation of acetate. Although another Fe(III)-reducing member of the betaproteobacteria, *Ferribacterium limneticum*, was previously described, this organism has since been lost. In addition to Fe(III) reduction, preliminary phenotypic characterization studies indicated that strain CR was also capable of oxidizing Fe(II) coupled to nitrate reduction. A recently published pure culture study of *Geobacter metallireducens* demonstrated that, similarly to strain CR, this organism was alternatively capable of coupling the oxidation of Fe(II) as well as U(IV) to the reduction of nitrate. Together these results indicate that metal-reducing bacteria may also be capable of oxidizing Fe(II) or other metals coupled to nitrate reduction.

The implications of such metabolic versatility on the long-term immobilization and stabilization of radionuclides such as uranium has yet to be determined.