Isolation and characterization of a novel Fe(II)-oxidizing *Alphaproteobacterium* from the Hanford 300 Area subsurface

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As part of an ongoing study of microbial Fe redox cycling in Hanford 300 Area subsurface sediments, a novel neutral-pH Fe(II)-oxidizing *Alphaproteobacterium* was isolated from Hanford formation groundwater using in situ incubation chambers ("i-chips") containing the insoluble Fe(II)-bearing mineral biotite as an energy source. After five months of in situ deployment, samples from i-chips were transferred into aerobic liquid medium with biotite the sole energy source. Following several transfers on this medium, the cultures were grown for three generations on medium to which soluble Fe(II) ($FeCl_2$) and air were added repeatedly over time. A pure culture was subsequently isolated on aerobic plates containing acetate and yeast extract. Sequencing of the 16S rRNA gene of the culture showed that it is >99% similar to various strains of *Bradyrhizobium japonicum*. The culture (referred to as *Bradyrhizobium* sp. strain 22, or simply "strain 22") was tested for the ability to grow chemolithoautotrophically with soluble and insoluble forms of Fe(II), as well as other inorganic electron donors. Strain 22 grew in organics-free medium with $FeCl_2$ or Fe(II)-NTA as the electron donor and oxygen or nitrate as the electron acceptor. The organism could also grow repeatedly with biotite (aerobic conditions) or reduced smectite (nitrate-reducing conditions) as the electron donor. Unlike other known chemolithoautotrophic strains of *Bradyrhizobium* (e.g. *B. japonicum* strain USDA 110), strain 22 is not able to grow by oxidation of hydrogen or thiosulfate. A draft whole genome sequence for strain 22 was obtained through the University of Wisconsin-Madison Biotechnology Center. The 7.4 MB sequence underwent de novo assembly and automated annotation through RAST, leading to identification of a total of 7003 coding sequences. The draft genome was searched for genes likely to be involved in chemolithoautotrophic growth with Fe(II). A complete Rubisco system for CO_2 fixation, analogous to that present in other chemolithoautotrophic strains of *Bradyrhizobium*, was identified. Although numerous *c*-type cytochromes were found in the genome, none of them showed homology to the type of outer membrane proteins (e.g. the *mtrAB* system) known to be associated with extracellular electron transfer in some Fe(III)-reducing or Fe(II)-oxidizing organisms. This conclusion was supported by PCR screening of strain 22 genomic DNA for *mtrAB* homologs, which gave negative results. Although verification of the latter finding is pending (by southern blot analysis), information available to date suggest either failure in the annotation of novel outer membrane *c*-type cytochromes, and/or that strain 22 uses a mechanism for Fe(II) oxidation distinct from other known Fe(II) oxidizers. Motivation for more detailed studies of the mechanisms for Fe(II) oxidation in strain 22 (together with other *Bradyrhizobium* isolates from Hanford) comes from the observation of relatively high proportions of *Bradyrhizobium*-related sequences (up to 5-10% of total sequences) in 16S rRNA gene clone libraries constructed with Hanford 300 Area sediments. Development of genetic tools to detect the presence and expression of genes associated with Fe(II) oxidation could provide insight into the role(s) of *Bradyrhizobium*-related organisms in in situ biogeochemical processes in 300 Area sediments. Future studies will employ differential gene expression (via RNAseq) and proteomics during organotrophic vs. chemolithoautotrophic growth with Fe(II) as a means for identifying key components of the Fe(II) oxidation system in strain 22.