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ABSTRACT TITLE: Electron Transfer Mechanisms for Microbial Iron Redox Cycle in Hanford Subsurface Sediments  

ABSTRACT: Iron (Fe) is the fourth most abundant element in the earth and present at between 3.5 and 8% (by weight) in the subsurface sediments of Hanford 300 area (300A). It represents a large reservoir of oxidant [i.e., Fe(III)] and reductant [Fe(II)] for microbial energy generation, in which Fe(III) reduction is coupled to oxidation of organic matter and probably methane, while Fe(II) oxidation is coupled to reduction of O₂, nitrate and CO₂. Critical to this Fe(II/III) redox cycling are the microbial electron transfer pathways that physically link the extracellular Fe redox reactions and the intracellular metabolic activities. These electron transfer pathways in the 300A microorganisms are, however, essentially unknown.

Heme-staining analyses of the Fe(III)-reducing bacterium Geobacter sp. FA1 (FA1) isolated from the 300A reveals that the abundance of a heme-containing protein, whose apparent molecular mass is close to that of OmCB of the model Fe(III)-reducing bacterium G. sulfurreducens PCA (PCA), increases under the Fe(III)-reducing condition. The PCA OmCB is an outer membrane, 12-heme c-type cytochrome that resides on the bacterial surface where it is involved in Fe(III) reduction. A gene encoding a putative outer membrane c-type cytochrome with multiple hemes was subsequently cloned and sequenced from the FA1 genome. Its deduced amino acid sequence is 38% identical to that of PCA OmCB and possesses 12 heme-binding motifs. This cloned gene is named as omcB and its protein product OmCB is hypothesized to be part of extracellular electron transfer pathways used for Fe(III) reduction by FA1. To test this hypothesis, current research focuses on (i) the role of FA1 OmcB in Fe(III) reduction, (ii) purification and characterization of FA1 OmcB and (iii) determination of the whole genomic sequence of FA1 through the JGI Microbial Isolates Sequencing Program.

c-Type cytochromes and multicopper oxidases play important roles in microbial Fe(II) oxidation. Analysis of a drafted whole genomic sequence for the chemolithoautotrophic Fe(II)-oxidizing bacterium Bradyrhizobium sp 22, which is also isolated from the 300A, has identified 75 putative c-type cytochromes and 6 putative multicopper oxidases. Further analyses have also identified the putative proteins that may be used for nitrate reduction and CO₂ fixation. To investigate whether these identified proteins are involved in Fe(II) oxidation that is coupled to nitrate reduction and CO₂ fixation, development of a genetic system for Bradyrhizobium sp 22 has been initiated.

A proteoliposome system is successfully developed to investigate the interfacial electron transfer reactions between the surface-exposed bacterial terminal metal reductases and Fe(III) minerals. Immuno-gold localization and proteolytic digestion consistently demonstrate that the topology of the bacterial reductases on the proteoliposomes is the same to that on the bacterial cells. By providing a continuous flow of electrons, the proteoliposome experiments demonstrate that conduction through the bacterial terminal metal reductases directly to Fe(III) minerals is sufficient to support in vivo, anaerobic, solid-phase Fe(III) respiration. This system will be used to characterize electron conductance properties of the pathways identified from the 300A microorganisms.

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