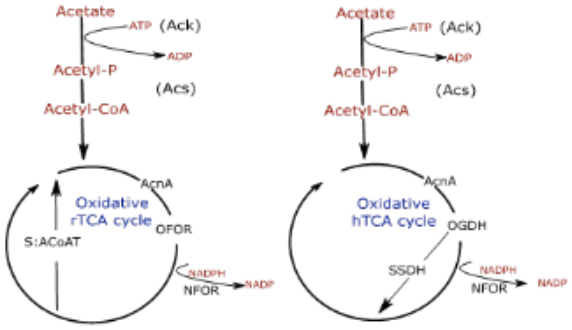
MICROBIAL MANGANESE(III) REDUCTION FUELED BY ANAEROBIC ACETATE OXIDATION. N. Szeinbaum[1], L. Hui[2], J. A. Brandes[3], M. Taillefert[2], J. B. Glass[2], and T. J. DiChristina[1], [1]School of Biological Sciences, Georgia Institute of Technology, Atlanta, GA (nadia@gatech.edu) [2]School of Earth and Atmospheric Sciences, Georgia Institute of Technology, [3]Skidaway Institute of Oceanography, Savannah, GA

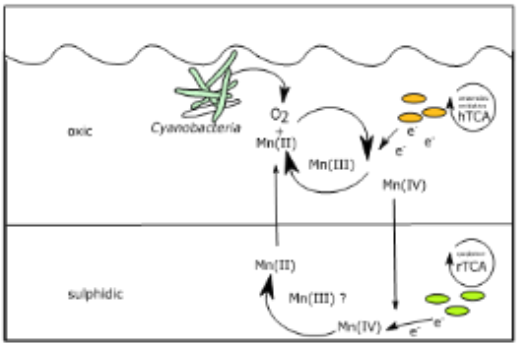
Background: Microbial manganese (Mn) cycling in marine and freshwater environments is generally assumed to consist of Mn^{2+} oxidation in oxic water columns and Mn(IV) oxide reduction in anoxic sediments [*1*] as the only two bioavailable Mn redox species. This dogma was recently overturned with the discovery that soluble Mn^{3+} dominates the soluble Mn pool at μM concentrations in suboxic environments [*2*]. Although Mn^{3+} is soluble, electron transport and protein secretion pathways involved in extracellular metal reduction are required for it to be used as an electron acceptor [*3*]. Acetate is the most abundant volatile fatty acid fueling Mn reduction in aquatic environments and is also one of the primordial organic carbon substrates for microbial life. Although biochemical pathways for anaerobic acetate oxidation have been studied for decades in methanogenic archaea and sulfate reducing bacteria, little is known about metal-reducing acetate oxidizers.

Methods: Anoxic enrichment cultures were established by inoculating a layer of suboxic Mn^{2+}-rich salt marsh sediment into sulfate-free media with soluble Mn^{3+}-pyrophosphate and acetate. Mn^{3+} reduction was monitored spectrophotometrically by absorbance at 480 nm. Samples for carbon isotopic analysis of total dissolved inorganic carbon (δ^{13}C-DIC) were analyzed by liquid chromatography-isotope ratio mass spectrometry (LC-IRMS). Whole genome sequences were compared using RAST together with KEGG, BioCyc, and NCBI databases for protein sequence similarity and domain analysis.

Findings: We isolated *Shewanella* strain MN-01 with 98% average nucleotide identity to *S. algae* and *S. haliotis*, members of a genus previously considered unable to oxidize acetate anaerobically. Strain MN-01 was able to oxidize acetate coupled to reduction of either soluble Mn^{3+} or Fe^{2+}, confirmed via ^{13}DIC production from ^{13}C1- and ^{13}C2-labeled acetate. Other *S. algae* strains were also capable of Mn^{3+} reduction with acetate, thus expanding the ecological niche of the *Shewanella* genus. Genomic comparisons among acetate-oxidizing and non-oxidizing *Shewanella* spp. revealed four distinct enzymes (aconitate hydratase 2 (AcnA), succinate semialdehyde dehydrogenase, and two NADPH:quinone oxidoreductases) present only in acetate-oxidizing strains, which may allow metal-reducing Gammaproteobacteria to use the heterotrophic, oxygen-tolerant TCA cycle (hTCA) anaerobically whereas Deltaproteobacteria use the reductive, oxygen-sensitive TCA (rTCA) cycle.



Implications: The ability of *Shewanella* to oxidize acetate using the hTCA cycle has evolutionary implications for the emergence of metal reduction in Proteobacteria. Deep phylogenetic origins of metal-reducing microbes support an early origin for Mn respiration, consistent with geological evidence for microbial Mn oxides as early as 2.9 Ga [*4*]. We propose that the enzymatic machinery for acetate oxidation coupled to metal reduction evolved under different oxygen regimes: (1) anaerobic Deltaproteobacteria (e.g. *Geobacter*) evolved metal reduction first under anoxic conditions by retaining the oxygen-sensitive enzymes of the rTCA cycle; (2) after Cyanobacteria began producing significant oxygen in the photic zone, facultative anaerobic Gammaproteobacteria (e.g. *Shewanella*) evolved metal reduction using the less oxygen sensitive hTCA cycle.



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