

Metabolic pathway(s) coupled to energy conservation in neutrophilic Fe-oxidizing bacteria. D. Emerson¹, R.A. Barco^{1,2}, J.J. Scott¹, and C. S. Chan³. ¹Bigelow Laboratory for Ocean Sciences, East Boothbay, ME (demerson@bigelow.org), ²University of Southern California, Los Angeles, CA, ³University of Delaware, Newark DE.

Introduction: Iron is one of the most abundant elements in solar system that can support the growth of chemosynthetic microbial communities, yet we are still trying to understand the primary metabolic pathway, or pathways, used by obligate Fe-oxidizing bacteria (FeOB) to conserve energy via Fe-oxidation. In response to the physicochemical challenges inherent for growth on iron at circumneutral pH, many FeOB produce morphologically unique structures that can coalesce to make intricate microbial mats that exhibit remarkable phylogenetic and morphological diversity on the micrometer scale [1]. Despite sharing a host of common phenotypic attributes, the prevalent FeOB found in freshwater versus marine iron mats differ substantially at the genetic level. In marine systems, members of the class Zetaproteobacteria are prevalent, while freshwater iron mats are dominated by members of order Gallionellales within the Betaproteobacteria.

Because FeOB produce an insoluble Fe-oxyhydroxide as a result of their metabolism, it is assumed they carry out extracellular electron transfer (EET) whereby electrons captured from Fe-oxidation at the cell surface are translocated across the periplasmic space to the electron transfer chain (ETC) on the cytoplasmic membrane. Comparative genomics has revealed some conserved features of the ETC among different marine and freshwater FeOB; however there does not appear to be a universal pathway. One gene of particular interest encodes an outer membrane cytochrome-containing porin that is a homolog to Cyc2, a protein originally identified in *Acidithiobacillus* as an iron oxidase. Homologs of the *cyc2* gene have been identified in all the 15 genomes currently sequenced from either marine (10 genomes) or freshwater (5 genomes) FeOB, as well as partial or near complete genomes from single amplified genomes or metagenomes from either marine or freshwater Fe(II)-fueled ecosystems. Cyc2 is a mono-heme porin that appears to belong to a family of outer membrane associated porin-cytochrome c proteins implicated in EET [2].

Proteomic analysis of the obligate marine Fe-oxidizer, *Mariprofundus ferrooxydans*, identified Cyc2 as one of the most abundant proteins in Fe(II)-grown cells [3]. Analysis of a new isolate, '*Ghiorsea bivora*', that can grow on either Fe(II) or H₂ as sole electron donor, revealed that the key uptake hydrogenases were only expressed under H₂-grown conditions, while Cyc2 was

found to be relatively abundant under either H₂- or Fe(II)-grown conditions. This suggests that Cyc2 is constitutively expressed. This would be consistent with *G. bivora* being primarily adapted for growth on Fe(II), with H₂-fueled growth providing a competitive advantage on occasions where H₂ was present in addition to Fe(II). Circumstantial evidence for this conjecture comes from an analysis of over 55,000 (non-human microbiome) amplicon-based 16S gene surveys of different microbiomes on Earth. A small subset of approximately 700 samples had sequences related to Zetaproteobacteria (mostly as singletons or doubletons), and of those only 17 samples had evidence of '*G. bivora*'. Thus, despite H₂ likely being a potential substrate in a number of the different sampled environments, '*G. bivora*' was limited to a few samples all consistent with Fe(II) being the principle electron donor. Interestingly, some of these habitats, like mild steel surfaces, and the ocean crust subsurface, are also locales where H₂ maybe present as a co-substrate.

References:

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