Cytochrome Cyc2: Role in Extracellular Electron Transfer

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Introduction: Iron-oxidizing bacteria (FeOB) represent one of the first identified groups of bacteria, dating back to the early 19th century (1). However, due to difficulties associated with cultivating FeOB, relatively little is known about the biochemical mechanisms involved in the extracellular-electron transfer (EET) reactions that make chemolithoautotrophic iron oxidation possible. These electrochemically-active microbes couple the oxidation of ferrous iron (Fe^{2+}) to the reduction of oxygen, nitrate, or water (in phototrophic iron oxidation) (2, 6), and may represent one of the most ancient metabolism on earth (3). Much of our knowledge of iron oxidation mechanisms stems from studies performed on iron-reducing bacteria, such as *Shewanella* and *Geobacter* sp., resulting in a model for iron oxidation, *MtoAB*, illustrated in figure 1; this model features a large 26-strand beta-barrel porin associated with a decaheme cytochrome (4), and is generally accepted for some of the model iron-oxidizing organisms; however, another model for iron-oxidation exists, the one that is represented by the protein *Cyc2*, a 16-strand beta-barrel porin fused to a monoheme cytochrome (figure 2) (5). Homologs of *Cyc2* are signifincatly more common across the bacterial domain than *MtoAB*, and are present in the genomes of known FeOBs, known iron-reducing bacteria, and organisms not previously associated with EET. *In situ* evidence is needed to substantiate the role of *Cyc2* in iron oxidation and delineate the functional differences between the two models.

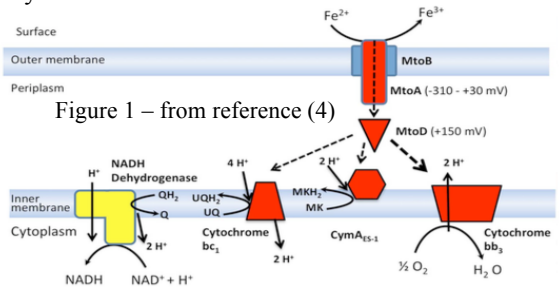

Figure 1 – from reference (4)

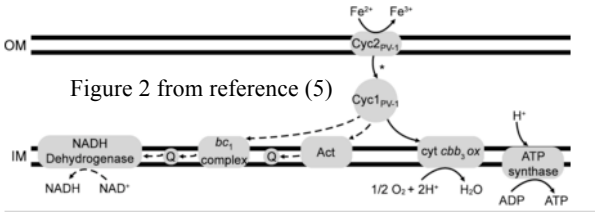

Figure 2 from reference (5)

This work will aim to characterize the expression of *Cyc2* during biological iron oxidation using reverse transcription coupled to quantitative polymerase chain reaction (RT-qPCR). Of particular interest are FeOB that are capable of deriving energy from compounds besides iron(II), such as the extreme acidophile *Acidithiobacillus ferroxidans*, which can couple sulfur oxidation to the dissimilatory reduction of iron(III) (7), and FeOB whose genomes encode both, *Cyc2* and *MtoAB* homologs, such as *Sideroxydans lithotrophicus*. The RT-qPCR approach will give us a glimpse into the transcriptional dynamics of different organisms subjected to various conditions and may shed some light on *Cyc2's* role in with respect to other forms of EET and other putative iron oxidase systems.

We also plan to address the possibly overlooked presence of *Cyc2* in environmental metatranscriptomic datasets, such as those reported by (8) and (9). Transcriptomic studies of FeRB and metatranscriptomic studies relevant to EET have particularly focused on the deca/dodecaheme cytochromes such as those present in *Geobacter* and *Shewanella* sp.. Thus, identification and quantification of *Cyc2*-related environmental transcripts may provide valuable insights into the proteins involved in EET and, specifically, iron oxidation.

Methods: In order to quantitatively measure the expression of putative EET components we will design primers for the *Cyc1* and *Cyc2* genes of *A. ferroxidans*. Primers will be designed for homologs of *Cyc1* and *Cyc2* of S. lithotrophicus, in adition to the primers for the *MtoABD* components. Cell cultures will be grown in media correspoding to the conditions in which transcript levels will be measured (that is, for measurements of *Cyc1* and *Cyc2* expression during anaerobic sulfur oxidation by *A. ferroxidans*, cells will be grown on sulfur and iron(III)). In addition to iron-oxidation/reduction measurements, RNA will extracted and reverse trasncribed into cDNA; that cDNA will be used as a template for quantitative polymerase-catalyzed amplification of selected genes.

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