Biochemical Mechanisms of Neutrophilic, Chemolithoautotrophic Iron Oxidation.

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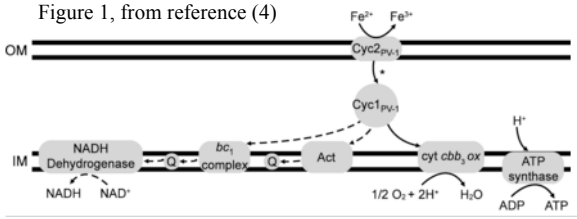
Introduction: Iron-oxidizing bacteria (FeOB) represent one of the first identified groups of bacteria, dating back to the early 19th century (1). However, due to difficulties associated with cultivating FeOB, relatively little is known about the biochemical mechanisms involved in the extracellular-electron transfer (EET) reactions that make chemolithoautotrophic iron oxidation possible. A putative pathway is illustrated in figure 1. 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), and may represent one of the most ancient metabolism on earth (3). A better understanding of the molecular mechanisms by which cells can link the inner-membrane-associated and periplasmic redox components to extracellularly-localized minerals and ions will provide valuable insights with respect to the evolution of iron respiration here on earth and possibly elsewhere.

Figure 1, from reference (4)



To better understand iron oxidation mechanisms, we aim to heterologously express, purify, and characterize a putative iron oxidase, *Cyc2*$_{PV-1}$, which is proposed to act as the initial electron acceptor from Fe^{2+}, in the respiratory pathway of a neutrophilic, chemolithoautotrophic *Mariprofundus ferroxydans* PV-1 (4). *Cyc2*$_{PV-1}$'s tertiary structure has been predicted and features a 16-strand β-barrel porin fused to a monoheme cytochrome (figure 2). This multi-domain structure may represent an adaptation to EET that is unique to, and possibly older than, the multi-gene Mtr iron-reductase system of *Shewanella oneidensis* MR-1 (5). Thus, the overarching question that we are addressing: '*What is the role of Cyc2 in the oxidation of iron, and what evolutionary relationship, if any, does it have to the Mtr system?*'

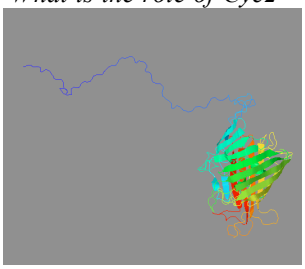
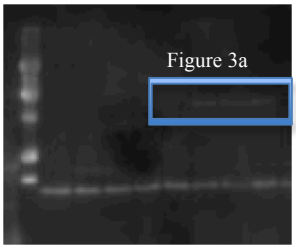
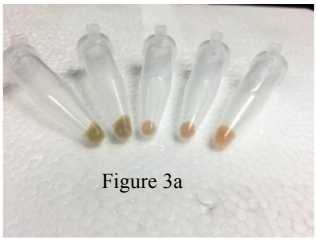


Figure 2. β-barrel porin modeled after a homologus structure in Pseudomonas aeruginosa. Cytochrome domain, disordered blue region, is unmodeled here.

Methods: This study will utilize biochemical and bioinformatical techniques. Our first task is expression of *Cyc2*$_{PV-1}$ in *E. coli*, followed by purification via column chromatography. After purification, we plan to proceed with a thorough biochemical characterization, measuring *Cyc2's* redox potential, iron oxidase activity, and possibly 3D structure via X-ray crystallography. We will also perform experiments to see whether heterologous expression of *Cyc2* confers iron oxidase activity to its expression strain, as has been observed previously with heterologous expression of the Mtr iron-reductase components (6). In addition, we will perform *in silico* characterization of *Cyc2*$_{PV-1}$ and its homologs that are present in the genomes of over 400 other organisms. This will be done with software such as "DomainTeam" (7) to identify potential clues to the evolution of *Cyc2*. We also plan to investigate the interactions between the cytochrome and porin domains of *Cyc2* by studying the co-evolution of the two domains, further informing our knowledge of *Cyc2's* structure/function (8).

Preliminary Data: Results suggest that *E. coli* express *Cyc2*$_{PV-1}$ with a native signal sequence, but with relatively low yield (figure 3a, 3 right lanes). It is unclear whether the protein is expressed with the cytochrome in its mature/properly folded form. Expression with a plasmid that confers constitutive expression of cytochrome c maturation genes to *E. coli* results in unusually red cell pellets, suggesting presence of an oxidized heme (figure 3b, 3 right tubes).





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