# *Marinobacter aquaeolei* gene expression studies for clues to neutrophilic Iron Oxidation

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Iron is the fourth most plentiful element on the Earths crust and occurs commonly on other planets such as Mars. In the near surface environment iron occurs in two readily interconvertible redox states $Fe^{2+}$ and $Fe^{3+}$ and is frequently associated with catalytic domains of proteins. This widespread occurrence suggests that iron played a key role in early life forms where it probably served as a key constituent in early prosthetic moieties. The capacity for Fe oxidation is broadly distributed among prokaryotes and the activities of Fe-oxidizing bacteria exert critical influence on many major elemental cycles including the C cycle. Despite their importance the fundamental biology of Fe oxidation

remains poorly understood. *Marinobacter aquaeolei* is a facultative mixotrophic Fe oxidizer that oxidizes ferrous iron under neutrophilic conditions and represents an ideal model bacterium to use for the study of Fe oxidation. To study the genes responsible for iron oxidation/ regulation in *Marinobacter* we constructed a fosmid library with ~40 kb inserts from *Marinobacter* DNA. When plated on iron containing medium, recombinants capable of iron oxidation formed orange colored colonies. We identified nineteen recombinants by this screening procedure and selected one for complete sequence analysis. Using a shotgun sequencing strategy, we identified three supercontigs plus a small contig. We employed directed sequencing strategies to obtain a fully closed fosmid sequence. The insert was then translated using the Getorf program in EMBOSS. Blast and pfam analysis identified the function of inferred orfs.
Based on these preliminary results the genes with a potential for iron oxidation or contributing towards iron regulation were identified as :oxygen independent coproporphyrinogen III oxidase, fnr protein, C4 dicarboxylate transporter, amino acid transporter/multicopperoxidase, hypothetical protein/nitrogen fixation protein, glutamyl tRNA reductase (hemeA gene), peptide chain release factor, hypothetical protein, methyltransferase/hemeK gene, hypothetical protein, nitrate /nitrite transporter, iron uptake protein, succinate dehydrogenase, hypothetical protein/putative membrane protein cytochrome C oxidase chain, C4 dicarboxylate and nitrogen regulation protein.Detailed annotation efforts are currently underway. Random mutagenesis of one of the clones showed that inactivation of the HemeA gene significantly reduced the coloration. The HemeA gene is involved in *de novo* haem biosynthesis, and suggest a possible role for Haem in Fe oxidation.