Identification and expression from Genes Involved in Iron and Lanthanide Homeostasis in *Methylobacterium extorquens* AM1

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Abstract

Methylobacterium extorquens is a model organism for the understanding of methylotrophic growth and a platform for production of biofuels and biodegradable plastics from methanol. Because methanol is abundant and inexpensive, it is an ideal carbon source for the production of these value added chemicals. Previous microarray data suggested that the methylotrophic carbon assimilation regulator, QscR, may not only control expression of genes required for methylotrophic growth, but may also modulate expression of iron uptake genes. This finding led us to investigate the iron requirements of *M. extorquens* during single- and multi-carbon growth, the genes involved in iron acquisition and storage, and the regulators that control these processes. Phenotypic analyses of *M. extorquens* indicate that the concentration of iron required to reach maximum growth is dependent on the type of exogenous iron supplied. To assess transcriptional regulation of genes predicted to be involved in iron homeostasis, transcriptional reporter fusion constructs were generated by fusing the promoter regions of interest to the fluorescent reporter Venus, a modified yellow fluorescence protein. Our results suggest that several of the selected promoters are differentially regulated not only by iron, but also by lanthanum, a rare-earth element involved in methanol assimilation. This suggests that the processes mediating iron uptake may also be involved in the sequestration and transport of lanthanides. Using gel electrophoresis mobility shift assays and transcriptional reporter fusions, future work will determine the relationship between different predicted iron regulators and sets of genes involved in iron metabolism, as well as the extent to which lanthanide and iron homeostasis overlap. These results will serve as a first step in determining which genes are involved in iron and lanthanide uptake and how these processes are regulated. Funding for this project was provided by San José State University.

Background

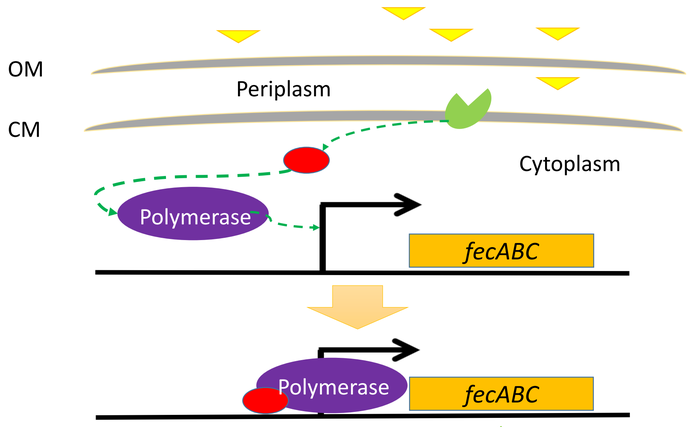
Figure 1 Methylotrophic colonies show, in some cases, better growth when lanthanum is present than in its absence

Metal Chelation and Regulation:

- Lanthanides and iron in their oxidized forms are insoluble metals that can be solubilized by organic acids, or chelated by metabolites secreted by organisms to take up iron into the cell
- Acquisition of lanthanides, just like iron, may be a siderophore mediated process
- In other organisms, such as *Escherichia coli*, separate pathways have been characterized for the uptake of ferric citrate and ferric sulfate

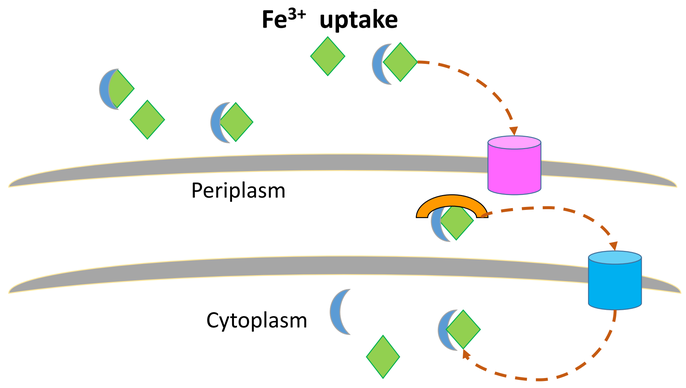
Figure 2

A. Possible model for the regulation of ferric citrate uptake by FecR and FecI



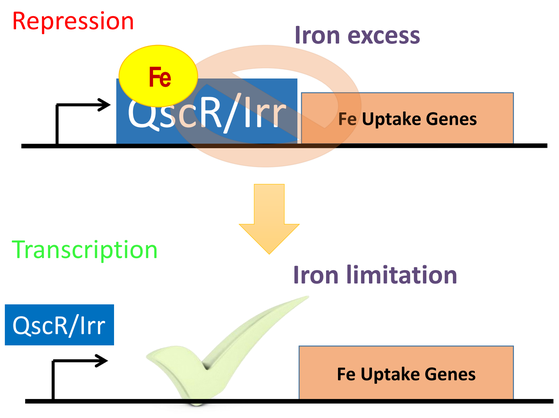
When ferric citrate is available, FecR senses it and activates the FecI sigma factor allowing RNA polymerase to bind to the promoter controlling expression from the iron uptake gene (*fecABC*)

B. Possible model for siderophore mediated iron Fe^{3+} uptake



When Fe^{3+} is available, secreted siderophores bind to the metal. A TonB-dependent siderophore receptor in the outer membrane senses the siderophore and transports it into the periplasm where it is paired with a binding protein that transports the siderophore-iron complex across the cytoplasmic membrane through an ABC transporter and into the cytoplasm, where the iron is released.

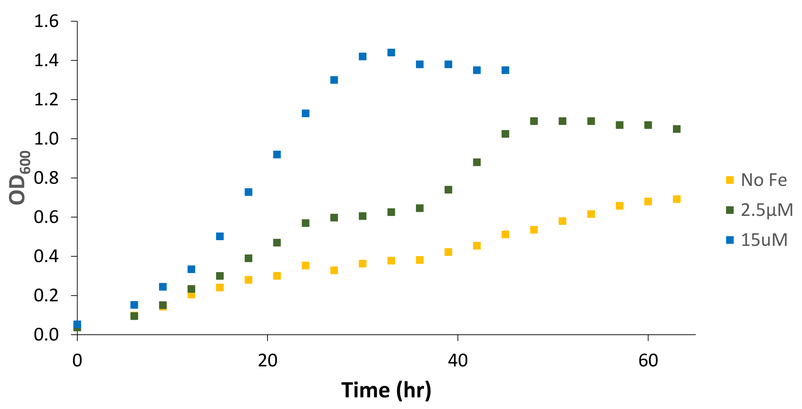
Figure 3 Possible model for the regulation of iron uptake genes by QscR and Irr



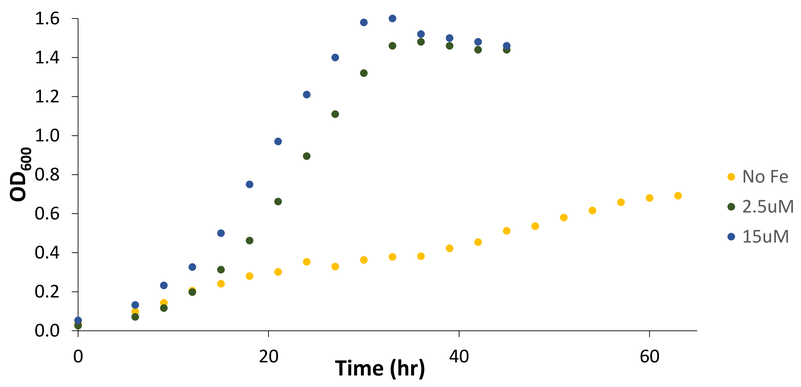
Results

Figure 4

A. Methanol growth of wild type with different concentrations of ferric sulfate



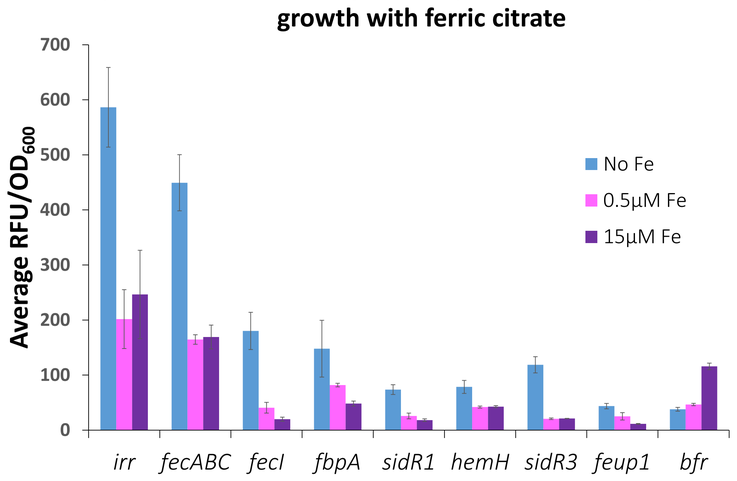
B. Methanol growth of wild type with different concentrations of ferric citrate



Conditions of iron limitation and excess were determined by growing cells in methanol medium with increasing iron concentrations. Growth with ferric sulfate required higher iron concentration to reach maximal growth.

Figure 5

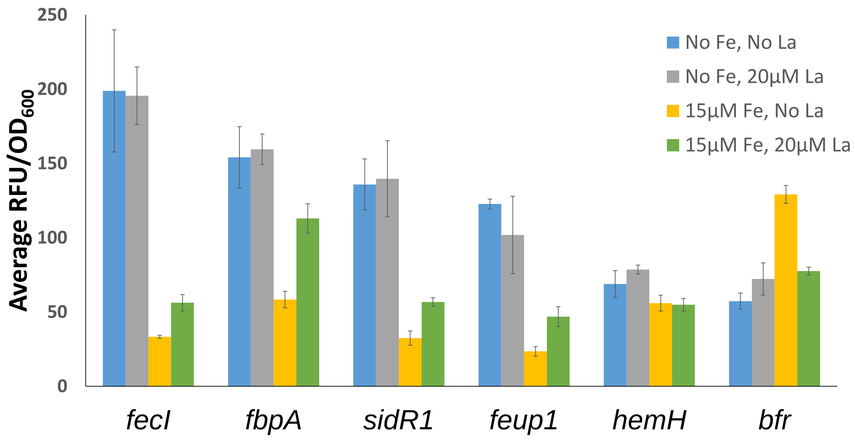
Expression from iron-dependent promoters during methanol growth with ferric citrate



- Expression from predicted iron uptake genes is upregulated when iron is limiting
- Expression from the *bfr* (iron storage) promoter is upregulated when iron is in excess

Figure 6

Lanthanum affects expression from some but not all iron regulated promoters



- Expression from sigma factor *fecI,* iron uptake *feup1,* siderophore receptor *sidR1*, and iron transporter *fbpA* promoters are upregulated in the presence of lanthanum when iron is in excess.
- Expression from iron storage promoter, *bfr* , is downregulated in the presence of lanthanum when iron is in excess.

Conclusions

- Lower concentrations of ferric citrate are needed to achieve the maximum growth rate and cell density when compared to ferric sulfate
- The promoter regions tested show differential regulation in response to increasing iron concentrations
- Some iron uptake genes respond to lanthanides as well, suggesting that lanthanide uptake may also be mediated by siderophores

Future Directions

- Determine which regulators control expression from which promoters using transcriptional reporter fusions
- Identify additional iron and lanthanide regulated genes using global transcriptomics and transposon mutagenesis
- Purify regulators and conduct gel electrophoresis mobility shift assays and microscale thermophoresis experiments to determine direct interactions between iron regulators with iron and lanthanum, and the promoters they regulate

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