Expression and purification of GxcA, a *c*-type cytochrome involved in metal respiration by the bacterium *Geothrix fermentans*

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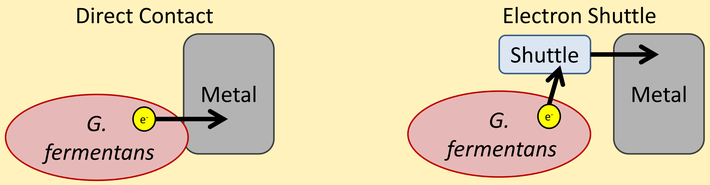
Abstract

The study of bacteria capable of respiring oxidized metals offers insights into the geochemical cycling of metals, including toxic heavy metal contaminants. These dissimilatory metal-reducing organisms couple oxidation of organic compounds with the reduction of substrates to gain energy. Improving understanding of the various bacterial metal-reducing strategies will increase our ability to produce renewable energy using microbial fuel cell technologies. The bacterium *Geothrix fermentans* has long been ignored in the study of metal respiration, but it has recently been shown to employ a unique strategy involving more than one biochemical pathway that appears tuned to use of high potential metals, such as uranium and manganese. Membranes isolated from Fe(III)-respiring *G. fermentans* contain high levels of a decaheme cytochrome, known as GxcA, which is suspected in electron transfer by *G. fermentans*. As a genetic system for *G. fermentans* is not yet available, GxcA was targeted for expression and purification. The DNA sequence for *GxcA,* containing an in-frame hexahistidine sequence, was first cloned into *E. coli,* using the inducible expression vector pETlite. Then, the recombinant plasmid was co-transformed into *E. coli* with pEC86, a plasmid that contains genes for the *ccm c*-type cytochrome maturation system. Colonies were screened for *c*-type cytochromes by redox difference absorption analysis and heme stain analysis. Future work will involve GxcA purification for redox and localization experiments to determine GxcA's potential role in *G. fermentans*.

The importance of *Geothrix*

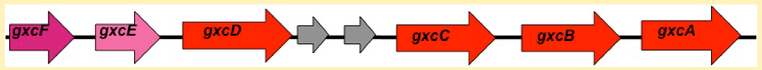
Geothrix belongs to the poorly studied, yet ubiquitous, soil phylum *Acidobacteria*. Although this phylum is mostly uncultivated, the isolate *G. fermentans* is available for study. This bacterium has been largely ignored in the study of metal-respiration, despite being found in metal-rich, arsenic-releasing, petroleum-contaminated and other anaerobic habitats alongside the model organisms *Shewanella* and *Geobacter.* Recently, the bacterium *Geothrix fermentans* been shown to employ a unique strategy involving more than one biochemical pathway to respire metals. Two of the hypothesized pathways are diagramed below.



Thus, *G. fermentans* provides a rare opportunity to study how environmentally relevant bacteria transfer electrons to metals and compete in the subsurface.

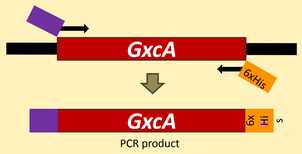
The importance of cytochromes

All known iron reducing bacteria contain 25 - 50 multiheme cytochrome genes. These encode proteins that are able to span membranes and cross long distances to transfer electrons to external metals. Membrane purification experiments examining the metal-reducing mechanism of *G. fermentans* identified key proteins which belong to the *gxcABCD* cluster. This cluster consists of 4 homologous decaheme cytochromes as shown below. GxcA was targeted for expression and purification to determine its contribution to *G. fermentans's* ability to use high potential metals.

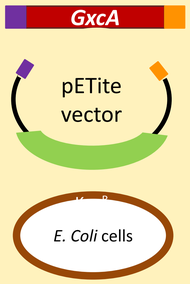


Cloning and Expressing GxcA

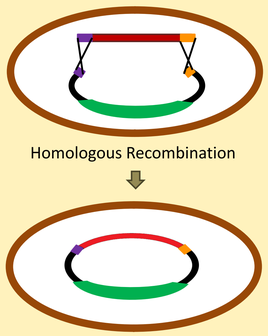
1. Amplify *GxcA* using primers that contain overlap with vector ends; one of these primers contains a 6xHis sequence .



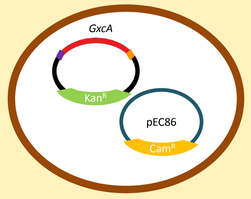
2. Mix PCR product, pETite vector, and *E. coli* cells



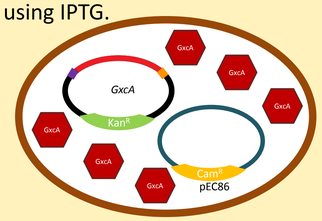
3. Transform by heat shock. The gene is inserted into the vector by homologous recombination within the cells. Select for recombinants on kanamycin agar plates.



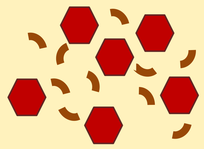
4. Co-Transform *E. coli* with the recombinant plasmid and pEC86, a plasmid that contains genes for the *ccm c*-type cytochrome maturation system required for active cytochromes. Select for co-transformed cells on kanamycin & chloramphenicol agar plates.



5. Induce protein production using IPTG.



6. Lyse cells by sonication.



7. Collect total membranes of colonies by centrifugation.

8. Analyze for *c*-type cytochromes by redox absorption difference analysis.

Acknowledgements

This project was supported by the University of Minnesota's Undergraduate Research Opportunities Program

Results

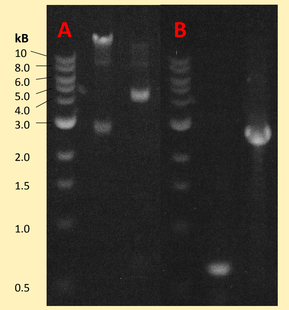


Figure 1 – Gel electrophoresis images of **A)** restriction enzyme digested plasmids (Successful insertion of *GxcA* is demonstrated by the 4.8 kb band in lane 2, as compared to no insertion in lane 1), and **B)** PCR amplification of the cloning site (Successful insertion of *GxcA* is demonstrated by the 2.8 kb band in lane 2 as compared to no insertion in lane 1)

Figure 2 – Pellets of overnight grown cultures of **A)** *E. coli* cells and **B & C)** *GxcA* transformed cells. *GxcA* cells are darker and pinker than plain *E. coli* cells, as *c*-type cytochromes are pink in color and bestow a reddish hue to cells.

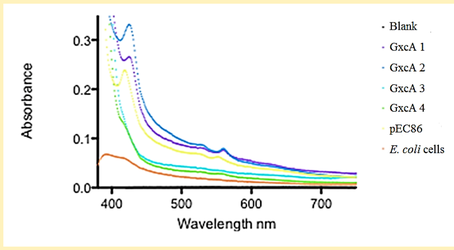


Figure 3 – Redox absorption difference graph of 4 *GxcA* clones, *E. coli* cells with pEC86, and WT *E. coli* cells. The presence of *c*-type cytochromes is indicated by characteristic peaks at 413, 522, and 552 nm as demonstrated by GxcA 1 and 2.

GxcA has been cloned into *E. coli*

- *GxcA* has been inserted into the pETite vector
- Wild-Type *E. coli* cells lack active production of *c*-type cytochromes, but transformed cells are expressing them

Future directions

- Confirm functional GxcA production by detection of heme in isolated proteins, and western blot detection using antibodies targeting the 6Xhis sequence
- Purify GxcA for future redox and localization experiments to determine its potential role in the electron transfer pathway of *G. fermentans*