Overexpression of the Pyruvate Kinase gene in *B. japonicum*

Sarah Popadowski
Department of Biochemistry, SUNY at Buffalo, Amherst, NY 14228

Introduction

Iron transport in *B. japonicum* is a complex process closely linked to manganese levels. It has been determined that the Irr protein is the major regulator of iron transport. Manganese is responsible for controlling iron transport through Irr degradation. The *mntH* gene is a major manganese transporter which is activated under manganese limited conditions. It has been shown in previous studies that under glycerol containing conditions, bacterial cells lacking the *mntH* gene will not grow in low manganese conditions, even with the presence of iron. However replacement of glycerol media with pyruvate subsequently allows the cells to grow. This suggests that in these mutants, the Pyruvate Kinase gene is somehow bypassed and is not functional. It is my job over this year and next to set up and carry out experiments which will explain this phenomenon.

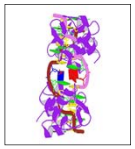

Figure 1. 3D model of the proposed structure the IRR protein.

Materials and methods

Bacterial cells from either the a wild type strain or a *mntH* mutant strain were added to growth media at a concentration of 1000000 cells/L. The growth of these cells were analyzed under varying amounts of iron and manganese via measuring light absorbance at 540nm. The results are shown in the next section. In order to over express the Pyruvate Kinase gene, primers encoding the gene for Pyruvate Kinase were designed and ordered. The segment of DNA of interest was then amplified using a polymerase chain reaction which takes the building blacks of DNA and a template in order to create more DNA. The product of my PCR experiment was subjected to separation and purification via a .8% agarose gel.

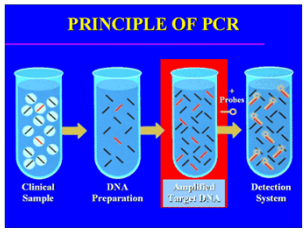

Figure 2. A depiction of how a Polymerase Chain Reaction is used to amplify a segment of DNA

After gel extraction, a ligation reaction was run in order to bind the segment of DNA into a pSK plasmid which could be taken up by e-coli cells via a transformation reaction which included heat shocking the cells and then an hour incubation. The cells were then plated on lb growth media containing Ampicilin and X-Gal. White blue selection was then achieved and five white colonies were selected for and placed in a small solution of liquid lb growth media and Ampicilin where they were incubated overnight. The next morning, the cells were spun down, and the plasmid extracted.

Results

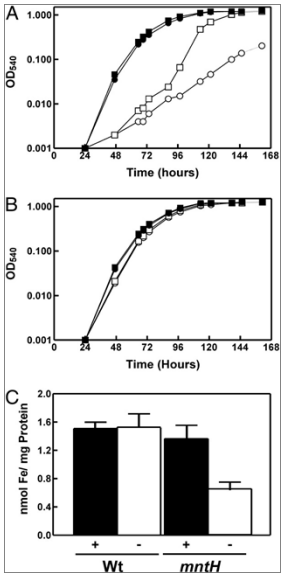

Figure 3. The above graphs show the results of several growth assays of bacteria under varying levels of manganese and iron. The growth of the cells was determined by measuring light absorbance at 540 nm

A. In the first assay both the wild type (closed) and the mutant strains (open) were placed in either unsupplemented (.3µM – circles) or iron containing conditions (20µM - squares) in the absence of manganese.

B. In the second assay Manganese was added at concentrations of 50µM (squares) or unsupplemented conditions of .4µM (circles)

C. Both strains were assayed for the amount of iron present as a function of the amount of protein present in each sample via absorption spectroscopy

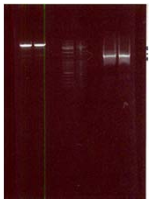

Figure 4. The gel analysis of my PCR product and a pET 14b frozen stock that has been digested by restriction enzymes BamH1 and NDE1.

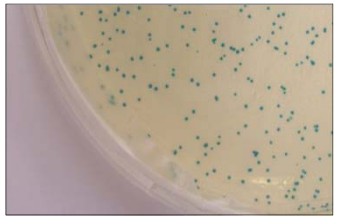

Figure 5. A close up photo of the e-coli stained plates showing blue white selection after a first transformation. The blue color indicates that the individual colony either did not take up the plasmid, or that it is nonfunctional.

Conclusions

The members of the lab who I work with have shown in previous studies that bacterial cells lacking the *mntH* gene will not grow in manganese deficient conditions containing glycerol. However using pyruvate as a carbon source allows the e-coli cells to grow in otherwise repressive conditions. With the addition of manganese (Figure 3B) it can be seen that the mutant strain seems to grow as well as the wild type. This suggests that the added pyruvate is somehow bypassing the Pyruvate Kinase gene to act as a fuel source. The presence of any resulting product after the PCR and the ligation reactions indicate that they were successful. The presence of blue and white colonies after plating indicates that the first transformation was successful in that the white colonies had a functional plasmid and the blue colonies did not. Sequencing of these results revealed that one out of the five chosen colonies produced plasmid containing the Pyruvate Kinase gene. However sequencing also indicated that this plasmid was impure. This could likely be the result of several different errors, including contamination of the frozen cells, or contamination from contact with a blue colony. So in order to make it viable, a second transformation was done with the plasmid for purification purposes. Blue white selection was again obtained and four white colonies were chosen for the purpose of setting up an overnight and plasmid extraction. The plasmids were subsequently sent for sequencing to confirm purity. Thus the gene encoding for Pyruvate Kinase has been successfully over expressed. In future studies we plan to then digest the plasmid utilizing the same restriction enzymes we used for the pET 14b sample, BamH1 and NDE1. We will run the sample on another gel.

Literature cited

O'Brian, Mark R. Hohle, Tom H. Puri, Sumant. "Control of bacterial iron homeostasis by manganese" PNAS 107.23 (2010): 10691-10695

Hohle TH, O'Brian MR (2009) The *mntH* gene encodes the major Mn(2+) transporter in *Bradyrhizobium japonicum* and is regulated by manganese via the Fur protein. Mol Microbiol 72:399–409.

Image of 3Irr. (de Rosa, M., de Sanctis, D., Rosario, A.L., Archer, M., Rich, A., Athanasiadis, A., Carrondo, M.A.) (2010) Proc.Natl.Acad.Sci USA **107:** 9088-9092

Acknowledgments

I would like to give special thanks to Tom Hohle for directing me and being especially patient with all my setbacks. Also I wish to thank everyone else at the lab, Sid, Sandra, Rosalba and for all their hard work and for making me feel truly accepted. I also would extend my deepest gratitude to Mark O'Brian for this wonderful opportunity and taking a chance on me. It's really their project.

For further information

Please contact *mrobrian@buffalo.edu*. More information on this and related projects can be viewed on the SUNY Buffalo Department of Biochemistry website.
http://medicine.buffalo.edu/content/medicine/faculty/profile.html?ubit=mrobrian