Effects of mutating the Mrub_1345 gene found in Meiothermus Ruber

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Effects of Site-Directed Mutagenesis on the *Meiothermus ruber* proC Gene

Brandon M. Wills and Dr. Lori R. Scott

**Introduction**

Proline is one of the important amino acids that plays a critical role in maintaining proper functioning in many organisms. According to Fichman et al., 2014, proline production gives certain types of cells increased tolerance to external stresses such as osmolality and temperature. Since the *Meiothermus ruber* bacteria live in high temperature environments, we thought this might prompt it to produce increased levels of proline. A variety of bioinformatics tools were used to analyze the Mrub1345 gene which suggested that it may be orthologous to a gene in *E. coli* called proC. Subsequently, we used complementation and site-directed mutagenesis to confirm Mrub1345 function. As shown in the figure below, the *E. coli* version of this enzyme is part of the proline biosynthesis pathway. This enzyme, called pyrroline-5-carboxylate reductase, is responsible for catalyzing the last reaction of this pathway, producing L-proline from (S)-1-pyrroline-5-carboxylate.

![Proline biosynthesis pathway](image)

Figure 1. Metacyc pathway showing the proline biosynthesis pathway found in *E. coli*. Image was taken from metacyc.org

The goal of this study is to use site-directed mutagenesis to swap out amino acids in the Mrub1345 gene and show that this has a significant effect on proline production. In this study, we hypothesized that the swapping of a highly conserved glycine residue within the Mrub1345 gene will cause the proline biosynthesis pathway to become non-functional and the cell will fail to grow in conditions in which proline is absent.

![Stratgene Lightning Quickchange site-directed mutagenesis](image)

Figure 2. Stratgene Lightning Quickchange site-directed mutagenesis

**Materials and methods**

- Obtained *E. coli* proC, proK1 and Mrub-1345 proK1 clones
- Confirmed insert identity via sequencing
- Transformed into null strains
- Complementation assay
- Sequenced strain used for complementation
- Site-directed mutagenesis G11D in wild type strain
- Retransformation into null strains
- Repeat complementation assay
- Sequenced to confirm mutation
- Repeat site-directed mutagenesis

**Results - Functional**

![Successful amplification of proC and Mrub1345 genes isolated from *E. coli* and Meiothermus ruber, respectively. Lane 1: 15µL, 1x ladder; lane 2: 15µL, *E. coli* proC M1; lane 4: 15µL, *E. coli*-proC M2; lane 6: 15µL, *M. ruber* M1; lane 8: 15µL, *M. ruber* M2.](image)

Figure 3. Successful amplification of proC and Mrub1345 genes isolated from *E. coli* and Meiothermus ruber, respectively. Lane 1: 15µL, 1x ladder; lane 2: 15µL, *E. coli* proC M1; lane 4: 15µL, *E. coli*-proC M2; lane 6: 15µL, *M. ruber* M1; lane 8: 15µL, *M. ruber* M2.

**Results - Annotation**

![Mutations induced in the Mrub1345 gene seemed to have no effect on proline biosynthesis. Plate A was prepared using minimal media; plate B was prepared using minimal media + proline. Sector 1: *Escherichia coli* K12 strain; Sector 2: proC null strain; Sector 3: proK1 + null; Sector 4: *E. coli* 2014-195-proK1; Sector 5: Mrub1345-proK1 mutation 1; Sector 6: Mrub1345-proK1 mutation 2; Sector 7: Mrub1345-proK1 mutation 3. Mutations for all three experiments were done.](image)

Figure 4. Mutations induced in the Mrub1345 gene seemed to have no effect on proline biosynthesis. Plate A was prepared using minimal media; plate B was prepared using minimal media + proline. Sector 1: *Escherichia coli* K12 strain; Sector 2: proC null strain; Sector 3: proK1 + null; Sector 4: *E. coli* 2014-195-proK1; Sector 5: Mrub1345-proK1 mutation 1; Sector 6: Mrub1345-proK1 mutation 2; Sector 7: Mrub1345-proK1 mutation 3. Mutations for all three experiments were done. Plate B saw growth on sectors 1, 4, 5, 6 and 7; Plate C saw growth on all 7 sectors that bacteria were spread on.

**Conclusions and Reflections**

Although we did not obtain the results we anticipated, this experiment demonstrated that mutating the G11 amino acid seems to have no effect on pyrroline-5-carboxylate reductase function. This came to us as a surprise since the bioinformatics analysis via BLAST showed that this residue was highly conserved in many different species of bacteria. One reason that this mutation may not have had an effect on proline biosynthesis is because there is another glycine residue at the 9th position that might be able to somehow maintain proper enzyme conformation even with the mutation. However, according to Betts et al. (2003), any mutational change that is made on a glycine residue should significantly alter protein structure. It's interesting to note that this was not the case when we swapped out the G11 of the proC gene with an aspartic acid residue. We are currently testing out another mutation induced via site-directed mutagenesis that swaps a threonine residue with an alanine residue. A mechanism found in an article written by Nociek et al. (2015) shows that a Thr226 is one of the active site residues that aids in catalysis of the substrate. By swapping this important residue, we are hoping to prove that the proC gene of *Meiothermus ruber* does in fact play an important role in proline biosynthesis. A BLAST analysis revealed that the mutation was present in the proC gene. Unfortunately, we did not have enough time to explore this mutation.

![Mechanism of pyrroline-5-carboxylate reductase in *N. meningitidis* and *S. pyogenes* showing the Thr226 residue.](image)

Figure 5. Mechanism of pyrroline-5-carboxylate reductase in *N. meningitidis* and *S. pyogenes* showing the Thr226 residue.

**Literature cited**

