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

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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 stressors such as osmolarity 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.

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 hypothesize 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.

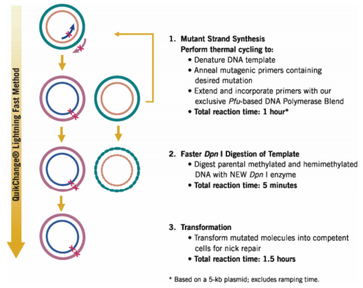

Figure 2. Stratgene Lightning Quickchange site-directed mutagenesis

Materials and methods

Obtained *E. coli proC*-pKt1 and *Mrub1345*-pKt1 clones

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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 - Annotation

RESULTS

Table 1: *E. coli proC* and *Mrub_1345* are orthologs

Bioinformatics tool used	*E. coli b_0386* gene (*proC*)	M. ruber *Mrub_1345* gene
BLAST *E. coli* against M. ruber	Score: 129 bits E-value: 1e-39	
CDD Data (COG category)	COG Number: COG0345 Pyrroline-5-carboxylate reductase	
	E-value: 9.14e-82	E-value: 2.27e-117
Cellular Localization	Cytoplasm of the cell	
TIGRfam – protein family	TIGR00112 Pyrroline-5-carboxylate reductase	
	E-value: 1.9e-101	E-Value: 2e-72
Pfam – protein family	Pf03807 (NADP oxidoreductase coenzyme F420-dependent & Pf14748 (Pyrroline-5-carboxylate reductase dimer)	
	E-values: 1.5e-24 2e-38	E-values: 4.9e-11 6.1e-35
Protein Database	3GT0 pyrroline-5-carboxylate reductase crystallized in *bacillus*	
	E-value: 3.09938E-72	E-value: 3.10687E-72

Table 1 summarizes the results from a variety of bioinformatics tools that were used to compare the *E. coli proC* gene to Mrub1345.

Results – Functional

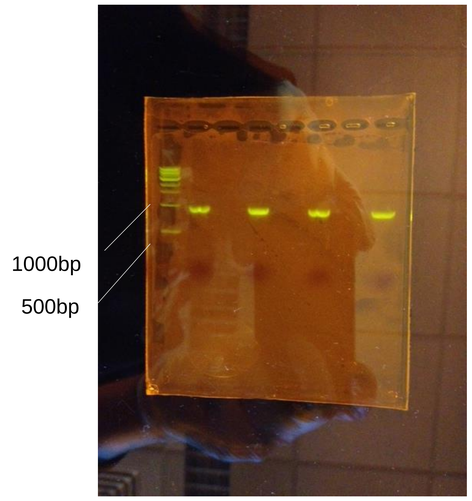

Figure 3. Successful amplification of *proC* and Mrub1345 genes isolated from E. coli and Meiothermus ruber, respectively. Lane 1: 10µL 1kb ladder; lane 2: 15µL E. coli *proC* M1; lane 4: 15µL E. coli *proC* M2; lane 6: 15µL *M. ruber* M1; lane 8: 15µL *M. ruber* M2.

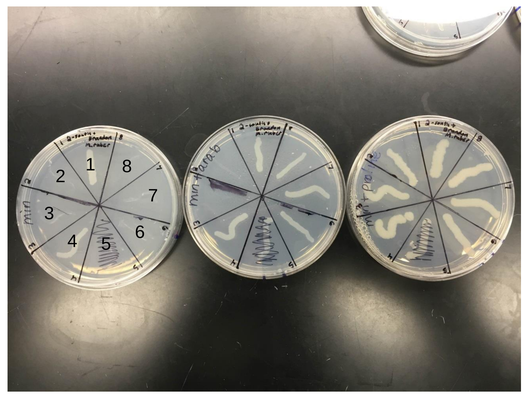

Figure 4. Mutations induced in the Mrub1345 gene seemed to have no effect on proline biosynthesis. Plate A was prepared using minimal media; plate B was prepared using minimal media + arabinose; plate C was prepared using minimal media + proline. Sector 1: *Escherichia coli K12* strain; Sector 2: *proC*- null strain; Sector 3: pKt1 + null; Sector 4: *E. coli* 2014-2015-pKt1; Sector 5: Mrub1345-pKt1 mutation 1; Sector 6: Mrub1345-pKt1 mutation 2; Sector 7: Mrub1345-pKt1 mutation 3. Mutations for all three experimental sectors were the point mutation G11D. Plates were incubated for a 24hr time period to allow for sufficient growth. Colonies were seen in sectors 1 and 4 on plate A; 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 Nocek *et. al* (2005) 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.

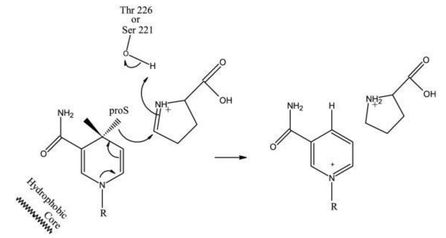

Figure 5. Mechanism of pyrroline-5-carboxylate reductase in *N. menegitides* and *S. pyogenes* showing the Thr226 residue.

Literature cited

Nocek B, Chang C, Li H, Lezondra L, Holzle D, Collart F, Joachimiak A. 2005. Crystal structures of delta1-pyrroline-5-carboxylate reductase from human pathogens Neisseria meningitides and Streptococcus pyogenes. J Mol Biol 354(1):91-106

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