Translating to “temperature-loving” and “red-pigmented”, *Meiothermus ruber*, is eubacteria that most successfully survives in temperatures near 60 degrees Celsius, like hot springs. This prokaryote is characterized as gram-negative, meaning the cell envelope is sequentially composed of an inner cytoplasmic cell membrane, peptidoglycan cell wall, and then a bacterial outer membrane. Unlike eukaryotic cell, prokaryotic DNA is circular, and some of the genes may be found on a plasmid, which is like a holder for additional genetic information.

There is little research on *M. ruber*’s biological processes, specifically the biosynthesis of proline. Studying *M. ruber* may help provide more knowledge of unknown microbes and their diverse biological pathways and processes. We wanted to determine if *M. ruber* uses the same biological pathways as model organisms like *Escherichia coli*. *E. coli* is a proline prototroph, as in, it can synthesize its own proline under minimal survival conditions. Previous research revealed structural similarities between *E. coli* and *M. ruber*, including the same protein family and well as two protein domains. Similar enzymes and protein location were predicted using bioinformatics tools. Structural similarities to *E. coli* suggest that *M. ruber* might follow similar biological pathways, specifically, proline synthesis. Proline is an essential amino acid in many bacteria that is used as a structural component for the changes in direction of a polypeptide chain. Proline’s role in physiological processes includes being used as an energy source and aids in maintaining osmolarity of the cell.

To study proline synthesis in *M. ruber*, plasmid DNA (pDNA) was isolated in eight different strains in *E. coli* hosts. These included wild-type and mutant type proC of *E. coli* and *M. ruber* in the presence and absence of the pKt1 plasmid. Isolation of the pDNA was followed by a polymerase chain reaction (PCR) of each strain to exponentially increase replication. This was done by denaturing the DNA strand to separate them for annealing primers. The primers bind to complementary base pairs on the single-stranded template which initiates extension by deoxyribonucleotides rapidly binding in the 5' to 3' direction. pDNA of the strains were loaded into lanes of an agarose gel submersed in a buffer solution. This technique, called agarose gel electrophoresis involves using electrical currents that pass through the buffer solution and negatively charge DNA migrates through the gel based on its molecular weight. Strands that are larger or longer in base pairs will move slower through the gel while smaller fragments will travel farther. Results are compared to a “molecular weight marker” that shows known strand lengths.

Selective media was utilized to assess the bacteria’s ability to survive in specific environments and confirm successful insertion. Because the pKt1 vector is antibiotic-resistant, *E. coli* that did not contain the inserted proC gene (proC+) would die if exposed to ampicillin and kanamycin. While ampicillin is an antibiotic that targets cell wall formation, kanamycin can cause protein malformation from mRNA misreading. Two antibiotic environments were used because proC is sensitive to both and successful insertion would be presented if growth was found in both conditions. Both are used to create valid and accurate results that confirm proC was inserted in the pKt1 plasmid. Minimal media was used as a control media because it contained only the necessary nutrients for bacteria to survive.

Once proC was confirmed, pDNA from each strain went through another cycle of PCR and agarose gel electrophoresis for extraction of DNA. Gel extraction involved excising relevant fragments of DNA with minimal gel for purification. The excised DNA was heated and
centrifuged until the gel had liquified, then purified through an elution column. The purified DNA was prepared and sent to the University of Iowa for sequencing.

While our sequences were being processed, we proceeded to use site-directed mutagenesis (SDM) for inserting a polyhistidine tag, to purify the proteins. PCR was done before incorporating the polyhistidine tag into the coding region of proC. This was done through a KDL reaction, which involves kinase to phosphorylate the 5’ ends for ligase to recircularize the linear DNA. A restriction enzyme (Dnpl) followed and removed methylated DNA leaving the purified strand. *E. coli* host cells are put through heat shock for the pKtl/proC to be taken up for transformation. Selective media of bacteria with pKtl/proC was used to confirm successful insertion by only allowing cells with the inserted gene to grow. Histidine tagging was used for SDM because of strong purification mechanism using immobilized metal-affinity chromatography (IMAC). The polyhistidine tag was inserted using a designed PCR primer that binds to the 3’ end of the proC.

Single, double, and triple deletion mutations in PAY176-178 region of the bacterium were also used to record loss of function. Complementation assays revealed that *M. ruber* showed loss of function only when there was a triple deletion mutation. *M. ruber* mutated bacteria were found to not grow on minimal media, when mutated *E. coli* suggesting a possible difference in proline synthesis. The bioinformatics tool, Expasy, was used to translate DNA sequences to amino acids and EMBOSS was used for pairwise analysis against the known *E. coli* proC+ sequence. Pairwise analysis of *E. coli* proC+ and our sample showed unsuccessful addition of a histidine tag. This was shown by the unwanted mutations on the COOH terminus of the protein.

Due to the meticulousness of the academic research, we assisted Dr. Lori Scott to maximize efficiency and accuracy of each experiment. We regularly discussed the research, while also carefully recording and analyzing the data collected. With the help of Dr. Lori Scott, we prepared materials for submission to research agencies.