May 12th, 11:00 AM - 1:00 PM

Reverse Gyrase is Not Necessary for Survival of Hyperthermophilic Archaeon Pyrococcus Furiosus

Farshid Taghizadeh

Michael S. Bartlett
Portland State University

Reverse Gyrase Is Not Essential For Survival Of Hyperthermophilic Archaeon *Pyrococcus furiosus*

Farshid Taghizadeh, Dr. Michael Bartlett

Portland State UNIVERSITY

Background:

Reverse gyrase is the only known topoisomerase protein with positive supercoiling activity on covalently-closed DNA. This positive supercoiling is required to prevent DNA from denaturation at high temperatures[1] (Figure 1). The gene that codes for this protein is present in all hyperthermophiles and absent from all mesophilic and thermophilic genomes, suggesting that this protein is the only hyperthermophile-specific protein.[2] To investigate if this protein is vital for the cells, we knocked out its gene from the genome of living organism, *Pyrococcus furiosus*. *Pyrococcus furiosus* is a hyperthermophilic, anaerobic archaeon that grows between 70°C to 103°C with an optimum growth temperature of 100°C.[3]

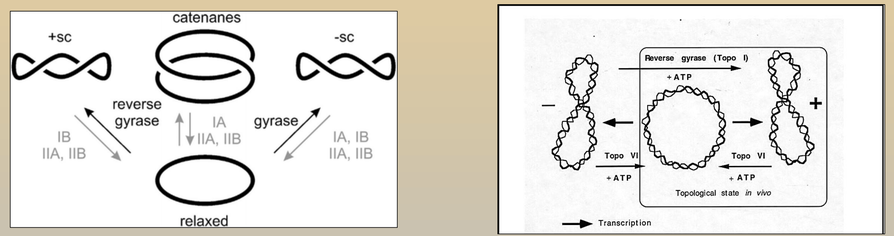


Figure 1. Left: General classification of topoisomerases. Right: Topodymanic in *Pyrococcus furiosus*

Methods:

Recombinant DNA was made using Gibson Assembly technique. In this technique, DNA fragments become assembled into one fragment by using a mixture of exonuclease, DNA polymerase, and DNA ligase enzymes (Figure 2). DNA fragments were included: left flanking region of Pf0495 gene (the gene that codes reverse gyrase), pyr F promoter, gdhP (uracil synthesis gene) and right flanking region. The recombinant DNA was sequenced to confirm the correct construction. This DNA which included a selection marker for ability to synthesize uracil, was cloned in *E. coli* and used to transform *Pyrococcus furiosus* strain 'COM1' (Figure 3). Uracil prototrophs were selected on growth medium lacking uracil, and the deletion of the target gene was confirmed using PCR. All cultures were incubated in water bath or air incubator at 90°C. To check for phenotypic changes, mutants and wild-type cells were inoculated into cellobiose medium and incubated at temperatures ranging from 75°C to 95°C with 5°C increments. We also performed heat shocks by incubating the cells at 105°C for one hour.

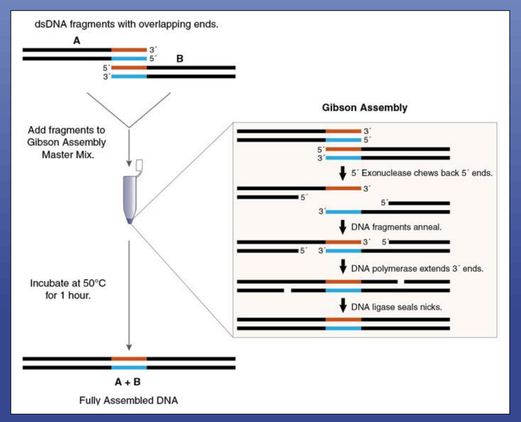


Figure 2. Gibson Assembly

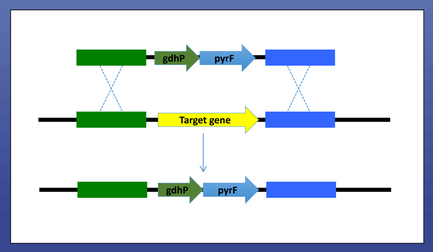
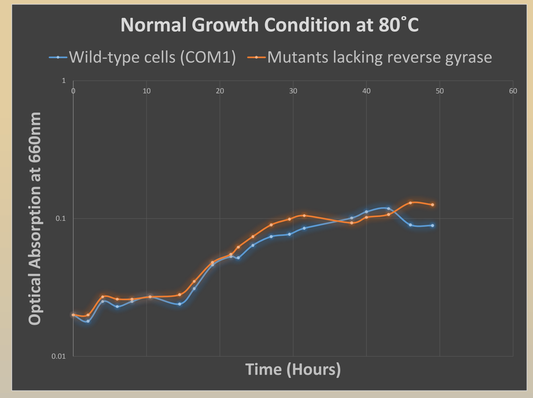
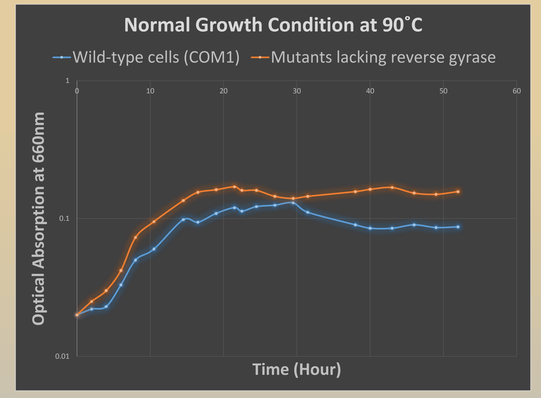


Figure 3. Knocking out a gene via double-crossover homologous recombination

Results:

Although no difference was observed between growth rates of the mutants and wild-type cells at temperatures ranging from 75°C-93°C , mutants do not survive in temperatures over 93°C. Below, two growth curves at normal conditions (80°C and 90°C) are shown as samples, along with the growth curve related to the heat shock at 105°C.





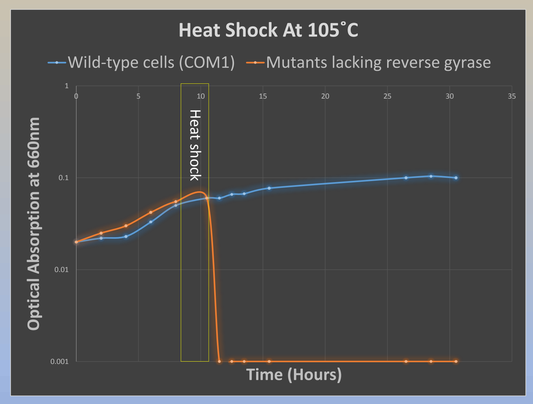


Figure 4. Upper left: Growth curves under normal condition at 80°C. Upper right: Growth curves under normal condition at 90°C. Bottom: Growth curves while undergoing a heat shock at 105°C for 1 hour.

Conclusions:

The fact that we were able to knock out the gene that codes for reverse gyrase shows that this protein is not essential for survival of *Pyrococcus furiosus* under normal conditions. In addition, mutants lacking reverse gyrase show the same growth rate as wild-type cells in temperatures from 75°C to 93°C which further strengthens the above statement. However, mutants don't survive in temperatures above 93°C which is consistent with the assumption that reverse gyrase is necessary to prevent denaturation of DNA at high temperatures.

References:

1. Atomi, H., Matsumi, R., & Imanaka, T. (2004). Reverse gyrase is not a prerequisite for hyperthermophilic life. *Journal of bacteriology*, *186*(14), 4829-4833.
2. Nadal, M., Jaxel, C., Portemer, C., Forterre, P., Mirambeau, G., & Duguet, M. (1988). Reverse gyrase of Sulfolobus: purification to homogeneity and characterization. *Biochemistry*, *27*(26), 9102-9108.
3. Borges, K. M., Bergerat, A., Bogert, A. M., DiRuggiero, J., Forterre, P., & Robb, F. T. (1997). Characterization of the reverse gyrase from the hyperthermophilic archaeon Pyrococcus furiosus. *Journal of bacteriology*,*179*(5), 1721-1726.