**MutL induced endonuclease activity in Cyanobacteria**

**Introduction**

Cyanobacteria are an ancient photosynthetic class of eubacteria with cosmopolitan distribution [1] . They are major producers of the Earth’s atmospheric oxygen and among the oldest organisms on the planet, having existed for more than 3 billion years. Cyanobacteria colonize a wide range of ecosystems, due to their ability to acclimate to a extreme environments. Cyanobacteria can be found in solid, air, dry rock, and aquatic systems, due to their ability to survive various adverse physiological growth conditions like desiccation, high temperatures, extreme pH, cold, osmosis, salt, light, nitrogen, and high salinity. Due to their age and robustness they serve the as the ideal candidates for investigating biological processes such as photosynthesis, respiration, photoregulation of gene expression, developmental gene rearrangements, and much more [2].

Cyanobacteria are known for their ability to perform as microbial factories that use the Earth's most ample natural resources; CO2, sunlight, and water for the production of chemicals. However, genetic instability, in cyanobacteria engineered for biotechnological purposes, occurs when using DNA recombination to repressess/regulate the expression of the recently popularized genes of industrial interest [3]. Therefore, a more exhaustive understanding of DNA recombination and repair in cyanobacteria could help increasing their robustness and the genetic stability of the engineered strains[3].

Repetitive sequences are prevalent features of genomes throughout the bacterial kingdom, and vary in factors such as mobility, length frequency, and spatial organization.

Commonly occurring sequences act as substrates for rearrangement of genomic regions, as well as deletion and duplication. They play an integral role in contributing to genome evolution and plasticity. High frequency sequences in bacteria can act as enforcers of genetic exchange, and participate in the regulation of genes and gene products[4].

Highly Iterative Palindrome-1 (HIP1) is an octamer palindrome (GCGATCGC) that appears in high frequencies in, almost exclusively, cyanobacterial genomes. HIP1 frequency can reach as high as one per 350nt. This ratio of occurence, suggest that multiple HIP1 motifs are associated with every single gene, on average. Such widespread over-abundance conserved over time suggests an important and functional role for HIP1 in the biology of cyanobacterial genomes. However, the molecular and functional roles of HIP1, if any, as well as the reasons for its high frequency, remain unclear[4] Understanding the role of HIP1’s functionality in Cyanobacteria may play a role in its biotechnological utility[4].

All living organisms possess a system for repair of non canonical nucleotide pairs or mismatches and small loops, named the methyl directed mismatch repair system. Failure of the MMR system at any stage can increase mutation frequency, leading to the development of various types of tumours. Wyrzykowski [8] demonstrates MMR in E.coli is conducted and dependent on the behavior of three proteins; MutH, MutS, MutL. Unlike MutL and MutS, MutH, the protein responsible for marking mismatched DNA near hemimethylated GmeATC/GATC, is not found in cyanobacteria.Thus, the process of identifying mismatched DNA by methylation in cyanobacteria is unclear, along with the roles of MutS and MutL. Elhai[6] proposed that for a phylogenetically distinct group of cyanobacteria, “MMR is directed by GmeC, where the methylated cytosine is usually contributed by a CGATCG-MTase that methylates the first cytosine, ultimately leading to frequent HIP1 sites.” This experiment will provide laboratory evidence for the existence of a GmeC- specific MMR in cyanobacteria, by testing whether MutL has GmeC- specific endonuclease activity in *Calothrix PCC 7103*.

**Experiment**

MMR corrects DNA damage by excising an extended single-stranded fragment of the newly synthesized DNA and then filling the resulting gap. The signal for the MMR is hydrolysis of the phosphodiester bond in one of the DNA strands. This experiment, following the lead of Monakhova, will be done in four parts; construction of plasmids containing the MutL gene from a species of Cyanobacteria, protein purification of MutL, and hydrolysis of plasmid DNA by MutL.

The first step is construction of the plasmids containing the rsMutL gene. The open reading frame of mutL gene will be amplified from *Calothrix PCC 7103* genomic DNA by PCR with Pfu DNA polymerase using gene-specific primers containing a 5’-overhang that introduced a restriction site. The primers would be designed based on the annotated complete genome sequence of *Calothrix PCC 7103.* The product of the PCR would be treated and cloned into a plasmid vector that contains the sequence.

The purification process of MutL would use methods described from *N. gonorrhoeae* [9], and includes cell lysis by sonication, centrifugation of the lystate, and chromatography of the resultant supernatant on Ni-NTA-agarose, to yield homogenous MutL protein.

Linear DNA substrates with the potential mismatch and hemimethylated GMeC/CG sites were generated, by methods proposed by Winkler [10], from PCR products amplified by Pfu DNA polymerase similarly as described earlier. The product of the PCR would be treated and cloned into a plasmid vector that contains the hemimethylated site. Hydrolysis of plasmid DNA by MutL would begin by incubating the MMR-plasmid with the MutL. DNA hydrolysis efficiency would be measured by the amount of formed nickel DNA (plasmid with a single-stranded break). The reaction mixture would be analyzed by electrophoresis[5].

**Discussion**

Results from this experiment have the potential to demonstrate that MutL from *Calothrix PCC 7103* is endonuclease capable of introducing single-single breaks in DNA[7]. This result would provide evidence to support the possibility of a GmeC- specific MMR system in cyanobacteria, and possiblying aid in increasing the stability of engineered cyanobacterial based industrial applications in the future. If MutL was unable to hydrolyze the DNA, it may suggest lack of involvement of MutL in MMR system. Potential pitfalls include being able to acquire *Calothrix PCC 7103,* and alternatives with similar phylogenetic profile may need to be considered.

The experiment could be more expansive and exhaustive if the effect of different divalent metal cations on DNA hydrolysis were also considered, so that rate of hydrolysis could be compared. The effect of ATP on MutL endonuclease activity could also be another variable to consider.

**References**

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