Molecular Cloning and Structural Studies on ATase from *Pyrococcus horikoshii* OT3.

R. Guru Raj Rao[1], K. Sekar[3], Chung-Jung Chen[2], J. Jeyakanthan[1*]

[1]Structural Biology & Bio-Computing Lab, Department of Bioinformatics, Alagappa University, Karaikudi - 630004, Tamil Nadu, India.

[2]Life Science Group, Scientific Research Division, National Synchrotron Radition Research Centre, Hsinchu, 30076, Taiwan.

[3]Department of Computational and Data Sciences, Indian Institute of Science, Bangalore - 560012, India.

* E-mail : jjkanthan@gmail.com

Purine biosynthesis is perceived as vital process because of their indispensable role involved in creating genetic material that is DNA and RNA. Amidophosphoribosyltransferase (ATase) catalyzes first step in de novo purine biosynthesis pathway facilitating that synthesizes of N(1)-(5-phospho-D-ribosyl)glycinamide from 5-phospho-alpha-D-ribose 1-diphosphate. Hence, inhibiting ATase is crucial due to its involvement in the regulation of uncontrollable cancer cell proliferation. In this study, the gene Amidophosphoribosyltransferase (ATase) is amplified using PCR from *P.horikoshii* OT3 genome as a template with gene specific primers cloned in pETM11 vector utilizing KpnI and NcoI restriction enzymes. The clone was transformed into *E.coli* DH5α and colony PCR was performed to assess the orientation. Double digestion of clone results in insert release, the clone was confirmed using sequencing. The protein was trypsin digested, confirmed using MALDI-TOF analysis as ATase enzyme. The clone was further transformed in *E.coli* BL21 DE3 cells for over expression of the protein; induced using IPTG and cells were harvested by centrifugation and sonicated. The lysate was heated at 75 C for 15 min and the supernatant was loaded on to a Nickel-NTA agarose column, and subjected to further purification using Superdex G200 column. The protein was subjected to 10% SDS-PAGE analysis and was found to be pure (free from contaminants) and the concentration of protein is 12 mg/ml. The protein was further subjected to crystallization using Hampton crystal screen kit, crystals are obtained in peg ion B-22 condition, which has to be further optimized to get diffract-able quality crystals. Further X-ray studies will be done to solve the structure.

Keywords: Cloning, crystallization, ATase, De-novo and purine biosynthesis.

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