In-vitro Studies on Aspartokinase for Designing Structure Guided Inhibitor against Lymphatic Filariasis

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Abstract

Aspartokinase (AK) catalyzes the first step in aminoacid biosynthesis pathway to produce essential aminoacids such as lysine, threonine, methionine and isoleucine in all microbes and plants. This pathway is essential for survival of all plants and microorganisms but found absent in mammals. Deletion of AK gene also causes fatal effect in microorganisms, thus making AK an attractive target for synthesis of new antibiotics. In this study, we target AK from *Wolbachia* endosymbiont of *Brugia malayi*; causative agent of lymphatic filariasis. AK gene encoding Aspartokinase from *Wolbachia* endosymbiont of *Brugia malayi* (wbm0441) was isolated and amplified using desired forward and reverse primers. The amplified product was cloned into pET30a+ vector and transformed into *E.coli* BL21 DE3 cells. *E.coli* containing target gene was induced using 1mm IPTG(Isopropylthio-beta-D-galctoside) concentration and the expressed protein was purified using various chromatography techniques such as Affinity, Ion exchange and Size Exclusion Chromatography. The purified homogenous protein was confirmed in 10% SDS-PAGE and western blot technique. Finally the protein was concentrated to 12 mg/ml using amicon concentrator and subjected to crystallization trials.Optimization of crystal condition is under progress.

Keywords: Lymphatic Filariasis, Aspartokinase, IPTG, Affinity Chromatography, Gel Filteration Chromatography, SDS-PAGE.

Acknowledgment: Authors greatly express their sincere gratitude to DST SERB/EMR/2016/000498 dt 26.09.2016, DST-FIST (SR/FST/LSI-667/2016) (C), DST-PURSE Phase-II (No. SR/PURSE Phase 2/38 (G), 2017), UGC- Innovative F.No. 14-13/2013 (Inno/ASIST) dt: 30.03.213. DAE-BRNS (35/14/02/2018-BRNS/35009), UGC-RA (No. F. 30-32/2016(SA-II) dt 18.04.2016), and MHRD-RUSA 2.0 (F.24-51/2014-U, Policy (TNMulti-Gen), Dept. of Edn. Govt. of India, Dt.09.10.2018, MOST 107-2923-B-213-001-MY3 and MOST 108-2311-B-213-001-MY3 to Dr. Chun-Jung Chen from Taiwan.