

Evaluation of ELP Tag Efficiency in Protein's Purification from Expression of IpaD-STxB Antigen in *E.coli BL21(DE3)*

Atefeh Ghasemi

1.M.sc.Student ,Department of Agronomy and Plant Breeding, College of Agriculture and Natural Resources University of Tehran, karaj, Iran.

One of the novel methods of recombinant protein purification and reducing the cost of production is the method does not require the use of chromatography. Elastin-like polypeptides are repeats of the amino acids ''VPGXG'' that undergo a temperature dependent phase transition and have utility in the purification of recombinant proteins but can also enhance the accumulation of recombinant proteins that are fused. To investigate the effect of the sequence on the efficacy of *IpaD-STxB* chimeric protein purification in total protein of transformed *E.coli BL21*. We have used a 29.7 kDa elastin-like polypeptide as a fusion partner for recombinant *IpaD-STxB* protein. In this study in order to assemble the pET28α-IpaD-STxB-Elp and pET28α-IpaD-STxB vector, the 810 nucleotide ELP fragment was first added to the 3'end of IpaD-STxB fragment using *SOEing PCR*.each fragment was cloned into PET28α vector for bacterial transformation. The transformation of these vectors *into E. coli BL21 (DE3)* which was confirmed by PCR and enzymatic incision. The expression of the antigen was evaluated. IpaD-StxB and IpaD-stxB-ELP genes were induced by IPTG, and after purification of the desired protein by the phase shift and his trap of IpaD-STxB-Elp and IpaD-STxB respectively, the produced IpaD-STxB recombinant protein was confirmed by SDS-PAGE and Western blot analyses. The amount of protein produced was evaluated by ELISA The purification was performed by added 2mM ammonium sulfate concentration and incubated at 37 ° C to precipitate the ElP-tagged protein. The *IpaD-STxB* target protein with a molecular weight of 55 kDa was found in SDS-PAGE. The pure protein reaction was confirmed with the anti-IpaD-StxB antibody in the western blot. The purified protein concentration was 40.5 mg / L. The ELISA test showed that the amount of purified protein by means of ELP was significantly higher than the pure protein derived from the His-Trap column. Elastin-Like Polypeptides can be recommended as a cost –and time –efficient purification tag and eliminated chromatography method for recombinant protein production in bacterial and herbal hosts.

Biography

Atefeh Ghasemi has graduated in B.S of agriculture-horticultural engineering from Shahre-Kord university at the age of 23 years and currently study agricultural Biotechnology in MS.C from the University of Tehran.

Presenting author details

Full name: Atefeh.ghsemi.ut.ac.ir
Contact number:
+989139850616
Twitter account:
Linked In account:ghasemi.bio23@gmail.com
Session name/ number:
Category: Poster presentation