Production of Recombinant Toxin-Coregulated Pilus TcpB from Vibrio cholera and Evaluation of Antigenicity

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Background & Objectives: *V. cholerae* is a typical non-invasive enteric pathogen which is spread by the faecal- oral route, that is, by the ingestion of contaminated food or water. TCP is essential for *V. cholerae* colonization of the small intestine both in an infant mouse model of cholera and during human infection. the aim of this work to design a recombinant vaccine of research in future.

Methods: In this study, The full coding sequence of tcpB was amplified by polymerase chain reaction (PCR) using specific primers containing BamHI and XhoI sites. The purified fragment and the pET-32a vector were digested by R.E. The fragment was ligated to the pET-32a vector. The ligation product was transformed into competent E. coli DH5α. For expression, the recombinant plasmid, tcpB-pET-32a, was transformed into competent BL21 (DE3) plysS. and gene expression was induced by IPTG. Then it was purified by Ni-NTA sepharose kit. The concentration of rtcpB was assayed by Bradford methods. Recombinant tcpB were further analyzed by Western Blot.

Results: The sequencing result was confirmed by Sanger methods and was same as tcpB gene. *Escherichia coli* BL21 (DE3) plysS was transformed with tcpB- pET-32a and gene expression was induced by IPTG. The expressed protein was purified by affinity chromatography by Ni-NTA resin. The concentration of purified protein was 3µg/ml. The integrity of product was confirmed by Western- Blot analysis using a mouse anti tcpB.

Conclusion: Our data showed that recombinant tcpB protein can be produced by pET-32a in *Escherichia coli* This protein as an antigen by sera in infected mice. Therefore, Recombinant tcpB has same properties with natural form of this antigen. The expression of recombinant proteins is a basical methods to vaccines could be designed.

Keywords: Toxin-Coregulated Pilus (TCP) Gene; *Vibrio cholerae*; Expression