Cloning and Expression of a Vibrio cholera Toxin Co Regulated Pilus a (TcpA), in Escherichia coli

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Background & Objectives: The toxin co-regulated pilus (TCP) has been identified as a critical colonization factor in both animal models and humans for Vibrio cholerae O1. The major pilin subunit, TcpA, is similar to type-4 pilins but TCP probably more appropriately belongs to a sub-class which includes the bundle-forming pilus of EPEC. The aim of this work to design a recombinant vaccine of research in future.

Methods: In this study, The full coding sequence of tcpA was amplified by polymerase chain reaction (PCR) using specific primers containing BamHI and XhoI sites. The purified fragment and the pGEX4T1 vector were digested by R.E. The fragment was ligated to the pGEX4T1vector. The ligation product was transformed into competent E.coli DH5α. For expression, the recombinant plasmid, tcpA-pGEX4T1, was transformed into competent E. coli BL21(DE3). and gene expression was induced by IPTG. Then it was purified by GST sepharose kit. The concentration of rtcpA was assayed by Bradford methods.

Results: The sequencing result was confirmed by Sanger Methods and was same as tcpA gene. Escherichia coli BL21 (DE3) was transformed with tcpA-pGEX4T1 and gene expression was induced by IPTG. The expressed protein was purified by affinity chromatography by GST resin. The concentration of purified protein was 6µg/ml.

Conclusion: Our data showed that recombinant TcpA protein can be produced by pGEX4T1 in Escherichia coli. The expression of recombinant proteins is a basical Methods to vaccines could be designed.

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