

## عنوان مقاله:

Insilico Design and Construction of a Chimeric Gene Comprising the A Subunit of Vibrio cholera Pilin and the B Subunit of Cholera Toxin

## محل انتشار:

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## خلاصه مقاله:

Background and Aims: Cholera is a lethal diarrheal disease that cause by Vibrio cholerae. Cholera toxin and colonization factor pili (tcpA) are the major virulence factor in V.cholerae pathogenesis. The B subunit of the enterotoxin (ctxB) which is responsible for toxin binding to eukaryotic cells and toxin-coregulated pili A (tcpA) that is essential for V.cholerae colonization, have immunogenic properties. Chimeric proteins carrying epitopes, linkers or adjuvant sequences could increase immunogenicity for recombinant antigens and can also elicit broad immune responses. The aim of this study was to design an immunogen against adherence and toxicity of V.cholerae. Materials and Methods: ctxB and tcpA genes were analyzed for rare codons and gene optimization was performed using optimization software In ۲۰۱۷. The half-life and protein instability index was determined. Secondary and tertiary structure was predicted and evaluated. Linear and conformational epitopes were predicted. Recombinant pET۲۸a/chimeric gene plasmid was transformed to E.coli BL۲۱ DE۳ and expression was induced with Isopropyl β-D-۱-thiogalactopyranoside (IPTG). The protein expression was evaluated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Results: Chimeric protein instability index was ۲۴.۰۴ and the codon adaptation index of chimeric constructs increased to ۰.۹. The tertiary predicted structure of the chimeric proteins confirmed and mRNA was stable. Conformational and linear epitopes were seen in both domains of the chimeric protein. Restriction analysis confirmed cloning of the gene into pET۲۸a vector. Expression of recombinant protein in E.coli led to the production of chimeric protein with ۳۷ k Da molecular weight. Conclusions: According to the results of bioinformatics and recombinant protein expression, the design chimeric protein could be used as an immunogen to evaluate the immunity against cholera.

## کلمات کلیدی:

,Vibrio cholerae, Virulence factors, Cholera toxin, Bioinformatic design, Chimeric gene

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