Purification and Immunological Characterization Evaluation of Recombinant Exotoxin A (Domains I, II)- Flagellin (N-terminal) Fusion Protein Isolated From Pseudomonas aeruginosa as Subunit Vaccine Candidate

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Background & Objectives: *Pseudomonas aeruginosa*, as an important bacterium in nosocomial infections, has several plasmids and chromosome mediated antibiotic resistance factors, and antibiotics have failed in treatment of P. aeruginosa infections. So because of high resistance, immunoprophlaxy and immunotherapy may be an effective methods for control and treatment of P. aeruginosa infections. Exotoxin A, the most toxic virulence factor, is an immunogenic protein that antibodies against it are very protective. The bacterial flagellum also acts as a strong immunogenic protein that induces antibody production by active and passive immunization. These antibodies neutralize bacterial motility and thus prevent infection dissemination. Therefore it seems that flagellin and Exotoxin A are promising factors for study as vaccine candidates. In this research, recombinant exotoxin A (Domains I, II)- flagellin (N-terminal) fusion protein was produced and its immunological characteristics were evaluated.

Methods: In this study, exotoxin A (Domains I, II)- flagellin (N-terminal) gene fusion was constructed by over lapping PCR Methods. Then, the ExoA-FliC fragment was ligated onto PET22b expression vector and ligation product was transformed into E. coli BL21. The protein expression was assessed by SDS-PAGE. The recombinant fusion protein was purified by Ni-NTA Affinity chromatography and it's identity and immunologic properties were evaluated by western blotting.

Results: The electrophoresis of overlapping PCR products on 1% agarose showed a 1722 bp band that was in accordance with ExoA-FliC fusion gene size. Sequencing of cloned gene was confirmed the ExoA-FliC fusion gene sequence. SDS-PAGE analysis revealed expression of recombinant protein with molecular weight of approximately 63kD that was in accordance with ExoA-FliC fusion protein.Optimization analysis showed that 2 hours induction with 1mM IPTG at 37C is the optimum condition for expression of recombinant protein. Evaluation of immunologic characteristics of recombinant fusion protein by western blotting showed that this protein is highly immunogenic and patients with P. aeruginosa infection contain antibody against this protein.

Conclusion: The results of this study suggested that recombinant Exotoxin A-flagllin fusion protein is a highly immunogenic protein and can be studied as candidate vaccine for *Pseudomonas aeruginosa* infections.

Keywords: *Pseudomonas aeruginosa*; Candidate Vaccine; Exotoxin A-Flagellin; Gene Fusion