Overlap Extension PCR a Simple and Reliable Way to Create ExoA-FliC Gene Fusion from Pseudomonas aeruginosa as Vaccine Candidate

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Background & Objectives: *Pseudomonas aeruginosa* (*P. aeruginosa*) is an important nosocomial infection agents. This bacterium has many antibiotic resistance factors, and antibiotics have failed in treatment of *P. aeruginosa* infections. *P. aeruginosa* has several virulence factors, each of these virulence factors play a key role in P. aeruginosa infections. Neutralization each of virulence factors can be neutralizes *P. aeruginosa* infections. The gene fusion techniques are used to produce a recombinant protein with multiple immunogenic functions. In this study, ExoA-FliC gene fusion and recombinant fusion protein as two important pathogenic factors, was product by Over Lapping PCR methods.

Methods: Genomic DNA was extracted with phenol – chloroform methods. ExoA and FliC genes were amplified separately by polymerase chain reaction (PCR). Overlapping PCR was performed in 25 µl volume by using of both ExoA and FliC PCR products as template DNA and ExoA forward and FliC reverse primers as pair primers. The gene amplification conditions were 95C(4min),30 cycles consisting of 94 C (1min),60 C (60S),72 C (1.5min) and an additional extension time at 72 C (10min).PCR products were electrophoresed on 1% agarose gel and bands were analyzed by gel document. The gel bands were carefully taken from gel. The gel containing fusion gene was recovered by using of MN (Macherey Nagel) purification kit The gene fusion was legated to PET22b victor, and then transformation of legation product to E. coli LB21 was performed. Protein expression was tested by SDS-PAGE.

Results: Overlapping PCR amplification of P. aeruginosa exotoxin A (Domains I, II) - flagellin (N-terminal) fusion resulted in a fragment of 1212 bp. Sequencing of the cloned gene confirmed the identity of PCR product. Expression by pET 22b vector and BL21 cells resulted in high level expression of recombinant protein that appeared as a dense band in SDS-PAGE analysis of cell lysates. The different methods such as multi-stage ligation and transformation are used for fusion gene construction. In this study, ExoA-FliC gene fusion was product by over Lapping PCR Methods (as a simple and rapid Methods).

Conclusion: The results indicated that overlap extension PCR is a simple, rapid and cost effective methods to create gene fusion and fusion protein preparation that can be used for production of multi valent vaccine candidates.

Keywords: *Pseudomonas aeruginosa*; Exotoxin A; Flagellin; Recombinant Protein FliC-Exo A