Original Article

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Production of a novel multi-epitope vaccine based on outer membrane proteins of *Klebsiella pneumoniae*

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Abstract

Klebsiella pneumoniae is a hospital-acquired pathogen that leads to various infections. Hence, efforts to develop an effective vaccine against that pathogen are well documented. Our interest is the production of the previously designed multi-epitope vaccine construct against the *K. pneumoniae* in a prokaryotic host. Therefore, a new construct containing the nucleotide sequence of the novel vaccine was successfully expressed in *Escherichia coli* and then purified by Ni-NTA spin column. The purified recombinant protein can be considered as potential vaccine candidate for wet-laboratory analysis aiming to fight *K pneumoniae.*

Keywords: Klebsiella pneumoniae, Multi-epitope vaccine, Vaccine production.

1. Introduction

Klebsiella pneumoniae is a member of Enteriobacteriacae, which lives in the human intestine flora (1). This bacterium causes hospital-acquired infections in patients undergoing certain diseases such as malignancy, diabetes mellitus (DM), hepatic and biliary tract disorders and alcoholism. Such pathological conditions may disturb the individual's immune responses and increase the chance of *K. pneumonia* infection. If these infections are not treated at early stages, they can lead to a high rate of morbidity and mortality (2).

Presence of lipopolysaccharides within the outer membrane of gram-negative bacteria makes a protective barrier that prevents the permeability of hydrophilic and hydrophobic elements (3). A series of channel proteins named the outer membrane proteins (Omps) are placed within the outer membrane of these bacteria to facilitate uptake of components, that are necessary for growth and function of the cell (4). In addition to transport function, Omps play an essential role in bacterial pathogenicity (5).

A number of Omps such as OmpA, OmpC, OmpW, OmpX and FepA from *K. pneumonia* have been recognized, which are able to induce robust humoral and cellular immune responses (6,7). Therefore, they are ideal candidates for vaccine development against K. pneumoniae.

Generally, vaccines provide protection against infectious diseases (8). Nowadays, most of the vaccines that are produced against *K. pneumonia* are based on native components such as fim-

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briae, capsular polysaccharides and lipopolysaccharides (9). It has been shown that these vaccines are unsuitable, because the immune responses triggered by *K. pneumonia* in nature are not optimally efficient to eliminate this pathogen. It is suggested that this pathogen can evade both innate and adaptive immune responses and are able to persist in the body even a life time (10). Therefore, the modified immunotherapies like an epitope-based vaccine is an efficient modality to fight against *K. pneumonia* (10).

Epitope-based vaccines consist of highly immunogenic protein sequences (indicated as T and/or B-cell epitopes) integrated into a single molecule (multi-epitope vaccines) can be recognized by the immune system. The immune responses that are induced by epitope vaccines include either cellular (induced by T-cells) or humoral (induced by B-cells) or a combination of both responses (11). Epitope-vaccines that induce strong cellular and humoral immunity usually contain cytotoxic *T lymphocytes* (CTLs) epitopes, helper *T lymphocytes* (Ths) epitopes and B-cell epitopes (12).

The currently available bioinformatics tools allow us to survey the target proteins of pathogens (including virus, bacterium or tumor antigen) for the presence of immuno-dominant B or/and T-cell epitopes to design efficient epitope vaccines (13).

A common bacterial system such as *E. coli* is widely used for the production of recombinant proteins. The advantages of *E. coli* as a recombinant host include high cell densities as well as product yields and possibility to modify the host genome quickly and precisely (14, 15, 16, 17).

A number of approaches can be applied for sub-cloning a protein-coding sequence of DNA into a pET vector for expression. All the pET expression vectors consist of translation stop codons in all three reading frames following the cloning and tag sites as well as a downstream T7 transcription terminator. pET vectors contain different regions near to the cloning sites that encode a number of peptide "tags", which carry out detection, localization or purification functions when fused with the target protein. The procedure of cloning will determine whether or not these "tags" or any extra amino acids from the plasmid are expressed as a fusion to the desired protein (18).

In our previous study, a multi-epitope vaccine, based on Omps of the *K. pneumonia,* was designed (19). In this study, the vaccine construct was produced in *E. coli* prokaryotic host.

2. Materials and methods

2.1 Recombinant vector preparation

The recombinant vector (pET23d+construct gene sequence) was obtained from Nedayefan (Tehran; Iran). The map for recombinant pET23d containing our construct is shown in Fig. 1.

2.2. Transformation

The recombinant vector

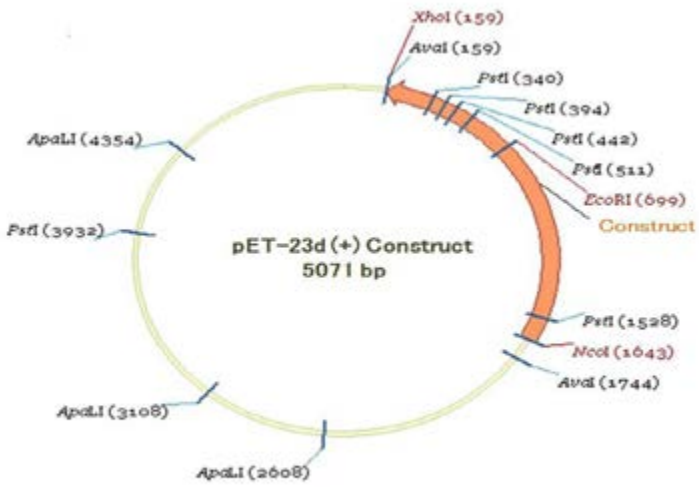

Figure1. The map of the Recombinant pET23d containing the gene sequence of the epitope vaccine.

(pET23d+construct gene sequence) was transformed into *E. coli* DE3 cells using TransformAid™ Bacterial Transformation Kit (Fermentas, EU).

2.3.Screening positive bacterial colonies (for recombinant plasmid)

The transformants (positive bacterial colonies) were screened as follows: at first, plasmids were extracted by AccuPrep® Nano-Plus Plasmid Mini Extraction Kit (Bioneer, Korea). Then, the DNA was digested using XhoI and NcoI FastDigest™ enzymes.

2.4. Agarose gel electerophoresis

The digested plasmid DNA was analyzed by electrophoresis through 1.5% agarose gel.

2.5. Protein expression

After culturing the recombinant cells at 37 ºC in LB medium containing 100 µg/ml ampicillin, the protein expression was induced as follows: 1 ml cultures (LB medium containing 100 µg/ml ampicillin) was inoculated with a transformed colony containing the recombinant vector. The cultures were incubated overnight at 37 °C to obtain a saturated culture. Then 20 ml of LB medium containing 100 µg/ml ampicillin was inoculated in a 50-ml flask with 200 µl of a saturated culture. The cultures were incubated for 2 hours at 37 °C. Each culture was induced by adding IPTG to a final concentration of 1.0 mM and the incubation was continued at 37 °C with aeration. At 4, 6 and 8 hours after induction, 1 ml of each culture was transferred to a microfuge tube, and the tubes were centrifuged at 4000 rpm speed for 5 minutes at room temperature. The supernatants were removed by aspiration. Each pellet was resuspended in 100 µl of 1x SDS gel-loading buffer and was analyzed using SDS-PAGE analysis.

2.6. SDS-PAGE analysis

The SDS-PAGE analysis of the pellet of *E. coli* DE3 containing recombinant vector (pET23d+construct gene sequence) after induction for 4, 6 and 8h was done to confirm the presence of the recombinant protein in the cytoplasm.

2.7. Western blot analysis

The expressed recombinant protein vaccine was confirmed by western blot analysis of the transformed *E. coli* cells. The expression of the protein was detected in the transformed cells using Anti-His6-Peroxidase. Anti-His6-Peroxidase is a mouse monoclonal antibody for the detection of histidine-tagged recombinant proteins, conjugated with peroxidase.

2.8. Purification of the poly histidine-tagged construct

The recombinant His-tagged construct was purified from *E. coli* DE3 lysates by applying Ni-NTA affinity column chromatography under denaturing condition (Qiagen, USA).

3. Results

3.1. Recombinant vector preparation and transformation of the construct

The recombinant vector (pET23d+construct gene sequence) was transformed into *E. coli* DE3, and the plasmid DNA was purified and digested. Agarose gel electrophoresis of the product confirmed the presence of the desired insert fragment for transformants (Fig. 2). Figure shows the presence of construct gene sequence (1500 base-pairs: bp) and pET23d (5000 bp).

3.2. Protein expression and molecular weight determination

After culturing the recombinant cells at 37 ºC in LB medium containing 100 µg/ml ampicillin, the T7 promoter was induced by 1 mM IPTG at 37 ºC for 4, 6 and 8h. The SDS-PAGE analysis of the pellets of *E. coli* DE3 containing recombinant vector (pET23d+construct gene sequence) after induction for 4, 6 and 8h confirmed the presence of the recombinant protein in the cytoplasm with a desired band at about 55 kDa, which was consistent with the expected molecular weight of the recombinant multi-epitope construct (Fig. 3). As the figure shows, expression of the construct after induction for 4h was higher than its expression after induction for 6 and 8h.

3.3. Western blot analysis

The expressed recombinant protein was confirmed by western blot analysis of the transformed *E. coli* cells (Fig. 4). 4h post-transformation samples were analyzed for presence of the protein vaccine using western blot. Our recombinant protein vaccine is ~55 kDa in size, which

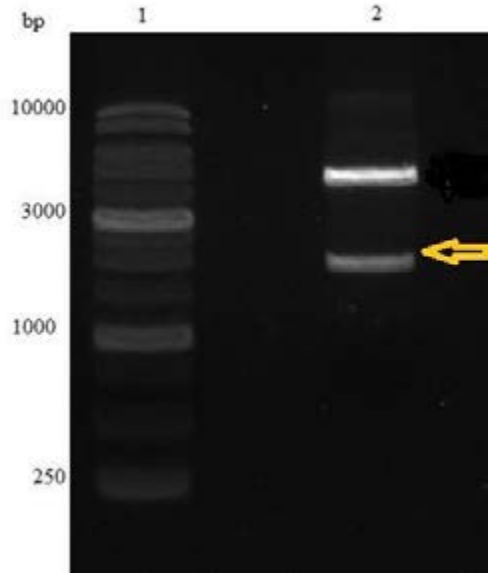

Figure 2. 1.5% Agarose gel electrophoresis of the digested recombinant vector (pET23d+construct gene sequence). Lane 1– 10 kb plus ladder, lane 2 – 4h post transformation cell pellet.

closely matched with the calculated values.

3.4. Protein purification

The presence of C-terminal His-tag at the end of the pET23d vector made it easy to purify recombinant construct using nickel-chelating resins in a denatured form. The recombinant His-tagged protein was successfully purified by applying Ni-NTA affinity column chromatography under denaturing condition. Monitoring of the protein purification by SDS-PAGE analysis represented a single band with a molecular mass of about 55 kDa as shown in Fig. 5.

4. Discussion

The bacillus *K. pneumoniae* has a world-wide distribution and is a significant microorganism responsible for human infections resulting in a high percentage of morbidity and mortality (2). Due to the increasing resistance to antibiotics, a variety of preventive and therapeutic approaches such as immunotherapy has been considered for fighting with this pathogen (20).

Traditional approaches for vaccine formulations, such as using killed or attenuated pathogen, possess a series of problems involving inflammation, failure to efficiently culture the pathogen, the probability of motivating an autoimmune reaction, and the requirement for refrigerated storage (21). To overcome these failures, epitope-based vaccines against pathogens can be a promising alternative.

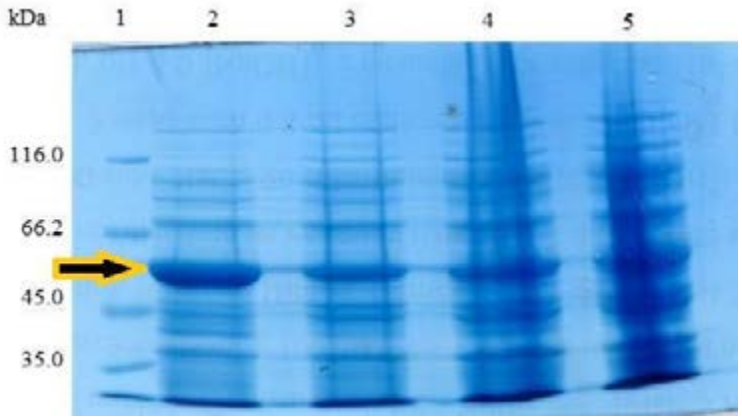

Figure 3. Construct (expected Mw: 55 kDa) run on SDS–PAGE and stained with Coomassie blue. Lane 1– molecular size marker, lane 2– transformed E. coli after inducing of expression for 4h using IPTG, lane 3– transformed E. coli after inducing of expression for 6h, lane 4– transformed *E. coli* after inducing of expression for 8h, lane 5– transformed *E. coli* before inducing of expression.

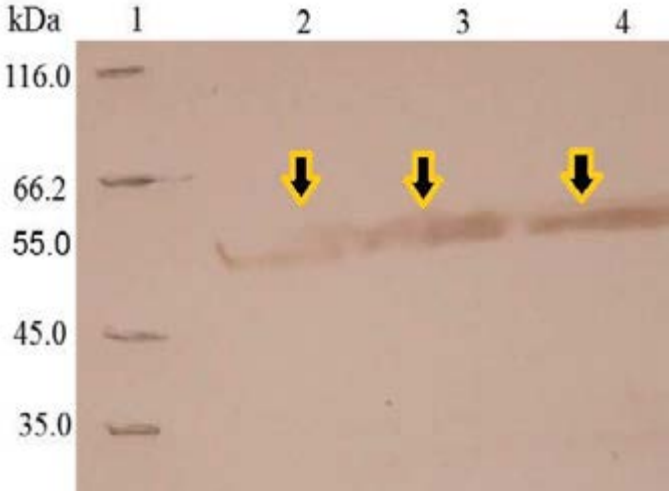

Figure 4. Western blot analysis. Lane 1– molecular size marker, lane 2 to 4- transformed *E. coli* after inducing of expression for 4h using IPTG.

Recently, utilizing a proteomics method, a number of immunogenic *K. pneumoniae* antigens, including OmpW, FepA, OmpC, OmpA, and OmpX have been introduced as candidates for designing protein and epitope-based vaccines (22). Advances in molecular biology, immunology and bioinformatics have accelerated the discovery of antigenic epitopes (23). In a previous research, we designed a multi-epitope vaccine construct against *K. pneumoniae* based on five Omp proteins using the in silico methods (19).

Administration of multi-epitope DNA vaccines may cause undesirable genetic modifications such as insertional mutagenesis and promotor's effects (24). In the present study, we have attempted to overcome disadvantages of DNA vaccine by producing the recombinant multi-epitope protein vaccine in the *E. coli* host.

5. Conclusion

In conclusion, we produced the previously designed multi-epitope vaccine candidate that is based on five Omp proteins of *K.pneumoniae* in the prokaryotic host. The produced construct can be considered as a novel vaccine candidate to fight the pathogen. In the next step, *in vitro* and *in vivo* experimental studies are essential for evaluation of our novel multi-epitope vaccine.

Acknowledgment

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Conflict of interest

None declared.

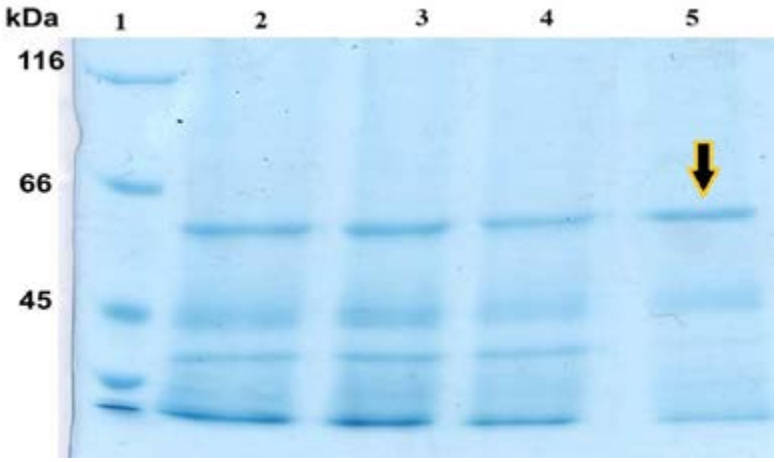

Figure 5. Construct following purification run on SDS–PAGE and stained with Coomassie blue. Lane 1– molecular size marker, lanes 2 to 4– unpurified protein, lane 5– purified protein.

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