

# Construction and assessment of a fusion protein from different antigens of uropathogenic *Escherichia coli* isolates as a new vaccine candidate against urinary tract infection



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## Introduction

Uropathogenic *Escherichia coli* (UPEC) are the most frequent pathogen causing urinary tract infections (UTIs) in humans. Nowadays, emerging of antibiotic resistance among UPEC isolates is considered as an important challenge in treatment of UTIs, making the development of an efficacious vaccine against these infections more urgent. FimH adhesin of type 1 pili, cytotoxic necrotizing factor type 1 (CNF1) and iron scavenger receptors have important roles in pathogenesis of UPEC isolates. In this study, a fusion gene of *fimH*, *cnf1* and iron receptor of UPEC isolates was constructed and its immunogenicity was evaluated in animal model.

## Materials and Methods

For construction of the fusion gene, different forms of FimH, CNF1 and Iron receptor were modeled using the I-Tasser server. The validity of the structures was evaluated using RAMPAGE and ProSA web. Modeled structures were docked to TLR-4 using Hex docking server. Depending on the energy value and pose of interaction, the best fusion form was constructed by overlap-PCR and subjected to cloning into pET28a vector and expressed in BL21 (DE3) host. Then, purification of the recombinant fusion protein was performed by Ni-NTA resin. Mice were immunized intranasally with 50 µg of the fusion protein. Then, the levels of serum immune responses in the vaccinated mice were measured by ELISA test.

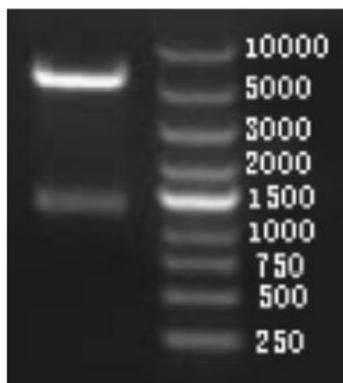


Fig 1. Confirmation of cloning the fusion gene by enzyme digestion

## Results

According to the bioinformatics studies, it was predicted that FimH.CNF1.Iron had the best interaction to TLR-4 and selected for further studies. Cloning of the fusion gene was confirmed by digestion (Fig. 1) and sequencing and its expression was evaluated on SDS-PAGE and confirmed by Western blot (Fig. 2). We observed that fusion protein FimH.CNF1.Iron induced a significantly higher total IgG than control mice ( $P < 0.05$ ). The fusion protein stimulated a mixed of Th1 (IgG2a) and Th2 (IgG1) immune responses against FimH, CNF1 and Iron antigens of the fusion protein.

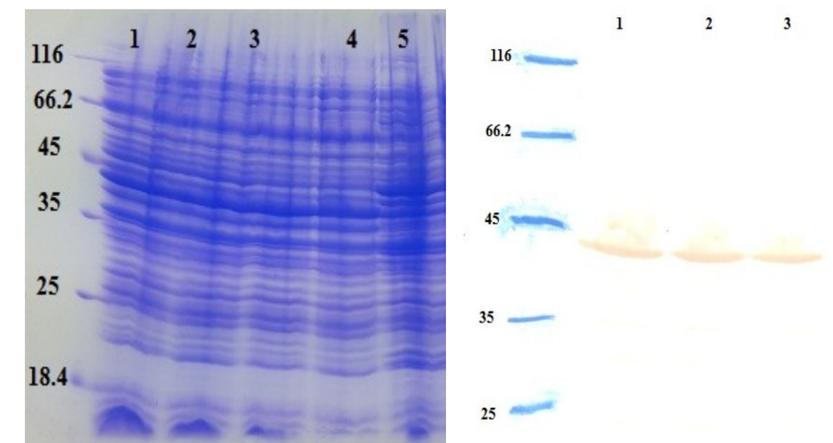


Fig 2. Confirmation of expression of the fusion gene by SDS-PAGE and Western blot. The line 5 in SSD-PAGE and line 4 in Western blot is negative control without IPTG inducer. The size of the fusion was approximately 42 KD.

## Conclusion

Development of effective vaccines to prevent or treat UTIs will have an important role in public health. Bioinformatics tools accelerate the analysis and design of fusion proteins as vaccine candidates. In this study we constructed and evaluated the immunogenicity of FimH.CNF1.Iron fusion protein for vaccination against UTI that could protect immunized mice of challenge with UPEC isolate. The stimulation of humoral immunity is essential for the generation of successful vaccines against UTI. We concluded that the immune responses against the constructed fusion protein in animal model were significant. Thus, our results propose new promising vaccine candidate based on the different antigens of UPEC isolates. Evaluation of the protection efficacy of the fusion protein as vaccine candidate is under study.