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A New Approach for Designing a Potentially Vaccine Candidate against Urinary Tract Infection by Using Protein Display on Lactobacillus Surface

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ABSTRACT

Background: The prevalence of Urinary Tract Infection (UTI) is really high in the world. *Escherichia coli* is a major agent of UTI. One of the strategies for decreasing UTI infections is vaccine development. As the attachment is a really important stage in colonization and infection, attachment inhibition has an applied strategy. FimH protein is a major factor during bacterial colonization in urinary tract and could be used as a vaccine. Thus, it was considered in this research as a candidate antigen.

Methods: The sequences of *fimH* and *acmA* genes were used for designing a synthetic gene. It was cloned to pET23a expression vector and transformed to *E. coli* (DE3) Origami. To confirm the expression of recombinant protein, SDS-PAGE and western blotting methods were used. Subsequently, recombinant protein was purified. On the other hand, *Lactobacillus reuteri* was cultured and mixed with FimH / AcmA recombinant protein. The rate of protein localization on lactobacillus surface was assessed using ELISA method.

Results: It was showed that the recombinant protein was expressed in *E. coli* (DE3) Origami and purified by affinity chromatography. Moreover, this protein could be localized on lactobacillus surface by 5 days.

Conclusion: In current study, a fusion recombinant protein was prepared and displayed on *L. reuteri* surface. This strain could be used for animal experiment as a competitor against Uropathogenic *E. coli* (UPEC). Using manipulated probiotics strains instead of antibiotic therapy could decrease the antibiotic consumption and reduce multi-drug resistant strains.

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Introduction

Urinary tract infections (UTIs) are among the most common bacterial infectious diseases encountered in clinical practice and account for significant morbidity and high medical costs (1). *Escherichia coli* are the most predominant pathogen causing 80-90% of community-acquired UTIs and 30-50% of nosocomially-acquired UTIs. Recurrent UTIs (RUTIs) are reported in 25% of women within 6 months of an acute UTI episode and pose a major problem (2). The *E. coli* pathotypes that cause extraintestinal infections are collectively called Extraintestinal Pathogenic *E. coli* (ExPEC) (3). One of these pathotypes is Uropathogenic *E. coli* (UPEC), the most common etiological agent of community-acquired UTIs. A number of virulence determinants facilitate the ability of UPEC to colonize the urinary tract and exert cytopathic effects, including type 1 fimbriae, P fimbriae, Dr adhesins, hemolysin, toxins like cytotoxic necrotizing factor 1, Sat and Vat, flagella, capsule polysaccharide, lipopolysaccharide O antigen, and TonB-dependent iron transport systems (4, 5). Antibiotics usually are effective in treating acute infections and are the primary means of prophylaxis for recurrent UTI patients. However, their value is being lessened by the emergence of increasing numbers of drug-resistant bacteria (6). Consequently, it is important that alternative prevention strategies be developed, and one approach, being actively explored, is the immunization of susceptible individuals to increase natural immunity against infection (7, 8). The components of vaccine should necessarily reflect the antigenic profile of bacteria responsible for the largest number of UTIs, which currently are various strains of *E. coli*, *Klebsiella* spp. and *Proteus* species (9). Type 1 pili, have an adhesion, fimH, at its tip. It has critical role in binding and colonization of UPEC on urothelial cells (uroplakins), and invasion of UPEC into the bladder epithelial cells (10). So, it could be a proper candidate antigen for designing a vaccine (11). On the other hand, preclinical and clinical reports have focused on lactobacillus strains, their possible prophylactic effects against experimental *E. coli* infection, and the use of these strains for the prevention of human urogenital infections (12). A kind of protein display (*Figure 1*) (13) was used in this study for designing a vaccine candidate against UTI.

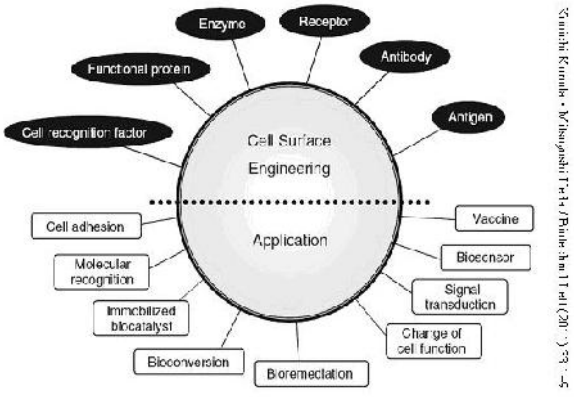

Figure 1. Protein Surface Display technology and its application

Materials and Methods

Designing the synthetic gene sequence

The sequences of target gene was designed according to *fim*H and *acm*A genes sequences which has been submitted in Genbank with accession number JF289169.1 and AF03-6720.1, respectively (*Figure 2*). It was 498 bp

and artificially synthesized by MWG Company (Germany).

Preparation of fusion recombinant protein FimH.AcmA

The pGEM / fimH.acmA vector was received from MWG Company and transformed to competent *E. coli* DH5α and propagated by following the manufacturer's instructions. Then, the fimH.acmA digested by *Nde*I and *BamH*I restriction enzymes (*Figure 3*) and inserted to pET21a expression vector under the T7 promoter with histidine Tag (His6) to generate proteins with His6 at the C-terminal of the protein.

CATATG[7]TGTAAAACCGCCAATGGTACCGCAATCCCTATTGGCGGTGGCAGCGCCAATGTTTATGTAAACCTTGC
GCCTGCCGTGAATGTGGGGCAAAACCTGGTCGTAGATCTTTCGACGCAAATCTTTTGCCATAACGATTACCCAG
AAACC[157]CACCACCACCACCACCAC[175]GAGGCAGCCGCTAAAGAGGCAGCCGCTAAAGAGGCAGCCGCTAAAGA
GGCAGCCGCTAAA[235]GACGGAGCTTCTTCAGCTGGAAATACTAATTCTGGTGGCTCGACAACCACAAATACGAA
TAATAATTCTGGAACCAATAGCAGTTCAACTACTTATACCGTCAAATCTGGTGATACTCTTTGGGGAATTTCACA
AAGATATGGAATTAGTGTCGCTCAAATTCAAAGTGCGAATAATCTTAAAAGTACCATTATCTACATTGGTCAAA
AACTTTTACTGACAGGTTCAGCTTCTTCTACAAATTCAGGTGGTTCA[487]TGATAAGGATCC

Figure 2. The sequence of chimeric gene

Underline letter shows restriction enzyme site of *Nde*I (at the first) and *BamH*I (at the end) of sequence. From number 7 to 156 and from 235 to 486 represent *fim*H and *acm*A sequence respectively. Also, number 157 to 174 shows six Histidine codons. Number 175 to 234 referred to linker sequence. The total length of sequence is 498 bp.

The Luria broth (LB) agar plates containing 100 µg/µl ampicillin were used for selection of transformed colonies. Some colonies were selected and following an overnight cultivation, were subjected to plasmid extraction. In order to verify the fidelity of the cloned chimeric fragment, the selected recombinant plasmid was subjected to sequencing (Macrogen, South Korea). The validated pET/fimH.acmA recombinant vector transformed to competent *E. coli* Origami as expression host. Recombinant *E. coli* Origami cells were grown overnight in LB medium containing ampicillin (100 µg/ml) at 37°C. On the following day, 5 ml of LB medium was inoculated with 200 µl of the overnight culture of origami. The inoculated culture was grown with agitation under aerobic conditions at 37°C till OD_{600nm} equal 0.5. Then, expression of the cloned gene was induced by different concentrations of IPTG (final concentration 0.5 mM). After incubation for 4 h, cells were harvested by centrifugation at 4°C and gene expression was analyzed by using SDS-PAGE and western blotting methods. The chimeric recombinant FimH.AcmA protein was purified by affinity chromatography according to a standard protocol.

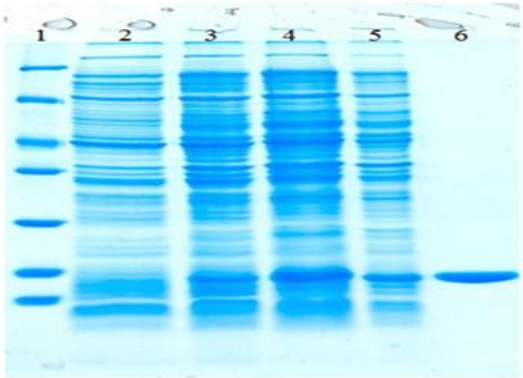

Figure 3. SDS-PAGE analysis of chimeric recombinant protein expression

Lane1: protein Ladder (Fermentas, Cat. No. SM0431: 14.4, 18.4, 25, 35, 45, 66.2, 116 KDa); lane 2: *E. coli* Origami (DE3) / pET / fim.acmA fusion, T0 induc-

tion; lane 3: *E. coli* Origami (DE3) / pET / fim.acmA fusion, T1 induction**;** lane 4: *E. coli* Origami (DE3) / pET / fim.acmA fusion, T4 induction (clone 1)**;** lane 5: *E. coli* Origami (DE3) / pET / fim.acmA fusion, T4 induction (clone 2); lane 6: Purified chimeric recombinant protein.

FimH.AcmA protein display on Lactobacillus sp. surface

Lactobacillus reuteri was purchased from Persian Type Culture Collection and was grown at 30°C in MRS broth or MRS agar (Oxoid) with 0.5% glucose as a standing culture. For binding of chimeric FimH.AcmA protein on *Lactobacillus* surface, the cells in 5 ml of exponentially growing *L. reuteri* were gently resuspended in 600 µl of fresh MRS broth after centrifugation and 200 µl of purified recombinant protein was added to those cells and incubated at 30°C for 2 h. The mixture was then centrifuged again at 9000 rpm for 5 min and the cell pellet washed three times with 1 ml of 1×PBS each time. The binding was then analyzed by ELISA and a stability study was assessed up to 120 h.

Results

Designing a chimeric gene and construction of pET/fimH.acmA

For designing a chimeric gene, the nucleotide related sequence of fifteen amino acids of N-terminal of FimH was selected. Then, the sequence of six histidine codons, linker and part of repetitive *acm*A sequence were added. After designing, the chimeric gene inserted to pET21a vector and the chimeric recombinant protein was expressed (*Figure 3*).

Evaluation of protein display on L. reuteri surface

After expression and purification of FimH.AcmA chimeric protein, it was combined with *L. reuteri* to attach on bacterial surface. The attachment was assessed by using ELISA method and shows the chimeric protein was attach to the *L. reuteri* and could be stable by 5 days (*Figure 4*).

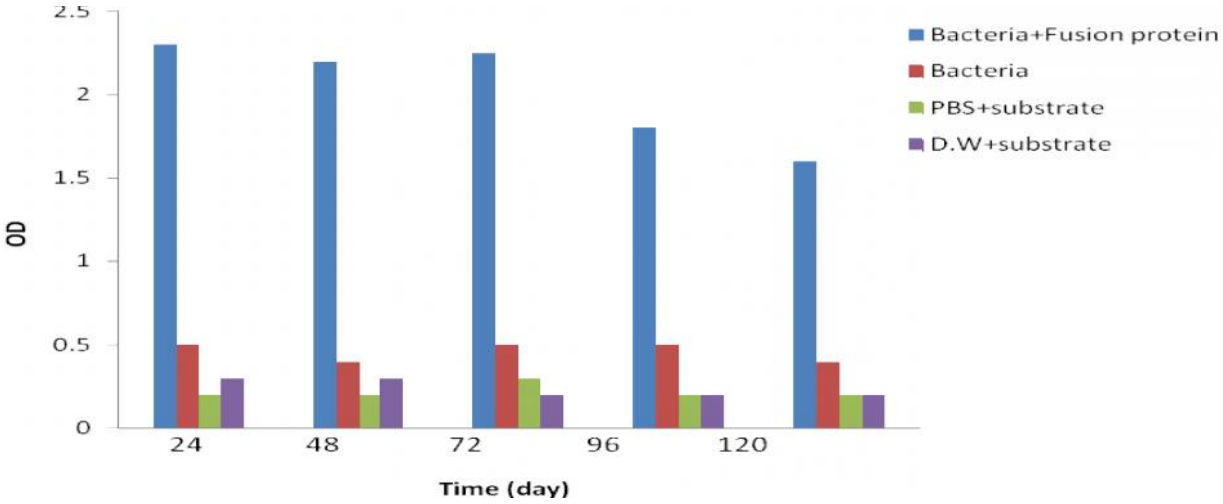

Figure 4. The assessment of protein stability on *L. reuteri* surface by ELISA

Discussion

Uropathogenic strains of *E. coli* (UPEC) are the predominant cause of community onset and healthcare-associated UTI. Consequently, understanding the compositional and pathogenetic details of UPEC is important for the development of therapeutic systems (14). Although it appears that a prior UTI fails to elicit a protective host immune response and uropathogen heterogeneity complicates vaccine design, data from animal model studies offer encouragement for successful UPEC vaccine development (15).

Bacterial adherence mediated by fimbriae is an essential prerequisite for colonization of the urinary tract. UPEC expresses a number of different adhesive organelles, including P, type 1, S, and F1C fimbriae. Type 1, or mannose-sensitive, fimbriae are produced by 80% of all uropathogenic *E. coli*. It is now well established that the expression of type 1 fimbriae by *E. coli* is a virulence factor for pathogenesis of urinary tract (16). So, the *fimH* gene sequence was used in our research. On the other hand, N-Acetylmuraminidase AcmA is an autolysin of *L. lactis* which is required for cell separation and is responsible for cell lysis during the stationary phase. The major peptidoglycan hydrolase (AcmA) of *L. lactis* consists of three domains: the N-terminal signal sequence, followed by an active domain and a C-terminal membrane anchor. Three repeated regions comprising of 44 amino acids are present in the C-terminal domain of the protein. These repeats are involved in bacterial cell wall binding and are separated by the intervening sequences which are highly enriched with serine, threonine and asparagine residues. Only one of these repeats is sufficient for cell wall binding (17). Therefore, one of these repetitive sequences was selected for designing the synthetic gene.

In this research, a lactobacillus was constructed to display FimH adhesin for preventing of UPEC attachment and colonization on bladder tissue. The administration of therapeutic molecules via mucosal routes offers several important advantages over systemic delivery such as reduction of secondary effects, easy administration and the possibility to modulate both systemic and mucosal immune responses (18). Moreover, direct delivery of the appropriate medical molecules to exert their effects at mucosal surfaces is a very efficient prophylactic and therapeutic strategy (19).

Conclusion

Lactobacilli are able to influence the human immune system in different ways. Together with the production of antimicrobial substances and the alteration of the host immune response, the ability to adhere to mucosal cells is one of the most important characteristics of lactobacilli. This property is fundamental for colonizing mucosa and in doing so to develop beneficial effects in displacing pathogens. Therefore, we tried to design a *lactobacillus* which displays an adhesin like FimH on its surface. Further investigations are required to determine the immunological effects when it is used in the bladder mucosa and the innate protective immune responses during UTI.

Acknowledgement

None declared.

Conflict of Interest

None declared conflicts of interest.

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