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Cloning of synthetic gene including antigens against Urinary Tract Infections in pET28a+ vector

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Abstract

There are many different bacterial infections in the world that patients are suffering from and research teams are trying to find suitable ways to prevent and treat them. Urinary Tract Infections (UTIs) are most important infections in the world, and they are more common among women because vaginal cavity is near to urethral opening. The aim of this study is cloning of synthetic gene include antigens against UTIs in pET28a+ vector. Antibiotic resistant has been increasing because of antibiotic overuse recently, so it shows the necessity of developing a vaccine against these infections. There for, it will be imperative to develop a vaccine instead of antibiotics. This infection causes by many organisms, most important of which are Uropathogenic Escherichia coli (UPEC), Proteus mirabilis and Klebsiella pneumoniae Uropathogenic Escherichia .coli is the most important microorganism that causes these infections more than other bacteria, so in developing a vaccine it is the most important one, that have to be considered. The synthetic Gene which was designed against these three bacteria including antigens which are important and common to cause these infections. This gene has involved 1293bp. It was ordered to Gene Ray Biotechnology. Primers were designed by Gene Runner. Gene and pET28a+ vector was checked by SnappGene. Synthetic gene was multiplied by PCR and cloned in pET28a+ vector. Construct was transformed into E. coli TOP10.The clone was confirmed by PCR, Digestion. This data indicates that this gene can be expressed and it might be a vaccine candidate to protect people from these infections in the future.

Keywords: Cloning, pET28a+, Urinary Tract Infections, Uropathogenic Escherichia coli

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Introduction

UTIs have received much attention in recent years due to its importance about antibiotic resistance. UTIs are the most common infection that people gain from hospitals (Stamm, 2002). The most important bacterial infection in renal transplant recipients is UTI and it causes major problems despite advances in organ transplantation (Satish, 2009). However kidneys are the most frequently transplanted organs and renal transplantation is the preferred method for treating people who get this infection with end-stage renal disease, post-transplantation urinary tract infection (UTI) is still a cause of morbidity. The Fact those UTIs is the most common infection in renal transplant recipients shows the importance of this issue (Schmaldienst and Hörl, 1998).Clinically, UTIs are distinguished as uncomplicated and complicated. Uncomplicated UTIs typically affect people who are healthy and have no structural or neurological urinary tract abnormalities. Uncomplicated UTIs are differentiated into lower UTIs (cystitis) and upper UTIs (pyelonephritis) (Hooton, 2012; Nielubowicz and Mobley, 2010; Hannan et al., 2012)

UTIs include infections of the urethra (urethritis), bladder (cystitis), ureters (ureteritis), and kidney (pyelonephritis) (Dielubanza and Schaeffer, 2011). Diagnosis of acute uncomplicated cystitis is based on that person medical history, taking into family health problems, sexual activity, and current symptoms (Rosen et al., 2007).

Epidemiology of UTI

One in 3 women suffers UTI requiring antibiotic treatment by age 24, and 50% of women experience at least once, UTI during their life (Dielubanza and Schaeffer, 2011).

The rate of cystitis is significantly higher in women than men. Specifically, transit bacterial from the urethral opening to the bladder in female with the shorter urethra, can be facilitated. Colonization of the vaginal by gastrointestinal pathogens can increase urinary tract infiltration (Weichhart et al., 2008). No treatment strategy has proven to be particularly effective in the prevention of UTI sequelae till now (Spencer, 2014). Escherichia. coli is the bacterial pathogen which is the most important responsible for UTI and pyelonephritis. Uropathogenic E. coli (UPEC) are hypothesized to originate in the rectal flora, spread across the perineum, and enter the bladder through the urethra (Wolfe, 2012; Sobel, 1997; Brading and Turner, 1994).

Vaccinology

An alternate strategy for the prevention of recurrent and chronic UTIs is the develop a knowledge of vaccines and explore it (Hopkins et al., 2007; Uehling et al., 2003; Foxman, 2014) Synthetic Gene this project is against three major bacteria that cause this infection. The present paper presents cloning of a gene includes antigens of Uropathogenic Escherichia .coli, Klebsiella, Proteus mirabilis against UTI. In general, this study may open a new avenue for treating this infection.

Materials and Methods

Primers was designed by SnappGene and Gene Runner software's ,with XhoI (reverse) and NcoI (forward) (Fermentase) restriction enzymes ,and it was ordered to Gene Fanavaran. The gene that was ordered to GeneRay Biotechnology was digested with HindIII (Fermentase) to make sure of the size of the gene. Gene was multiplied PCR (Eppendorf ,Master Cycler Gradient). The PCR product was recovered from the gel and purified with Roche kit according to recommendation. pET 28a+ vector was extracted with High pure plasmid Isolation Kit (Roche) (Figure3). pET28a+ vector and PCR product were double digested by XhoI and NcoI (Fermentase). PCR product was purified again and Ligation was done. Transform to competent-cell (Top10) was done, and it was cultured on LB agar (5mL) containing 50 μg/mL of kanamycin. It was kept overnight and after that single clone was gotten from Liquid culture. They were cultured in MacConkey's Agar. Finally selected colonies' constructs were analyzed by PCR, Digestion and Sequencing.

Result

The result of digestion with Hind III is shown in Figure1 and the outcome of PCR is shown in figure 2. Extraction of pET28+ vector was done the result of which is shown in Figure 3. The colony which was confirmed by digestion is dem-

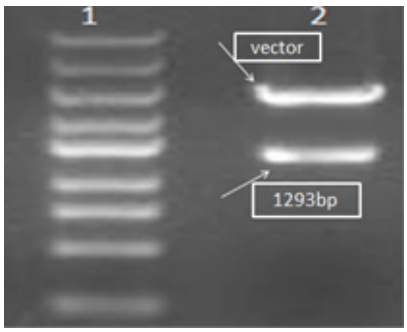

Figure 1: Electrophoresis of gene and vector digested by HindIII on Agarose Gel(1%)
Lane 1 1kbLadder
Lane2 gene and vector

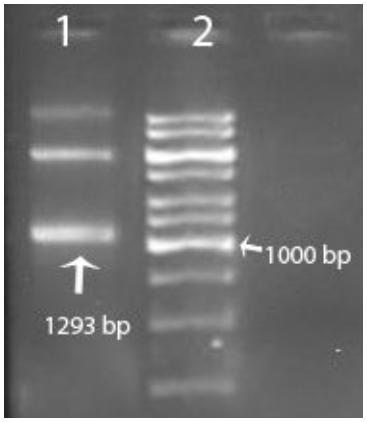

Figure 2: Electrophoresis of Polymerase chain reaction on Agarose Gel (1%)
Lane1 1kb Ladder
Lane2 PCR product

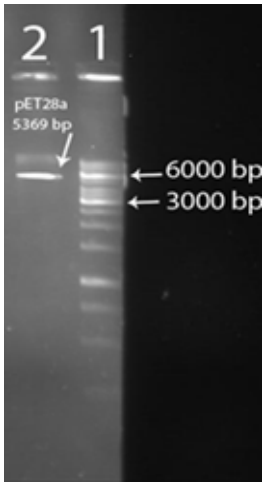

Figure 3: Electrophoresis of extracted pET28a+ vector on Gel Agarose (1%)
Lane1 1kb Ladder
Lane2 Extracted pET28a+ vector

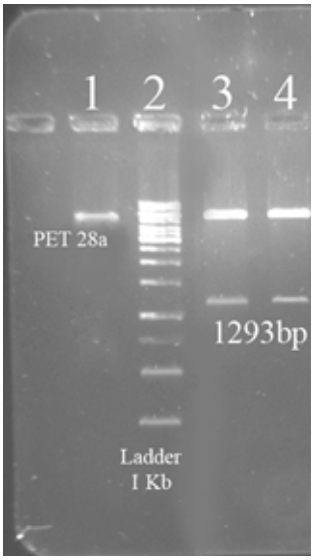

Figure 4: Electrophoresis of Double Digestion of construct with NcoI, XhoI Enzymes
Lane 1 pET28a+ vector
Lane2 1kb Ladder
Lane 3,4 Double Digestion

Table1: primers

No	Primer name	Primer Sequences (5'-3')
1	Forward (NcoI)	CATGCCATGGCAATCAATCCATTC
2	Reverse (XhoI)	CCGCTCGAGCGGACCTGGACC

Table 2: Polymerase Chain Reaction

Steps	Time	Temperature
Initial denaturation	5 min	95°C
Denaturation	1 min	95°C
Annealing	1 min	60°C
Extension	1 min	72°C
Repeat	30 time	_
Final extension	10 min	72°C

onstrated in Figure 4. Table 1 refers to primers which were designed and ordered, while Table 2 indicates program of PCR.

Discussion

Multidrug-resistant uropathogenic organisms have been becoming an expanding public health threat, as Enterobacteriaceae family members increasingly acquire extended-spectrum β-lactamases (ESBLs) such as cefotaximases (CTX-Ms) and oxacillinases (OXAs), AmpC-type β-lactamases and carbapenemases (Flores Mireles et al., 2015; Foxman, 2002). Study showed, high incidence of UTI by UPEC among patients of UTI One of the important criteria for developing an ideal vaccine target against UPEC is its wide distribution among clinical UPEC isolates. Other studies from Iran show that the isolation rate of UPEC was 57.4% and 74.6% (Farajnia et al., 2009; Mashouf et al., 2009). The increasing of antibiotic resistance in UPEC strains in the world is the major cause for an increasing requirement for vaccine development against UTI. Over the past 20 years, several vaccination approaches have been explored, encompass the use of heat-killed whole bacteria, bacterial cell extracts, and purified UPEC-associated virulence factors as antigens. Vaccination of women using a vaginal suppository containing 10 heat-killed strains of uropathogenic bacteria showed much promise in recent years (Hopkins et al., 2007; Uehling et al., 2003).

In conclusion this project has studied and worked for the first time to describe a construct as a candidate vaccine in the future. Furthermore expression and immunological studies are required for evaluation of this gene as a novel and safe vaccine candidate against UTI caused by UPEC Proteus mirabilis and Klebsiella pneumoniae.

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