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Original Article

Cloning of *Clostridium perfringens* Iota Toxin Gene in *Escherichia coli*

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ABSTRACT

Iota toxin is produced by *Clostridium perfringens* type E. This toxin causes antibiotic-associated enterotoxemia in lambs and calves. Iota toxin is a binary toxin that has two components including Ia (the enzyme component) and Ib (the binding component). Ib binds to the surface receptor of target cells and translocate Ia into the cytosol of cells. The aim of this study was to clone toxigenic epitope of iota a gene in *E. coli* strain Top10. In this study, the phenol–chloroform method was used for the extraction of the whole genomic DNA. The toxigenic epitope of iota a gene was amplified by polymerase chain reaction (PCR). The PCR product was ligated into the pTZ57R/T vector cloning site. Then, based on the TA-cloning method, the product was cloned in competent *E. coli* strain Top10. Colony PCR was used to screen bacterial colonies transformed with recombinant plasmids. The presence of 446-bp fragment on agarose gel showed that the toxigenic epitope of iota a gene of *C. perfringens* has been cloned in *E. coli* strain Top10.

Keywords: Cloning, *Clostridium perfringens*, Iota toxin, *E. coli*, TA-cloning

Clonage du gène iota-toxine du *Clostridium perfringens* chez

Résumé: Iota-toxine est produite par le Clostridium perfringens de type E. Cette toxine provoque une entérotoxémie associée aux antibiotiques chez les agneaux et les veaux. L'iota-toxine est une toxine binaire qui a deux composants dont Ia (le composant enzymatique) et Ib (le composant de liaison). Ib se lie au récepteur de surface des cellules cibles et translocation Ia dans le cytosol des cellules. Le but de cette recherche était le clonage de l'épitope toxigénique du gène iota a dans la souche *d'E. coli* Top10. Dans ce travail de recherche, la méthode phénol-chloroforme a été utilisée pour l'extraction de l'ADN entier. L'épitope toxigénique de iota est un gène amplifié par PCR. Le produit de PCR a été ligaturé dans le clonage du vecteur pTZ57R / T et a été ensuite cloné dans *E. colistrain* Top10 selon la méthode de clonage TA,. L'analyse PCR des colonies a été utilisée pour cribler des colonies bactériennes qui ont été transformées avec des plasmides recombinants. La présence d'un fragment de 446 pb sur legel d'agarose a montré que l'épitope toxigénique de iota, un gène de *C. perfringens*, a été cloné dans la souche d'*E. coli* Top10.

Mots-clés: clonage, *Clostridium perfringens*, iota-toxine, *E. coli*, TA-clonage

INTRODUCTION

Clostridium perfringens is a Gram-positive, spore-forming, anaerobic, and rod-shaped pathogenic bacterium that causes food poisoning and gas gangrene (Pilehchian Langroudi, 2015; Bakhshi et al., 2016; Aziminia et al., 2016). *C. perfringens* strains produce 17 different toxins, and based on the production of four major toxins (alpha, beta, epsilon, and iota), they are classified into the five types of A, B, C, D, and E (Pilehchian Langroudi et al., 2013; Czeczulin et al., 1996). Iota toxin is produced by *C. perfringens* type E and causes antibiotic-associated enterotoxemia in lambs and calves (Brynestad et al., 1997; McDonel, 1986). This toxin is a binary toxin composed of an enzyme component (Ia) and a binding component (Ib) (Sakurai et al., 1997, 2009; Petit et al., 1999; Barth et al., 2004; Aktories and Wegner, 1989). Ib binds to a surface receptor of the target cell and transmits Ia into its cytosol and there Ia ADP-ribosylates actin. These components lack toxic activity alone, but they can show cytotoxic, lethal, and dermonecrotic activities in binary combinations (Sakurai et al., 2009; Barth et al., 2004). Therefore, Iota a (47.5 kDa) is an ADP-ribosyltransferase, and iota b (94 kDa) binds to the surface of target cells and facilitates Ia entrance into the cytosol (Vandekerckhove et al., 1987; Perelle et al., 1997; Stiles et al., 2002). It is worth mentioning that these two components are transferred to the early endosome, where acidification cause the initiation of cytosolic entry of Ia (Nagahama et al., 2011; Hale et al., 2004). Then, Ia binds to G-actin in the cytosol of the target cell and ADP-ribosylates it, thereby, Ia inhibits the polymerization of actin, and eventually, intoxicates cells (Sakurai et al., 2009; Nagahama et al., 2011; Blöcker et al., 2001). The aim of this study was to clone toxigenic epitope of iota toxin gene of *C. perfringens* type E via the TA-cloning method in *E. coli* strain Top10.

MATERIALS AND METHODS

The toxigenic epitope region of *C. perfringens* iota gene was cloned by the TA-cloning method in *E. coli* strain Top10 that was acquired from Razi Vaccine and Serum Research Institute. The plasmid pTZ57R/T, PCR Master Mix Kit, InsTA Clone TM PCR Cloning kit, and 100 bp Plus DNA size markers were purchased from SinaClon Company.

Bacterial cultivation and DNA extraction. *C. perfringens* was cultured anaerobically at 37 °C in the liver extraction media. After 5 h, this media was centrifuged and the supernatant was removed and discarded; the phenol–chloroform method was used for the extraction of the whole genomic DNA.

PCR amplification of Ia gene. The toxigenic epitope region of Ia gene was amplified by PCR using specific primers (Table 1).

Table 1. Sequences of the primers of the epitope region of Ia gene used in polymerase chain reaction

Primer	Sequence	Temperature (°C)	Product size (bp)
Forward	5' ATG TCT TCA AAA AAA GAA GAT 3'	58	44
Reverse	5' CAG TTG CAG GAA CAT TAG TAT A 3'	56	

Taq DNA polymerase was used for amplification to add adenosine triphosphate residue to the 3' ends of the double stranded PCR product. The 25-µl volume of PCR mix included 12.5 µl of PCR Master Mix (CinnaGen PCR Master Mix, 2X, CAT. NO.: PR8252C), 1 µl (100 ng) of template DNA, 1 µl (0.4 µM) of each primer, and 9.5 µl of deionized distilled water. The amplification reaction consisted of an initial denaturation at 95 °C for 5 min prior to 30 cycles of denaturation at 95 °C for 1 min, annealing at 52°C for 1 min, extension at 72 °C for 1 min, followed by a final extension at 72 °C for 15 min (Table 2). The PCR product was then analyzed by electrophoresis on 2% agarose gel.

Cloning of the PCR product. In this study, the TA-cloning method was used. The amplified toxic epitope region of Ia was ligated into the Linearized pTZ57R/T vector using the InsTA Clone TM PCR Cloning kit (K1213) according to the manufacturer's protocol

(Table 3). The ligation results were analyzed by the electrophoresis of ligation mix on 1% agarose gel. Recombinant plasmids were transformed into competent *E. coli* strain Top10 according to the above-mentioned instructions in the kit. Screening of recombinant bacterial colonies was performed via resistance to ampicillin and colony PCR using the primers listed in Table 1. The empty cloning vector was used as the negative control. The reaction components and protocol of this PCR were the same as the primary PCR described above.

Table 2. Protocol of polymerase chain reaction

Temperature program		Time	Temperature (°C)
Initial denaturation		5 min	95
Amplification (30 cycles)	Denaturation	1 min	95
	Annealing	30 s	52
	Extension	30 s	72
Final Extension		2 min	72

Table 3. Required compounds for ligation

Compound	Content
pTZ57R/T (0.17 pmol ends vector)	3 µl
Ligation buffer 5x	6µl
PCR product	4 µl
Free nuclease water	16 µl
DNA ligase T4	1 µl
Total volume	30 µl

RESULTS

The whole genome of *C. perfringens* type E was extracted and PCR was used for the isolation and amplification of toxigenic epitope region of iota a gene. Then, the PCR product was analyzed by electrophoresis on 2% agarose gel, and a 446-bp band was observed on the gel (Figure 1). After the ligation of PCR product into the 2887-bp pTZ57R/T cloning vector, ligation accuracy was analyzed via the electrophoresis of ligation solution on 1% agarose gel and a 3333-bp fragment was observed. After transformation, the bacterial colony growth on Lysogeny Broth agar medium containing ampicillin confirmed the transformation of E. coli strain Top10 by pTZ57R/T vector that contained ampicillin resistance gene (Figure 2). Colony PCR on the transformed bacterial colonies containing the recombinant pTZ57R/T cloning vector using the specific primers listed in Table 1 confirmed the presence of a 446-bp DNA fragment after the electrophoresis of its product on 2% agarose gel (Figure 3).

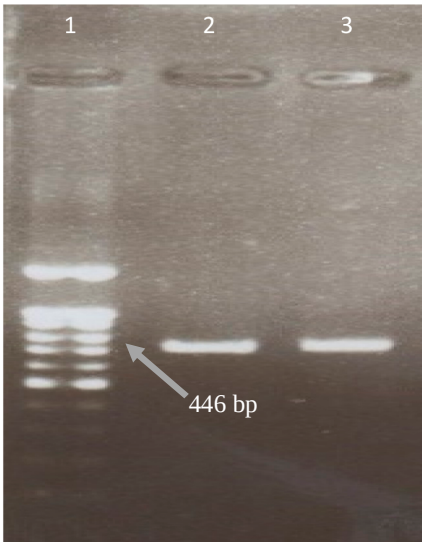

Figure 1. Polymerase chain reaction amplification of toxigenic epitope of iota toxin gene. Lane 1:100 bp DNA size marker; Lanes 2 and 3: toxigenic epitope of Ia gene (446 bp).

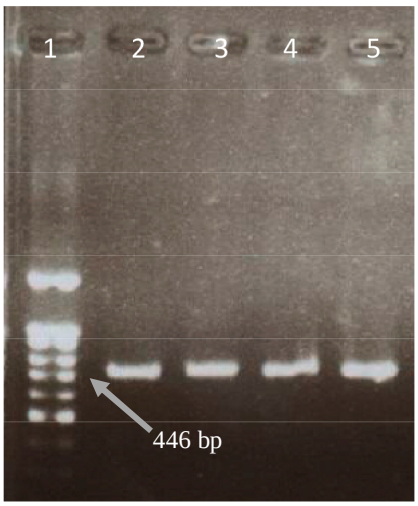

Figure 2. Colonies of transformed *E. coli* strain Top10 on Lysogeny Broth agar medium with and without ampicillin

Figure 3. Colony polymerase chain reaction products. Lane 1: 100 bpDNA size marker; Lane 2-5: Ia gene (446 bp)

DISCUSSION

In spite of using traditional methods in the production of effective vaccines, the large-scale production of such vaccines from different Clostridium species needs highly safe conditions and precise quality control steps. Therefore, the production of these candidate vaccines in a safe host is beneficial (Nijland et al., 2007; Aziminia et al., 2016). Recombinant DNA technology has enabled researchers to produce vaccines for diseases that could not be treated using the traditional methods, infections caused by *C. perfringens* species are a case in point (Nijland et al., 2007; Aziminia et al., 2016). Accordingly, the first stage of producing such vaccines is to have a pure gene bank. Therefore it is important to prepare safe gene banks and clone the genes of such toxins in safe host cells such as E. coli. and *C. perfringens*. Producing multiple toxins, strong pathogens can engender various diseases in humans and animals. In this study, the TA cloning technique was used. This technique does not need to restrict enzymes and it is easier and quicker than the conventional cloning techniques. The technique is supported by the hybridization of complementary base pairs (adenine and thymine) on different DNA fragment ends, which become ligated in the presence of ligase (Ming-Yi and Celso E., 2000). For this purpose, the linearized pTZ57R/T vector was employed, the ends of which have a 3'-T overhang. Because Taq polymerase has terminal transferase-like activity, so adds a single 3'-Adenin to blunt-ended of double-stranded DNA (Ming-Yi and Celso E., 2000; Hadjeb and Berkowitz, 1996). Most PCR products that are amplified by Taq polymerase have a single adenosine overhang at both 3'-ends. To directly clone such PCR products, a linearized “Tvector” can be used, which has a single tyrosine overhang at both 3'-ends (Ming-Yi and Celso E., 2000; Hadjeb and Berkowitz, 1996). Thus, it is clear that the complementarity between their 3'-ends allows the direct ligation of Taq-amplified PCR products into the T-vector (Ming-Yi and Celso E., 2000). Another advantage of this technique is that without white-blue method, recombinant colonies can be screened from the colonies in medium containing ampicillin by Colony-PCR. Our study showed that the toxigenic epitope region of alpha iota gene of *C. perfringens* can be cloned by the TA cloning technique in competent *E. coli* Top10 and that recombinant colonies can be screened by colony PCR.

Ethics

I hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest.

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