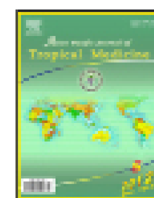
Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm



Document heading doi:

Molecular cloning of virB12 gene of *Brucella melitensis* 16M strain in pET28a vector

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ARTICLE INFO

Article history:
RReceived 7 November 2011
Received in revised form 27 January 2012
Accepted 15 March 2012
Available online 20 July 2012

Keywords:
Brucella
Cloning
virB12
pET28a
pJE1.2

ABSTRACT

Objective: To clone the virB12 gene in pET28a expression vector for production of recombinant protein to be used as antigenic component for future serological test development. **Methods:** *Brucella melitensis (B. melitensis)* 16M strain was cultured and bacterial DNA was extracted by Bioneer AccuPrep® Genomic DNA Extraction Kit. Oligonucleotide primer pair was designed based on *Brucella* virB12 gene sequence with BamHI and HindIII restriction site at 5′ end of the forward and reverse primers, respectively. DNA amplification was performed using PrimSTAR® HS DNA polymerase and the PCR product was purified by DNA AccuPrep®Gel Purification Kit. Purified DNA was cloned into pJET1.2 cloning vector. VirB12 gene fragment was excised from pJET1.2 using BamHI/HindIII and subsequently subcloned into pET28a (+). **Results:** *Brucella* virB12 gene was successfully cloned in pJET1.2 and then in pET28a (+) plasmids. PCR and restriction enzyme digestion confirms the procedure. **Conclusion:** We cloned and expressed the *Brucella* virB12 gene which could be used as antigenic component for specific serological assay development.

1. Introduction

Brucellosis is a widespread zoonotic infectious disease that is acquired by humans primarily through contact with infected abortion-related animal tissues and contaminated dairy products. It can have diverse clinical manifestations with symptoms that overlap with other diseases[1-3]. Owing to the fact that bacteriological methods are not sensitive enough for brocellusis detection[4,5], serological tests are often the preferred method for diagnosis of Brucellosis in both humans and animals[6]. Since the available serological tests detect circulating antibodies to bacterial lipopolysaccharide, these tests suffer from extensive cross reaction with other Gram-negative bacteria; therefore, interest in finding alternative and more specific bacterial antigen to detect brucellosis is on the rise[7-10].

Several proteins from *Brucella melitensis (B. melitensis)* were found to induce an antibody response in infected animals and humans[11-13]. It was recently reported that VirB12 protein, a surface-localized protein of *Brucella* spp. elicit antibody responses in both experimentally and naturally infected animals[2,14]. The aim of this study was cloning of virB12 gene in pET28a expression vector for production of recombinant protein to be used as antigenic component for future serological test development.

2. Materials and methods

2.1. Bacterial strains, plasmids

B. melitensis 16M strain was procured from Pasteur institute of Iran. *Escherichia coli (E. coli)* strains DH5 α and BL21 (DE3) were received from Novagene Co., Cloning vector pJET1.2 and expression vector pET28a were obtained from Fermentas and Invitrogen Co., respectively.

2.2. Culture of bacteria

B. melitensis was cultured in *Brucella* broth and *E. coli* was grown in Broth and agar Luria-Bertani medium. When antibiotic selection was necessary, the above media were supplemented with appropriate concentrations of antibiotic.

2.3. Genomic DNA extraction

Genomic DNA of *B. melitensis* 16M strain was extracted by Bioneer AccuPrep® Genomic DNA Extraction kit. Quality

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and purity of the extracted DNA were assessed using agarose gel electrophoresis and spectrophotometerically.

2.4. Primer design

A pair of oligonucleotide was designed based on the virB12 gene sequence of *B. melitensis* 16M strain obtained from Gene Bank (Accession no. AF226278) with BamHI and HindIII restriction site at the 5′ end of the forward and reverse primers, respectively (Table 1).

Table1

Primers designed for amplification of the virB12 gene of *B. melitensis*.

virB12	Primer
Forward virB12	AGTGGATCCATGCGCACATTGGTTATGGTCGC
Reverse virB12	CACAAGCTTGATATCCACGCGCCTGTTCAGG

The attached restriction enzyme sites are underlined.

2.5. PCR amplification

The PCR reaction mixture included 2 μL of bacterial genomic DNA (containing 100 ng), 150 nM dNTPs, and 1× PCR buffer (containing of $MgCl_2$), 1.25 units of PrimSTAR® HS DNA polymerase and 40 picomoles each of the forward and reverse primers in 50 μL final volume. PCR amplification was performed by the following parameters: denaturing at 94 ℃ for 40 s, annealing at 58 ℃ for 30 s and extension at 72 ℃ for 30 s in an Eppendorf master gradient thermocycler. PCR product was electrophoresed on 1.5% (w/v) agarose gel, stained with ethidium bromide and visualized under UV transilluminator.

2.6. Gene cloning

DNA band was sliced under long wave UV and recovered by Bioneer AccuPrep®Gel Purification Kit. Recovered DNA was ligated into pJET1.2 cloning vector at 22 ℃ for 1 h with T4 DNA ligase (Fermentas Co.). The ligation product was transformed into *E. coli* DH5α strain competent cells and dispersed onto LB agar plates containing 100 μg/mL ampicillin.

After 16-18 h incubation at 37 ℃, colonies on the agar plate that contained recombinant plasmids were detected. For confirmation, PCR amplifications were performed on these colonies using primers specific for virB12 gene and pJET vector and colonies containing the recombinant plasmid were selected. Recombinant plasmid was extracted by Bioneer AccuPrep® Plasmid Extraction Kit and digested by BamHI and HindIII restriction enzymes (Fermentas Co.). Following electrophoresis, the DNA digested bands were purified by Bioneer AccuPrep®Gel Purification Kit. Fragments of BamHI and HindIII digestes were subcloned in HindIII and BamHI digested pET28a (+) expression vector and transformed into *E. coli* DH5α competent cells and spread onto LB agar plates containing 30 μg/mL kanamycin. Then, the recombinant plasmid pET28a-virB12 was confirmed by PCR, restriction enzymes digestion and sequencing which was performed by a commercial company using universal forward and reverse T7 promoter and terminator primers (TAG Copenhage A/S Symbion, Denmark).

3. Results

Brucella virB12 gene was amplified from genomic DNA of B. melitensis 16M strain. The PCR products analyzed on 1.5 % (w/v) agarose gel displayed a single band with the correct size (513 bp) pertaining to the amplification of the virB12 gene (Figure 1).

The PCR product was cloned in pJET1.2 plasmid. Colonies containing the pJET - virB12 recombinant plasmid were confirmed by PCR using VirB12 gene and pJET 1.2 primers.

Recombinant plasmid pJET-virB12 was successfully digested by BamHI and Hind III restriction enzymes. The digested band (virB12 gene) was extracted and subcloned into pET28a expression vector. Presence of the inserted gene was confirmed by PCR method, using primers designed according to the sequence of the pET28a and virB12 gene and digestion of pET28a-virB12 recombinant plasmid by BamHI and HindIII (Figure 2). Negative control was pET28a vector alone which displayed a lower molecular weight band relative to the recombinant plasmid. Finally, the integrity and orientation of virB12 in construct were confirmed by DNA sequencing.

Results show a 513 bp fragment corresponding to the *B. melitensis* virB12 gene was successfully cloned and transformed to the bacterial expression vector pET28a.

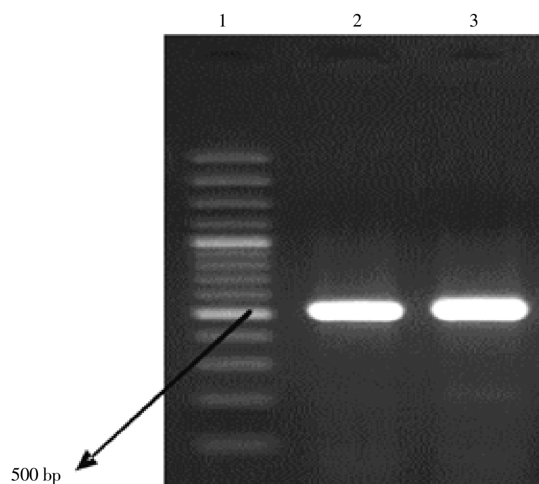

Figure 1. Electrophoresis of the amplified virB12 gene on 1.5% (w/v) agarose gel. Lane 1: 100 bp Plus DNA ladder. Lane 2, 3: Single expected band of virB12 gene (approximately 513 bp).

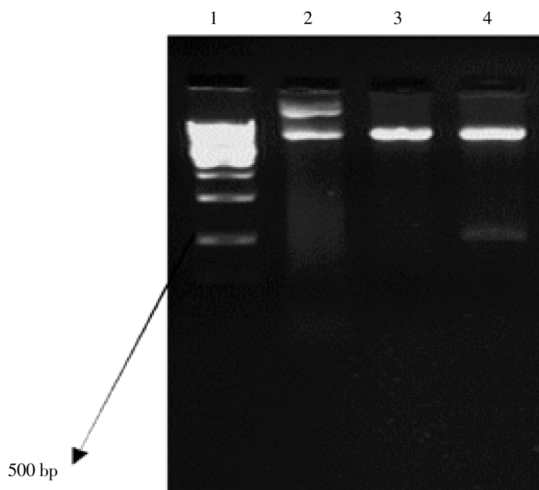

Figure 2. Analysis of enzymatic digestion of recombinant plasmid on 1.5% (w/v) agarose gel. Lane 1: DNA ladder. Lane 2: virB12-pET28a recombinant plasmid. Lane 3: Mono digestion of virB12-pET28a recombinant plasmid with BamHI. Lane 4: Double digestion of virB12-pET28a recombinant plasmid with BamHI and HindIII.

4. Discussion

Brucellosis is an infectious disease that is endemic in most developing countries[15,16]. Rapid and accurate diagnosis of Brucellosis has an important role in effective treatment of patients and improvement of public hygiene. The routine serological tests that are often used are based on detection of anti-lipopolysaccharide antibodies. In these serological tests, cross reaction occur between *Brucella* and many other Gram negative bacteria[7].

Elucidation of an antigenic component for diagnosis and vaccination of brucellosis infection serves as a valuable tool. There are several studies on different proteins of *Brucella* as antigenic component including the OMP 31 kDa, 28 kDa and 26 kDa periplasmic proteins from *B. melitensis*[4,9,13].
The proteins that are located on the surface of bacteria have traditionally been used as useful antigens for diagnostic and vaccine component. For this reason, in the present study, cloning of the virB12 gene of *Brucella* that is located on bacterial surface was designed.

Previous study indicated that natural hosts infected with *Brucella* produce an antibody response to the protein encoded by the virB12 gene[2]. VirB12 protein is a part of the type IV secretion system that is encoded by the virB locus and is located on the surface of *Brucella*. *Brucella* cell surface proteins have shown to elicit an immune response related to the protection of the host which can also potentially be used for diagnostic purpose[2,14].

In this study, the virB12 gene is cloned using DH5 α *E. coli* as a host which doesn't contain the T7 RNA polymerase and the expression vector pET28a was used that is a powerful system for cloning and expression of recombinant proteins in *E. coli*. The nucleotide sequence of virB12 gene cloned in this study was consistent with the sequence of virB12 gene as published in the GeneBank. Cloning site in the pET28a also contained His Tag sequences for detection and purification.

In conclusion, we were able to clone the virB12 gene of *B. melitensis* in expression vector for protein expression in order to be used in future study as antigenic component.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgment

This study was supported by grant from Tehran University of Medical Sciences.

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