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ORIGINAL ARTICLE

Cloning and expression of Brucella cyclic β-1, 2 glucan transporter gene (cgt)

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ABSTRACT

Background: Brucellosis is an important cosmopolitan infection disease caused by organisms belonging to the genus Brucella. The cgt gene (cyclic β-1, 2 glucan transporter gene) is a virulent factor in Brucella genus. The present study was conducted with the aim of cloning and expression of Brucella cgt gene.

Materials and methods: Brucella melitensis cgt gene was amplified from extracted chromosomal DNA by PCR, then PCR product was cloned into pTZ57R and subcloned into pGEMEX-1 expression vector, then expressed in JM109 E.coli strain. Recombinant protein was confirmed by western blot analysis using patient's serum.

Results: The PCR product was cloned in pTZ57R plasmid via T/A cloning method. Recombinant plasmid was digested by BamHI and SacI restriction enzymes, the released band was purified and subcloned into pGEMEX-1 expression vector. Then, sample cells were lysed using lyses buffer and sonicated, then electrophoresed on SDS-PAGE. Protein bands were transferred on nitrocellulose membrane and reacted by patient's serum and detected by HRP conjugated anti human antibody.

Conclusion: We cloned and expressed Brucella abortus cyclic ß-1, 2-glucan transporter gene (cgt) which is an important agent in brucellosis. Using cgt gene mutant may be an effective way for inhibiting or decreasing the pathogenicity of bacteria.

Keywords: *Brucella, Cyclic beta 1-2 glucan transporter, Recombinant protein*.
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INTRODUCTION

Brucellae are pathogenic zoonotic intracellular bacteria. Scientists and clinicians face challenges with human brucellosis including understanding the pathogenic mechanisms, identification of markers for disease severity, treatment response, and development of new therapeutic regimens (1). B. melitensis and B. abortus are main pathogenic species and cause enormous economic losses (2).

The genus of Brucella is based on antigenic variation contains seven species including: B. melitensis (pathogen for sheep and goats), B. suis (pathogen for hogs), B. abortus (pathogen for cattle), B. ovis (pathogen for sheep), B. canis (pathogen for dogs), B. neotomae (pathogen for

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wood rats) and B. maris (pathogen for marine mammals) (3). Phylogenetically, Brucellae are classified according to the α2 subdivision of Proteobacteria, which includes Agrobacterium, Rickettsia, Rhodobacter, and Rhizobium (4). The Brucella genome consists of two circular chromosomes without plasmids, suggesting a remarkable difference compared to the single chromosome of many bacteria (5).

The cgt gene of pathogenic B. abortus like Sinorhizobium meliloti nodule development (ndvA) and Agrobacterium tumefaciens chromosomal virulence (chvA) mutants is associated with the presence of cyclic ß-1, 2-glucan in the periplasm (2,6). B. abortus cgt contains a 1800 bp open reading frame coding for a predicted membrane protein of 600 amino acids.

Vaccination against brucellosis has varying degrees of success in controlling disease but is pathogenic for human (7).

The presence of cyclic β-1, 2 glucan (cgt) in the periplasm of bacterial membrane may stabilize membrane proteins against improper assembly or disassembly. Some reports suggest that the cgt must be transported into the periplasmatic space to exert its action as a virulent factor. The construction of mutants B. abortus do not transport cyclic β-1,2 glucan to the periplasmic, as shown by the absence of anionic cyclic glucan, and the display reduced virulence in mice and defective intracellular multiplication in Hela cells (6).

The aim of the present study was cloning and expression of Brucella cgt gene in laboratory scale for further studies on immunity in human brucellosis.

MATERIALS and METHODS

B. melitensis was cultured in laboratory and DNA was extracted by boiling method, briefly, bacterial suspension was transferred to micro tube and lysed by lyses buffer (330 mM glucose, 10mM Tris, 5 mM $MgCl_2$, 2% Triton X-100, 2% SDS) for 1 hour at 37°C and boiled for 10 minutes. Bacterial lysate was centrifuged at 5000 g for 5 minutes and supernatant transferred into a new micro tube and subjected for PCR amplification.

For PCR amplification, a pair of oligonucleotide was designed based on brucella cgt gene sequence (Accession AY237159) with SacI and BamHI restriction site on 5` end of forward and reverse primers, respectively (cgt F 5́–CAG CTC ATG TGC GTT GCT AAA AAT CAT-3́ and cgt R 5́–GGA TCC CTA AGC GAC CGC GCC TTT GAC-3´). PCR reaction was included 2 µL of bacterial genomic DNA (containing 100 ng), 150 nM dNTPs, and 40 Pico moles each of forward and reverse primers, 1.5 mM $MgCI_2$, 1 X PCR buffer and 1.25 units of Taq DNA polymerase in 50 µL final volume. PCR amplification was performed by following parameters: denaturing at 94°C for 30 seconds, annealing at 62.5°C for 60 seconds and extension at 72°C for 60 seconds.

For gene cloning, PCR product was electrophoresed on 1% low melting point agarose gel and DNA band was sliced under long wave UV (260 nm) and recovered by DNA extraction kit (Fermentas Cat. No k0513). Recovered DNA was cloned into pTZ57R cloning vector via T/A cloning method. Briefly, EcoRV blunt digested pTZ57R was 3` tailed using dTTP by terminal deoxy nucleotidyl transferase (Eun 1996) and 3` A tailed PCR product was ligated to it (8). The ligation reaction was transformed into Ecoli DH5α strain competent cells (9) and dispensed on LB agar plates containing 100 µg/ml ampicillin, 20µg/ml X-gal and 2mM IPTG. There were grew some white/blue colonies on agar plate and white colonies containing recombinant plasmids were selected. Recombinant plasmid was extracted (10) and digested by SacI and BamHI, electrophoresed and released band (cgt gene) was purified by DNA extraction kit (Fermentas Cat. No k0513). The gene was sub cloned in BamHI and SacI digested pGEMEX-1 plasmid and transformed in E.coli,

JM109 strain competent cells. Recombinant pGEMEX-1 was cultured in LB medium containing 100 µg/ml ampicillin. Cultures in the logarithmic phase (at OD_{600} = 0.6) were induced with 1 mM IPTG. Samples were collected 3 and 7 hours after induction. Cells were lysed in 5 X sample buffer (100 mM Tris–HCl (pH 8), 20% glycerol, 4% SDS, 2% β-mercaptoethanol, 0.2% bromophenol blue and analyzed by 12% SDS-PAGE. The gel was stained with Coomassie brilliant blue R-250 (11). The un-induced control culture was analyzed in parallel.

Gel was transferred on nitrocellulose membrane and analyzed by western blot. Briefly, membrane was incubated by patient's serum as primary antibody and detected by anti human IgG HRP conjugated as secondary antibody. Finally, di amino benzidine (DAB)/H_2O_2 was used for color development as antigen-antibody reaction.

RESULTS

Brucella cgt gene was amplified and PCR product was electrophoresed on 1% agarose gel and shown in figure 1.

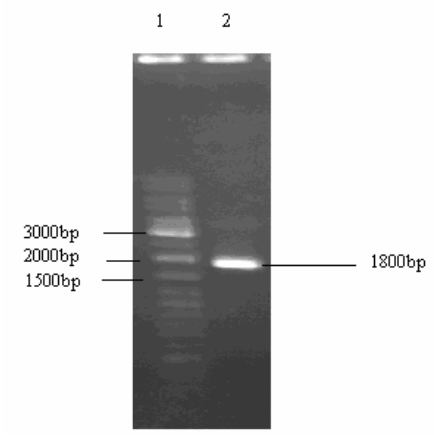

Figure 1. 1% agarose gel electrophoresis. Lane 1, 100 bp DNA ladder marker. Lane 2, 1800 bp as PCR product of cgt gene.

Gene cloning: The PCR product was cloned in pTZ57R plasmid via T/A cloning method (figure 2). Recombinant plasmid was digested by BamHI and SacI restriction enzymes, the released band (cgt gene, figure 3) was purified and subcloned into pGEMEX-1 expression vector. Figure 4 shows SacI and BamH1 restriction enzymes digested pGEMEX-1 recombinant plasmid. *Western blotting:* Sample cells were lysed using lyses buffer and sonicated, then electrophoresed on SDS-PAGE. Protein bands were transferred on nitrocellulose membrane and reacted by patient's serum and detected by HRP conjugated anti human antibody.

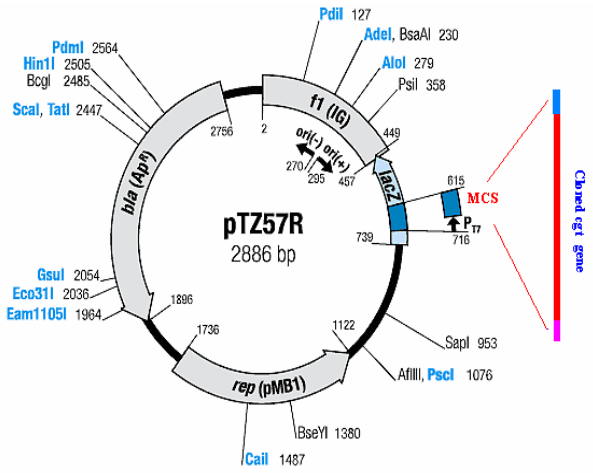

Figure 2. Map of pTZ57R plasmid

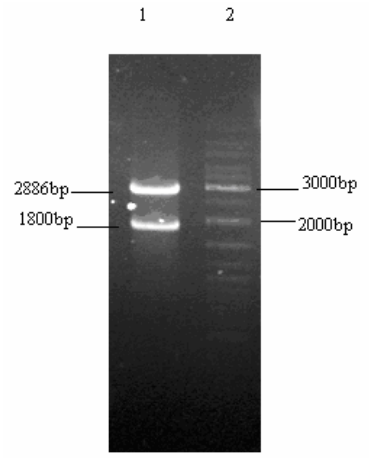

Figure 3. 1% agarose gel electrophoresis. Lane 1, Recombinant pTZ57R digested with SacI and BamH1 restriction enzymes. Lane 2, 100bp DNA ladder marker.

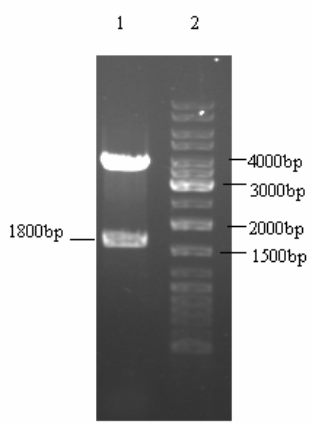

Figure 4. 1% agarose gel electrophoresis. Lane 1, Recombinant pGEMEX-1 digested with SacI and BamH 1 restriction enzymes. Lane 2, 100bp DNA ladder marker.

DISCUSSION

The cgt gene of pathogenic B. abortus like Sinorhizobium meliloti nodule development (ndvA) and Agrobacterium tumefaciens chromosomal virulence (chvA) mutants (6) is associated with the presence of cyclic ß-1, 2-glucan transporter in the periplasm (2). Roset et al characterized this molecule by nuclear magnetic resonance spectrometry analysis, thin-layer chromatography, and DEAE-Sephadex chromatography (12). On the other hand, B. abortus cgt mutants do not accumulate anionic cyclic ß-1, 2-glucan and have reduced virulence (13).

The construction of mutants B. abortus do not transport cyclic β-1, 2 glucan to the periplasmic (14). A popular vaccine is the attenuated strain of B. abortus S19 obtained spontaneously from the virulent strain B. abortus 2308 (15). B. abortus S19 has lost some essential unknown mechanism of virulence. Despite this fact, the vaccinal strain conserves some degree of virulence, being pathogenic for humans (16), and produces abortion and persistent infection in adult vaccinated cattle. Vaccination with B. abortus S19 is used only for sexually immature animals (15). Some reports suggest that the presence of cyclic ß-1, 2-glucan transporter in the bacterial periplasm may stabilize membrane proteins against improper assembly or disassembly (17).

In this report we cloned and expressed B. abortus cyclic ß-1, 2-glucan transporter gene (cgt) which is an important agent in brucellosis. Using cgt gene mutant may be an effective way for inhibiting or decreasing the pathogenicity of bacteria.

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