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Expression of Infectious Bovine Rhinotracheitis Virus Glycoprotein B in Bacterial Cell

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Abstract: Bovine Herpesvirus 1 (BHV-1) belongs to the genus of *Varicellovirus* and the family of *Herpesviridae*, which contains three main gB, gC and gD genes and can cause different respiratory, reproductive and nervous system disorders in cows. In order to cloning of the coding region of gB gene of IBR virus, PCR product of the open reading frame of the gene from IBR-MDBK cell line was amplified by PCR. A 723 bp PCR product of the gB gene with *EcoR*I, *Sal*I restriction sites were subcloned of pTZ57R/T and digested by the mentioned endonucleases. Digested insert cloned in to pGEX-4T-3 and transfected in *E. coli* cells. For the expression of gB protein, the pGEX-4T-3 recombinant vector was transformed and then induced in BL21 (DE3) strain of *E. coli* competent cells using IPTG, the presence of gB expressed protein was shown in immunoblotting and SDS-PAGE system. With respect to the remarkable frequency of infection to IBR in Iran and the necessity of controlling it through vaccination with recombinant vaccines of thymidine kinase, manufacturing and applying the recombinant gB protein are vital goals in recognition and distinction between infection and responses caused by vaccine.

Key words: IBR virus, gB protein, pGEX-4T-3 vector, protein expression, SDS-PAGE, immunoblotting

INTRODUCTION

Bovine Herpesvirus 1 (BHV-1), a member of the *Alphaherpesvirinae* subfamily (Meurens *et al*., 2004), classified in list B of the Office International des Epizooties (Winkler *et al*., 2000), is an important viral pathogen of cattle that causes two major disease syndromes: Infectious Bovine Rhinotracheitis (IBR) and infectious pustular vulvovaginitis (Taylor *et al*., 1998; Meurens *et al*., 2004). Secondary bacterial infections resulting in bronchopneumonia and death are common (Winkler *et al*., 2000). Although, IBR was eradicated in several European countries, still causes economic losses for the European and the U.S. beef industries (Meurens *et al*., 2004). In the nations, where, BHV-1 has not been eradicated, control and eradication programs are associated with vaccination strategy with live attenuated or inactivated vaccines (Taylor *et al*., 1998).

The glycoprotein C, D, E, G and I, in besides UL49 h and thymidine kinase proteins are involved in viral virulence and are useful targets for diagnosis, prevention or antiviral treatment (Smith *et al*., 1994; Van Engelenburg *et al*., 1994; Young and Smith, 1995; Van Oirschot *et al*., 1996; Schwyzer and Ackermann, 1996; Liang *et al*., 1996, 1997; Kaashoek *et al*., 1998). Most herpesvirus glycoproteins are present in the viral envelope and are important for virus-host interactions, also necessary for a productive BHV-1 infection of bovine cells. However, gB, gC and gD have been known as the major BHV-1 envelope glycoproteins (Winkler *et al*., 2000; Meurens *et al*., 2004). The lack of information on isolated BHV-1 in Iran, obviously seems.

The aim of this study is based on cloning and expression of BHV-1 gB gene to obtain glycoprotein B of isolated virus in Iran. The preparing recombinant protein will be applied in coming up studies to design a Dot-ELISA kit for detecting and distinguishing infected and vaccinated cows, also this recombinant protein can be use as an antigen to prepare monoclonal antibody.

MATERIALS AND METHODS

Sample, plasmids and bacterial strains: The extracted DNA from IBR virus isolated in Iran was selected to be cloned. Plasmid pTZ57R/T (Ins T/A clone PCR Cloning

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kit, Fermentas) and *E. coli* strain JM107 (Fermentas) were used for initial cloning, sequencing and maintenance of DNA fragment. For recombinant protein production, a prokaryotic expression vector pGEX-4T-3 (Pharmacia) was used. The recombinant pGEX-4T-3 (pGEX-4T-3-gB) is transformed into *E. coli* BL21 (DE3) (Fermentas) as host strain. The required antibiotics were added to LB media according to the reference recommendation (Sambrook and Russell, 2001).

Primers design: Perimers were designed according to the published sequence for gB gene of IBR (accession number: DQ006853.1). The forward primer, gB F: 5-TTA GAA TTC GGC GTC ATC TAC AAG G-3 contain *EcoR*I site. Reverse primer, gB R: 5-AAA TAT GTC GAC GGT GAA GCG GAA AGT TCC C-3 contain recognition site for *Sal*I. The restriction enzyme sites (underlined) were added to the primers for subsequent cloning procedure.

Gene amplification of gB (encoding the glycoprotein B): The amplification reaction was performed in 50 μL reaction mixtures containing 0.1 mM of each dexoynucteotide, 15 pmol of each primer, 50 mM Kcl, 10 mM Tris-Hcl (pH = 9), 2 mM $Mgcl_2$, 10% dimethyl sulfoxide (DMSO, Sigma), 1.5 U of Taq DNA polymerase (Sigma) and 40 ng of template DNA. The PCR reaction was carried out in a PCR programmed thermocycler (Eppendrof, Mastercycler 5330, Eppendorf-Nethel-Hinz GmbH, Hamburg, Germany Co.) using the thermal profiles: initial cycle 94°C for 9 min, followed by a further 35 cycles: denaturation at 95°C for 60 sec, annealing at 58°C for 60 sec and extension by polymerase at 72°C for 60 sec. The final cycle was run at 72°C for 7 min (Ros and Belak, 1999). The PCR product was analyzed by electrophoresis in 1% agarose gel in 1 X TBE buffer and visualized by ethidium bromide staining on UV transilluminator. The PCR product was purified by High pure PCR product purification kit (Roche applied science) according to the manufacturer recommendation.

Cloning of gB gene: The PCR product was digested with *EcoR*I and *Sal*I and ligated to pTZ57R/T and pGEX-4T-3, which were digested by the same restriction enzymes, using T4 DNA ligase (Invitrogen) at 14°C over night. *E. coli* JM107 and *E. coli* BL21(DE3) competent cells were prepared by calcium chloride method and were used for transformation of pTZ57R/T-gB and pGEX-4T-3-gB vectors, respectively. The transformed bacteria were selected by screening the colonies on LB media containing antibiotic. The suspected colony was further analyzed by restriction enzyme digestion and PCR (Sambrook and Russell, 2001).

Expression and purification of recombinant glycoprotein B: *Escherichia coli* strain BL21 (DE3) transformed with the recombinant pGEX-4T-3 plasmid was grown at 37°C in 2× YT medium to an Optical Density of 0.8-1.0 ($OD_{600\,nm}$) before induction with 1 mM IPTG for 4 h. Bacterial cells were harvested and lysed by sonication in Phosphate-Buffered Saline (PBS, 10 mM sodium phosphate, pH 7.3; 150 mM NaCl). Triton X-100 was then added to a final concentration of 1% and the lysates were incubated for 30 min at 0°C and subsequently clarified by centrifugation at 12,000 g for 30 min at 4°C. The clarified supernatants were passed over glutathione-Sepharose 4B column (Pharmacia) that was equalized with PBS. The GST fusion protein-bound column was washed by PBS over 10 column volumes and eluted with reduced glutathione (10 mM) for three column volumes. Quantity of the purified recombinant gB protein was analyzed by Bradford methods and subsequently its quality was assayed by SDS-PAGE 12% (2.5 μg $well^{-1}$). In order to analyze the cross-reaction between fused segment of gB protein with infected sera, an *E. coli* BL21(DE3) containing pGEX-4T-3 a vector was induced by IPTG.

Immunoblot analysis: For western blot analysis, 0.5 μg of purified recombinant Tax protein was used per well. As a negative control, the bacterial lysate from induced *E. coli* BL21(DE3) contain pGEX-4T-3 vector was analyzed by westen blot. The gel was blotted on to Polyvinylidine Difluoride (PVDF Membrane, Roche Diagnostics GmbH) membrane using transfer buffer containing 25 mM Tris (pH = 8.3), 192 mM glycine and 20% methanol at 55 v for 1 h at 4°C. The blotted membrane was blocked with 3% (w v^{-1}) BSA in TBST buffer (0.5 M Nacl, 0.02 M Tris pH = 8.5, 0.05% Tween 20) for 1 h at Room Temperature (RT). Membrane was incubated for 2 h at 37°C with IBR-infected cow serum, diluted 1:25, respectively. Negative serum from apparently health cow that had negative results in PCR and ELISA was used as control. After reaction the primery antibody, the blotted membranes were washed three times with TBST and incubated with peroxidase conjugated anti-bovine IgG (Sigma) at a 1:2500 dilution in TBST. The blots were then washed three times with TBST and reaction were developed by Diamino Benzidine (DAB) solution (Sigma).

RESULTS

The recombinant plasmid (pGEX-4T-3-gB) was sequenced by specific primers and Sanger sequencing method (Macrogen, Korea). The sequencing result was confirmed by comparing with databases and using Basic Local Alignment Search Tool (BLAST) software.

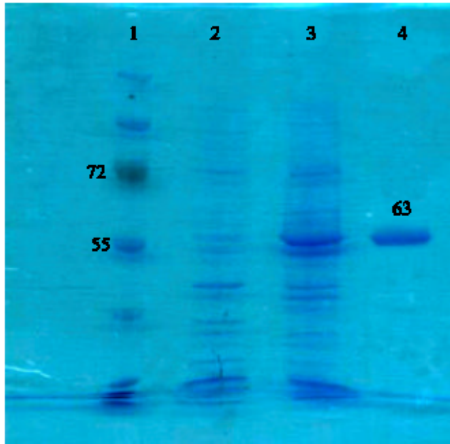

Fig. 1: Expression of recombinant gB protein and its purification (Lane 1, Protein marker; Lane 2, pGEX-4T-3-gB befor induction; Lane 3, pGEX-4T-3-gB after induction; Lane 4, Purified gB recombinant protein)

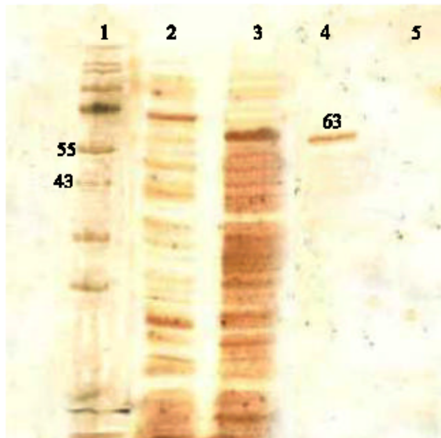

Fig. 2: Western blot analyses against recombinant gB protein by IBR-infected serum (Line 1, Protein marker; Lane 2, Western blotting pGEX-4T-3-gB befor induction; Lane 3, Western blotting pGEX-4T-3-gB after induction; Lane 4, Western blotting by negative control serum; Lane 5-9, Western blotting by infected sera; Lane 10, Western blotting reaction between the expressed pGEX-4T-3 and positive serum)

Expression of pGEX-4T-3-gB in *E. coli* BL21(DE3) induced and the expressed protein was purified by glutathione-Sepharose 4 B column (Fig. 1). The result showed that the best conditions for recombinant gB protein expression can be achieved when 1 mM of IPTG and OD_{600} = 0.9 for 3 h was used.

To determine the reactivity of recombinant protein gB, the purified recombinant protein was assayed by western blotting method. The infected cattle serum (which, had previously shown positive serological result based on ELISA and AGID) was used.

A negative serum from disinfected cattle used as a control. Figure 2 showed the specific interaction between positive serum and purified recombinant gB protein. There was no reaction between the expressed pGEX-4T-3 in *E. coli* BL21(DE3) and IBR infected serum (Lane 5 in Fig. 2).

DISCUSSION

Herpes virus infections have been reported from all continents and most countries in the world. Because of the certain nature of these kind of latent infections and diseases caused by the causative agent of the infection, non-appearance of significant clinical signs during the existence of latent virus in body as well as complicated immunohistochemical, histopathologic and laboratory diagnosis of these diseases, herpes virus infections have a worldwide spread in different countries. The results of many studies having been conducted on these viruses in human and animals have provided a widespread field for studying genetic variability, laboratory diagnosis, epidemiological studies and finally, appropriate strategies for preventing herpes virus infections. Among cattle herpes virus infections, the bovine herpes virus type 1 and different diseases caused thereby are significant and hence, many widespread researches have been made on diagnosis, control and prevention methods for these diseases with using serologic and molecular biologic methods.

Among BHV-1 structure genes, the ones coding for glycoproteins gB, gC and gD are considered as major and relatively protected genes and today most molecular biological diagnostic methods have been designed based on PCR, cloning and on the basis of detection of these genes (Yan *et al*., 2008). gB gene plays main role in penetrating BHV-1 virus into a host cell and it acts as major viral antibody against protective immune response in natural infections due to virus. Those antibodies acting against glycoprotein B are considered as a main diagnostic arm in most diagnostic serum tests for infections with BHV-1 (Gao *et al*., 1994; Kramps *et al*., 1994; McGeoch and Cook, 1994; Ros and Belakm 1999, 2002).

Anyway, one of the main goals of this examination, which was tracing of the coding gene of gB protein of IBR

in this virus, achieved for the first time in Iran and the presence of the corresponded gene was confirmed with the help of sequencing of the fragment.

With respect to this point that primers applied for identification of the gB gene in this study involve the main part of encoding frame of the gene thus, from the beginning the primers were designed for cloning and gene expression of gB in the way that the amplified fragment could be able to be cloned in different vectors such as cloning and expressing vectors.

The second goal of this study was cloning of the mentioned gene in each of the cloning vector (pTZ57R/T vector) and expressing vector (pGEX-4T-3). The cloning of this gene in the cloning vector after sequencing and comparing resulted sequences to other known sequences of the gB gene available in Genebank indicates the success in cloning the gene into the related vector. Such vector have the capacity to be proliferated in the competent bacterial cells, to be digested because of several sites for restriction enzymes, to be extracted and to be inserted in the expressing vectors. The last finding was derived by cloning the coding gene of gB protein of IBR in the expressing vector of pGEX-4T-3 for the first time in Iran and the presence of expressing protein was confirmed through SDS-PAGE and immunoblotting system.

With respect to the remarkable frequency of infection to IBR in Iran and the necessity of controlling it through vaccination with recombinant vaccines of thymidin kinase, manufacturing and applying the recombinant gB protein are vital goals in recognition and distinction between infection and responses caused by vaccine.

As the amplified fragment by PCR involves all the domains of gB and be placed in the expressing frame based on first designs of primers and has successfully been cloned in the expressing vector of pGEX-4T-3 so, the expression of this gene and the preparing recombinant protein will be applied in near future for designing Dot-ELISA kit for detection of antibodies against gB antigen of IBR in infected and vaccinated cows.

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