Journal of Biotechnology 270 (2018) 51–60

Contents lists available at ScienceDirect

Journal of Biotechnology

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

The construction of recombinant *Lactobacillus casei* expressing BVDV E2 protein and its immune response in mice

Anjuman Ara Bhuyan[a,b], Atta Muhammad Memon[a], Ali Akbar Bhuiyan[c,d], Li Zhonghua[a], Bingzhou Zhang[a], Shiyi Ye[a], Li Mengying[a], Qi-Gai He[a,*]

[a] State Key Laboratory of Agricultural Microbiology, Ministry of Education, College of Animal Science and Veterinary Medicine, Huazhong Agricultural University, Wuhan, 430070, China
[b] National Institute of Biotechnology, Savar, Dhaka, 1349, Bangladesh
[c] Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction, Ministry of Education, College of Animal Science and Veterinary Medicine, Huazhong Agricultural University, Wuhan, 430070, China
[d] Bangladesh Livestock Research Institute, Savar, Dhaka, 1341, Bangladesh

ARTICLE INFO

Keywords:
BVDV
E2 protein
Lactobacillus casei
Immune responses
Mucosal vaccine
Protection

ABSTRACT

Bovine viral diarrhea virus (BVDV) is the etiological agent of BVD causes substantial economic losses and endemic in world-wide cattle population. Mucosal immunity plays an important role in protection against BVDV infection and *Lactobacillus casei* is believed as an excellent live vaccine vector for expressing foreign genes. In this study, we have constructed a novel recombinant *L. casei*/pELX1-E2 strain expressing the most immunogenic E2 antigen of BVDV; using growth phage dependent surface expression system pELX1. The expression of E2 protein was verified by SDS-PAGE, Western blotting, and Immunofluorescence microscopic analysis. The immune responses triggered by the E2 producing recombinant *L. casei* were evaluated in BALB/c mice revealed that oral and intranasal (IN) administration of the recombinant strain was able to induce a significantly higher level of specific anti-E2 mucosal IgA and serum IgG as well as the greater level of cellular response by IFN-γ and IL-12 than those of intramuscular (IM) and control groups of mice. However, IN inoculation was found the most potent route of immunization. The ability of the recombinant strain to induce serum neutralizing antibody against BVDV and reduced viral load after viral challenge indicated better protection of BVDV infection. Therefore, this recombinant *L. casei* expressing E2 could be a safe and promising mucosal vaccine candidate against BVD.

1. Introduction

Bovine viral diarrhea (BVD) is the most significant infectious endemic disease in world-wide cattle population and causes substantial economic losses for its negative effect on production and general health conditions (Cowley et al., 2012). Due to high prevalence, persistence and clinical consequences of BVD, it is the greatest substantial infectious diseases in livestock industry (Moennig et al., 2005). Although the clinical conditions for acute infection range from asymptomatic to mild diarrhea but due to the ability to induce lymphopenia as well as immunosuppression, it allows for secondary infection which is the most devastating resultant inevitably fatal (Lieblertenorio et al., 2004). Bovine viral diarrhea virus (BVDV) is a single-stranded positive-sense RNA virus belonging to the pestivirus genus within the Flaviviridae family. The major envelope glycoprotein of BVDV is *E2* gene which is the most immunogenic portion of this virus and plays key role to attach with the

Abbreviations: µg, microgram; µL, microliter; ANOVA, analysis of variance; bp, base pair; BSA, bovine serum albumin; BVDV, bovine viral diarrhea virus; CFU, colony forming units; Ct, cycle threshold; CVCC, China Catalogue of Veterinary Cultures; DMEM, Dulbecco's modified eagle's medium; dpc, days post challenge; dpi, day post immunization; E2, enveloped glycoprotein 2; ECL, enhanced chemiluminescence; EDTA, ethylenediaminetetraacetic acid; ELISAe, nzyme-linked immunosorbent assay; FITC, fluorescein isothiocyanate; HRP, horseradish peroxidase; IgA, immunoglobulin A; IgG, immunoglobulin G; IL-12, interleukin-12; IFN-γ, interferon gamma; Amp, ampicillin; IM, intramuscular; IN, intranasal; kDa, kilo dalton; LAB, lactic acid bacteria; MRSG, Mann Rogosa Sharpe with 10% sterile glucose; MDBK, Madin-Darby Bovine Kidney; mg, milligram; min, minute; mL, milliliter; MW, marker molecular weight marker; nm, nanometer; NS, non-significant; OD, optical density; PBS, phosphate buffer saline; PBST, phosphate buffer saline with 0.5% tween 20; pmol, pico mole; qRT-PCR, quantitative real time polymerase chain reaction; rpm, rotation per minute; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; sec, second; SPSS, software package of statistical analysis in social science; $TCID_{50}$, 50% tissue culture infective dose of virus; TMB, 3,3′,5,5′-Tetramethylbenzidine; TNBS, trinitrobenzenesulfonic acid; TSB, tryptone Soy Broth; w/v, weight/volume

* Corresponding author at: State Key Laboratory of Agricultural Microbiology, College of Veterinary Medicine, Huazhong Agricultural University, Wuhan, China.
E-mail addresses: aab_bau@yahoo.com (A.A. Bhuyan), memonatta80@webmail.hzau.edu.cn (A.M. Memon), aab76_blri@yahoo.com (A.A. Bhuiyan), lzh1990@webmail.hzau.edu.cn (L. Zhonghua), abing0313@webmail.hzau.edu.cn (B. Zhang), yeshiyi11@outlook.com (S. Ye), 790642535@qq.com (L. Mengying), he628@mail.hzau.edu.cn (Q.-G. He).

https://doi.org/10.1016/j.jbiotec.2018.01.016
Received 23 October 2017; Received in revised form 24 January 2018; Accepted 24 January 2018
Available online 31 January 2018
0168-1656/

receptor to enter into the host cell (Omari et al., 2013). Antibody directed against E2 protein induces neutralizing antibody and plays preeminent role in the defense against BVDV infection (Thomas et al., 2009).

Vaccines are the most profitable tools for prophylaxis of infectious diseases which have saved millions of lives. On the other hand, innate mucosal immunity stops the entry of pathogenic microorganisms and neutralizes the adverse consequences. Furthermore, it regulates the adaptive immune responses and generates a memory response. The initial infection sites of BVDV is oronasal mucosa and this mucosa-centric viral infection is the foundation of mucosal vaccine to prevent the virus from establishment, replication, and circulation to other tissues through local immune response (Brownlie, 1990). Several advantages of mucosal vaccination over the conventional vaccination were established by many other studies (Bermúdez-Humarán et al., 2011; Hazebrouck et al., 2009). Mucosal vaccination is comparatively easy administration, less chance of hypersensitivity reaction and cost-effective. Nevertheless, there is one difficulty for mucosal as well as oral or nasal vaccination is that denaturing of antigen in the stomach and intestinal tract environment causing unavailability to stimulates intestinal mucosa and sets of lymphnodes. To overcome this problem, live expressing vector can be used which can survive in the adverse environment and deliver the antigen. Thus, evolving of a safe mucosal vaccine for BVDV becomes decade demand which can protect locally and systemically from infection.

Lactobacillus casei (L. casei) is the gram-positive bacterial vehicle which can play double role as adjuvant and vector to induce significant enhance of exogenous antigen on cellular and systemic immune system makes them more attractive as a vaccine vector (Seegers, 2002). Nowadays, lactic acid bacteria (LAB) are considered as more effective live vaccine-carrier than *Salmonella, Shigella*, and *Listeria* (Shata and Hone, 2001). Due to the ability to enhance immune response, expressing reactive antigen by recombinant, intrinsic adjuvanticity and cytokine-inducing properties; *L. casei* becoming increasing interesting as heterologous expressing molecules (Lee et al., 2016). The ability of *Lactobacillus casei* BL23 was proved to attenuate TNBS significantly which resulted in increased survival rates, reduced animal weight loss, reduced bacterial translocation to liver and the prevention of damage to the large intestine (Leblanc et al., 2011). Thus, the use of commensal bacteria *L. casei* has abundant beneficial effect on health which will be the additional benefit for the vaccinating host. Insufficient expression of the protein may be the main adverse influence of gram-positive bacterial vector which can be minimized by using surface expression system. The use of well deliberate surface anchoring system pELX1 which is consists of P_{SlpA} protein from the S-layer of *L. acidophilus* has been reported in the successful construction of recombinant strain (Chen et al., 2014; Lin et al., 2015). This pELX1 system causes growth phase-dependent expression in homologous host but consecutive promoter in heterologous host and serves as attachment of extracellular protein, and interacts with the epithelial cell layer.

In this study, we have constructed efficient E2 antigen-expressing recombinant BVDV mucosal vaccine candidate. To achieve this, we have used *L. casei* MCJ strain as an antigen delivering carrier and pELX1 plasmid as a surface expressing vector. The immunogenicity of the constructed candidate was detected in BALB/c mice to induce a local, cellular and humoral response by using different immunizing routes of vaccination followed by a challenge experiment.

2. Materials and methods

2.1. Ethics statement

In the present study, all the experimental methodology on animals were performed in strict accordance with the guideline for experimental animals approved by the Animal Experimental Ethics Committee of the College of Veterinary Medicine, Huazhong Agricultural University, China. The authors state their agreement to publication ethics. This study does not include any experiment performed on human participants.

2.2. Vectors, bacterial strains and growth conditions

Heterologous gene expression vector “pELX1” was used for the surface expression of BVDV E2 gene in *L. casei* strain consisted of ampicillin resistant determinant and a 277-bp promoter as well as surface anchor motif which is a part of the S-layer protein of *L. acidophilus* (P_{SlpA}) as a precursor gene. The vector system and the *L. casei* competent cells were received as a gift from Professor Yunxiang Liang of State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan, China. The standard plasmid for qRT-PCR was constructed by means of the pMDT-18T vector (TAKARA). In this study, *E. coli* DH5α and BL-21cells were used for cloning and expression respectively which grew in Tryptone Soy Broth (TSB) broth containing 10 μg/mL ampicillin incubated at 37 °C with 180 rpm rotation. *L. casei* was grown in Mann Rogosa Sharpe with 10% sterile glucose (MRSG) media containing 10 ng/mL of erythromycin at 30 °C without shaking.

2.3. Construction of recombinant L. casei/pELX1-E2

The reference strain of BVDV-1 (NADL-AV67) was purchased from China Catalogue of Veterinary Cultures (CVCC), Beijing, China to amplify *E2* gene and also for further challenge experiment. It was propagated at 37 °C with 5% CO_2 and maintained in Madin-Darby Bovine Kidney (MDBK) cells using Dulbecco's modified Eagle's medium (Gibco, Invitrogen), 100 μg/mL streptomycin, 100 IU/mL penicillin, and 10% heated-inactivated fetal bovine serum (Gibco, Invitrogen) which is free from BVDV antigen or antibody. The enveloped glycoprotein BVDV *E2* gene was amplified from viral cDNA using *E2* specific designed forward primer, (P1) 5′-CCGGCCATGGATGCACTTGGATTGCAAACCTGAA-3′ containing *NcoI* (underlined) and reverse primer (P2) 5′GGCCGGTACCCCTAAGGCCTTCTGTTCT3′ containing *KpnI* (underlined) by means of the following RT-PCR conditions; 94 °C for 5 min, 32 cycles of 94 °C for 50s, 55 °C for 50s, 72 °C for 1 min and final extension for 10 min at 72 °C. The purified PCR product of *E2* gene was digested with *NcoI* and *KpnI* restriction endonucleases and inserted into the corresponding sites of the pELX1 expression vector following transforming into *E. coli* DH5α and designated as BpELX1-E2.

After confirmation of plasmid construction, electroporation was performed as previously described by Chen et al., 2014 (Chen et al., 2014) and designated as *L. casei*/pELX1-E2. As a control, *L. casei* containing empty plasmid pELX1 was generated using the same procedure and designated as *L. casei*/pELX1. The presence and integrity of the constructions of *L. casei*/pELX1-E2 were carried by SDS-PAGE, RT-PCR analysis, and sequencing. For more confirmation, 408 universal primers for pELX1 containing *EcoR-I* and *BamH-I* restriction sites were designed (U1) sense 5′-CCGGGAATTCAAGCGGTAGGTGAAATATTAC-3′ (U2) antisense 5′-GGCCGGATCCAGCTTGCGTTTGATTTTC-3′) which generates a 550bp-amplicon in the case of an empty plasmid.

2.4. Immunoblotting and immunofluorescence microscopy

Recombinant *L. casei*/pELX1-E2 and *L. casei*/pELX1 cells were cultured in fresh MRSG media in 1:100 ratio containing 10 ng/mL of erythromycin at 30 °C for overnight and fraction of cultured bacteria was took-out every two hours and measured OD_{600}(optical density at 600 nm) absorbance value. Then the cell lysate was prepared following previously described protocol (Li et al., 2010) whereas BpELX1-E2 cell lysate was prepared according to the previously described protocol by Hashish et al., 2013 (Hashish et al., 2013). For Immunoblotting analysis, the gel from 12%SDS-PAGE was transferred to nitrocellulose membrane and in-house anti E2 monoclonal antibody was used as probe for nitrocellulose membrane at 1:1000 ratio and HRP conjugated