UDC 575
DOI: 10.2298/GENSR1502499D
Original scientific paper

GENERATION OF DIVALENT DNA VACCINE BASED ON *p39* AND *shiga-like toxin 2* (*stx2*) GENES

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Doosti A., P. Ghasemi-Dehkordi, M. Kargar, A.Sharifi (2015): *Generation of divalent DNA vaccine based on p39 and shiga-like toxin 2 (stx2) gene-* Genetika, Vol 47, No. 2, 499-507.

The virulence factors such as shiga-like toxin (Stx) and immunogenic P39 protein in *Escherichia coli* and *Brucella melitensis* are related to disease of digestive system in human worldwide. In the present study the *stx2* and *p39* genes were cloned into expression plasmid pEEF1D-FLAG (pcDNA 3.1^{+}) as a divalent DNA vaccine candidate. The Enterohemorrhagic *E. coli* ATCC 3081 and smooth virulent *B. melitensis* strain M5 were obtained and cultured on specific media. Bacterial DNA was extracted from colonies and was used for *p39* and *stx2* genes amplification by PCR. The amplified products on 2% agarose gel electrophoresis were revealed 285 and 1220 bp fragments for *stx2* and *p39* genes, respectively. Each amplified genes were T/A cloned into pGEM-T easy vector and pGEM-T-stx2 and pGEM-T-p39 were produced. The *stx2* and *p39* genes were sub-cloned in linearized expression vector (pcDNA 3.1^{+}) using *Hind*III, *Xho*I and *Xba*I restriction enzymes and pCDNA3-stx2-p39 was generated. This final construct was confirmed by PCR and enzymes digestion. The results were showed *stx2* and *p39* genes were sub-cloned, successfully into pcDNA 3.1^{+} to generate pcDNA 3.1^{+}-stx2-p39 recombinant vector. According to these findings novel recombinant pcDNA 3.1^{+}-stx2-p39 construct that was produced in this study could be useful as DNA vaccine candidate in animal models against shiga-like toxin producing *E. coli* and virulence *B. melitensis* strains in future studies.

Key words: Brucella melitensis, Cloning, *Escherichia coli*, P39, PCR, Stx2

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Abbreviations:
E. coli: *Escherichia coli*; *B. melitensis*: *Brucella melitensis*; Stx: Shiga-like toxin; P39: 39-kilodalton protein

INTRODUCTION

Bacterial infection special gastrointestinal bacteria such as *Brucella melitensis* (*B. melitensis*) and *Escherichia coli* (*E. coli*) are one of important concerns in digestion system, immune functions, and general health in the worldwide people. *B. melitensis* is an aerobic Gram negative coccobacillus bacterium from the *Brucellaceae* family and it is the most virulent *Brucella* spp. that is the causative agents of brucellosis in both humans and animals special in sheep and goats. This bacterium is facultative intracellular pathogens with short rods shape and contains three biovars (biovars 1, 2 and 3). In infected sheep and goats the symptoms are abortions, stillbirths, and the birth of weak offspring (DELVECCHIO *et al.*, 2002). *B. melitensis* is a significant problem in under-developing countries and this pathogen is prevalent in southern and eastern edges of Mediterranean basin, particularly in Libya, Egypt, Tunisia, Syria, and in the Arabian Peninsula and Iran. The distribution of *B. melitensis* in Iran is varying in different areas of the country and biovar 1 is responsible for the disease in Isfahan, Khorasan, Guilan (north), Khoozestan (south), Yazd (central), and Kermanshah (west) provinces, whereas in Tehran and Azarbaijan the biovars 1, 2 and 3 are the responsible ones (ZOWGHI *et al.*, 2008). The 39-kilodalton protein (P39) is immunodominant protein and have important role in *Brucella* family infections (TIBOR *et al.*, 1998). The *p39* gene antigen of *B. melitensis* encoding periplasmic binding protein (PBP) and combined with CpG motifs as an adjuvant has been shown to elicit a Th1-type immune response (AL-MARIRI *et al.*, 2001; BHATTACHARJEE *et al.*, 2006).

Shiga-like toxin 1 (stx1) and Shiga-like toxin 2 (stx2), also known as verotoxins produced by enterohemorrhagic *E. coli* (EHEC) and Shiga-like toxin-producing *E. coli* (STEC) such as *E. coli* O157:H7. These zoonotic food-borne pathogens are commonly associated with gastroenteritis, abdominal cramps and diarrhea which can be bloody, hemorrhagic colitis (HC), haemolytic-uremic syndrome (HUS) and in some cases death can occur. *Stx* genes in *Shigella dysenteriae* are located on the chromosome but these genes in *E. coli* are carried by lysogenic bacteriophages and can be acquired by horizontal gene transfer (O'BRIEN *et al.*, 1992; MAURO and KOUDELKA, 2011). EHEC infections caused by *E. coli* O157 as well as non-O157 strains have global distribution but in Iran have varying rates in different years and studied groups (SALMANZADEH-AHRABI *et al.*, 2005; ALIKHANI *et al.*, 2007; BONYADIAN *et al.*, 2010; JAFARI *et al.*, 2012). Shiga toxins consist of two subunits include single A and B subunits. The B subunit of Stx1 and Stx2 binds to Gb3, which has been identified as the receptor for the Stx family (TAHAMTAN *et al.*, 2010; SHARIFI-YAZDI *et al.*, 2011; DOOSTI *et al.*, 2011). The B subunit pentamer binds to globotriaosyl ceramide receptors on the cell membrane while A subunit acts as an RNA N-glycosidase enzyme, that catalyzes the release of an adenine at position 4324 in 28S rRNA of eukaryotic cells and inhibits protein synthesis. Furthermore, A and B subunits of *stx1* and *stx2* in their nucleotide sequences and amino acid showed 57 to 60% and 55 to 57% homology, respectively (SATO *et al.*, 2003; DOOSTI *et al.*, 2011).

In recent years, many vaccines are invented against bacterial pathogens like DNA vaccine. DNA vaccine is a kind of vaccines that induces immune responses by injecting it with genetically engineered DNA and could immunization against diseases caused by various pathogens such as bacteria, viruses, protozoa, and even use for tumors and illnesses with genetic

origins (LUO *et al.*, 2006; SUN *et al.*, 2012). DNA vaccines have many advantages include posing no risk of infection, induction of a long-lived immune response, elicit both humoral and cell mediated immunity, better stability than live attenuated vaccines, not required refrigeration, easy preparation, and cheap. The limitations of DNA vaccine are low but this type of vaccination would be restricted to pathogens with a distinctive protein immunogen, extended immunostimulation leads to chronic inflammation, and may increase slight risk of potentially disrupting normal cellular processes (LUO *et al.*, 2006; SUN *et al.*, 2012). For production of DNA vaccine virulence factors such as *p39* and *stx2* genes in *B. melitensis* and *E. coli*, respectively could be selected and these genes are important in pathogenicity and immune responses of these bacteria (DHANASHREE and SHRIKAR MALLYA, 2008; SUN *et al.*, 2012). So, the present study was performed to generate of divalent DNA vaccine based on *stx2* and *p39* genes of *E. coli* and *B. melitensis*, respectively by cloning of these genes into expression vector pEEF1D-FLAG (pcDNA 3.1+).

MATERIALS AND METHODS

Bacterial culture and plasmid preparation

EHEC *E. coli* ATCC 3081 and smooth virulent *B. melitensis* strain M5 were maintained as frozen glycerol stocks were obtained from the Razi Institute (Karaj, Iran) and Microbiology laboratory of Islamic Azad University of Shahrekord Branch, respectively. *B. melitensis* were cultured into *Brucella* agar (Becton Dickinson Microbiological Systems, Franklin Lakes, NJ, USA) and *E. coli* ATCC 3081 were cultured on MacConkey agar and were incubated at 37°C in a 5% CO_2 atmosphere until OD 600 reaches 0.9-1. Also, pGEM-T easy vector and pcDNA 3.1^+ (both Invitrogen, San Diego, CA) were used as cloning and expression vectors, respectively. *E. coli* strain Top10F' (Invitrogen, Carlsbad, USA) were cultured in 5 mL of Luria-Bertani (LB) broth at 37°C overnight and were used for cloning and plasmid preparation.

Bacterial DNA extraction

Bacterial genomic DNA was extracted from colonies of *E. coli* and *B. melitensis* using DNP™ Kit (CinnaGen, Iran) according to the manufacturer's recommendation. The quality of total extracted DNA was measured by NanoDrop ND-1000 (PeqLab) at a wavelength of 260/280 nm according to the method described by Sambrook and Russell (SAMBROOK and RUSSELL, 2001).

Gene amplification

In the present study for amplification of *stx2* and *p39* genes specific oligonucleotide primers were designed according to the published sequences. The sequences of these primers included Stx2-F: 5'-ACGAAGCTTATGAAGAAGATGTTTATGGC-3' and Stx2-R: 5'-ACGCTCGAGGTCATTATTAAACTGCAC-3' (accession number: KJ158456.1) containing *Hind*III and *Xho*I restriction sites, respectively and P39-F: 5'-TAGTCTAGACGCCTGTTGCCAATGC-3' and P39-R: 5'-GCGCTCGAGTTATTTTGCGGCTTCAAC-3' (accession number: JF918761.1) containing *Xba*I and *Xho*I restriction sites, respectively. For genes amplification the polymerase chain reaction (PCR) was performed using Thermal Cycler (Mastercycler Gradient, Eppendrof, Germany) in a final reaction volume of 50 µL in 0.5 mL tubes containing 1 µg of DNA sample, 1 µM of each forward and reverse primers, 2 mM $MgCl_2$, 200 mM dNTPs, 5 µL of 10X PCR buffer, and 1 unit

of *Taq* DNA polymerase (all Fermentas, Germany). PCR temperature conditions involved an initial denaturation at 95°C for 5 minutes, followed by 32 cycles; denaturation at 94°C for 1 min, annealing at 60°C for 1 min, extension at 72°C for 1 min, and a final extension at 72°C for 5 min was carried out at the end of the amplification program. The amplified products were detected in 2% agarose gel electrophoresis and the gel was stained with ethidium bromide and photograph was obtained under UVIdoc gel documentation systems (Uvitec, UK).

Cloning of stx and p39 genes and plasmid construction

The amplified genes were purified from the gel using QIAquick Gel Extraction Kit (Qiagen, Inc.) according to manufacturer's protocol. The extracted amplified products were T/A cloned in pGEM-T easy vector (Invitrogen, San Diego, CA) and the recombinant vectors were transformed into *E. coli* TOP10F' competent cells using calcium chloride solution and under heat shock (42°C) for 90s. Then, competent cells were cultured in LB agar media (Merck Co., Germany) containing Ampicillin antibiotic (100 μg/mL), IPTG (0.1 M) and Xgal (20 mg/mL) for screening of recombinant vectors at 37°C overnight. The white colonies were selected to preparation of matrix and were cultured again at 37°C overnight in LB broth media (containing Ampicillin antibiotic). The recombinant vectors were extracted from bacterial cells using Plasmid Mini Extraction Kit (BIONEER, South Korea) using manufacturer's instructions and PCR and restriction enzymes analysis were used for confirmation of cloning.

Sub-cloning of stx and p39 genes into expression vector pcDNA 3.1$^+$

The 285 and 1220 bp fragments of *stx2* and *p39* genes were removed from recombinant pGEM-T easy vectors (pGEM-T-stx2 and pGEM-T-p39) by *Hind*III-*Xho*I and *Xba*I-*Xho*I double digestion, respectively and were sub-cloned in linearized pcDNA 3.1$^+$ expression vector (5428 bp) by these enzymes to produce pcDNA 3.1$^+$-stx2 and pcDNA 3.1$^+$-p39, respectively. Then, each recombinant linearized pcDNA 3.1$^+$ using T4 ligase enzyme was ligated to generate the final recombinant expression vector (pcDNA 3.1$^+$-stx2-p39). This construct was confirmed by PCR using specific oligonucleotide primers for *stx2* and *p39* genes and *Hind*III and *Xba*I restriction enzymes digestion.

RESULTS

The amplified *stx2* and *p39* genes on 2% agarose gel were revealed 285 and 1220 bp, fragments, respectively (Figure 1). These genes were T/A cloned using *Hind*III-*Xho*I and *Xba*I-*Xho*I double digestion into pGEM-T easy vector (3015 bp) and pGEM-T-stx2 and pGEM-T-p39 were produced, successfully. For confirmation of gene cloning pGEM-T-stx2 and pGEM-T-p39 recombinant vectors were digested by *Hind*III, *Xho*I, and *Xho*I restriction enzymes. Then, pGEM-T-stx2, pGEM-T-p39, and expression vector were digested and linearized with above restriction enzymes and *stx2* and *p39* genes were sub-cloned into pcDNA 3.1$^+$ using T4 ligase enzyme and finally pcDNA 3.1$^+$-stx2-p39 recombinant expression construct were generated, successfully. The results of enzyme digestion of pcDNA 3.1$^+$-stx2-p39 construct on 2% agarose gel electrophoresis were revealed 1505 and 5428 bp for stx2-p39 consolidated segment and pcDNA 3.1$^+$ vector (without inserted gene), respectively (Figure 2).

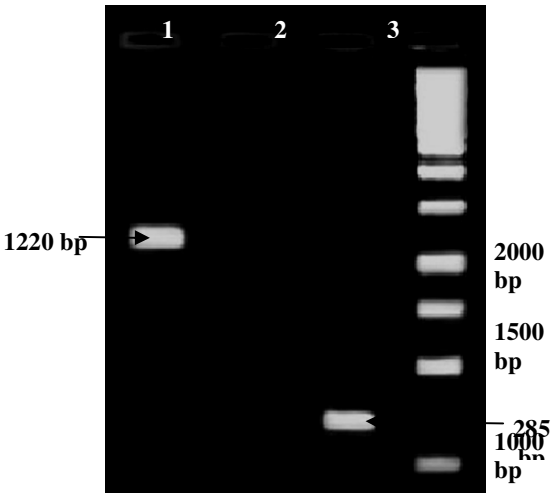

Figure 1. The amplified *stx2* and *p39* genes of *E. coli* and *B. melitensis* on 2% agarose gel electrophoresis (Lane M is 1 kb DNA ladder (Fermentas, Germany), lanes 1 and 3 are amplified *p39* (1220 bp) and *stx2* (285 bp) genes, respectively and lane 2 is negative (no DNA) control)

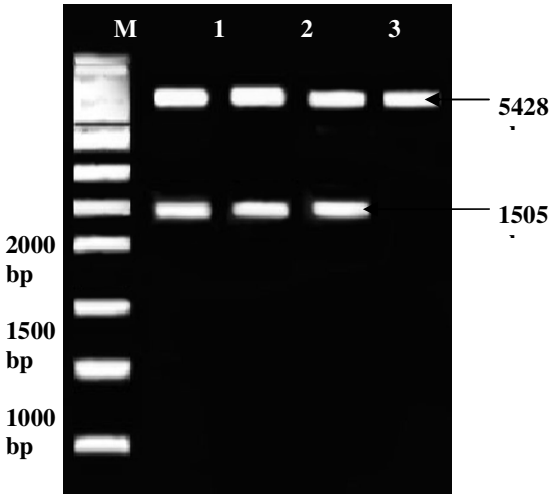

Figure 2. The enzymes digestion of pcDNA 3.1^+-stx2-p39 recombinant construct (Lane M: 1 kb DNA marker (Fermentas, Germany), lanes 1 to 3 are digested pcDNA 3.1^+-stx2-p39 construct to pcDNA 3.1^+ vector (5428 bp) and stx2-p39 consolidated segment (1505 bp) by *Hind*III and *Xho*I restriction enzymes, and lane 4 is pcDNA 3.1^+ without cloned gene)

DISCUSSION

In recent years, generations of new vaccines to prevent bacterial pathogens are developed. In this study, a novel recombinant pcDNA 3.1^+-stx2-p39 expression vector was generated as a divalent DNA vaccine candidate. In order to achieve this goal first *stx2* and *p39* genes of *E. coli* and *B. melitensis*, respectively were amplified using specific oligonucleotide primers and were cloned successfully in pGEM-T easy vector. Then pGEM-T-stx2 and pGEM-T-p39 recombinant plasmids using *Hind*III, *Xho*I and *Xba*I restriction enzymes and T4 ligase enzyme were sub-cloned in linearized pcDNA 3.1^+ expression vector, successfully and pCDNA3-stx2-p39 construct were generated. In recent decades, many studies performed to produce DNA vaccines and their applications against bacterial pathogens. KALUS *et al.* (1999) are used combination vaccinia vaccines and dual-gene vaccinia vaccines to enhance antigen-specific T-cell immunity via T-cell costimulation and indicated that direct bearing on the design of vaccine clinical trials for infectious agents and/or tumor associated antigens (KALUS *et al.*, 1999). AL-MARIRI *et al.* (2001) evaluated the potential of P39 recombinant proteins and BFR with CpG ODN as adjuvant in inducing a Th1 response and the efficiency of these vaccines to protect BALB/c mice against an infectious *B. abortus* evaluated and concluded that mice protected at 8 weeks but after BFR vaccination protection not observed. Their study showed that p39 factor that used in this study could stimulate the immune system as vaccine. LUO *et al.* (2006) constructed a DNA vaccine pcDNA3.1-Omp16 to investigate the potentiality of an Omp16-based DNA vaccine in protection against *Brucella* infection and concluded that *Brucella* DNA vaccine can elicit a higher cellular immune response and provide great protection for the host against *Brucella* infection. Their method same to the present work but we used *stx2* and *p39* genes of *E. coli* and *B. melitensis* to generate pCDNA3-stx2-p39 construct as a divalent DNA vaccine candidate. TU *et al.* (2015) in another study was cloned *stx1* region (stx1a and stx1b) from *E. coli* O157:H7 into pET-32a with a single promoter, successfully, but in the present research, we cloned *stx2* and *p39* genes to pcDNA 3.1^+ expression vector. CHATTOPADHYAY *et al.* (2003) were cloned and expressed *stx2* gene of STEC *E. coli* O157:H7 to pUC 18 and pUC 19 vectors. MEHREN *et al.* (2000) in a pilot study evaluated the dual gene recombinant Avipox vaccine containing both carcinoembryonic antigen (CEA) and B7.1 transgenes in patients with recurrent CEA-expressing adenocarcinomas and showed it is associated with the induction of a CEA specific T-cell response. CAPOZZO *et al.* (2003) were cloned B subunit of *stx2* to the pCDNA 3+ as a DNA vaccine against *E. coli* producing Hemolytic-Uremic Syndrome in a murine model. Moreover, ACHESON *et al.* (1995) were cloned B subunit of *stx2* gene to the pET9 for expression and purification of this gene and in study of BENTANCOR *et al.* (2009) the A and B subunits of *stx2* of *E. coli* were cloned to pGEM-T easy vector successful and their method and cloning vector same to the present work. DOOSTI *et al.* (2009) were evaluated the immunogenicity and the protective efficacy of a divalent fusion DNA vaccine encoding both the *B. melitensis* Omp31 protein and P39 protein and who designated pCDNA3 recombinant vector as a divalent DNA vaccine. Their study was showed that intramuscular injection of this divalent DNA vaccine based on the *omp31* and *p39* genes elicited cellular immune responses in Balb/c mice. In another study the heavy chain (HC) domain of *Clostridium botulinum* neurotoxin gene was cloned successful in *E. coli* using T/A cloning technique in PCR 8/GW/TOPO vector as a gene vaccine candidate (DOOSTI, 2013). In addition, SUN *et al.* (2012) generated a divalent DNA vaccine based on Sia10 and OmpU and this vaccine induced cross protection against *Streptococcus iniae* and *Vibrio anguillarum* in Japanese flounder. DNA vaccines could activate both cellular and immune

response to protect the host from the pathogens. To summarize, the recombinant pCDNA3-stx2-p39 DNA construct produced in the present study can be used as a DNA vaccine candidate against shiga-like toxin producing *E. coli* and virulence *B. melitensis* strains in future researches and the efficiency of this divalent DNA vaccine could evaluated in animal models.

ACKNOWLEDGEMENTS

The authors would like to express their deepest gratitude to the Razi Institute (Karaj, Iran) and Microbiology laboratory of Islamic Azad University of Shahrekord Branch for preparation of bacterial strains. This article was obtained from a research project (Grant num. 92/12/6) and under supervision of Biotechnology Research Center of Islamic Azad University of Shahrekord Branch.

Received January 09th, 2015

Accepted April 22th, 2015

REFERENCES

ACHESON, D.W., S.A. DE BREUCKER, M. JACEWICZ, L.L. LINCICOME, A. DONOHUE-ROLFE, A.V. KANE, G.T. KEUSCH (1995): Expression and purification of Shiga-like toxin II B subunits. Infect. Immun. *63*(1): 301-308.

ALIKHANI, M.Y., A. MIRSALEHIAN, B. FATOLLAHZADEH, M.R. POURSHAFIE, M.M. ASLANI (2007): Prevalence of enteropathogenic and Shiga toxin-producing *Escherichia coli* among children with and without diarrhea. J. Health Popul. Nutr. *25*: 88-93.

AL-MARIRI, A., A. TIBOR, P. MERTENS, X.D. BOLLE, P. MITCHEL, J. GODEFROID, K. WALRAVENS, J.J. LETESSON (2001): Protection of BALB/c mice against *Brucella abortus* 544 challenge by vaccination with bacterioferritin or P39 recombinant protein with CpG oligodeoxynucleotide as adjuvant. Infect. Immun. *69*: 4816-4822.

BENTANCOR, L.V., M. BILEN, R.J.F. BRANDO, M.V. RAMOS, L.C.S. FERREIRA, GHIRINGHELLI P.D., M.S. PALERMO (2009): A DNA vaccine encoding the enterohemorragic *Escherichia coli* Shiga-like toxin 2 A2 and B subunits confers protective immunity to Shiga toxin challenge in the Murine model. Clin. Vaccine Immunol. *16*(5): 712-718.

BHATTACHARJEE, A.K., M.J. IZADJOO, W.D. ZOLLINGER, M.P. NIKOLICH, D.L. HOOVER (2006): Comparison of protective efficacy of subcutaneous versus intranasal immunization of mice with a *Brucella melitensis* lipopolysaccharide subunit vaccine. Infect. Immun. *74*(10): 5820-5825.

BONYADIAN, M., H. MOMTAZ, E. RAHIMI, R. HABIBIAN, A. YAZDANI, M. ZAMANI (2010): Identification & characterization of Shiga toxin-producing *Escherichia coli* isolates from patients with diarrhea in Iran. Indian J. Med. Res. *132*: 328-331.

CAPOZZO, A.V.E., V.P. CREYDT, G. DRAN, G. FERNANDEZ, S. GOMEZ, L.V. BENTANCOR, C. RUBEL, C. IBARRA, M. ISTURIZ, M.S. PALERMO (2003): Development of DNA vaccines against hemolytic-uremic syndrome in a murine model. Infect. Immun. *71*(7): 3971-3978.

CHATTOPADHYAY, U.K., S. GUPTA, S. DUTTA (2003): Search for Shiga toxin producing *Escherichia coli* (STEC) including 0157: H7 strains in and around Kolkata. Indian J. Med. Microbiol. *21*(1): 17-20.

DELVECCHIO, V.G., V. KAPATRAL, P. ELZER, G. PATRA, C.V. MUJER (2002): The genome of *Brucella melitensis*. Vet. Microbiol. *90*(1-4): 587-592.

DHANASHREE, B., P. SHRIKAR MALLYA (2008): Detection of shiga-toxigenic *Escherichia coli* (STEC) in diarrhoeagenic stool & meat samples in Mangalore, India. Indian J. Med. Res. *128*: 271-277.

DOOSTI, A. (2013): Cloning of the gene encoding neurotoxin heavy chain of *Clostridium botulinum* in *E. coli*. J. Microbial World *5*(13): 77-84.

DOOSTI, A., P. GHASEMI-DEHKORDI, G.R. JAVADI, S. SARADRI, M.A. SHOKRGOZAR (2009): DNA vaccine encoding the *Omp31* gene of *Brucella melitensis* induces protective immunity in BALB/c mice. Res. J. Biol. Sci. *4*(1): 126-131.

DOOSTI, A., M. KARGAR, M. GOLSHAN (2011): Cloning of *Stx*2 B subunit gene from Enterohemorrhagic *Escherichia coli* O157:H7 to the expression vector (Pcdna 3.1+) as DNA vaccine candidate. Middle East J. Sci. Res. *9*(5): 616-620.

TU, W., T. LI, Q. WANG, K. CAI, X. GAO, H. WANG (2015): A simple method for expression and purification of Shiga toxin 1 (Stx1) with biological activities by using a single-promoter vector and native signal peptide. Biotechnol. Appl. Biochem. DOI: 10.1002/bab.1398.

JAFARI, A., M.M. ASLANI, S. BOUZARI (2012): *Escherichia coli*: a brief review of diarrheagenic pathotypes and their role in diarrheal diseases in Iran. Iran. J. Microbiol. *4*(3): 102-117.

KALUS, R.M., J.A. KANTOR, L. GRITZ, A. GOMEZ YAFAL, G.P. MAZZARA, J. SCHLOM, J.W. HODGE (1999): The use of combination vaccinia vaccines and dual-gene vaccinia vaccines to enhance antigen-specific T-cell immunity via T-cell costimulation. Vaccine *17*(7-8): 893-903.

LUO, D., B. NI, P. LI, W. SHI, S. ZHANG, Y. HAN, M. LIWEI, Y. HE, Y. WU, X. WANG (2006): Protective immunity elicited by a divalent DNA vaccine encoding both the L7/L12 and *Omp16* genes of *Brucella abortus*. Infect. Immun. *74*(5): 2734-2741.

MAURO, S.A., G.B. KOUDELKA (2011): Shiga toxin: expression, distribution, and its role in the environment. Toxins *3*: 608-625.

O'BRIEN, A.D., V.L. TESH, A. DONOHUE-ROLFE, M.P. JACKSON, S. OLSNES, K. SANDVIG, A.A. LIDBERG, G.T. KEUSCH (1992): Shiga toxin: Biochemistry, genetics, mode of action, and role in pathogenesis. Curr. Top. Microbiol. Immunol. *180*: 65-94.

SALMANZADEH-AHRABI, S., E. HABIBI, F. JAAFARI, M.R. ZALI (2005): Molecular epidemiology of *Escherichia coli* diarrhea in children in Tehran. Ann. Trop. Med. *25*: 35-39.

SAMBROOK, J., D.W. RUSSELL (2001): Molecular cloning: A laboratory manual, 3rd edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor.

SATO, T., T. SHIMIZU, M. WATARAI, M. KOBAYASHI, S. KANO, T. HAMABATA, Y. TAKEDA, S. YAMASAKI (2003): Distinctiveness of the genomic sequence of Shiga toxin 2-converting phage isolated from *Escherichia coli* O157:H7 Okayama strain as compared to other Shiga toxin 2-converting phages. Gene *309*: 35-48.

SHARIFI-YAZDI, M.K., A. AKBARI, M.M. SOLTAN DALLAL (2011): Multiplex polymerase chain reaction (PCR) assay for simultaneous detection of Shiga-like toxin (*stx1* and *stx2*), intimin (*eae*) and invasive plasmid antigen H (*ipa*H) genes in diarrheagenic *Escherichia coli*. Afr. J. Biotechnol. *10*: 1522-1526.

SUN, Y., M. ZHANG, C.S. LIU, R. QIU, L. SUN (2012): A divalent DNA vaccine based on Sia10 and OmpU induces cross protection against *Streptococcus iniae* and *Vibrio anguillarum* in Japanese flounder. Fish Shellfish Immunol. *32*(6): 1216-1222.

TAHAMTAN, Y., M. HAYATI, M.M. NAMAVARI (2010): Prevalence and distribution of the *stx1*, *stx2* genes in Shiga toxin producing *E. coli* (STEC) isolates from cattle. Iran. J. Microbiol. *2*(1): 8-13.

TIBOR, A., I. JACQUES, L. GUILLOTEAU, J.M. VERGER, M. GRAYON, V. WANSARD, J-J. LETESSON (1998): Effect of *p39* gene deletion in live *Brucella* vaccine strains on residual virulence and protective activity in mice. Infect. Immun. *66*(11): 5561-5564.

VON MEHREN, M., P. ARLEN, K.Y. TSANG, A. ROGATKO, N. MEROPOL, H.S. COOPER, M. DAVEY, S. MCLAUGHLIN, J. SCHLOM, L.M. WEINER (2000): Transgenes in patients with recurrent CEA-expressing containing both carcinoembryonic antigen (CEA) and B7.1 pilot study of a dual gene recombinant Avipox vaccine adenocarcinomas. Clin. Canc. Res. *6*: 2219-2228.

ZOWGHI, E., A. EBADI, M. YARAHMADI (2008): Isolation and identification of *Brucella* organisms in Iran. Iranian J. Clin. Infect. Dis. *3*(4): 185-188.

GENERISANJE DIVALENTNE DNK VAKCINE ZASNOVANO NA *p39* I *shiga-like toxin 2* (*stx2*) GENU

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Izvod

U ovim ispitivanjima izvršeno je kloniranje *stx2* i *p39* gena u ekspresioni plazmid pEEF1D-FLAG (pcDNA 3.1^+) kao kandidata za divalentnu vakcinu. Dobijeni su Enterohemorrhagic *E. coli* ATCC 3081 i vrsta umereno virulentnog soja *B. melitensis* i gajeni na specifičnim podlogama. Izolovana je bakterijska DNK iz kolonija i korišćena za umnožavanje *p39* i *stx2* gena amplificijom primenom PCR reakcije. Amplifikovani produkti su separisani na 2% agaroznom gelu i dobijeno je 285 odnosno 1220 fragmenata za *stx2* i *p39* gene. Svaki amplifikovani A/T gen kloniran je u pGEM-T vektor i pGEM-T-stx2 i pGEM-T-p39 su proizvedeni. *Stx2* i *p39* geni su subklonirani u linearizovan eksresioni vektor (pcDNA 3.1^+) korišćenjem *Hind*III, *Xho*I *i Xba*I restrikcionih enzima i generisan je CDNA3-stx2-p39. Finalni konstrukt je potvrđen reakcijom PCR i digestijom. Rezultati pokazuju da su reakcije bile uspešne. Prema dobijenim rezultatima dobijen je novi recombinantni pcDNA 3.1^+-stx2-p39 konstrukt koji može da bude koristan kao kandidat DNA vakcine kod animalnih sistema prema ispitivanim sojevima patogena.

Primljeno 09.I 2015.
Odobreno 22. IV. 2015.