Survey of Protection of Recombinant Cell Surface Protein 31kDa from Brucella melitensis in BALB/c Mice

Article *in* Journal of Pure and Applied Microbiology · March 2012

CITATIONS
2

READS
157

7 authors, including:

Leila Azimi
Iran University of Medical Sciences
56 PUBLICATIONS **535** CITATIONS
SEE PROFILE

Nima Khoramabadi
Tarbiat Modares University
29 PUBLICATIONS **101** CITATIONS
SEE PROFILE

Esmaeil Asli
Razi Vaccine and Serum Research Institute
14 PUBLICATIONS **28** CITATIONS
SEE PROFILE

Naser Harzandi
Islamic Azad University Karaj Branch
1 PUBLICATION **2** CITATIONS
SEE PROFILE

JOURNAL OF PURE AND APPLIED MICROBIOLOGY, March 2012. Vol. **6**(1), p. 69-73

Survey of Protection of Recombinant Cell Surface Protein 31kDa from *Brucella melitensis* in BALB/c Mice

Liela Azimi[1,2], Nima Khoramabadi[3], Ashraf Mohabati Mobarez[3], Esmaeil Asli[2], Naser Harzandi[2], Reza Mirnejad[4] and Abdolaziz Rastegar Lari[1]*

[1]Antimicrobial Resistance Research Center, Tehran University of Medical Sciences, Tehran, Iran.
[2]Department of Microbiology, Karaj Branch, Islamic Azad University, Karaj, Iran.
[3]Department of Bacteriology, Tarbiat Modares University, Tehran, Iran.
[4]Molecular Biology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran.

(Received: 28 May 2011; accepted: 14 July 2011)

Brucellosis is an one of important zoonotic disease with enormous economic significance. *Brucella* causes infection in several animal species and humans. Prevention of the disease is necessary for eradication human and animal infection. Characterization and evaluation of different antigens of *Brucella* cells has a key role in progression of prevention programs. Here, we report the production and purification of recombinant 31kDa cell surface protein *Brucella melitensis* (BCSP31) and conjugated to detoxify LPS of *Brucella melitensis* for evaluation of its immunogenicity in BALA/c mice. In Tarbiat Modares University, *Brucella* 31kDa cell surface protein gene was cloned in pET28a (+) vector and expression in *E coli* BL21 (DE3) with 1mM IPTG and recombinant protein was purified by Ni-NTA agarose resins. Recombinant proteins were eluted with 250mM imidazol. Imidazol removed by dialysis. Proteins were assayed by Western-blotting and rBCSP31 was probed by *Brucella* rabbit anti serum. Purified protein injected to 32 semiannual BALB/c mice for survey of its protection effect. Percentage of clearance and log unit protection in injected mice showed the significant protection against colonization of *Brucella melitensis* in spleen of mice. BCSP31 were successfully cloned, expressed and purified. Injections of this recombinant protein can protection of mice against colonization in spleen versus of challenge strain.

Key words: *Brucella melitensis*, rBCSP31, Protection.

Brucellosis is one of important zoonotic disease and economic concern due to decreased productivity, increased numbers of abortions and weak offspring in livestock[1]. Human infections are commonly acquired through the usage of unpasteurized milk and another dairy products[1]. Microorganisms belonging to the genus of *Brucella* are cause of this disease[2]. They cause infection in several animal species and humans[2, 3]. Prevention of the disease and opportune diagnosis of new cases are essential for eradication of human and cattle infection. Characterization and evaluation of different antigens of *Brucella* cells has a key role in progression of this aim.

* To whom all correspondence should be addressed.
Tel.: 82943183; Fax: 82943183
E-mail: lari@tums.ac.ir

Identifying the antigens of the etiological agent that are able to stimulate the host defensive is so important in infection diseases such as brucellosis[4]. Bacterial outer membranes are composed of different components though proteins have more important role to stimulate immune response[1]. Surface proteins of *Brucella* contains outer membrane proteins (OMPs) and Brucella cell surface proteins (BCSPs) are more attractive for researchers because of their immunogenic characterization[3]. Cloning, expression and evaluation of these Brucella proteins can help to find new methods in diagnosis and serum evaluation of immune response. This method may confirm the advantage of OMPs and BCSPs in eliciting the immune responses[5].

BCSP31 is one of the immunogenic proteins in *Brucella* cells. BCSP31 can induce immune responses that are detectable in immunogenic tests and therefore it would be useful to induct immune system for different aims[6].

Here, we cloned, expressed, purified, and evaluated Brucella cell surface protein 31kDa (BCSP31), which is one of immunogenic Brucella proteins. Then, recombinant BCSP31 (rBCSP31) injected to BALB/c mice for surveillance of its protection. Aim from in this study was clone and express of gene of BCSP31 from B. melitensis and evaluate the immunogenicity of it in BALB/c mice

MATERIALS AND METHODS

Bacterial strains, media, and growth conditions

Bacterial strain *B. melitensis* 16M were obtained Tarbiyat Modares Uiversity, Iran. *B. melitensis* was routinely cultured on *Brucella* agar at 35°C for 72 hours. *E. coli* DH5α were used as non-expression host and *E. coli* BL21 (DE3) were selected as expression host. *E. coli* strains were cultured in Luria-Bertani broth (LB) (Liofilchem) or LB agar when antibiotic selection was needed Ampicillin (at 50 µg ml^{-1}) (Sigma) and Kanamycin was added to the agar and broth (at 30µg ml^{-1}) (Sigma).

PCR amplification

Bacterial DNA from cultures of *B. melitensis* 16M grown 72 hours was extracted *(AccuPrep*® DNA Extraction Kit, Bioneer). The region encoding the *Brucella* 31 kDa cell surface protein (BCSP31) (AE008917), consisting of 990bp. A primer complementary to the 5' end of BCSP31 gene 5' ACTGGATCCATGAAATT CGGAAGCAAAATCC 3' was designed to contain a *Bam*H1 restriction site (underlined). The reverse primer, 5'AATCTCGAGTTATTTCAGCACGCCC GCTTC 3' with *Xoh*1 restriction site (underlined). Primers were designed based on the nucleotides sequence of the BCSP31 gene from *B. melitensis* 16M (AE008917). BCSP31 gene was amplified with Primestar®HS DNA polymerase (TaKaRa) which has high fidelity. PCR was conducted in a final volume of 25 µl that contained 0.75 µl of ^{2}50µM dNTP, 5µl of 5x buffer, 0.1 µM of each forward and reverse primers, 0.15 µl of Primestar®HS DNA polymerase, 1 µl of *B. melitensis* genome and 17.25 µl of PCR water. The PCR program was designed as 1 cycle of 98°C for 3-5 min; 30 cycles of 98°C for 10 s, 60°C for 10 s and 72°C for 1 min; and 1 cycle of 72°C for 5-10 min then refrigeration at 4°C.

Cloning of BCSP31 gene

The amplified blunt-ended BCSP31 gene from *B. melitensis* 16M was directly cloned into pJET1.2/blunt (clone JET® PCR cloning kit, Fermatase) as entry vector. Recombinant pJET1.2 was transformed chemically into competent *E. coli* DH5α. Recombinant plasmids were extracted by *AccuPrep*® Plasmin Mini Extraction Kit (Bioneer) and examined by restriction digestion and sequenced. One positive entry plasmid was selected for ligation into destination vector pET28a (+) (Novagen) which was digested by *Bam*H1 and *Xho*1.Prior to this step the fragment inserted between *Bam*H1 and *Xho*1 sites in pET28a (+) followed by conventional assays such as PCR and restriction digestion.

Expression of BCSP31 in *E. coli*

Recombinant pET28a (+) transformed into competent expression host *E.coli* BL21 (DE3). Expression was induced with 1mM IPTG.

Purification

Recombinant protein was purified via denaturation procedure using 8M urea to dissolve proteins which are present as inclusion bodies with Ni-NTA agarose resins. Purified proteins were assayed by western-blotting and rBCSP31 was probed by *Brucella* rabbit anti serum.

Western blot analysis of the expressed recombinant proteins

The tank electroblotting transfer of proteins from the polyacrylamide gel to a

nitrocellulose membrane was carried out using Tris/glycine buffer with 20% methanol. Then remains procedure was done according to general western blot protocol.

Injection and challenge

Recombinant proteins was injected to female, semiannual BALB/c mice for evaluating effluence of that. We had 6 groups of mice, including of:

Grup1: negative control (PBS was injected)
Group2: positive control (*Rev*1 was injected)
Group3: positive control (*S*19 was injected)
Group4: rBCSP31 was injected

Each group contains of 8 mice and subcutaneous injection with 0/5ml volume was use for immunization of that. Immunization was use in 0, 14th and 28th day. Mount of usage antigen was 10µg in each injection.

Mice were challenged with 5×104 CFU virulence *Brucella melitensis* 16M that was injected IP style. Surveillance of spleens of mice was done after 4 weeks.

RESULTS

Genome DNA prepared from *B. melitensis* was use as template in the PCR by using Primestar®HS DNA polymerase. Results show that single band with correct molecular weight has been amplified for BCSP31 gene.

Cloning of the *B. melitensis* 16M BCSP31 gene

Screening of the recombinant pJET1.2 plasmids were carried out with restriction digestion and sequenced. Insertions were excised from recombinant pJET1.2 with positive results and subcloned into pET28a (+). Cloned plasmids were examined with restriction digestion and colony PCR.

Expression of BCSP31 in *E. coli*

Expression of induced cells was checked, at hourly intervals up to 4 hours after induction with 1mM IPTG .

Table 1. Log10 detected bacteria in spleen of mice

Group	Log10 detected bacteria in mice spleens	Log unit protection
Negative control (PBS)	5.06 ± 0.13	0
Rev1	2.10 ± 0.12	2.96
S19	2.41 ± 0.13	2.65
rBCSP31(L/m)	3.72 ± 0.38	1.37

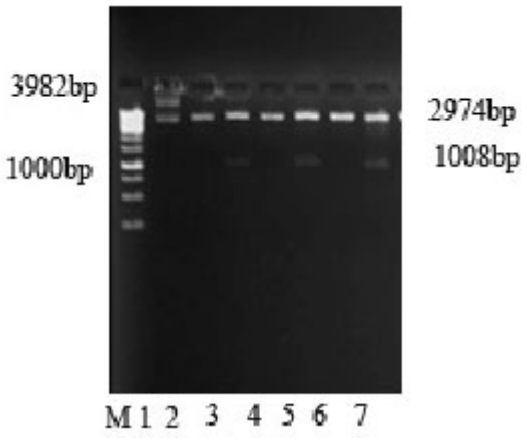

Fig. 1. Double digestion and mono digestion of recombinant pJET1.2 (M: 1kb DNA ladder, 1: undigested recombinant pJET1.2, 2-4-6: mono digested recombinant pJET1.2, 3-5-7: double digested recombinant pJET1.2

Western blotting

Purified recombinant proteins were analyzed with Western blotting after Dialysis.

Challenge

Purification of rBCSP31that was done. Results of challenge were showed in table1.

Percentage of clearance was obtained with (1 - T/C)×100. T: $\log_{10}$ number of test groups.

C: $\log_{10}$ number of negative control group.

Log unit protection was obtained with ($\log_{10}$ number of test groups – $\log_{10}$ number of negative control group).

Mann-Whitny test showed that difference in detected bacteria between negative control groups and group that was received rBCSP31 ($P \leq 0.001$). These results showed the effect of usage of rBCSP31.

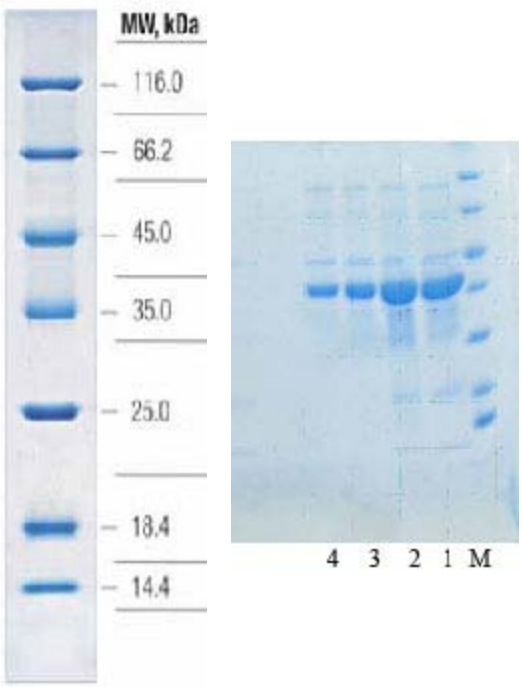

Fig. 2. Expression was checked, at hourly intervals up to 4 hours after induction. BCSP31 molecular weight has been changed because additional nucleotides were designed in primers for enzyme restriction sites. (M: marker, 1: 1 hour, 2: 2 hours, 3: 3 hours, 4: 4 hours) SDS-PAGE showed best expression after 3-4 hours

Fig. 3. Western blotting of BCSP31[3]

DISCUSSION

Brucella cause of widespread zoonotic disease that call brucellosis and can infect variety of livestock and wildlife[7]. Producing of an effective human vaccine and improved animal vaccines and diagnostic tests would significantly assist prevention of brucellosis[3].

*Rev*1 and *S*19 are *Brucella* standard vaccines which used generally. They are live attenuated bacteria strains. These vaccines have some problems, such as weak inducible for immune system and have potential of human infection[8-10]. Based on this data, the discovery of immunogenic bacterial constituent is vital component of subunit vaccine and diagnostic development process[3]. Surface proteins of the bacteria have been reported to play important role during *Brucella* infection and inducing immune response. Generally, successful vaccines against their bacteria primarily induce antibodies against surface structure. These surface proteins of the bacteria have been thought of as useful antigens for development of both diagnostic reagent and vaccine candidates[3].

Preparing proteins in naturally form of *Brucella* need high amount of bacterial culture and purification of them costs a lot[11]. For these reasons, we obtained BCSP 31 from recombinant technique that is able to prepare amount of target protein.

In the preset study, we cloned and expressed BCSP 31. This protein has potential to stimulate immune responses and can be assayed in serum evaluation to detect disease.

The gene of BCSP31 was PCR synthesized and cloned into the pET28a (+) expression system. The system provides a benefit for optimal expression of this expression of *Brucella melitensis* 16M membrane proteins using alternative destination vectors for different purposes. For expression of BCSP31 gene pGEX-4T-1 vector was used in another study and the results as well as pET28a (+) that was used in our research has better expression[12] . Another researcher used pETDEST42 as a expression vector (Invitrogen) for *Brucella* Omps[3] and the results were satisfied. These expressed recombinant proteins have been designed to be fused with 6-His tags at their N terminal. In this study, all expressed proteins were confirmed by SDS-PAGE. Purify recombinant proteins with Ni-

NTA agarose resins prepared us truth folding proteins. Purified proteins checked by western blotting. This recombinant surface protein will be further examined in vivo model to test their immune stimulation. Winter used of S-LPS and porin complex of

Brucella abortus with (log unit protection 1.76) but without (log unit protection 1.33) adjuvant that was minor in comparison our results (log unit protection 1.37) [13]. In another study use of rOmp31for immunization of mice and obtained log unit protection was 1.16 that also was minor in comparison our results (log unit protection 1.37). Until now, we do not have any report about use of any component for immunization of mice with result near to standard vaccine but our result approximately was near. Surely, this study can follow up with prevalence of immune response, switch of cellular immune respond and also activation of different type of T-cells.

ACKNOWLEDGEMENTS

Authors thank Tarbiyat Modares University and Azad University. Karaj branch for cooperation and Mr. A. Bakhtiyari and Haniyeh Aghababa for helping to do this project

REFERENCES

1. Thavaselvam, D., *et al*., Cloning and expression of the immunoreactive Brucella melitensis 28 kDa outer-membrane protein (Omp28) encoding gene and evaluation of the potential of Omp28 for clinical diagnosis of brucellosis. *J Med Microbiol.* **59**(Pt 4): p. 421-8.
2. Vizcaino, N., *et al*., Cloning, nucleotide sequence, and expression of the Brucella melitensis omp31 gene coding for an immunogenic major outer membrane protein. *Infect Immun*, 1996; **64**(9): 3744-51.
3. Ding, X.Z., *et al*., Cloning, expression, and purification of Brucella suis outer membrane proteins. *Protein Expr Purif*, 2005; **40**(1): 134-41.
4. Gonzalez, M., *et al*., Cloning, expression and immunogenicity of the translation initiation factor 3 homologue of *Brucella abortus*. *Immunobiology*, 2009; **214**(2): 113-20.
5. Cloeckaert, A., *et al*., Major outer membrane proteins of Brucella spp.: past, present and future. *Vet Microbiol*, 2002; **90**(1-4): 229-47.
6. Halling, S.M., *et al*., Deletion of the BCSP31 gene of Brucella abortus by replacement. *Infect Immun*, 1991; **59**(11): 3863-8.
7. Araceli Contreras-Rodriguez1, Mohamed Naguieb Seleem2, Gerhardt G. Schurig2, Nammalwar and S.M.B.A.L.-M. Sriranganathan, Cloning, expressionand characterization of immunogenic aminopeptidaseN from *Brucella melitensis*. *FEMS Immunol Med Microbiol*, 2006; 252-256.
8. J.M.Blasco, A review of the use of B.melitensis Rev 1 vaccine in adult sheep and goats. *Prev. Vet . Med*, 2003: 275-283.
9. Meeting, R.o.W., The Development of New / Improved Brucellosis vaccines. Report of WHO Meeting, WHO / EMC/ZDI/ Geneva, Switzerland, 1997.
10. Wilson, T., *Brucella SPP.*, in Topley & wilson Microbiology& Microbiali Nfections. 2005. p. 1719-1745.
11. Mahajan, N.K., *et al.*, Immunogenicity of major cell surface protein(s) of Brucella melitensis Rev 1. *Vet Res Commun*, 2005; **29**(3): p. 189-99.
12. CAO Xiao-an, Q.C.-q., ZHOU Ji-zhang,LIN Guo-zhen, High-Dissolved Expression of BCSP31 Gene of Brucella abortus in *E. coli Journal of Jilin Agricultural University*, 2007.
13. Winter AJ, R.G., Duncan JR, Eis MJ, Widom J and Ganem B, Effectiveness of natural and synthetic complexes of porin and O polysaccharide as vaccines against Brucella abortus in mice. *Infect Immun.ology*, 1988; **56**: 2808-18.