International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
DOI: 10.22192/ijarbs Coden: IJARQG (USA) Volume 7, Issue 7 -2020

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2020.07.07.007

Cloning, expression, purification and immunochemical characterization of *Brucella abortus* 28kDa *Omp* encoding gene

Deepti Singh[1], Sharad Kumar Yadav[2], Lalita Sharma[3]
College of Biotechnology,
Uttar Pradesh Pandit Deen Dayal Upadhayay Pashu Chikitsa Vigyan Vishwavidyalaya Ewam Go-Anusandhan Sansthan (DUVASU), Mathura 281001 India

Present address:
[1] PhD scholar (*deepti2722@gmail.com*),
[2] Professor, Department of Veterinary Microbiology (*sharadyadav944@gmail.com*)
[3] Ph.D. Scholar DUVASU Mathura (*Sharma.lalita.108@gmail.com*)

Abstract

Brucellosis, a zoonotic disease is caused by Gram negative intracellular bacteria and leads to heavy economic looses to human and dairy animals in India as well as other developing countries. For effective disease prevention and control, early and precise diagnosis of disease is important. At present, the diagnosis of brucellosis in India is done by conventional serological test (rose Bengal test or standard tube agglutination test) based on the antibody detection against lipopolysacchide (LPS) or whole bacterial lysate. These tests may give false positive results due to cross reactivity to other pathogenic organisms. The present study was designed to develop a recombinant outer membrane protein, which may be used to develop the sensitive and specific diagnostic tests for bovine brucellosis. During the present study, the omp28 gene of *Brucella abortus* was amplified by PCR amplification using specific primers. The amplified omp28 gene was digested with the restriction enzymes (BamHI and HindIII) and ligated into the pET32b(+) vector. Thereafter, the ligated product was transformed into the expression host i.e. *E. coli* BL21 competent cells. The recombinant cells were confirmed by blue white screening, colony PCR. The omp28 protein was purified by Ni-NTA chromatography and further confirmed by western blotting with positive control bovine serum. This recombinant omp28 protein may be used for the development of suitable diagnostic test such as ELISA or latex agglutination test.

Keywords: Brucellosis, *Brucella abortus*, omp28, PCR, ELISA.

1. Introduction

Microbes belonging to the genus *Brucella* are Gram negative, facultative intracellular bacteria of zoonotic importance affecting animals as well as human beings all over the world (Dhama *et al.*, 2013; Kim *et al.*, 2016; Gadaga *et al.*, 2016; Kumar *et al.*, 2016; Ciftci *et al.*, 2017). Most of *Brucella* species are species specific as *B. abortus* mainly infect cattle and buffaloes. Disease is transmitted from dam to offspring before or at the birth, through milk, by sexual contact, direct physical contact particularly trouncing, from polluted environments, and from eating spoiled raw meat mainly placenta and birth products. The disease is usually clinically characterized by abortion in the last trimester of

gestation, retention of placenta, weak calves and reduced milk production in the female whereas orchitis and epididymitis with sterility in male (Radostits *et al.*, 2000). In India as per the recent study conducted by Singh *et al*., 2015, the economic losses due to brucellosis are US$ 3.4 billion. Because of huge economic losses and zoonotic impact of brucellosis, efforts have been made to prevent the disease through the use of live vaccines. Control of brucellosis is completely dependent upon reliable methods for the identification of *Brucella* infections in livestock as well as in humans. The lipopolysaccharide (LPS) of smooth *Brucella* species is the strongest antigen on comparison with the other antigenic molecules. But tests based on anti-LPS antibodies give false positives due to cross-reactivity with several other Gram-negative bacteria viz., *Yersinia enterocolitica* O:9, *Salmonella* species, *Escherichia coli* O157 due to the high structural similarity of the O-polysaccharide (OPS) structures (Corbel *et al.*, 1985; Kittelberger *et al*., 1995; Weynants *et al*., 1996; Nielsen *et al.*, 2004; Munoz *et al.*, 2005; Ko *et al*., 2012; Mirkalantari, *et al*., 2017). Therefore, various cytoplasmic or surface components of *Brucella spp* such as outer membrane protein (Chaudhuri *et al.*, 2010; Kaushik *et al.*, 2010; Lim *et al.*, 2012; Simborio *et al.*, 2016) has been investigated in detail for the purpose of disease diagnosis and development of vaccine. On the basis of their molecular weight, *Brucella* OMPs are divided into three groups viz.; group 1 (94 kDa), group 2 (41–43 kDa) and group 3 (30 kDa) (Verstreate *et al*., 1982). The OMP 28 of 28 kDa belongs to group 3 antigens and it is a conserved and immunodominant protein in the periplasm and this protein has been a target molecule for detection of anti-*Brucella* antibodies and development of vaccines (Cloeckaert *et. al*., 2001; Chaudhuri *et al.*, 2010; Lim *et al.*, 2012). Therefore, the present study was conducted to isolate and purify the Omp28 protein from *Brucella abortus*.

2. Materials and Methods

Procurement of Brucella

Brucella *abortus* strain 19 was used in the present study to clone and express 28kDa omp gene.the strain was provided by Dr. Amit Kumar, principal Investigator of Indian Immunological Limited, Hyderabad sponsored clinical trial for Brucella *abortus* reduced dose vaccine at Department of Veterinary Microbiology, DUVASU, Mathura.

Isolation and quantification of B. *abortus* DNA

The genomic DNA was extracted from the Brucella *abortus* strain 19 culture (lndian Immunological Limited, Hyderabad) by phenol chloroform method (Sambrook and Russel, 2001). The qualitative analysis of genomic DNA was checked by ethidium brormide stained agarose gel electrophoresis using a submarine horizontal electrophoresis apparatus. Quantification of DNA was done by the NanoDrop@ ND-1000 Spectrophotometer was used to measure the DNA concentration. The 'nucleic acid" was selected on the application module and the further measurements were performed according to the user manual.

PCR Amplification and cloning of omp28 gene

A set of following specific primers were used for the amplification of OMP28 gene: F:5'GACGAACGGAATTTTTCCAATCCC 3' and R:3'TGCCGATCACTTAAGGGCCTTCAT 5'.

The PCR was performed for 33 cycles at 94^{o}C for 30 seconds, 57^{o}C for 30 seconds and 72^{o}C for 50 seconds. Inorder to overproduce the 28 kDa of B. *abortus*, the amplified DNA was cloned into expression vector pET32b(+).The amplified PCR product was digested with the help of BamHI and HindIII restriction enzymes to facilitate cloning. The vector was also double digested with the same enzymes, so as to facilitate a directional cloning of the gene into the vector. The digested vector and PCR product was purified using the DNA gel extraction Kit. The eluted product was ligated to the pET32b(+) vector with T4 DNA ligase. The newly constructed vector was transformed into calcium treated *E.coli* BL2l (DE3) competent cells.

Induction of expression and purification of recombinant protein

E.coli cells harboring pET32b(+) plasmid were grown for 4hrs in LB medium till the cell density reached approximately 0.5 OD_{600}. The cells were then supplemented with 1mM IPTG and allowed to grow further at 37^{o}C for 3hrs. Now the culture sample was centrifuged at 6000g for 30 min to pellet the cells and the supernatant was discarded. The cell pellet was resuspended in 2 ml urea lysis buffer for 1-2 hrs. Inorder to pellet the debris and collect the clear spernanat. The cell lysate was further centrifuged at 12000g for 15 min. the supernatant was mixed with

Ni-NTA agarose slurry and the lysate-resin mixture was then loaded on to an empty 10 ml column. The column was washed thrice with denaturing wash buffer and finally eluted four times with denaturing elution buffer. Renaturation of the denatured recombinant protein was allowed by dialysing the eluted product against PBS (pH 7.2) for 36 hrs at 4^0C with three changes in the buffer. The eluted protein was analyzed with SDS-PAGE.

SDS- PAGE analysis of Omp28 protein

The proteins were subjected to electrophoresis on SDS- PAGE using a 12% polyacrylamide separating gel stacked by a 5% polyacrylamide stacking gel having composition as described by Laemmli (1970). Protein samples were removed from -20^0C and mixed with 6x sample buffer. Now the mixture was boiled at 100^0C for 5 min. Gels were electrophoresed at a constant current initially at 24mA and then at 35mA until the blue dye front had nearly run out of the gel. After that the gel was submerged in the staining solution (Coomasie Brilliat Blue R-250) for l h followed by overnight destaining in destaining solution.

Immunochemical characterization of Omp28 protein

The electrophoresed proteins on the polyacrylamide gel were electroblotted to a nitrocellulose membrane using a Mini Trans-Blot@ Cell (BioRad, USA) according to a standard protocol. The electrophoretic transfer was conducted at a constant current of 45mA for 3 hrs. After transfer the membrane was washed in TBS-T (10mM TrisHCl, 100 mM NaCl, 0.1% Tween at pH7.4) and then blocked with 3% non-fat milk extract in TBS-T for l hr. The primary antibody was positive control for brucellosis so the membrane was exposed to primary antibody at 1:100 dilutions in TBS-T for 1hr at 37^0C. Now membrane was washed with TBS-T three times for ten minutes and was incubated with secondary antibodies rabbit anti bovine IgG conjugated with horseradish peroxidase (Sigma, USA) at 1:2000 dilution for 1 h at 37^oC. The membranes were washed three times for ten minutes in TBS-T. Omp28 bands were visualized by a colorimetric method using 3,3'-diarninobenzidine tetrahydrochloride (DAB) as the substrate and hydrogen peroxide solution.

3. Results

Omp28 gene of *Brucella abortus* was amplified, cloned into pET-32b vector and expressed in E. coli BL21 (DE3) as a recombinant protein. The results obtained in the present study are documented under the following sub headings:

3.1 *Extraction of Genomic DNA*

Genomic DNA was extracted from *Brucella abortus* strain 19 by phenol chloroform extraction method. The extracted DNA was further analyzed for qualitative analysis by ethidium bromide stained 1% agarose gel electroplroresis. The DNA sample was visualized as high molecular size distinct band (Fig. 1). The purity and concentration of DNA samples as ratio of molar absorbance coefficient at wavelengths 260nm and 280nm (A_{260}/A_{280}) and found between 1.7 and 1.9. This genomic DNA was used as template for amplification of gene of interest by polymerase chain reaction.

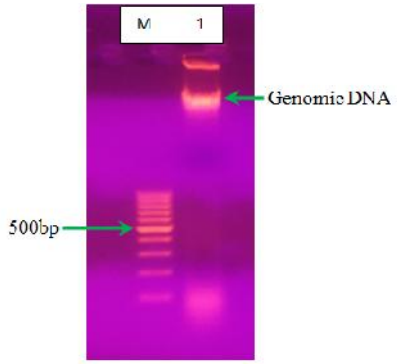

Figure 1: Genomic DNA extracted using phenol chloroform extraction method from *Brucella abortus* strain 19
Lane M : GeneRuler 100bp plus DNA Ladder (Fermentas)
Lane 1 : Genomic DNA of *Brucella abortus*

3.2 *PCR amplilication of omp28 gene*

PCR amplification of omp28 gene was carried out by using specific primer set. The agarose gel electrophoresis of PCR product on 1 percent gel along with 100bp molecular size DNA marker revealed specific amplicon of size 753bp (Fig. 2).

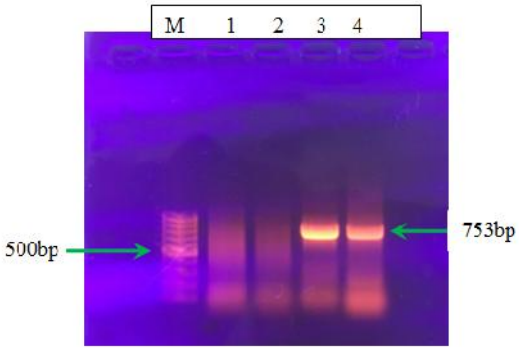

Figure 2: Amplification of *omp*28 gene from genomic DNA of *Brucella abortus*
Lane M : GeneRuler 100bp plus DNA Ladder (Fermentas)
Lane 1, 2: Negative Control
Lane 3, 4 : Amplified Product (753bp)

3.3 *Cloning of omp28 gene with pET 32b(+) vector*

pET 32b(+) vector and purified PCR product were digested with the same restriction enzymes, BamHI and Hindlll. The digested omp28 gene and pET 32b(+)vector were again purified by GeneJet gel extraction kit and ligated by T4 DNA ligases. Ligation mix was used to transform *Escherichia coli* BL21 (DE3) competent cells. Transformed cells were seeded on solid LB medium with IPTG and ampicillin and incubated for 24-36 hrs. for colony development. These colonies were screened for the presence of insert into vector by colony PCR and later on confirmed by plasmid PCR.

3.4 *Expression of recombinant omp28 mature protein using prokaryotic system*

For protein expression, the recombinant plasmid containing omp28 gene in *Escherichia coli* BL21 (DE3) cells were inoculated into 200 ml LB broth medium supplemented with ampicillin (50 mg/ml). When OD_{600} of the broth reached around 0.6 - 0.8 (approximately 4 hrs growth), the expression was induced with 1mM IPTG and broth was further incubated for 4hrs. On induction of the *E. coli* BL2l cells harboring the recombinant plasmid with 1mM IPTG for 4 hrs produced a predominant band of approximately 28 kDa protein on SDS-PAGE (Fig.3).

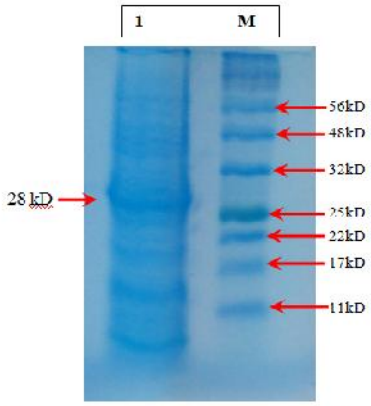

Figure 3 : *Brucella abortus omp*28 gene expression in *Escherichia coli* DH5α Cells
Lane M : Color Prestained protein marker broad range (New England Biolabs)

3.5 Immunoreactivity of recombinant Omp28 protein

The expression of *Brucella abortus* omp28 protein in BL21 cells was confirmed by western blotting using known bovine Brucella positive serum. The recombinant omp28 protein was separated on SDS-PAGE gel together with a protein molecular weight marker, and then transferred to a nitrocellulose membrane. Upon transfer of recombinant protein on nitrocellulose membrane and probing with the bovine Brucella positive serum and HRPO conjugated anti-bovine antibody, the colour developed with chromogen substrate, indicated that the 28kDa protein corresponding to recombinant protein (Fig. 4). This indicated that the expressed protein was of Brucella specific epitope.

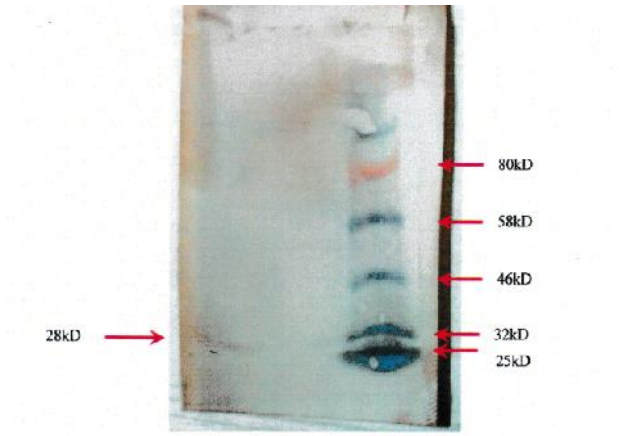

Figure 4 : Western Blot analysis of recombinant Omp28 kDa protein with *Brucella* positive serum

4. Discussion and Conclusion

Brucellosis, a zoonotic infection, is also recognized as an emerging public health disease that is endemic in most regions of the developing countries including India. The source of transmission of human brucellosis is animals. In cattle and buffaloes, it is mainly caused by *B. abortus* i.e. responsible for almost 90-95% cases of bovine brucellosis with exceptional incidence of *B. melitensis*. Moreover, being intracellular bacteria it is always difficult to diagnose and treat the infection in both human and animals (Kumar et al., 2016). In spite of such veterinary and public health importance of Brucella infections, the sensitive and specific diagnostic test for this disease is limited. Moreover, the live attenuated *Brucella ahortus* vaccines that are currently available induce a high-titer antibody response against the O-polysaccharide of the LPS, which interferes with the serologic diagnosis of the disease. Among non-lipopolysaccharide antigen, the outer membrane proteins (OMPs) of Brucella spp. were initially identified in the early 1980s and have been extensively characterized as potential immunogenic and protective antigens (Golshani *et al*., 20l5a; Abkar *et a1*.,2015; Simborio *et a1*.,2016; lm *et a1*,, 2016). In addition, antibodies against a few recombinant antigens, such as Omp25, Omp28 and Omp31, have been studied and identified as potential diagnostic markers (Yizcatno *et al*., 1996; Lim *et al*., 2012a; 2012b; Gomez 2016).

Outer membrane protein (Omp) can be a good diagnostic candidate due to their antigenicity and immunogenicity. Moreover, these Omps may also produce species specific responses that can be of further diagnostic value (Kumar *et al.*,2016). There are 5-7 major Omps in Brucella and out of these, Omp28 is considered as *Brucella abortus* specific and can be of diagnostic value as for the success of a diagnostic test it should be sensitive and specific and for that either monoclonal antibodies or recombinant Omp protein are recommended for the purpose. High level expression of Brucella Omp28 was achieved in *E. coli* system. Contrary to previous reports (Kumar *et al*., 2008; zygmunt *et al*., 2002) we could detect majority of expressed as inclusion bodies in the cytosol of the cell. Formation of inclusion bodies is a common feature when protein expresses at a high level. Aggregate formation increased the stability of the expressed protein. Expressed OMP28 protein could be purified after several days of induction without degradation of the protein. Keeping all these facts in view, the present study was taken up to characterize immunogenicity of omp28 gene for diagnosis of bovine brucellosis. For detection of Brucella DNA, various genes were targeted with different specific

primers along with different thermocyclic conditions (Gupta *et al*., 2006; Fretin *et al*., 2008; Huber *et al*., 2009; Singh *et al.*, 2010). In the present study, the amplification of omp28 gene from *Brucella abortus* was achieved by PCR using specific primers (Lim et al., 2012). As expected, the amplicon of 753 bp was amplified. The PCR amplified omp28 gene was cloned into pET32b(+) vector and transformed into *E. coli* BL21 (DE3) competent cells. Transformed cells were plated onto LB plate containing ampicillin. The individual colonies were confirmed by colony PCR and Plasmid PCR using same primers and thermocyclic conditions. The recombinant *E. coli* clone containing gene of insert was grown in LB medium and induced by IPTG and incubated for 4 hrs in shaking conditions. The harvested cells after lysis were subjected to SDS-PAGE for analysis expressed proteins. The SDS-PAGE analysis revealed the presence of distinct protein band of rOmp28 with molecular weight of approximately 28 kDa after staining with commassive brilliant blue. The desired protein band was absent in uninduced clone. This molecular weight was agreed with the theoretically molecular weights prediction of the expressed rOMP28 fusion protein. Similar findings have also been reported in case of *E. coli* expression system by various workers (Kumar et al., 2008; Lim et al., 2012a). After expression with IPTG, the recombinant omp28 protein was purified under denatured conditions using 8M urea. Cells were lysed by lysis buffer and clear cell lysate was purified by Ni-NTA chromatography. The recombinant protein that bound to the Ni NTA agarose was eluted using a pH gradient. Omp28 protein was expressed with N-terminal histidine tag, and thereby could be purified by using. Ni-NTA agarose resin. High purity of recombinant protein was obtained by this method. Use of histidine tag as a fusion partner has also been used for affinity purification of recombinant protein (Thavaselva et a1., 2010; Lim et al., 2012a; Simborio et at., 2015). The purified protein's purity was checked by SDSPAGE analysis by staining with Coommassie blue. Protein estimation was done by Bradford method, where the concentration of recombinant protein was approximately 3.1 mg protein/ml. The immunoreactivity of the expressed protein was confirmed by western blotting. The blots of rOmp28 reacted with Brucella-positive bovine serum.

In conclusion, it can be inferred that the recombinant Omp28 protein of *Brucella abortus* was successfully expressed in E coli expression system and the yield of recombinant Omp28 protein was high. This is suitable for bulk production of antigen in a very short period of time and also takes into account the safety aspects associated with handling *Brucella abortus* in the laboratory for preparation of LPS antigen. The rOmp28 protein can also be used for the development of field test or pen side detection and latex agglutination test to avoid the expenditure of costly equipments.

Acknowledgments

The authors thank the Dean of the College of Biotechnology and the Hon'ble Vice Chancellor, Uttar Pradesh Pandit Deen Dayal Upadhayay Pashu Chikitsa Vigyan Vishvidhyalaya Evum Go-Anusandhan Sansthan Pandit Deen Dayal Upadhayay Pashu Chikitsa Vigyan Vishvidhyalaya Evum Go-Anusandhan Sansthan (DUVASU), Mathura, India, for providing funds and necessary facilities to conduct this study.

References

1. Abkar, M.; Lotfi, A.S.; Amani, J,; Eskandari, K.; Ramandi, M.F.; Salimian, J.; Brujeni, G.N.; Alamian, s.; Kamali, M. and Koushki, H. (2015) Survey of omp19 immunogenicity against *Brucella abortus* and *Brucella melitensis*, influence of nanoparliculation versus traditional immunization . *Vet Res Commun*..39:217-228.
2. Chaudhuri, P.; Prasad, R.; Kumar, V. and Gangaplara, A,K. (2009) Recombinant oMP28 antigen based indirect ELISA for serodiagnosis of bovine brucellosis. *Mol Cell Probes*,24: 142145.
3. Ciftci, A.;Ica, T.;Savasan, S.;Sareyupoglu, B.;Akan. M. and Diker, K.S. (2017) Evaluation of PCR methods for detection of Brucella strains from culture and tissues. Trop Anim Health *Prod. doi 10.1007/sl 1250-A11-1256-l.*
4. Cloeckaert, A,; Baucheron, S.; Vizcaino, N. and Zygmunt, M.S. (2001) Use of recombinant BP26 protein in serological diagnosis of *Brucella melitensis* infection in sheep. *Clin. Diagn. Lab. Immunol*., 8: 772-775.
5. Corbel, M.J. and Brinley-Morgan, W.J. (1984) Genus Brucella. ln Bergey's Manual of Systematic Bacteriology (ed). *Williams and Wilkins*,pp 371-388
6. Dhama, K.; Rajagunalan, S.; Chakraborty, S.; Verma, A.K.; Kumar, A.; Tiwari, R. and Kapoor, S. (2013). Food-borne pathogens of Animal origin-Diagnosis, prevention and control and their zoonotic significance- A review. *Pak. J. Bioi. Sci*.,l6(20): 1076-85,

7. Fretin, D.; Whatmore, A.M.; Al-Dahouk, S.; Neubauer, H.; Garin-bastuji, B. and Albert, D. (2008) *Brucella suis* identification and biovar typing by real-time PCR. *Vet. Microbiol*. 131:376-385.
8. Gadaga, B.M.; Etter, E.M.; Mukamuri, B.; Makwangudze, K.J.; pfukenyi, D.M. and Matope, G, (2016) Living at the edge of an interface area in Zimbabwe: cattle owners, commodity chain and health workers' awareness, perceptions and practices on zoonoses. *BMC Pubt Hlth*, l6(l):84.
9. Golshani, M.; Rafati, S. and Bouzari, S. (2015a) ln *silico* design, cloning and high level expression of L7/L12-T Omp31 fusion protein 6f Brucella antigens. Res pharma science, l0(5):436-445.
10. Gomez, L.A.; Alvarez. F.I.; Fernandez, P.A.; Flores, M.A.; Molina, R.E.; coloma, R.F. and oflate A'A, (2016) Immunogenicity and Protective Response Induced by Recombinant Plasmids Based on the BABl-0267 and BABI_0270 Open Reading Frames of Brucella abortus 2308 in BALB/c Mice. *Front Cell infect Microbiol*.,6: 117.
11. Gupta, V.K.; Verma, D.K.; Singh, K.; Kumari, R.; Singh, S.V. and Vihan, V.S. (2006) Single_ step PCR for detection of Brucella melitensis from tissue and blood of goats. Small *Rumi. Res*. 66:169-174.
12. Huber, B.; scholz, H.c.; Lucero, N. and Busse, H,J. (2009) Development of a PCR assay for typing and subtypingof Brucella species. Int. J. Med. Microbiol.299 563-573
13. Im, Y.B,; Jung, M.; Shin, M.; Kim, S. and yoo, H.S. (2016a) Expression of cytokine and apoptosis-related genes in bovine peripheral blood mononuclear cells stimulated with *Brucella abortus* recombinant proteins. *Vet Res*.,47 30.
14. Kaushik, P.; Singh, D.K.; Kumar, S.V.; Tiwarim A,K,; Shuklam, G,; Dayal, S. and Chaudhuri, P. (2010) Protection of mice against *Brucella abortus* 544 challenge by vaccination with recombinant OMP28 adjuvanted with CpG oligonucleotides. *Vet Res Commun*.,34:119- 132.
15. Kim, H.N.; Hur, M.; Moon, H.W.; Shim, H.S,; Kim, H.; Ji, M.;Yun, Y.M.; Kim, S.Y.; Um, J.,Lee, Y.S. andHwang, S.D. (2016) First Case of Human Brucellosis Caused by Brucella melitensis in Korea. Ann Lab Med.,36(4):390-392.
16. Kittelberger, R.F.; Hansen, H.M.; Penrose, M.; delisle, G.W.; Letteson, J.J. and Carin-Bastujim B,, (1995) Serological cross reactivity between *Brucella abortus* gene and serological evaluation of the 1-rhodalton antigen that it encodes. *Clin Diagn Lab Immunol*.,2:263-267.
17. Ko, K.Y.; Kim, J.W.; Her, M.; Kang, S.l.; Jung. S.C.; Cho, D.H. and Kim, LY. (2012) Immunogenic proteins of *Brucella abortus* to minimiize cross reactions in brucellosis diagnosis. *Vet. Microbiol*., 156: 37 448.
18. Kumar, A.; Gupta, V.K.; Verma, A.K.; Sahzad; Kumar, V.; Singh, A. and Reddy, N.C.P, (2016) Seroprevalence and risk factors associated with bovine brucellosis in western Uttar Pradesh. *lndia. lndian J Anim Sci*, 86(2): 131-135.
19. Kumar, A.; Gupta, V.K.; Verma, A.K.; Sahzad; Kumar, V.; Singh, A. and Reddy, N.C.P, (2016) Seroprevalence and risk factors associated with bovine brucellosis in western Uttar Pradesh, lndia. lndian J Anim Scl, 86(2): 131-135.
20. Kumar, S.; Tuteja, U.; Kumar, A. and Batra, H.V. (2008) Expression and purification of the 26 kDa periplasmic protein of *Brucella abortus*: a reagent for the diagnosis of ovine brucellosis. *Biotechnol Appl Biochem*., 49 213-218.
21. Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T 4. Nature. 227 : 680-685.
22. Lim, J.J.; Kim, D.H.; Lee, J.J.; Kim, D.G.; Min, W.; Lee, H.J.; Rhee, M.H.; Chang, H.H. and Kim, S. (2012a). Evaluation of Recombinant 28 kDa Outer Membrane Protein of *Brucella abortus* for the Clinical Diagnosis of Bovine Brucellosis in Korea. *J. Vet. Med. Sci*.,74(6): 687-691.
23. Mirkalantari, S.; Zarnani, A.H.; Nazari, M.; Irajian, G.H. and Anrirmozafar, N. (2017) *Brucella melitensis* VirBl2 recombinant protein is a potential marker for serodiagnosis of human brucellosis. *Ann Clin Microbiol Antimiqob*., l6:8.
24. Munoz, P.M.; Marin, C.M.; Monreal, D.; Gonzalez, D.; Garin-Bastuji, B.; Diaz, R.; Mainar Jaime, R.C.; Moriyon, I. and Blasco, J.M. (2005) Efficacy of several serological tests and antigens for diagnosis of bovine brucellosis in the presence of false-positive serological results due to *Yersinia enterocolitica* O:9. *Clin. Vaccine Immunol*,12: l41-151.
25. Nielsen, K.; Smith, P.; Widdison, J.; Gall, D.; Kelly, L.; Kelly, W. and Nicoletti, P. (2004) Serological relationship between cattle exposed to *Brucella abortus*, *Yersinia enterocolitica* O;9 and *Escherichia coli* O157: H7. *Vet. Microbiol*., 100:25-30.
26. Radostits, E.D.; Gay, C,C. and Inchcliff; K.W. (2000) Veterinary Medicine, Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and

Horses.9th ed. W.B. Saunders Company Ltd. New York, pp 867-882.
27. Sambrook, J.; Fritsch, E.F. and Maniatis, T. (1989) Molecular cloning: a laboratory manual.2nd ed. Cold Spring Harbor Laboratory Press
28. Simborio, H.L.T,; Reyes, A.W.B.; Hop, H.T.; Arayan, L.T.; Min, W.; Lee, H.J.; Lee, J.J.; Chang, H'H' and Kim, S. (2016) Immune Modulation of Recombinant OmpA against *Brucella abortus* 544 Infection in Mice. *J. Microbiol. Biotechnol*.,26(3): 603-609.
29. Singh, M.; Singh, D.K.; Shivaramu, K.V.; Biswas, R.; Rawat, S.; Boral, R.; Singh, S. and Cheema, P.S. (2010) Serum as clinical specimen in PCR for diagnosis of ovine brucellosis. *Indian J. Anim. Sci*.80(l): l7-18
30. Thavaselvam, D.; Kumar, A.; Tiwari, S,; Mishra, M. and Prakash, A. (2010) 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,5g: 421-428
31. Verstreate, D.R.; Creasy. M.T.; Caveney, N.T,; Baldwin, C.L.; Blab, M.W. and Winter, A.J. (1982) Outer membrane proteins of *Brucella abortus*: isolation and characterization. *Infect. Immun*., 35: 979 -989.
32. Vizcar'no, N.; Cloeckaert, A.; Zygrnunt, M.S. and Dubray, G. (1996) Cloning, nucleotide sequence and expression of the *Brucella melitensis* omp3 I gene coding for an immunogenic major outer membrane protein. *Infect Immun*, 64:3744-31.51 .
33. Weynants, V.; Tibor, A.; Denoel, P.; Saegerman, C.; Godfroid, J. and Letesson, J.J. (1996) Evaluation of an ELISA using *Yersinia* outer membrane proteins of the involvement of *Yersinia enterocolitica* O:9 in the false positive serological responses observed in bovine brucellosis diagnostic tests. *Vet Microbial*., 48 : 101-112.
34. Zygmunt, M.S.; Baucheron. S.; Vizcaino, N.; Bowden. R.A. and Cloeckaert, A. (2002) Single step purification and evaluation of reconrbinantBP26 protein for serological diagnosis of *Brucella ovis* infection in rams. *Vet Microbiol*., 87: 213-220.

Access this Article in Online

Website: www.ijarbs.com

Subject: Biotechnology

Quick Response Code

DOI:10.22192/ijarbs.2020.07.07.007

How to cite this article:

Deepti Singh, Sharad Kumar Yadav, Lalita Sharma. (2020). Cloning, expression, purification and immunochemical characterization of *Brucella abortus* 28kDa *Omp* encoding gene. Int. J. Adv. Res. Biol. Sci. 7(7): 61-68.
DOI: http://dx.doi.org/10.22192/ijarbs.2020.07.07.007