

Original Article

***In silico* analysis of Omp25 and BLS *Brucella melitensis* antigens for designing subunit vaccine**

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ABSTRACT

Brucellosis is a well-known infection in domestic animals which caused by *Brucella* bacterium. Due to serious economic and medical consequences of this disease, various efforts have been made to prevent the infection through the use of recombinant vaccines based on *Brucella* outer membrane protein (OMP) antigens. The objectives of the present study were cloning, sequencing and epitope prediction of Omp25 and BLS genes as two major *Brucella melitensis* antigens. The full-length open reading frame (ORF) of Omp25 and BLS genes were amplified and cloned into pTZ57R/T vector. Phylogenetic analysis of sequenced genes showed that both genes were nearly similar in different *Brucella* species. Several online prediction softwares were used to predict B and T-cells epitopes, secondary and tertiary structures, antigenicity ability and enzymatic degradation sites. Bioinformatic tools used in the current study were confirmed by the results of three different experimental epitope predictions. Bioinformatic analysis identified five and two B-cell and two and one T-cell epitopes for Omp25 and BLS antigens, respectively. Finally, according to the antigenicity ability and proteosomal recognition site common B and T-cell epitope was predicted for Omp25 (154-162 amino acids) and BLS (37-48 and 119-139 amino acids). Results of this study might be useful for recombinant vaccine development.

Keywords: *Brucella melitensis*, Omp25, BLS, Bioinformatics analysis, Recombinant vaccine

INTRODUCTION

Brucellosis, a common zoonotic disease, concerns veterinarians as well as public health authorities in developing countries (Karthik et al., 2013). This infection caused by the genus *Brucella* that can infect domestic animals primarily (Cutler et al., 2005). Brucellosis is characterized by abortion and reduced fertility in animals, and also by chronic infections with

symptoms such as undulant fever, arthritis and osteomyelitis in human (Pappas et al., 2006). *Brucella* outer membrane proteins (OMPs) are cell specific surface antigens which have remarkable immunogenicity characteristics (Gupta et al., 2012). OMPs are excellent candidates in production of recombinant vaccines against Brucellosis (Gupta et al., 2012). These cell surface antigens are classified into two main groups consisting of OMP2a and OMP2b, and OMP25

and OMP31 (Gupta et al., 2012). OMP25 antigen is one of the virulent factors and the major antigen involved in survival of *Brucella* and found to be highly conserved among different *Brucella* species (Cloeckeaert et al., 1996; Edmonds et al., 2002). *Brucella* lumazine synthase (BLS) as another *Brucella*'s antigen has important immunogenic characteristics and can be used effectively as an adjuvant when covalently attached to a foreign antigen (Cassataro et al., 2005). Immune system produces antibodies which specifically attach to identified region of antigens, named epitope (Berzofsky, 1985). Epitopes may be classified as B (continuous and discontinuous) and T-cell (MHC I and MHC II) epitopes (Zhang et al., 2012). Continuous or linear epitopes are made up of consecutive amino acids whereas the discontinuous epitopes constitute the spatially folded amino acids which lie far away in the primary sequence. T-cell epitopes are antigenic peptide strings recognized by T-cells receptors (Chen et al., 2011). B and T-cell epitopes could be predicted using computational tools for the vast applications in the area of antibody production, immunodiagnosics, epitope-based vaccine design and selective de-immunization of therapeutic proteins and in autoimmunity (Steere et al., 2011). These tools are more cost effective and feasible and could help to increase the efficiency of experiments with lowering costs and increasing accuracy (Gu and Bourne, 2009). Currently, several epitope prediction softwares are available. The first generation of these prediction softwares were supported by motif-based algorithm (Chen et al., 2011), antigen's primary amino acid sequence (Hopp and Woods, 1981) or other physiochemical protein characteristics. Recently more sophisticated methods using various machine learning based algorithms have been developed based on support vector machines (Donnes and Elofsson, 2002), hidden markov models (Noguchi et al., 2002) and artificial neural networks (Buus et al., 2003). The objectives of the present study were cloning, sequencing and epitope prediction of two candidate *B. melitensis* antigens Omp25 and BLS and confirmation

of predicted epitopes with results of experimental epitope prediction studies.

MATERIALS AND METHODS

Bacterial strains, growth conditions and isolation.

B. melitensis strain Rev 1 was obtained from the *Brucella* culture collection (Razi Institute, Mashhad, Iran) and cultured as described (Delpino et al., 2007). DNA was extracted using a DNA extraction kit (Bioneer, Korea). The quality and purity of the extracted DNA were analyzed by agarose gel electrophoresis and NanoDrop ND-100 spectrophotometer (Thermo, USA), respectively. *Escherichia coli* strain DH5 α was used as host for cloning. pTZ57R/T (Thermo, USA) was used as T/A cloning vector for cloning and sequencing of amplified genes.

PCR amplification. *B. melitensis* Rev1 genomic DNA was used as template for amplification of full length open reading frame of Omp25 (642 bp) and BLS (477 bp) genes, using EX Taq DNA polymerase (Takara, Japan). Specific primers with restriction sites at the 5' ends (underlined) were designed using Primer Premier 5 (PREMIER Biosoft International), according to the available nucleotide sequences on the NCBI GenBank database (Table 1).

Table 1. The specific primers with restriction sites

Gene	Primer sequences (5' → 3')	Restrictin enzyme	Lenght (bp)
Omp25	F: <u>CCATGG</u> ATGCGCACTCTTC	<i>NcoI</i>	642
	R: <u>GAATTC</u> GAACTTG TAGCCGATGCC	<i>EcoRI</i>	
BLS	F: <u>GAATTC</u> ATGAACCAAAGCTGTCCC	<i>EcoRI</i>	477
	R: <u>GGATCC</u> TTATCAGACAAGCGCGGG	<i>BamHI</i>	

Polymerase Chain Reaction (PCR) was carried out, using the Personal Cycler™ thermocycler (Biometra, Germany) with the reaction mixture containing 2.5 μ l of 10X PCR buffer, 2 μ l MgCl₂ and 2 μ l dNTPs, 0.5 μ l of the DNA solution (50 to 100 ng/ μ l), 1.5 μ l of mix primer (5 pmol/ μ l) and 0.125 U/ μ l of EX Taq DNA polymerase, and some deionized water up to a final

volume of 25 μ l. The PCR program was performed with an initial denaturation step at 94 °C for 6 min followed by 30 cycles of denaturation at 94 °C for 30 sec, annealing at 62°C for 30 sec and extension at 72 °C for 45 sec, and a final extension at 72 °C for 10 min.

Cloning. PCR products were purified from the agarose gel by Ron's Agarose Gel Mini prep Kit (BioRon, Germany) according to the manufacturer's instruction. Purified PCR products were ligated into pTZ57R/T cloning vector by T/A cloning according to the manufacturer's instruction (Ins T/A clone™ PCR Product Cloning Kit, Thermo, USA). Competent cell preparation and transformation steps were performed (Sambrook and Russell, 2001). Recombinant vectors were transformed into competent *E.coli* DH5 α . The recombinant clone(s) harboring plasmid DNA with inserts were screened based on their ampicillin resistance. The fidelity of *E.coli* DH5 α transformants was verified by PCR reaction using M13 universal primers. Recombinant plasmids were purified using the Ron's Plasmid Mini Kit (BioRon, Germany) and confirmed by restriction enzyme digestion. Purified plasmids were subjected to sequencing at Bioneer company (South Korea). Obtained sequences were analyzed by homology search and aligned with other reported Omp25 and BLS genes using Basic Local Alignment Search Tool (BLAST) and CLC Main workbench 5.5 software (CLC bio), respectively.

Prediction of the secondary and tertiary structure. The secondary and tertiary structures of Omp25 and BLS were predicted using the improved self-optimized prediction method (SOPMA) software (http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_sopma.html) (Geourjon and Deleage, 1995) with four conformational states (helices, sheets, turns and coils) of candidate genes were analyzed and 3D Ligand Site, the online ligand-binding site prediction server (<http://www.sbg.bio.ic.ac.uk>) (Wass and Sternberg, 2009), respectively.

Servers and software for B and T-cell epitopes prediction. B and T-cell epitopes of candidate genes were predicted using different servers and software like

as: ABCpred, BepiPred, BCPred, SVMTrip and LEPS for B-cell prediction and IEDB, SYFPEITH, PropredI and Propred for T-cell prediction.

Confirming the bioinformatic analysis approach.

In order to confirm our predicted outputs, the results of three experimental epitope prediction studies (Table 1) were evaluated by bioinformatic tools which used in present study.

Characterization of epitopes. Final B and T-cell predicted epitopes were evaluated using the VaxiJen 2.0 server (<http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html>) for the alignment-independent prediction of protective antigens. In addition, enzymatic degradation sites, Mass (Da) and pI were determined using the Protein Digest server (<http://db.Systemsbiology.net:8080/proteomicsToolkit/proteinDigest.html>).

RESULTS

PCR amplification, Cloning and Nucleotide sequencing analysis. The OMP31, OMP25 and BLS genes from *B. melitensis* Rev1 were amplified. The accuracy of these fragments visualized on agarose gel electrophoresis (Figure 1).

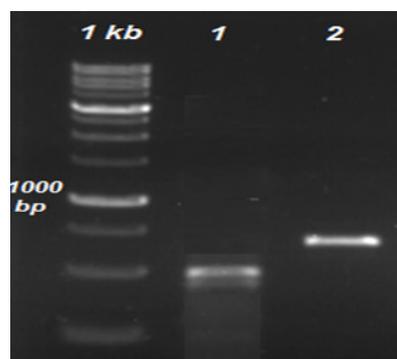


Figure1. PCR products of OMP25 and BLS genes
1: BLS with the length of 477 bp
2: OMP25 with the length of 642 bp

Amplified products were successfully ligated into pTZ57R/T cloning vector and transformed into competent *E. coli* DH5 α cells. After selection of positive colonies using Colony-PCR, the integrity of the recombinant plasmids were confirmed by restriction enzyme digestion. The sequencing of recombinant

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
omp25	1		99.38	99.38	99.22	99.53	99.53	99.38	99.38	99.07	99.84	99.84	99.84	99.84	100.00	
B.suis bv. 1 str. S2.CP006961.1	2	4		100.00	99.84	99.84	99.84	99.69	99.69	99.38	99.22	99.22	99.22	99.22	99.38	
B.suis VB122.CP003128.1	3	4	0		99.84	99.84	99.84	99.69	99.69	99.38	99.22	99.22	99.22	99.22	99.38	
B.canis str. Oliveri.HG803175.1	4	5	1	1		99.69	99.69	99.53	99.53	99.22	99.07	99.07	99.07	99.07	99.22	
B.pinnipedialis B2/94.CP002078.1	5	3	1	1	2		100.00	99.84	99.84	99.53	99.38	99.38	99.38	99.38	99.53	
B.suis ATCC 23445.CP000911.1	6	3	1	1	2	0		99.84	99.84	99.53	99.38	99.38	99.38	99.38	99.53	
B.abortus A13334.CP003176.1	7	4	2	2	3	1	1		100.00	99.69	99.22	99.22	99.22	99.22	99.38	
B.abortus S19.CP000887.1	8	4	2	2	3	1	1	0		99.69	99.22	99.22	99.22	99.22	99.38	
B.abortus X79284.1	9	6	4	4	5	3	3	2	2		98.91	98.91	98.91	98.91	99.07	
B.melitensis strain 0331.JX627633.1	10	1	5	5	6	4	4	5	5	7		100.00	100.00	100.00	99.84	
B.melitensis NI.CP002931.1	11	1	5	5	6	4	4	5	5	7	0		100.00	100.00	99.84	
B.melitensis M5-90.CP001851.1	12	1	5	5	6	4	4	5	5	7	0	0		100.00	99.84	
B.melitensis M28.CP002459.1	13	1	5	5	6	4	4	5	5	7	0	0	0		100.00	
B.melitensis ATCC 23457.CP001488.1	14	1	5	5	6	4	4	5	5	7	0	0	0	0		99.84
B.melitensis bv. 1 str. 16MAE/008917.1	15	0	4	4	5	3	3	4	4	6	1	1	1	1	1	

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
BLS	1		100.00	100.00	100.00	100.00	100.00	100.00	99.79	99.79	99.79	99.79	99.79	99.79	99.58	
B.abortus A13334.CP003177.1	2	0		100.00	100.00	100.00	100.00	100.00	99.79	99.79	99.79	99.79	99.79	99.79	99.58	
B.melitensis 16MAE/008918.1	3	0	0		100.00	100.00	100.00	100.00	99.79	99.79	99.79	99.79	99.79	99.79	99.58	
B.pinnipedialis B2/94.CP002079.1	4	0	0	0		100.00	100.00	100.00	99.79	99.79	99.79	99.79	99.79	99.79	99.58	
B.canis HSK A52141.CP003175.1	5	0	0	0	0		100.00	100.00	99.79	99.79	99.79	99.79	99.79	99.79	99.58	
B.canis ATCC 23365.CP000873.1	6	0	0	0	0	0		100.00	99.79	99.79	99.79	99.79	99.79	99.79	99.58	
B.abortus S19.CP000888.1	7	0	0	0	0	0	0		100.00	99.79	99.79	99.79	99.79	99.79	99.58	
B.canis str. Oliveri.HG803176.1	8	0	0	0	0	0	0	0		99.79	99.79	99.79	99.79	99.79	99.58	
B.suis bv. 1 str.CP006962.1	9	1	1	1	1	1	1	1	1		100.00	99.58	99.58	99.58	99.37	
B.suis VB122.CP003129.1	10	1	1	1	1	1	1	1	1	0		99.58	99.58	99.58	99.37	
B.melitensis NI.CP002932.1	11	1	1	1	1	1	1	1	1	2	2		100.00	100.00	99.37	
B.melitensis M5-90.CP001852.1	12	1	1	1	1	1	1	1	1	2	2	0		100.00	99.37	
B.melitensis M28.CP002460.1	13	1	1	1	1	1	1	1	1	2	2	0	0		100.00	
B.melitensis ATCC 23457.CP001489.1	14	1	1	1	1	1	1	1	1	2	2	0	0	0		99.37
B.ovis ATCC 25840.CP000709.1	15	2	2	2	2	2	2	2	2	3	3	3	3	3	3	

Figure 2. Pairwise comparison between candidate genes and other Brucella spp.

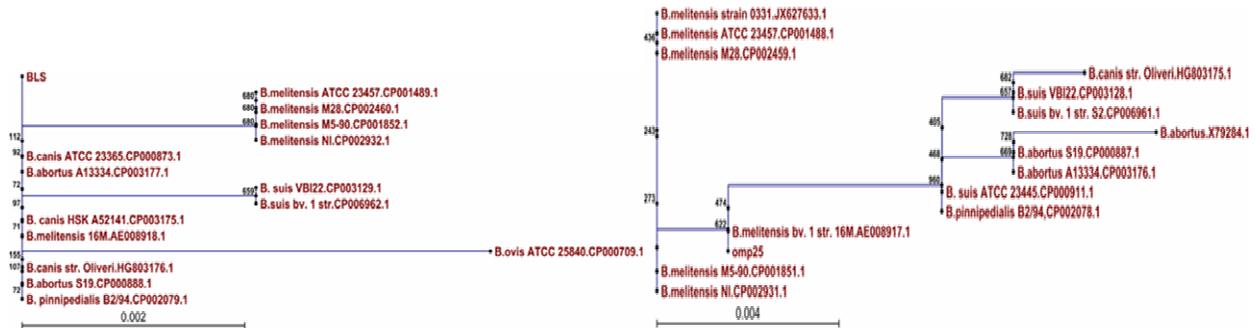


Figure 3. Phylogenetic tree between B.melitensis Rev 1 and other Brucella spp.

plasmids performed with specific primers as well as universal M13 primer. Sequences have been checked thoroughly and then published in NCBI gene bank with accession numbers of KJ193850 and KJ401344 for Omp25 and BLS genes, respectively. Obtained sequences were analyzed using BLAST and CLC Main workbench 5.5 software program. These sequences were aligned with other OMPs and BLS sequences from different Brucella species. Results showed that the BLS gene sequence was found to have 100% homology with the previously reported *B. melitensis* and *B. abortus* sequences and 99.58% homology with *B. ovis*. Omp25 gene sequence was found to be identical with the *B. melitensis* 16M and had lower similarity observed between Omp25 of *B. melitensis* Rev1 and *B. canis* (Figure 2). Upper triangle showed percent identity and down triangle showed differences in each matrix. In addition, the phylogenetic tree drawn for confirming the genetic distance matrix results. These analysis showed that the BLS gene sequence had higher similarity with the reported *B. melitensis* and *B. abortus* and also, Omp25 gene sequence was found to be like the *B. melitensis* 16M, as similar as pairwise comparison matrix results.

Prediction of the secondary structure. In order to assess the antigenic features of the candidate proteins, secondary structures were predicted using SOPMA server software. The results revealed that the proportion of random coils, β turns, α helices and extended strands (β folds) accounted for 51.64, 2.82, 20.66 and 24.88% in Omp25 and 25.32, 6.96, 51.90 and 15.82% in BLS. A greater proportion of extended strands and random coils present in the structure of the protein corresponded with an increased likelihood of the protein forming an antigenic epitope. The Omp25 have more antigenic ability compare to BLS.

Confirming the bioinformatic analysis. In order to validate B and T cell predicted softwares used in the present study, three antigens whose epitopes were determined experimentally (<http://www.iedb.org>) were selected and their epitopes were predicted using bioinformatic tools. Results revealed that our *in silico* predicted epitopes were similar to obtained founding of experimental studies for all of selected antigens (Table 2).

Table 2. Training bioinformatics software that used in present study

Antigen	Predicted epitopes	Experimental epitopes	Reference
GroEL ¹ of Yarsinia	28-42,78-92,178-185,275-290, 315-336 ,430-440,526-545	316-326	Yamaguchi et al,1996
Dnak ² of Brucella	40-67,78-92,210-227,357-370,523-537, 609-640	617-637	Vizcaino et al,1996
SOD ³ of Brucella	44-50, 70-86,134-153,147-165	75-86,136-150,149-162	Tabatabai et al,1994

1. Heat Shock Protein 60, 2. Heat Shock Protein 70, 3. Sodium Oxide Dismutase.

Similar epitopes between predicted epitopes using bioinformatics tools and experimental studies were specified by bold and were underline.

Prediction of the B and T-cell epitopes. The B-cell epitopes and MHCI (A-0101, A0201 and B-2705) and MHCII (DRB1-0101 and DRB1-0401) class of T-cell epitopes were predicted using online softwares. In each software epitopes with the highest score were selected. Moreover, five epitopes for Omp25 and three epitopes for BLS as final B-cell and four epitopes for Omp25 and three epitopes for BLS as final T-cell were chosen (Table 3).

Table 3. Final B and T-cell epitopes were predicted in this study

Gene	No.	Final B-cell epitope	Final T-cell epitope
Omp25	1	²⁶ AIQEQQPPVAPVEVAPQYS ₄₄	¹ MRTLKSLVIV ₁₀
	2	⁵⁹ NKAKTSTVGSIKPDDWKAGAF ₇₉	¹⁴ LLPFSATAF ₂₂
	3	⁸⁸ QIVYGVGDAGYSWAKKSKDGLEVK ₁₁₂	¹²² RVGYDLNPVMPY ₁₃₂
	4	¹⁴⁶ NNGLDDESKFRVGTAGAGLE ₁₆₆	¹⁵⁴ KFRVGTAG ₁₆₂
	5	¹⁷⁵ LEAKLTDNILGRVEYRYTQYGNKNYDLAGTT VRNKLDL ₂₀₂	²⁰⁶ RVGIGYKF ₂₁₃
BLS	1	³⁷ AAKTGGsVEVEI ₄₈	²⁸ ARKSFVAELAAKT ₄₀
	2	⁵² PGAYEIPLHAKTLARTGRYAAIV ₇₄	⁶⁷ TGRYAAIVGAA ₇₆
	3	¹¹⁹ FHESKEHHDFSHAHFKVKGV ₁₃₉	¹⁴³ AALQIVSERSRIA ₁₅₅

Table 4. Protein Digestion analysis of final B-and T-cell epitopes

Gene	NO.	B-cell epitopes	Mass (Da)	pI	Undigested enzyme
<i>Omp25</i>	1	AIQEQQPPVAPVEVAPQYS	2020.27	3.8	Trypsin, Clostripain, Cyanogen Bromide, Iodose Benzoate, AspN, Trypsin K, Trypsin R.
	2	NKAKTSTVGSIKPDDWKAGAF	2221.5	9.5	Clostripain, Cyanogen Bromide, Staph Protease, Trypsin R.
	3	QIVYGVEGDAGYSWAKKSKD GLEVK	2728.05	6.2	Trypsin R, ProlineEndopept, Cyanogen Bromide, Clostripain.
	4	NNGLDDESKFRVGTAGAGL E	2236.38	4.54	Cyanogen Bromide, ProlineEndopept.
	5	LEAKLTDNLRVEYRYTQYG NKNYDLAGTTVRNKLDL	4407.91	8.3	ProlineEndopept, Iodose Benzoate, Cyanogen Bromide.
<i>BLS</i>	1	AAKTGGSVEVEI	1160.29		Clostripain, Cyanogen Bromide, Iodose Benzoate, Proline Endopept, Chymotrypsin, Trypsin R, AspN, Cyanogen Bromide, Iodose Benzoate, Trypsin R, Clostripain
	2	FHESKEHHDFSHAHFKVKGVE	2532.76	6.55	
		T-cell epitopes	Mass(Da)	pI	Undigested enzyme
<i>Omp25</i>	1	RVGYDLNPVMPY	2423.65	5.83	Trypsin K, Iodose Benzoate, Staph Protease
	2	KFRVGTAG	1021.19	11	AspN, Staph Protease, ProlineEndopept, Cyanogen Bromide
	3	RVGIGYKF	939.13	9.9	Cyanogen Bromide, ProlineEndopept, Iodose Benzoate, AspN, Staph Protease

Characterization of epitopes. The results of Protein Digest server analysis for determination of mass (Da), pI and enzymatic degradation site showed in table 4. Results indicated that most of the predicted epitopes were lack of proteosomal recognition site. The candidate proteins identified as an antigen by VaxiJen 2.0 server (threshold 0.5) whit score 0.82 and 0.54 for *Omp25* and *BLS*, respectively. Also, the antigenicity of the final B and T-cell predicted epitopes was shown in table 5. Furthermore, the results of VaxiJen 2.0 analysis indicated that eight epitopes and two predicted epitopes had antigenicity ability for *Omp25* and *BLS*, respectively. The 3D structure of predicted epitopes with antigenicity ability were illustrated using 3D Ligand Site server (figure 4) after the antigenicity test of the final B and T-cell predicted epitopes.. 3D structure analysis also showed that all predicted B and T-cell epitopes located on the outside of the candidate antigens.

DISCUSSION

Several studies predicted epitopes of desired antigen by computationally approaches and used these results in experimental study in the aim of epitopic based

vaccine design (Simon et al., 2010). In this study, two dominant *B. melitensis* Rev1 antigens (*Omp25* and *BLS*) were candidate for cloning, molecular analysis and epitopic prediction. Results showed that all candidate genes successfully cloned and molecular analysis revealed that *Omp25* and *BLS* sequences of *B. melitensis* Rev1 are nearly similar to other *Brucella* spp. The strong nucleotide identity of these genes may be due to the high degree of genetic relatedness of these species (Rajagunalan et al., 2013). Comprehensive bioinformatics analyses were conducted on candidate antigens by online B and T-cell epitopic prediction servers. To confirm the results of our bioinformatics approaches, we analyzed three different antigens and compared obtained computational outputs with the experimental results. The bioinformatics analysis for GroEL, Dnak and SOD antigens predicted successfully experimentally achieved epitopes. The final epitopic prediction results proposed eight and two epitopes were predicted for *Omp25* and *BLS* proteins with antigenicity ability which was reported for the first time as *in silico* epitopes, respectively. The results of secondary and tertiary analysis showed that the common predicted B and T-cell epitopes were located

Table 5. The antigenicity of predicted epitopes

Gene	No.	Final B-cell epitope	VaxiJen score	Final T-cell epitope	VaxiJen score
<i>Omp25</i>	1	²⁶ AIQEPPVPAPVEVAPQYS ₄₄	0.66	¹ MRTLKSLVIV ₁₀	-0.1*
	2	⁵⁹ NKAKTSTVGSIKPDDWKAGAF ₇₉	0.7	¹⁴ LLPFSATAF ₂₂	0.23*
	3	⁸⁸ QIVYGVGEDAGYSWAKKSKD GLEVK ₁₁₂	1.2	¹²² RVGYDLNPVMPY ₁₃₂	0.7
	4	¹⁴⁶ NNGLDDESKFRVGTAGAGLE ₁₆₆	1.1	¹⁵⁴ KFRVGTAG ₁₆₂	1.38
	5	¹⁷⁵ LEAKLTDNILGRVEYRYTQYGKNYDLAGTTVRNKLD ₂₀₂	0.92	²⁰⁶ RVGIGYKF ₂₁₃	1.8
<i>BLS</i>	1	³⁷ AAKTGGSVEVEI ₄₈	2.26	²⁸ ARKSFVAELAAKT ₄₀	0.28*
	2	⁵² PGAYEIPHLAKTLARTGRYAAIV ₇₄	0.22*	⁶⁷ TGRYAAIVGAA ₇₆	0.12*

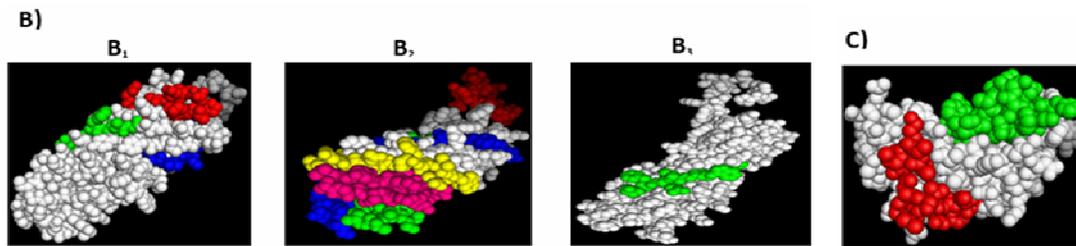


Figure 4. B-cell, T-cell and common predicted epitopes for *Omp25* (B) and *BLS* (C). B1: B-cell epitopes: 26-44, 59-79, 88-112, 146-166 and 175-202 showed by red, green, blue, yellow and pink color, respectively. B2: T-cell epitopes: 122-132, 154-162 and 206-213 identified by red, green and blue, respectively. B3: The same epitope between B- and T-cell epitopes: 154-162 identified by green. C: B-cell epitopes: 37-48 and 119-139 showed by red and green color, respectively.

in the random coil regions on the surface structure of candidate antigens. Random coil regions are located on the surface of the protein, where it is necessary for the surface structure to make appropriate binding to ligands, and have a high possibility of forming epitopes (Li et al., 2013). Epitope should lack of enzymatic recognition site, in order to prevent degradation of peptide (Toes et al., 2001). So, predicted B and T-cell epitope analyzed for enzymatic degradation and results revealed that the proposed epitopes in this study did not have enzymatic digestion sites for some cell dominant enzymes. Brucellosis is a common zoonotic disease that can infect domestic animals and till today there is no recombinant vaccine for it. Protein recombinant vaccines are feasible than live or poor bacterial vaccine because it can be produced in large scale and also are safe for recipient host. Therefore, the aim of the present

study was to clone, sequence, epitope prediction of two candidate *B. melitensis* antigens *Omp25* and *BLS* and confirmation of predicted epitopes with results of experimental epitope prediction studies in order to designing suitable recombinant vaccine. Phylogenetic analysis showed that both genes were nearly similar in different *Brucella* species and common B and T-cell epitope was predicted for *Omp25* (154-162 amino acids) and *BLS* (37-48 and 119-139 amino acids). In vitro synthesis of determined peptides and experimental validation are essential for using predicted epitope as an effective vaccine against *Brucella* pathogen. In this regards our laboratory has already initiated research in this direction.

Ethics

I hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest.

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References

- Berzofsky, J.A., 1985. Intrinsic and extrinsic factors in protein antigenic structure. *Science* 229, 932-940.
- Buus, S., Lauemoller, S.L., Worning, P., Kesmir, C., Frimurer, T., Corbet, S., Fomsgaard, A., Hilden, J., Holm, A., Brunak, S., 2003. Sensitive quantitative predictions of peptide-MHC binding by a 'Query by Committee' artificial neural network approach. *Tissue Antigens* 62, 378-384.
- Cassataro, J., Estein, S.M., Pasquevich, K.A., Velikovskiy, C.A., de la Barrera, S., Bowden, R., Fossati, C.A., Giambartolomei, G.H., 2005. Vaccination with the recombinant Brucella outer membrane protein 31 or a derived 27-amino-acid synthetic peptide elicits a CD4+ T helper 1 response that protects against Brucella melitensis infection. *Infect Immun* 73, 8079-8088.
- Chen, P., Rayner, S., Hu, K.H., 2011. Advances of bioinformatics tools applied in virus epitopes prediction. *Virol Sin* 26, 1-7.
- Cloeckaert, A., Verger, J.M., Grayon, M., Zygmunt, M.S., Grepinet, O., 1996. Nucleotide sequence and expression of the gene encoding the major 25-kilodalton outer membrane protein of Brucella ovis: Evidence for antigenic shift, compared with other Brucella species, due to a deletion in the gene. *Infect Immun* 64, 2047-2055.
- Cutler, S.J., Whatmore, A.M., Commander, N.J., 2005. Brucellosis--new aspects of an old disease. *J Appl Microbiol* 98, 1270-1281.
- Delpino, M.V., Estein, S.M., Fossati, C.A., Baldi, P.C., Cassataro, J., 2007. Vaccination with Brucella recombinant DnaK and SurA proteins induces protection against Brucella abortus infection in BALB/c mice. *Vaccine* 25, 6721-6729.
- Donnes, P., Elofsson, A., 2002. Prediction of MHC class I binding peptides, using SVMHC. *BMC Bioinformatics* 3, 25.
- Edmonds, M.D., Cloeckaert, A., Elzer, P.H., 2002. Brucella species lacking the major outer membrane protein Omp25 are attenuated in mice and protect against Brucella melitensis and Brucella ovis. *Vet Microbiol* 88, 205-221.
- Geourjon, C., Deleage, G., 1995. SOPMA: significant improvements in protein secondary structure prediction by consensus prediction from multiple alignments. *Comput Appl Biosci* 11, 681-684.
- Gu, J., Bourne, P.E., 2009. *Structural Bioinformatics*, Wiley.
- Gupta, V.K., Vohra, J., Kumari, R., Gururaj, K., Vihan, V.S., 2012. Identification of Brucella isolated from goats using PstI site polymorphism at Omp2 gene loci. *Indian J Anim Sci* 82, 240-243.
- Hopp, T.P., Woods, K.R., 1981. Prediction of protein antigenic determinants from amino acid sequences. *Proc Natl Acad Sci U S A* 78, 3824-3828.
- Karthik, K., Rathore, R., Verma, A.K., Tiwari, R., Dhama, K., 2013. Brucellosis – still it stings? *Livestock Technol* 2, 8-10.
- Li, Y., Liu, X., Zhu, Y., Zhou, X., Cao, C., Hu, X., Ma, H., Wen, H., Ma, X., Ding, J.B., 2013. Bioinformatic prediction of epitopes in the Emy162 antigen of. *Exp Ther Med* 6, 335-340.
- Noguchi, H., Kato, R., Hanai, T., Matsubara, Y., Honda, H., Brusica, V., Kobayashi, T., 2002. Hidden Markov model-based prediction of antigenic peptides that interact with MHC class II molecules. *J Biosci Bioeng* 94, 264-270.
- Pappas, G., Papadimitriou, P., Christou, L., Akritidis, N., 2006. Future trends in human brucellosis treatment. *Expert Opin Investig Drugs* 15, 1141-1149.
- Rajagunalan, S., Kumari, G., Gupta, S.K., Kumar, A., Agarwal, R.K., Rawool, D.B., Singh, D.K., 2013. Molecular characterization of Omp31 gene of Indian field isolates of Brucella melitensis. *Indian J Anim Sci* 83 673-677.
- Sambrook, J., Russell, D.W., 2001. *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
- Simon, G.G., Hu, Y., Khan, A.M., Zhou, J., Salmon, J., Chikhlikar, P.R., Jung, K.O., Marques, E.T., August, J.T., 2010. Dendritic cell mediated delivery of plasmid DNA encoding LAMP/HIV-1 Gag fusion immunogen enhances T cell epitope responses in HLA DR4 transgenic mice. *PLoS One* 5, e8574.
- Steere, A.C., Drouin, E.E., Glickstein, L.J., 2011. Relationship between immunity to Borrelia burgdorferi outer-surface protein A (OspA) and Lyme arthritis. *Clin Infect Dis* 52 Suppl 3, s259-265.
- Toes, R.E., Nussbaum, A.K., Degermann, S., Schirle, M., Emmerich, N.P., Kraft, M., Laplace, C., Zwinderman, A., Dick, T.P., Muller, J., Schonfisch, B., Schmid, C., Fehling, H.J., Stevanovic, S., Rammensee, H.G., Schild, H., 2001. Discrete cleavage motifs of constitutive and immunoproteasomes revealed by quantitative analysis of cleavage products. *J Exp Med* 194, 1-12.
- Wass, M.N., Sternberg, M.J., 2009. Prediction of ligand binding sites using homologous structures and conservation at CASP8. *Proteins* 77 Suppl 9, 147-151.
- Zhang, W., Liu, J., Zhao, M., Li, Q., 2012. Predicting linear B-cell epitopes by using sequence-derived structural and physicochemical features. *Int J Data Min Bioinform* 6, 557-569.