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In silico cloning and bioinformatics study of *Brucella melitensis* Omp31 antigen in different mammalian expression vectors

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Abstract *Brucella melitensis*, as a pathogenic gram-negative intracellular bacterium, causes brucellosis in animals and humans. According to literature, the *B. melitensis* outer membrane protein 31 (Omp31) is considered as an important vaccine candidate against brucellosis. The aim of the current study was to compare three different expression constructs containing *B. melitensis* Omp31 antigen using bioinformatics analysis approaches to facilitate choosing the best immunogenic construct. The coding sequence of Omp31 gene was PCR amplified, TA cloned and sequenced. The obtained DNA sequence was *in silico* cloned in pcDNA3.1/Hygro (+), pcDNA3.1/His A and pSecTag2/Hygro mammalian expression vectors using CLC Main Workbench 5.5 software. The Omp31 gene was successfully cloned into the pTZ57R/T vector, and recombination was confirmed by colony PCR and sequencing. Comparison of the obtained Omp31 sequence with other Omp31 gene sequences showed high similarities. Bioinformatics analysis of three different mammalian expression vectors harboring Omp31 gene made it possible to choose the best immunogenic structure for further studies in order to design effective DNA vaccines against brucellosis.

Keywords: bioinformatics, *Brucella melitensis*, *in silico* cloning, Omp31

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Introduction

Brucellosis, an infectious disease affecting various mammals, is caused by different species of the genus *Brucella* and remains endemic in many developing countries around the world (Habtamu et al., 2013). Abortions due to brucellosis imposed important economic losses to livestock industry. *Brucella melitensis* and *B. abortus* are the pathogenic species of *Brucella* genus, mainly involved in ovine and bovine brucellosis, respectively (Cloeckaert et al., 1996).

Vaccination is the most efficient tool for brucellosis control. To avoid the drawbacks of live-attenuated vaccines, it is essential to identify *Brucella* sp. protective antigens to implement in future subcellular vaccines. *B. melitensis* Rev 1 and *B. abortus* S19 and RB51 are attenuated strains that have been used for brucellosis control in domestic animals. Live vaccines have shown serious side effects which have restricted their use as ideal vaccines. In addition, these vaccines were not fully efficacious and hampered the field diagnosis of infection (Yang et al., 2005). These vaccines exhibited other disadvantages like inducing abortion in pregnant animals, remain pathogenic for humans and resistance to streptomycin (Cassataro et al., 2005). However, no effective and safe vaccine against human brucellosis has been developed so far.

Recent studies have focused on unraveling the mechanisms of protective immunity and development of an effective brucellosis vaccine (He and Xiang, 2010; Yang et al., 2013; Yang et al., 2011). Immunity against *Brucella* requires Th1 responses that are characterized by production of gamma interferon. The VIOLIN web-based vaccine database has identified 46 proteins that are conserved in all *Brucella* genomes with any sequence similarity with human or mouse proteins (He and Xiang, 2010). The outer membrane proteins (Omps) of *Brucella* sp. were initially identified in the early 1980s (Bowden et al., 1995; Dubray and Bezard, 1980) and have been extensively characterized as potential immunogenic and protective antigens (Cassataro et al., 2007; Cloeckaert et al., 1995). Three major Omps; Omp25 (25-27 kDa), Omp2b (36-38 kDa) and Omp31 (31-34 kDa), have been identified (Cloeckaert et al.,

2002), classified based on their apparent molecular mass (Cloeckaert et al., 1995).

It has been shown that antibodies against Omp31 (isolated from naturally or experimentally infected sheep) could protect mice against *B. melitensis* and *B. ovis* challenges. Therefore, the Omp31 has been suggested as a potential and interesting candidate for vaccine development against brucellosis.

Development of epitopic vaccines using experimental approaches is very costly due to the specific needs of molecular biology and immunological procedures. Epitope prediction has become possible by employing bioinformatics tools. Bioinformatics approaches are relatively rapid and low-cost methods and can replace the experimental methods, at least in part. The present study was a preliminary attempt for using the Omp31 gene; as an immunodominant antigen; to design an effective vaccine against brucellosis. The aim of this study was to choose the most suitable immunogenic construct by nucleotide analysis, *in silico* cloning, molecular characterization and bioinformatics study of *B. melitensis* Omp31 antigen in three different expression constructs.

Materials and Methods

DNA extraction and PCR amplification

Total DNA was extracted from purified *B. melitensis* strain Rev 1 Bactria using DNeasy Blood & Tissue Mini spin column DNA extraction kit (Qiagen, Germany) according to the manufacturer's instructions. Primers were designed using CLC Main Workbench 5.5 (CLC bio, Denmark) software based on published Omp31 gene sequences deposited in the NCBI GenBank database and were synthesized by Macrogen Co. (South Korea). PCR reaction was performed in a 25 µL volume containing 100 ng of template DNA, 0.5 µM of each primer 2.5 mM $MgCl_2$, 200 µM of each dNTPs, 1× PCR buffer and 1.5 unit of *pfu* DNA polymerase (Thermo Fisher Scientific, USA). Amplification reaction was performed using the following thermal profile: hot start at 94°C for 5 min, 35 amplification cycles (94°C for 40 sec, 55°C for 40 sec, and 72°C for 40 sec.), followed by a 72°C final extension for 10 min. The PCR products were analyzed by electrophoresis on 1% agarose gel in TBE buffer and visualized by SYBR® Safe (Invitrogen, UK) staining and UVidocGel Documentation system (SYNGNE, UK).

Cloning into pTZ57R/T vector

After electrophoresis of the PCR products, 723bp PCR products were purified from the agarose gel by QIAquick Gel Extraction Kit (Qiagen, Germany) according to the manufacturer's instruction. Because of removal of 3´-A overhangs necessary for TA cloning by proofreading polymerases like *pfu* DNA polymerase, 3´-A overhangs added to fragments. Tailed PCR products were ligated into pTZ57R/T vector (Fermentas, Germany) based on TA cloning scheme according to the manufacturer's instructions. A 1:3 (vector to insert) molar ratio was used. For transformation, 15 µL of ligation reaction product was added to 150 µL of competent cells from *Escherichia coli* strain DH5-α which was performed by calcium chloride method (Sambrook and Fritsch, 1989) and placed on ice for 40 minutes. Then the mixture was incubated at 42 ºC for 90 s and immediately was placed on ice for 5 minutes. 1 mL of LB antibiotic free medium was added to the transformed cells and incubated at 37 ºC for 2 hours with shaking. Recombinant plasmid was grown overnight at 37 ºC on a LB agar plate with ampicillin (100 µg/mL), X-Gal (Fermentas, Germany) and IPTG (Fermentas, Germany) for blue-white screening. After overnight incubation, plate was placed at 4 ºC for 2 hours and cells from white colonies were harvested and cultured on antibiotic containing LB agar plates.

Confirmation of recombination

Recombination was confirmed by colony PCR with Omp31 gene specific primers. Cells harboring the recombinant plasmid were cultured in antibiotic the containing the LB medium for 16 hours at 37 ºC in a shaker incubator. GeneJET Plasmid Miniprep Kit (Fermentas, Germany) was used to purify plasmids from *E. coli* DH5α according to the manufacturer's instructions. Recombinant and native plasmids were compared by electrophoresis of 3 µL of extracted plasmid on a 1% agarose gel. The recombinant plasmid was submitted for nucleotide sequencing (Macrogen, Korea) using universal primers.

In silico cloning into mammalian expression vectors

After primary analysis, the obtained Omp31 sequence was *in silico* cloned into three different mammalian expression vectors, pcDNA3.1/Hygro (+), pcDNA3.1/His A and pSecTag2/Hygro, using CLC main workbench software (CLC bio, Denmark). For this purpose, three pairs of primers were designed by CLC main workbench software (CLC bio, Denmark) with restriction sites according to multiple cloning sites of each vector. Finally, three constructs composed of designed fragme-

nts in desired plasmids were built and further analyzed by bioinformatics tools. Hydrophobicity plot of each protein construct was drawn which characterizes their hydrophobic and hydrophilic characteristics that may be useful in predicting membrane-spanning domains, potential antigenic sites and regions that are likely exposed on the protein surface (Kyte and Doolittle, 1982). ProtParam tool (http://expasy.org/tools/protparam.html) was employed for estimation and determination of deduced protein properties such as molecular weight, theoretical pI, amino acid composition, total number of negatively and positively charged residues, half-life, instability and aliphatic indices.

Bioinformatics study

The obtained nucleotide sequence of Omp31 gene was submitted to the BLAST search at NCBI server (http://www.ncbi.nlm.nih.gov/blast) for comparison with the sequences in the GenBank. For detailed analysis, all closely related sequences between published sequences were aligned using the CLC main workbench software (CLC bio, Denmark) by ClustalW2 multiple sequence alignment program. Phylogenetic and molecular evolutionary analyses were conducted using the CLC main workbench software (CLC bio, Denmark) by bootstrap test with 100 replications to estimate the confidence of branching patterns of the UPGMA tree. Furthermore, pairwise comparisons were performed to clarify the pairwise distances and percent identities. Four conformational states (helices, sheets, turns and coils) of Omp31 protein secondary structure were predicted using the improved self-optimized prediction method (SOPMA) software (https://npsa-prabi.ibcp.fr/cgi-bin/secpred_sopma.pl) (Geourjon and Deleage, 1995).

Prediction of the B-cell and T-cell epitopes

The B and T-cell epitopes of Omp31containing gene constructs were predicted using IEDB server at http://tools.iedb.org/main/analysis-tools. This site provides a collection of tools for prediction and analysis of immune epitopes. BepiPred linear epitope prediction method (Larsen et al., 2006) was used for predicting of the linear B-cell epitopes. The MHC class I and II binding sites were predicted using the recommended IEDB method for human and mouse source species.

Results

PCR amplification and TA cloning

The designed primers amplified an Omp31 gene fragment with 723 bp length (Figure 1a). A-tailed PCR products were successfully ligated into pTZ57R/T vector by TA cloning scheme. Colonies generated strong bands after Colony PCR, indicating recombination process was done as expected (Figure 1b). Because of different size of two native and recombinant plasmids, they were compared by electrophoresis on 1% agarose gel (Figure 1c). Nucleotide sequencing of recombinant plasmid; designated as pTZ57-Omp31; confirmed recombination and sequence data have been published on

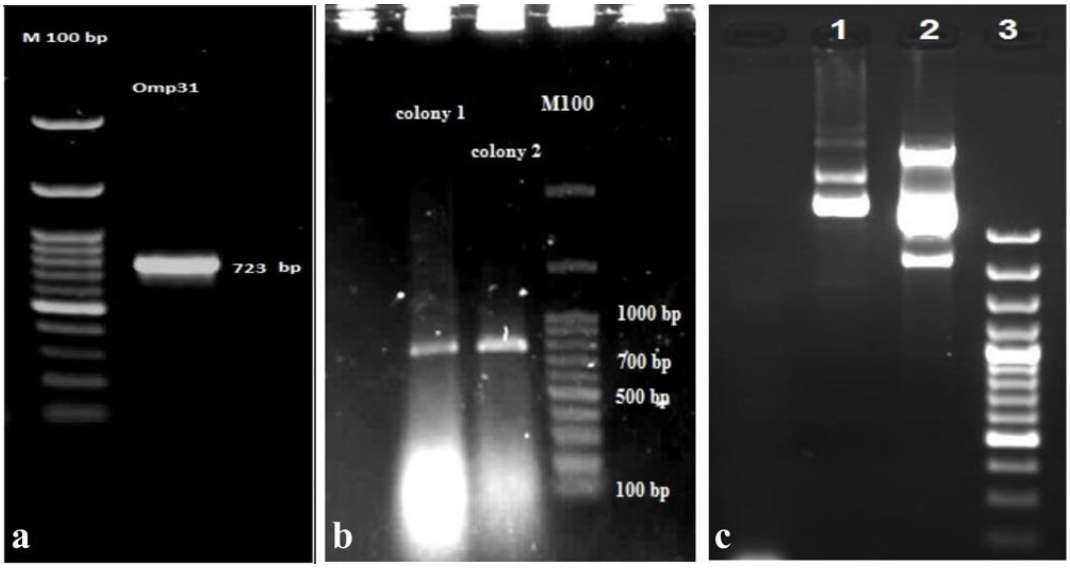


Figure 1. a: Amplified *Brucella melitensis* Rev1 Omp31 gene, b: Amplification of *B. melitensis* Rev1 Omp31 gene by colony PCR and c: Comparison of recombinant pTZ57-Omp31 (lane 1) versus native pTZ57R/T plasmid (lane 2). Lane 3 represents M100 plus size marker.

NCBI gene database with the accession No. KX100580.

In silico cloning and mammalian expression constructs design

A pair of gene-specific primers with specific restriction sites (according to vector and insert restriction map), initiation and termination codons was designed for each construct. No termination codon was placed in pSecTag2A/Hygro reverse primer (Table 1).

The Omp31 fragments were successfully *in silico* cloned into the three mammalian expression vectors using the CLC Main Workbench 5.5 (Figure 2a). the Omp31 fragment, inserted into pcDNA™3.1/Hygro (+), contained a Kozak consensus sequence (**A**CC**ATGA**) with an ATG initiation codon for proper initiation of translation. To ensure proper expression of recombinant protein in pcDNA™3.1/His vector, the Omp31 gene was cloned in frame with the ATG at base pairs 920–922 and created a fusion with the N-terminal polyhistidine tag, Xpress epitope, and the enterokinase cleavage site. Also for pSecTag2/Hygro A, interested fragment inserted in frame with the initiation ATG of the N-terminal Ig kappa chain leader sequence and the C-terminal myc epitope/polyhistidine tag. Inserts in two pcDNA3.1 vectors contained a stop codon for proper termination of gene of interest while it was not required for pSecTag vector (Table 1). Figure 2b shows the con-

Table 1 .PCR primers of *Brucella melitensis* Rev1 Omp31 gene for different constructs

Vector	Primer	Primer sequence*	Enzyme
pcDNA3.1/Hygro +	pc1-F	CCCAAGCTTGCCACC**ATG**AAGTCCGTAATTTTGGC	*Hind*III
	pc1-R	CGCGGATCCGGCAA**TTA**GAACTTGTAGTTCAGACCG	*Bam*HI
pcDNA3.1/His A	pc2-F	CCCGGATCCGCCAC**ATG**AAGTCCGTAATTTTGGC	*Bam*HI
	pc2-R	CGCGAATTCGGCAA**TTA**GAACTTGTAGTTCAGACCG	*Eco*RI
pSecTag2A/Hygro	Ps-F	CCCAAGCTTG**ATG**AAGTCCGTAATTTTGGCGTCC	*Hind*III
	Ps-R	CGCGGATCCGACATGAACTTGTAGTTCAGACCG	*Bam*HI

*Restriction sites are underlined, initiation and termination codons are shown in boldface.

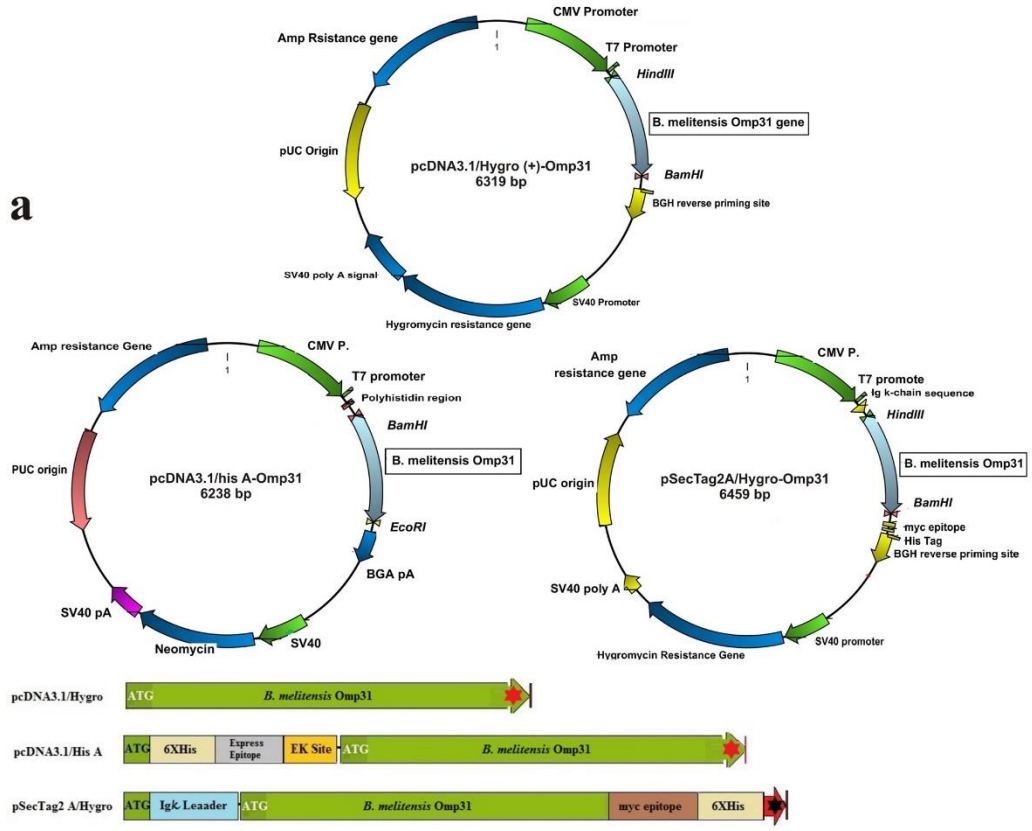


Figure 2. a: Three different *Brucella melitensis* Rev1 Omp31 inserted mammalian expression constructs, b: Three different structures of proteins derived of different expression constructs

structions of proteins derived from three different vectors. Various physico-chemical properties of the deduced translated Omp31 protein of each vector were computed using the ProtParam program with the results shown in Table 2. To elucidate properties of the studied proteins, a hydrophobicity plot for each protein sequence was produced based on Kyte and Doolitlle (Kyte and Doolittle, 1982) algorithm (Figure3).

Bioinformatics analysis

Blast analysis of the Omp31 identified sequence showed high similarity with the published sequences of Omp31 in other strains and species. The Omp31 sequence show-

Table 2 .Physico-chemical properties of *Brucella melitensis* Rev1 Omp31 protein derived from ProtParam

	PcDNA3.1/Hygro +	PcDNA3.1/His +	pSecTag2A/Hygro
Number of amino acids	240	277	318
Molecular weight	25309.1	29444.7	33734.7
Theoretical pI	5.22	5.82	5.45
Total number of negatively charged residues (Asp + Glu)	24	29	33
Total number of positively charged residues (Arg + Lys)	19	23	24
Formula	$C_{1134}H_{1723}N_{297}O_{354}S_4$	$C_{1304}H_{1984}N_{358}O_{407}S_8$	$C_{1501}H_{2307}N_{407}O_{466}S_7$
Total number of atoms	3512	4061	4688
Extinction coefficients	47900	49390	58900
Estimated half-life	30 hours (mammalian reticulocytes, *in vitro*). >20 hours (yeast, *in vivo*). >10 hours (Escherichia coli, *in vivo*).	30 hours (mammalian reticulocytes, *in vitro*). >20 hours (yeast, *in vivo*). >10 hours (Escherichia coli, *in vivo*).	30 hours (mammalian reticulocytes, *in vitro*). >20 hours (yeast, *in vivo*). >10 hours (Escherichia coli, *in vivo*).
Instability index	8.61	10.01	21.46
Aliphatic index	74.00	68.70	80.09
GRAVY	-0.090	-0.249	-0.106

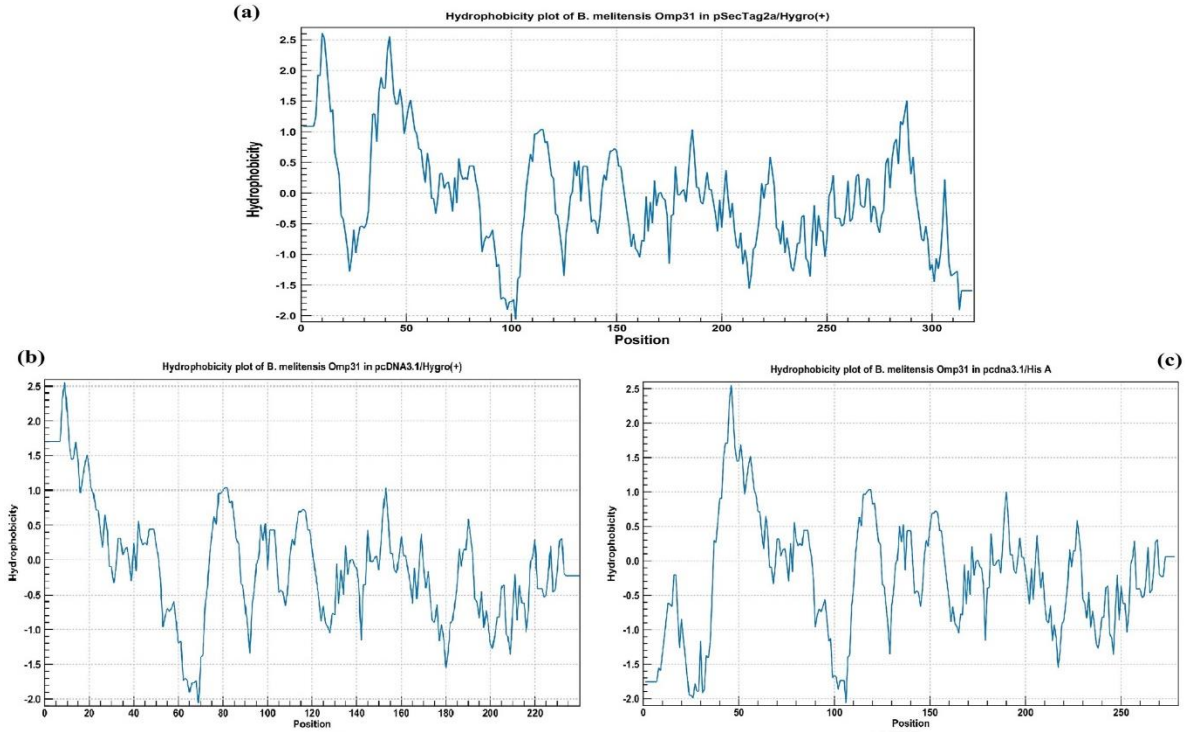
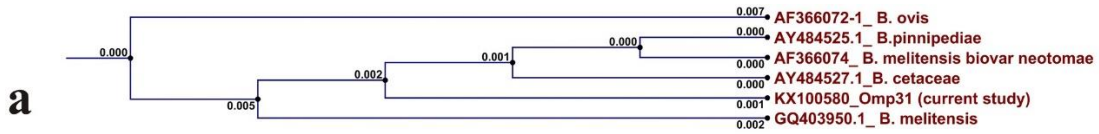


Figure 3. Hydrophobicity plots of the *Brucella melitensis* Rev1 Omp31 (windows size: 11) of different constructs. a: pSecTag2 A/Hygro- Omp31, b: pcDNA3.1/Hygro (+)- Omp31, c: pcDNA3.1/His A- Omp31



b

	1	2	3	4	5	6
AF366074_ B. melitensis biovar neotomae	1		100.00	100.00	99.86	99.59	98.76
AY484525.1_ B.pinnipediae	2	0.00		100.00	99.86	99.59	98.76
AY484527.1_B. cetaceae	3	0.00	0.00		99.86	99.59	98.76
KX100580_Omp31 (current study)	4	0.00	0.00	0.00		99.45	98.62
GQ403950.1_ B. melitensis	5	0.00	0.00	0.00	0.01		98.34
AF366072-1_ B. ovis	6	0.01	0.01	0.01	0.01	0.02	

Figure 4. a: Phylogenetic tree showing the relationships of *B. melitensis* Rev1 Omp31 sequence obtained from current study and other species, b: Comparison of *Brucella melitensis* Rev1 Omp31 sequences with other species showing identities and distances.

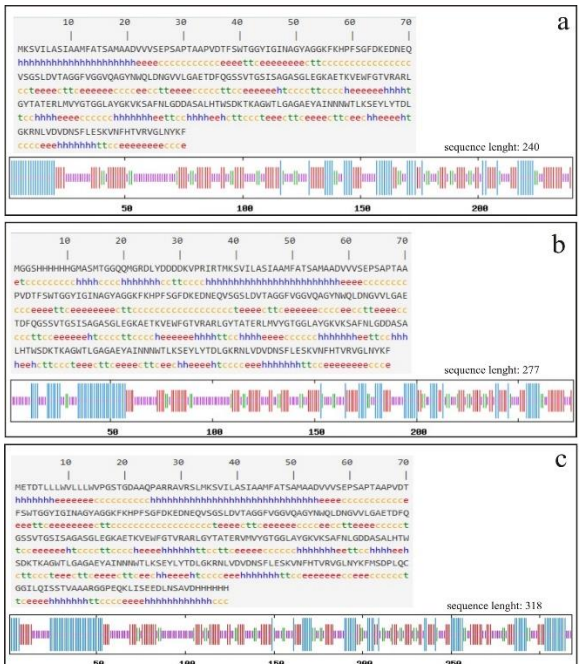


Figure 5. Secondary structure prediction results for the *Brucella melitensis* Rev1 Omp31 protein of different vectors, a: pcDNA3.1/Hygro (+), b: pcDNA3.1/His A and c: pSecTag2 A. Lines in different colors represent different secondary structures: Blue (h): α helix; Green (t): β turn; Red (e): extended strand and Purple (c): random coil

ed a high relationship to each of the known sequences of the Omp31 gene (Figure 4a). No consistent difference was found in the nucleotide sequence of *B. melitensis* Rev 1 in this study and the published sequences (Figure 4b).

Various physico-chemical properties of the studied proteins were computed using the ProtParam program. The ProtParam analysis results are shown in Table 2. Figure 5 shows the secondary structure of the Omp31 protein which was predicted using the improved self-optimized prediction (SOPMA) method.

Prediction of epitopes

The B-cell and T-cell epitopes predictions were performed using the IEDB online software. The Omp31 protein is composed of 241 amino acid residues. Table 3 shows the predicted continuous antibody epitopes from protein sequences. Values of calculated scores for each residue in B-cell epitope are shown in Figure 6. The larger score for the residues might indicate a higher probability for that residue to be part of the epitope (those residues are colored in yellow on the graphs). Table 4 depicts some of the predicted peptides that bind to the MHC class I and class II molecules with the lowest scores. A small numbered percentile rank indicated high affinity. The predictions were made using the IEDB analysis resource (Consensus tool) which combines predictions from ANN aka NetMHC for MHCI (Lundegaard et al., 2008; Nielsen et al., 2003) and MHCII (Wang et al., 2008; Wang et al., 2010) binding predictions.

Discussion

In this study, the Omp31 gene was *in silico* cloned into 3 mammalian expression vectors to create expression constructs. Bioinformatics and immunoinformatics tools were employed to determine the molecular characteristic and immunogenicity of the designed expression constructs' deduced proteins against *Brucella* infection. Some studies have evaluated the immunogenicity and protective efficacy of the recombinant Omp31 protein in combination with adjuvants and have shown that it could be protective against *B. melitensis* infection by eliciting a Th1 response mediated by CD4+ T cells (Cassataro et al., 2005; Vahedi et al., 2011; Yang et al.,

Table 3. B-cell predicted epitopes of *Brucella melitensis* Rev1 Omp31 derived from three constructs with lower scores using IEDB server

No	pcDNA3.1/Hygro (+)		pcDNA3.1/His A		pSecTag 2A/Hygro	
	Sequence of peptide	Length	Sequence of peptide	Length	Sequence of peptide	Length
1	$_{24}$VSEPSAPTAAPVDTFSW$_{40}$	17	$_{1}$MGGSHH$_{6}$	6	$_{1}$M$_{1}$	1
2	$_{50}$GYAGGKFKH$_{58}$	9	$_{14}$SMTGGQQMGRDLYDDDDKVPR$_{34}$	21	$_{17}$GSTGDAAQPARR$_{28}$	1
3	$_{61}$SGFDKEDNEQVSGSLDV$_{77}$	17	$_{60}$VVSEPSAPTAAPVDTFSW$_{77}$	18	$_{56}$VVSEPSAPTAAPVDTFSW$_{73}$	1
4	$_{82}$FVGG$_{85}$	4	$_{82}$I$_{82}$	1	$_{78}$IG$_{79}$	2
5	$_{87}$QAG$_{89}$	3	$_{87}$GYAGGKFKH$_{95}$	9	$_{82}$AGYAGGKFKH$_{91}$	1
6	$_{91}$NWQ$_{93}$	3	$_{98}$SGFDKEDNEQVSGSLDVT$_{115}$	18	$_{94}$SSFDKEDNEQVSGSLDVT$_{111}$	1
7	$_{104}$TDFQGSSVTGSISAGASGLEGKAETK$_{129}$	26	$_{117}$GGFVGGVQAG$_{126}$	10	$_{113}$GGFVGGVQAG$_{122}$	1
8	$_{155}$GLA$_{157}$	3	$_{128}$NWQ$_{130}$	3	$_{124}$NWQ$_{126}$	3
9	$_{166}$NLGDDASAL$_{174}$	9	$_{140}$ETDFQGSSVTGSISAGASGLEGKAETK$_{166}$	27	$_{132}$V$_{132}$	1
10	$_{176}$TWSDKTKAGWTLGAG$_{190}$	15	$_{192}$GLAY$_{195}$	4	$_{134}$G$_{134}$	1
11	$_{192}$EYA$_{194}$	3	$_{203}$NLGDDASALHTWSDKTKAGWTLGAG$_{227}$	25	$_{136}$ETDFQGSSVTGSISAGASGLEGKAETKV$_{163}$	2
12	$_{200}$T$_{200}$	1	$_{229}$EYA$_{231}$	3	$_{176}$T$_{176}$	1
13			$_{237}$T$_{237}$	1	$_{188}$GLAYG$_{192}$	5
14					$_{199}$NLGDDASALHTWSDKTKAGWTLGAGAEYAIN$_{229}$	3
15					$_{233}$T$_{233}$	1
16					$_{278}$LQ$_{279}$	2
17					$_{290}$VAAARGGPEQKLIS303	1
18					$_{306}$DLNSA$_{310}$	5

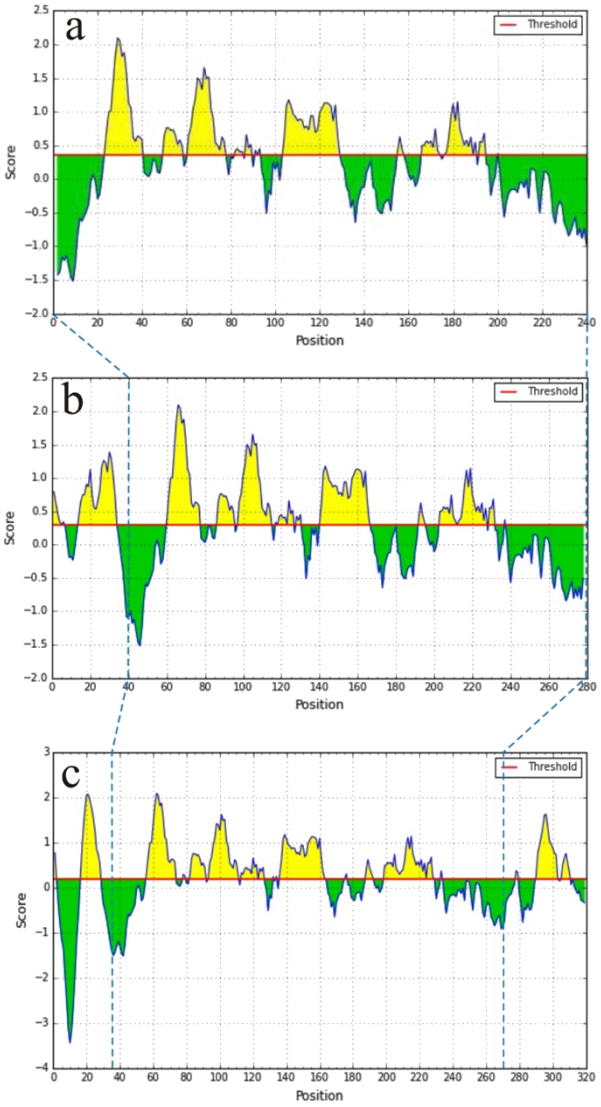


Figure 6. Diagram of *Brucella melitensis* Rev1 Omp31 predicted residue scores for linear epitope prediction using IEDB Analysis Resource derived from different constructs, a: pcDNA3.1/Hygro (+), b: pcDNA3.1/His A, c: pSecTag2 A (window size: 7)

2013). In a study by Golshani et al. (2014) a new truncated protein vaccine candidate was constructed with the help of *in silico* studies and protein modeling. The analysis performed on native Omps by Habtamu et al. (2013) revealed that the Omp31 protein was one of the major immunodominant proteins in *B. melitensis* as previously described by Bowden et al. (1995). They concluded that it might be useful for vaccination against sheep and goat brucellosis and for developing new generation vaccines (Dhama et al., 2008). Vahedi et al. (2011) explored the Omp31 as one of the most important vaccine candidates against *B. melitensis* and produced it as a fusion protein expressed by *E.coli*.

The pcDNA3.1/Hygro (+) vectors derived from pcDNA3.1 are nonfusion vectors which are designed for high-level stable and transient expression in mammalian hosts. On the other hand, the pcDNA3.1/His vectors are fusion vectors which must be cloned in frame with the ATG at base pairs 920–922 to ensure proper expression of the recombinant protein. This will create a fusion with the N-terminal polyhistidine tag, Xpress epitope, and the enterokinase cleavage site. The pSecTag2/Hygro A is an expression vector designed for high level expression and secretion of foreign proteins in mammalian hosts. Proteins expressed from pSecTag2/Hygro are fused at the N-terminus to the murine Ig kappa chain leader sequence for protein secretion and at the C terminus to a peptide containing the *c-myc* epitope and six tandem histidine residues for detection and purification.

The ProtParam relies on the "N-end rule", which relates the half-life of a protein to the identity of its N-terminal residue; the prediction is given for 3 model organisms (human, yeast and E. coli). The N-end rule states that the *in vivo* half-life of a protein is a function of the nature of its amino-terminal residue (Bachmair, 1986). Because the N terminus amino acid of all 3 deduced proteins are methionine, therefore, based on the N-end rule, they will be stable for about 30 hours in mammalian cells. The instability index provides an estimate of the stability of protein of interest in a test tube. Values greater than 40 indicate that the protein may be unstable *in vitro*. These values were derived from an analysis that found a significant difference in the occurrence of certain dipeptides between stable and unstable proteins. Based on the instability index, the analysis by ProtParam revealed that Omp31 can be classified as a stable protein (Instability index: 8.61-21.46). Amongst studied constructs, the protein derived from pSecTag2/Hygro A had the maximum value (21.46) which can be assumed as the most unstable protein while the protein derived from pcDNA3.1/Hygro was the most stable protein (8.61). The protein aliphatic index is regarded as a positive factor for the increase in the thermos-stability of globular proteins. The result from the aliphatic index analysis results were consistent with the previously described results from instability index. The higher aliphatic index is related to lower instability index and higher stability.

A GRAVY (Grand Average of hydropathicity) score is the relative value for the hydrophobic residues of proteins. This index indicates the solubility of the proteins: positive GRAVY protein is hydrophobic while negative GRAVY protein is hydrophilic (Kyte and Doolittle, 1982). As derived from ProtParam analysis, Omp31 gained a negative GRAVY score so it can be inferred

Table 4a. Analysis of the MHC-I and MHC- II T-cell epitopes of *Brucella melitensis* Rev1 Omp31 protein of pcDNA3.1/Hygro (+)

Class	Source Species	Allele	Peptide	Score*
MHC-I	Human	HLA-B	$_{191}$AEYAINNNW$_{199}$	0.1
		HLA-A	$_{149}$MVYGTGGLAY$_{158}$	0.1
		HLA-A	$_{36}$DTFSWTGGY$_{44}$	0.15
		HLA-B	$_{191}$AEYAINNNW$_{199}$	0.15
		HLA-B	$_{191}$AEYAINNNWTL$_{201}$	0.2
	Mouse	H-2-Db	$_{193}$YAINNNWTL$_{201}$	0.1
		H-2-Db	$_{193}$YAINNNWTLKSEYL$_{206}$	0.1
		H-2-Dd	$_{28}$SAPTAAPVDTF$_{38}$	0.2
		H-2-Kk	$_{203}$SEYLYTDLGKRNL$_{215}$	0.2
MHC-II	Human	HLA-DQA1	$_{1}$MKSVILASIAAMFAT$_{15}$	0.01
		HLA-DQA1	$_{2}$KSVILASIAAMFATS$_{16}$	0.01
		HLA-DQA1	$_{3}$SVILASIAAMFATSA$_{17}$	0.04
		HLA-DQA1	$_{4}$VILASIAAMFATSAM$_{18}$	0.05
		HLA-DRB1	$_{3}$SVILASIAAMFATSA$_{17}$	0.07
		HLA-DRB1	$_{6}$LASIAAMFATSAMAA$_{20}$	0.09
		HLA-DRB1	$_{1}$MKSVILASIAAMFAT$_{15}$	0.11
	Mouse	H2-IAd	$_{12}$MFATSAMAADVVVSE$_{26}$	0.04
		H2-IAd	$_{11}$AMFATSAMAADVVVS$_{25}$	0.12
		H2-IAd	$_{13}$FATSAMAADVVVSEP$_{27}$	0.14
		H2-IAb	$_{22}$VVVSEPSAPTAAPVD$_{36}$	0.3

*Score: percentile rank (Low percentile rank = good binders)

Table 4b. Analysis of the MHC-I and MHC- II T-cell epitopes of *Brucella melitensis* Rev1 Omp31 protein of pcDNA3.1/His A

Class	Source Species	Allele	Peptide	Score
MHC-I	Human	HLA-A	$_{175}$ARLGYTATERLMVY$_{188}$	0.1
		HLA-A	$_{176}$RLGYTATERLMVY$_{188}$	0.1
		HLA-A	$_{214}$WSDKTKAGWTLGA$_{226}$	0.1
		HLA-A	$_{177}$LGYTATERLMVY$_{188}$	0.1
		HLA-B	$_{228}$AEYAINNNWT$_{236}$	0.1
		HLA-A	$_{186}$MVYGTGGLAY$_{195}$	0.1
	Mouse	H-2-Lb	$_{66}$APTAAPVDTF$_{75}$	0.1
		H-2-Db	$_{230}$YAINNNWTLKSEYL$_{243}$	0.1
		H-2-Dd	$_{230}$YAINNNWTL$_{238}$	0.1
		H-2-Kd	$_{191}$GGLAYGKVKSAFNL$_{204}$	0.1
		H-2-Db	$_{227}$GAEYAINNNWTL$_{238}$	0.1
MHC-II	Human	HLA-DQA1	$_{38}$MKSVILASIAAMFAT$_{52}$	0.01
		HLA-DQA1	$_{39}$KSVILASIAAMFATS$_{53}$	0.01
		HLA-DQA1	$_{40}$SVILASIAAMFATSA$_{54}$	0.04
		HLA-DQA1	$_{41}$VILASIAAMFATSAM$_{55}$	0.05
		HLA-DRB1	$_{40}$SVILASIAAMFATSA$_{54}$	0.07
		HLA-DRB1	$_{43}$LASIAAMFATSAMAA$_{57}$	0.09
		HLA-DQA1	$_{44}$ASIAAMFATSAMAAD$_{58}$	0.09
	Mouse	H2-IAd	$_{49}$MFATSAMAADVVVSE$_{63}$	0.04
		H2-IAd	$_{48}$AMFATSAMAADVVVS$_{62}$	0.12
		H2-IAd	$_{50}$FATSAMAADVVVSEP$_{64}$	0.14
		H2-IAd	$_{51}$ATSAMAADVVVSEPS$_{36}$	0.18
		H2-IAd	$_{47}$AAMFATSAMAADVVV$_{61}$	0.28

Table 4c. Analysis of the MHC-I and MHC- II T-cell epitopes of *Brucella melitensis* Rev1 Omp31 protein of pSecTag2A/Hygro

Class	Source Species	Allele	Peptide	Score
MHC-I	Human	HLA-B	$_{1}$METDTLLLW$_{9}$	0.1
		HLA-B	$_{224}$AEYAINNNW$_{232}$	0.1
		HLA-A	$_{182}$MVYGTGGLAY$_{191}$	0.1
		HLA-A	$_{69}$DTFSWTGGY$_{77}$	0.15
		HLA-B	$_{224}$AEYAINNNW$_{232}$	0.15
	Mouse	H-2-Lb	$_{62}$APTAAPVDTF$_{71}$	0.1
		H-2-Kb	$_{266}$RVGLNYKFMSDPL$_{278}$	0.1
		H-2-Kb	$_{260}$LNYKFMSDPLQCG$_{281}$	0.1
		H-2-Db	$_{223}$GAEYAINNNWTL$_{234}$	0.1
		H-2-Kd	$_{187}$GGLAYGKVKSAFNL$_{200}$	0.1
		H-2-Db	$_{226}$YAINNNWTLKSEYL$_{230}$	0.1
MHC-II	Human	HLA-DQA1	$_{34}$MKSVILASIAAMFAT$_{48}$	0.01
		HLA-DQA1	$_{35}$KSVILASIAAMFATS$_{49}$	0.01
		HLA-DQA1	$_{36}$SVILASIAAMFATSA$_{50}$	0.04
		HLA-DQA1	$_{37}$VILASIAAMFATSAM$_{51}$	0.05
		HLA-DRB1	$_{36}$SVILASIAAMFATSA$_{50}$	0.07
		HLA-DRB1	$_{39}$LASIAAMFATSAMAA$_{53}$	0.09
		HLA-DQA1	$_{40}$ASIAAMFATSAMAAD$_{54}$	0.09
	Mouse	H2-IAd	$_{45}$MFATSAMAADVVVSE$_{59}$	0.04
		H2-IAd	$_{44}$AMFATSAMAADVVVS$_{58}$	0.12
		H2-IAd	$_{46}$FATSAMAADVVVSEP$_{60}$	0.14
		H2-IAd	$_{47}$ATSAMAADVVVSEPS$_{61}$	0.18
		H2-IAd	$_{43}$AAMFATSAMAADVVV$_{57}$	0.28

that Omp31 is a hydrophilic protein. According to Kyte and Doolittle (1982), integral membrane proteins typically have higher GRAVY scores than do globular proteins. Although this score is another helpful piece of information, it cannot reliably predict the structure without the help of hydropathy plots. There are some methods for evaluation of the degree of interaction of polar solvents such as water with specific amino acids. In these methods a hydrophobicity plot is created that is a quantitative analysis of the degree of hydrophobicity or hydrophilicity of amino acids in a protein. For example, extension of about 20 amino acids with positive charges indicates that, these amino acids may be part of alpha-helix spanning across a lipid bilayer, which is composed of hydrophobic fatty acids (Kyte and Doolittle, 1982).

Specific needs to molecular biology and immunological procedures are needed when developing epitope vaccines based on experimental research. Epitopes are classified as B and T-cell epitopes (Zhang et al., 2012). The majority of peptides examined as vaccine candidates correspond to continuous epitopes (Chen et al., 2011). Parameters such as hydrophilicity, flexibility, accessibility, turns, exposed surface, polarity and antigenic propensity of polypeptides chains have been correlated with the location of continuous epitopes (Chen et al., 2011). This has led to a search for empirical rules that would allow the position of continuous epitopes to be predicted from certain features of the protein sequence. In the present study, bioinformatics analyses were conducted on the *Brucella melitensis* Omp31 antigen using the IEDB online B and T-cell epitope prediction servers. For predicting the linear B-cell epitopes, the BepiPred Linear Epitope Prediction method was used (Larsen et al., 2006). The BepiPred predicts the location of linear B-cell epitopes using a combination of a hidden Markov model and a propensity scale method (Larsen et al., 2006). The residues with scores above the threshold are predicted to be part of an epitope and colored in yellow on the graph (Figure 6) (where Y-axis depicts the residue scores and X-axis shows the residue positions in the sequence). The values of the scores are not affected by the selected threshold (Larsen et al., 2006). Comparison of the result of B cell epitope prediction in three vectors showed that the number of predicted epitopes with higher probability in the pSecTag2A vector was more than that of the pcDNA/3.1 His and Hygro. Our results of T cell epitope prediction (Table 4a, b and c) showed that some amino acids and residues (24-40, 193-200) could be a common epitope in human and mouse species, and also with the ability to stimulate both cell-mediated and humoral immunity. Most of them were similar for three investigated vectors

although the number of epitopes with lower score (good binder) in pSecTag 2 A was more than that in others.

Conclusions

This study was a preliminary trial for production of an effective vaccine against *B. melitensis* using the Omp31 protein. The results showed that Omp31 can be used as an immunogenic candidate gene against *B. melitensis* Rev1 in all three expression vectors as a DNA vaccine, although pSecTag2 was more suitable for this purpose. However, further experimental and field studies are necessary to explore and compare the immunogenicity and cross-protectivity of these Omp31 expression constructs against *B. melitensis*.

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همسانه‌سازی *In silico* و مطالعه بیوانفورماتیکی آنتی‌ژن Omp31 *بروسلا ملی‌تنسیس* در پلاسمیدهای بیانی پستانداری مختلف

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چکیده برو سلا به عنوان یک باکتری گرم منفی درون سلولی بیماریزا، باعث ایجاد بیماری برو سلوزیس (تب مالت) در انسان و حیوانات می‌شود. بر اساس مقالات موجود پروتئین ۳۱ کیلودالتونی غشای خارجی (Omp31) بروسلا ملی‌تنسیس به عنوان یک کاندید مهم جهت طراحی واکسن علیه بروسلوزیس مورد توجه می‌باشد. هدف این مطالعه مقایسه سه ساختار بیانی متفاوت شامل آنتی‌ژن Omp31 برو سلا ملی‌تنسیس، با استفاده از آنالیزهای بیوانفورماتیکی جهت تسهیل در انتخاب بهترین ساختار ایمنی‌زا می‌باشد. برای این منظور توالی کدکننده ژن Omp31 توسط واکنش‌های زنجیره‌ای پلیمراز (PCR) تکثیر و بعد از انجام همسانه سازی TA، مورد توالی‌یابی قرار گرفت. توالی DNA بدست آمده با استفاده از نرم افزار CLC Main Workbench بصورت *in silico* در سه وکتور بیانی پستانداری pcDNA3.1/Hygro (+)، pcDNA3.1/His A و pSecTag2/Hygro وارد گردید. ژن Omp31 با موفقیت در وکتور pTZ57R/T همسانه‌سازی شد و نوترکیبی آن توسط colony PCR و توالی‌یابی مورد تأیید قرار گرفت. مقایسه توالی بدست آمده از ژن Omp31 با دیگر توالی‌های موجود شباهت زیادی را نشان داد. آنالیزهای بیوانفوماتیکی سه وکتور متفاوت بیانی پستانداری حامل ژن Omp31 امکان انتخاب بهترین ساختار ایمنی‌زا را جهت مطالعات بیشتر، به منظور طراحی واکسن‌های DNA مؤثر بر علیه بیماری بروسلوزیس، فراهم آورد.