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CONSTRUCTION, EXPRESSION AND PURIFICATION OF *BRUCELLA SPP.* RECOMBINANT PROTEINS L7/L12 AND SODC IN *E. COLI*

Abstract. Brucellosis is still an important public health problem as long as natural reservoirs of infection exist. Currently, live attenuated vaccines based on strains S19, RB51 and Rev1 are used for the prevention of brucellosis in animals, the main disadvantage of which is virulence for humans. However, animal immunization programs should be implemented to reduce the incidence of humans. The development of safe and effective new generation vaccines using "omix" technology is a promising direction of vaccinology. A number of immunogenic Brucella proteins that elicit both a humoral and cellular immune response has been identified. The aim of these research was to optimize the expression and purification conditions of the *Brucella spp.* recombinant proteins L7/L12 and SodC. As a result, expressing plasmids pET/Br-L7/L12 and pET/Br-SodC were obtained. The parameters of target genes expression in *E. coli* were established and the method for purification of recombinant proteins was optimized. Purification of the L7/L12 protein was performed under hybrid conditions on HisPur agarose using a binding buffer containing 6 M guanidine hydrochloride, a wash buffer with 20 mM imidazole and an elution buffer with 300 mM imidazole. Protein SodC was purified under denaturing conditions with the addition of 1 % Triton X-100 and 1 % sodium deoxycholate to the lysis buffer. Inclusions were solubilized with a buffer containing 8 M urea and 5 mM imidazole. The target protein was eluted from HisPur agarose with buffer containing 8 M urea and 100 mM imidazole. The use of modified purification protocols made it possible to obtain purified recombinant proteins with a yield of 13 mg/L for the L7/L12 protein and 10 mg/L for the protein SodC, respectively. The specificity of the proteins was confirmed by a Western blot. Immunization of mice with recombinant proteins led to the production of specific antibodies, the titer of which in ELISA was 1:20480 and 1:20480, respectively.

Key words: *Brucella spp.*, ribosomal protein L7/L12, superoxide dismutase, expression, protein purification.

Introduction. Brucellosis is one of the widespread zoonotic diseases causing great economic damage to agriculture. According to the Joint FAO Expert Committee, brucellosis of livestock is prevalent in almost the whole world and since animals with brucellosis are a source of infection for humans, this disease is a high degree of danger [1-4]. The annual identification of farm animals and people reacting to brucellosis in certain regions of Kazakhstan indicates an extremely unstable situation for this infection and the real possibility of forming foci of brucellosis with varying degrees of activity of manifestations of epizootic and epidemic processes in farms [5,6]. In this regard, this infection is a serious problem for veterinary and medical science.

Today, recombinant proteins having antigenic and immunogenic properties are widely used in the development of prophylactic and diagnostic preparations, as well as candidates for vaccine against various zoonotic diseases. A lot of work has been devoted to the study of immunogenic proteins of *Brucella spp* and a number of proteins have been discovered, such as: L7/L12, SodC, BP26, BCSP31, Omp16, Omp19, Omp31, which have immunogenic properties that can be used for diagnostic purposes. These proteins are conserved and their identity is 100 % between *Brucella* species [7-12].

In the study of Brucella antigens capable of inducing cellular immunity, a 12 kDa protein was detected that causes lymphocyte proliferation and is a L7/L12 ribosomal protein. It was shown that purified recombinant L7/L12 protein produced in *E. coli* stimulates CD4 T cell immunity in mice infected with *B. abortus*. It was also shown that immunization of mice with the recombinant ribosomal protein L7/L12 protects them from control infection with *B. abortus*. According to published data, L7/L12 is an immunodominant *B. abortus* protein that elicits a cellular immune response (Th1 and CD8 + T cells) [13-15].

The periplasmic protein SodC (Cu-Zn superoxide dismutase) is one of the main enzymes of the antioxidant system of microorganisms, which is considered as one of the universal mechanisms of the pathogenesis of infectious diseases, and indicators reflecting shifts in the levels of antioxidant enzymes are key factors in predicting the outcome of the disease. The researchers determined the protective effect of the antioxidant enzyme SodC *B.abortus*, expressed in significant induction of T-cell proliferation and production of gamma-interferon in infected mice. Thus, vaccination of mice with *E. coli* cells expressing the SodC *B.abortus* enzyme formed a defense against brucella infection. The use for this purpose of plasmid DNA, including the SodC gene *B.abortus*, also induced a humoral and cellular immune response against the causative agent of brucellosis [8,16].

The purpose of these studies was the construction of expression vectors, the expression and optimization of the purification conditions of the recombinant *Brucella spp* L7/L12 and SodC proteins, as well as the production of specific sera to them. Recombinant proteins and their specific sera will be used in the development of a vector anti-brucellosis vaccine based on sheep pox virus.

Materials and methods. *Bacterial strains.* The studies used the vaccine strain *B.abortus* S19 obtained from the laboratory of the collection of microorganisms of the Research Institute of Biological Safety Problems RK ME&S – Science Committee, Kazakhstan. *B.abortus* S19 genomic DNA was isolated using the PrepMan Ultra kit (Applied Biosystems, USA). For manipulation with plasmid DNA, *E. coli* strain TOP10 (Invitrogen, USA) was used. For bacterial expression, E. coli T7 express strain (New England Biolabs, USA) was used.

Construction of expression cassettes and obtaining producer strains. The *Brucella* genes *L7/L12* and *sodC* were amplified with the genomic DNA *B.abortus* S19 using pairs of primers FP-*L7/L12*-5'-CGCAT*ATG*GCTGATCTCGCAAAGATCGT-3', RP-*L7/L12*-5'-CGCTCGAGCTTGA GTTCAACCTTGGCGCCA-3' and FP-*SodC*-5`-CGCC*ATGG*TTAAGTCCTTATTTATTGC-3`, RP-*SodC*-5'-CGCTCGAGTTCGATCAC GCCGCAGGCAAAA-3', respectively.

Amplification was performed in 50 µl containing 5 µl of 10 × PCR buffer (Qiagen), 1 µl of 10 mM dNTPs (New England Biolabs, USA), 0.1 µl of DNA, 1 µl of each primer (20 pMol/µl), 0.5 µl of Taq DNA polymerase (2.5 units, Qiagen). Amplification conditions: 94 ºC 5 min; then 30 cycles of 94 ºC, 1 min, 50 ºC, 1 min, 72 ºC, 2 min, and 1 cycle of 72 ºC, 7 min.

The obtained products were digested with respective enzymes *NdeI – XhoI* (L7/L12), *NcoI – XhoI* (SodC) and cloned into the plasmid vector pET28b(+) (Novogen, USA).

As a result, recombinant plasmids pET/Br-L7/L12 and pET/Br-SodC were obtained containing the sequence coding the SodC protein fragment (1-173 aa) and the C-terminal peptide LEHHHHHH; and the sequence encoding the L7/L12 protein fragment (21-144 aa), the N-terminal peptide MSSHHHHHHSS and the C-terminal peptide LEHHHHHH. Plasmids were sequenced to verify the integrity of the inserts.

Plasmids were transformed into *E.coli* cells, strain T7 express (New England Biolabs, USA). As the result *E. coli* clones, producers of the recombinant proteins SodC and L7/L12, were obtained.

Gene expression. To expression the genes of the target proteins L7/L12 and SodC, bacterial cells were grown in LB-kan (Luria-Bertani broth, containing 50 µg/mL of kanamycin) at 37 °C on a shaker (250 rpm) to optical density OD600 = 0.6-1.0. Gene expression was induced by addition IPTG to final concentration 1 mM to the bacterial suspension with subsequent incubation for 4 h at 37 °C. The cells were harvested by centrifugation at 5000 × g for 15 min and stored at - 70 °C until further use. Aliquots

selected before and after induction were examined by PAGE electrophoresis. The solubility of the recombinant protein was determined using a BugBuster master mix reagent (Novagen, USA) according to the manufacturer's instructions.

Protein L7/L12 purification. Cell pellet was resuspended in NB buffer (50 мМ NaH_2PO_4, 300 мМ NaCl, 6 М guanidine hydrochloride, 10 мМ imidazole, pH 7,4) at the rate of 5 ml of buffer per 1 g of crude cell pellet. Suspension was incubated in ice for 30 min and sonicated. Cell lysate was clarified by centrifugation at 3000 g for 15 min. The supernatant (soluble protein fraction) was filtered through a 0.22 μm membrane and uploaded to HisPur ™ Cobalt Superflow (Thermo Scientific, USA) agarose pre-equilibrated with NB buffer. The agarose resin was washed using NW buffer (50 mM NaH_2PO_4, 300 mM NaCl, 20 mM imidazole, pH 7.4), the protein was eluted with NE buffer (50 mM NaH_2PO_4, 300 mM NaCl, 300 mM imidazole, pH 7.4). The pure protein fraction was dialyzed against 10 times the volume of the buffer (20 mM PBS, 300 mM NaCl, pH 7.4) overnight at 4°C.

Protein SodC purification. Protein purification was performed as [17] with modifications. Cells were resuspended in buffer 1 (100 mM Tris HCl pH 8.0, 150 mM NaCl, 1 % Triton X-100, 1 % Sodium deoxycholate) at the rate of 15 ml per 1 g of crude cell pellet. Lysozyme was added to the resulting suspension to a final concentration of 1 mg/ml. Cell lysis was performed by freezing twice at - 70 °C and thawing at 37 °C of the suspension. The cell lysate was incubated at 4 °C overnight, then centrifuged at 4 °C for 15,000 × g for 15 min. The pellet was washed successively with buffer 2 (100 mM Tris HCl, pH 8.0, 150 mM NaCl, 1 % Triton X-100), buffer 3 (100 mMTris HCl, pH 8.0, 1 M NaCl), buffer 4 (50 mM Tris HCl, pH 7.5). The pellet was resuspended in buffer 5 (20 mM PBS, pH 7.4, 300 mM NaCl, 8 M urea, 5 mM imidazole, pH 7.4) and incubated at 4°C overnight to completely solubilize. The dissolved inclusion fraction was filtered through a 0.22 μm membrane. Protein purification was carried out using HisPur ™ Cobalt Superflow agarose. After sorption of protein, resin was washed with DW buffer (50 mM NaH_2PO_4, 300 mM NaCl, 8 M urea, 5 mM imidazole, pH 7.4). Protein was eluted with DE buffer (50 mM NaH_2PO_4, 300 mM NaCl, 8 M urea, 100 mM imidazole, pH 7.4). Refolding of the protein was performed by dialysis sequentially against buffer 7 (20 mM PBS, 300 mM NaCl, 4 M urea, pH 7.4), buffer 8 (20 mM PBS, 300 mM NaCl, 2 M urea, pH 7.4), buffer 9 (20 mM PBS, 300 mM NaCl, 1 mM DTT (dithiothreitol), pH 7.4) and buffer 10 (20 mM PBS, 300 mM NaCl, pH 7.4). Protein concentration was determined by the method of Lowry et al. [18], using BSA as a standard.

Polyacrylamide gel electrophoresis and Western blot. Electrophoretic analysis of polypeptides was performed in 12 % SDS-PAGE under denaturing reducing conditions according to Laemmli [19]. For visualization of proteins, Coomassie G-250 staining was used. For Western blot analysis, proteins were transferred onto a nitrocellulose membrane and detected as described in [20] using anti-His (Cterm)/AP antibodies (Invitrogen, USA) and sera from sick cattle.

Obtaining specific serum to recombinant proteins. Animal experiments were carried out in accordance with applicable national and international legislation. The protocol was approved by the Bioethics Commission of the RIBSP RK ME&S of the Republic of Kazakhstan (No. 6 dated September 25, 2017).

To obtain specific sera, outbred white mice (females, 6-8 weeks old, weight 18-20 g) were immunized with a target protein. Proteins were prepared as follows: purified protein was mixed with Montanide Gel 01 (SEPPIC, USA) in a ratio of 9:1 (v/v). The final protein concentration was 150 μg/ml. Before administration of the drug, animals were bled to obtain normal serum. Immunization was carried out subcutaneously four times in a dose of 30 μg of protein. 14 days after the last injection, the animals were bled. The serum was tested in ELISA (enzyme-linked immunosorbent assay).

ELISA. 96-wells plates were coated with the 2 mg/ml of affinity purified recombinant proteins in 0.05 M carbonate-bicarbonate buffer, pH 9.6 (100 μL/well), and incubated overnight at 4 °C. Plates were washed four times with TBST buffer (150 мМ NaCl, 20 мМ tris-HCl, pH 7.5, 0,1 % tween-20) and blocked with TBST containing 5 % fat free dry milk for 1 h at 37 °C. Double dilutions of test sera in the blocking buffer, were added to wells (100 μL/well). Plates were incubated for 1 h at 37 °C and washed three times with TBST. Anti-mouse immunoglobulin IgG conjugated to alkaline phosphatase (1:5000) was added (100 μL/well) and the plates were incubated for 1 h at 37 °C. After washing, the substrate for alkaline phosphatase (pNPP, Sigma, USA) was added into each well (100 μL). The plates were incubated

for 30 min. Optical density was read at 405/630 nm on ELISA plates reader ImmunoChem-2100. Cut-off values were determined using the mean optical density values from negative control sera plus three standard deviations.

Results and discussion. *Construction of recombinant plasmids.* A comparative analysis of the amino acid sequences of the L7/L12 and SodC proteins showed their high identity (95-100 %) for *Brucella spp.* Using the SignalP [21] and TMHMM2.0 [22] software, the signal peptide was established for the SodC protein, while transmembrane domains were absent in both proteins (figure 1).

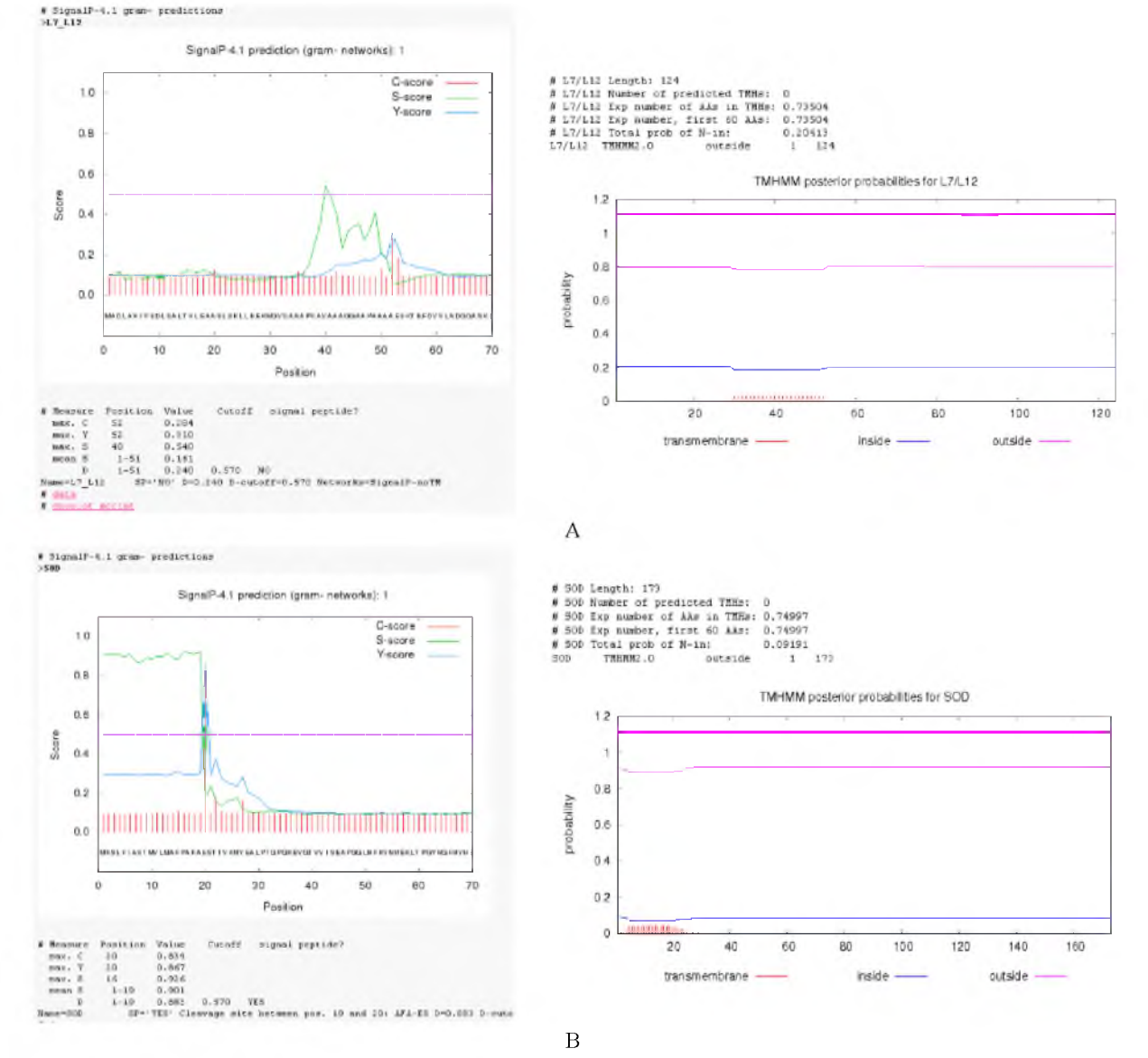


Figure 1 – The results of the analysis of the amino acid sequences of the target proteins for the presence of signal peptides and transmembrane domains: A – L7/L12, B – SodC

Amplified DNA fragments encoding the L7/L12 and SodC genes were cloned into the pET28b(+) vector. As a result, plasmids expressing recombinant proteins flanked at the N- and/or C-terminus by 6HIS oligopeptides were obtained (figure 2). The predicted molecular weights of SodC and L7/L12 proteins were 19.87 and 15.77 kDa, respectively. The obtained plasmids were transformed into *E. coli* T7 cells.

Analysis	Entire Protein
Length	152 aa
Molecular Weight	15774.20
1 microgram =	63.395 pMoles
Molar Extinction coefficient	5690
1 A[280] corr. to	2.77 mg/ml
A[280] of 1 mg/ml	0.36 AU
Isoelectric Point	6.10
Charge at pH 7	-5.07

```
  1  MGSSHHHHHH SSGLVPRGSH MADLAKIVED LSALTVLEAA ELSKLLEEKW
 51  GVSAAAPVAV AAAGGAAPAA AAEEKTEFDV VLADGGANKI NVIKEVRALT
101  GLGLKEAKDL VEGAPKAVKE GASKDEAEKI KAQLEAAGAK VELKLEHHHH
151  HH
```

A

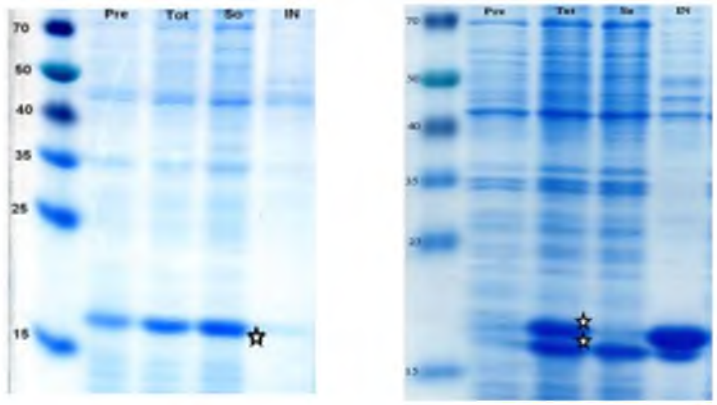
Analysis	Entire Protein
Length	188 aa
Molecular Weight	19875.26
1 microgram =	50.314 pMoles
Molar Extinction coefficient	5360
1 A[280] corr. to	3.71 mg/ml
A[280] of 1 mg/ml	0.27 AU
Isoelectric Point	6.37
Charge at pH 7	-3.75

```
  1  MKSLFIASTM VLMAFPAFAE STTVKMYEAL PTGPGKEVGT VVISEAPGGL
 51  HFKVNMEKLT PGYHGFHVHE NPSCAPGEKD GKIVPALAAG GHYDPGNTHH
101  HLGPEGDGHM GDLPRLSANA DGKVSETVVA PHLKKLAEIK QRSLMVHVGG
151  DNYSDKPEPL GGGGARFACG VIEDKLAAAL EHHHHHH*
```

B

Figure 2 – Prediction and analysis of amino acid sequences of recombinant proteins L7/L12 (A) and SodC (B)

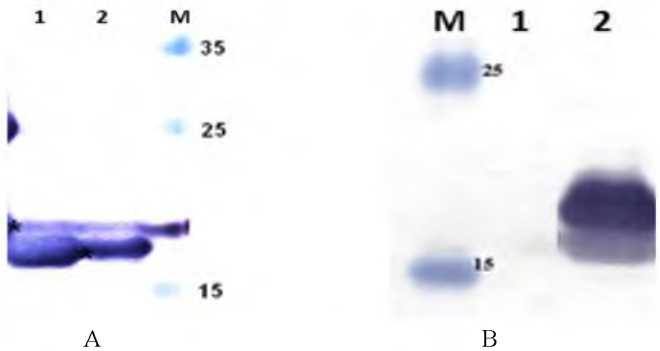
The expression of genes encoding recombinant proteins. Induction of target genes expression resulted in the production of L7/L12 (figure 3A) and SodC (Figure 3B) proteins. The molecular weight of the recombinant proteins corresponded to the calculated values (figures 2, 3). As a result of gene SodC expression, two protein products were formed (figure 3B, Tot), which is associated with the presence of a signal peptide in the sequence of the recombinant protein. Modified protein (without signal peptide) was in the soluble protein fraction (figure 3B, So), while unmodified protein formed inclusions bodies (figure 3B, IN).



A B

Figure 3 – Electrophoretic analysis of proteins of the cell lysate of E. coli strain T7, transformed with plasmids pL7/L12 (A) and pSodC (B): Pre – cell lysate before induction, Tot – after induction IPTG, So – soluble proteins, IN – inclusions. Recombinant proteins are marked with asterisks

The expression of the target Brucella L7/L12 and SodC proteins was confirmed by the Western blot using anti-His-antibodies (figure 4). Recombinant proteins also interacted with sera from brucellosis sick cattle, which confirms their specificity (figure 5).



A B

Figure 4 – Immunoblotting of proteins of the cell lysate of *E. coli* strain T7, transformed with recombinant pET plasmids, using serum to polyhistidine. A:1 – cell lysate prior to induction of L7/L12; 2 – after induction of IPTG; B:1 – cell lysate prior to SodC induction; 2 – after induction of IPTG

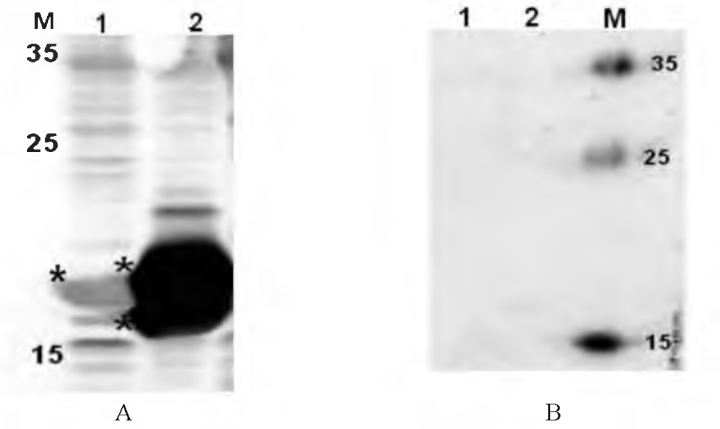


Figure 5 – Immunoblotting of proteins of the cell lysate of E. coli strain T7, transformed with recombinant pET plasmids, using serum from cattle with brucellosis: A – serum from cattle with brucellosis, B – normal cattle serum. 1 – cell lysate L7/L12; 2 – cell lysate SodC. Recombinant proteins are marked with asterisks

B. abortus Cu-Zn супероксиддисмутаза (SodC) была идентифицирована Beck et al. (1990) [23]. Using western blot, Betsy et al. (1990) proved that superoxide dismutase is found in most *Brucella* strains and species except *B. neotomae* and *B. suis* biovar 2 [24]. Both SodC and L7/L12 are immunodominant proteins and induce antibody production. Rajagunalan et al. (2014) in their studies, found antibodies to the recombinant protein L7/L12 of *B. melitensis* 16M in the sera from patients with acute brucellosis [25]. Proteins SodC and L7 / L12 were used in the development of DNA and vector vaccines. Recombinant vaccines elicited an immune response in animals [13, 14, 26].

Protein purification L7/L12. At the first stage, the purification of L7/L12 protein was carried out under native conditions (figure 6).

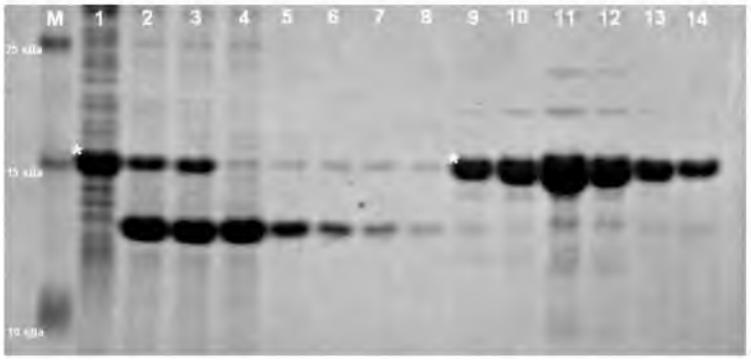


Figure 6 – Electrophoretic analysis of the recombinant protein L7/L12 during the cleaning process: M – molecular weight marker; 1 – total cell lysate; 2 – cell lysate after treatment with lysozyme; 3 – cell lysate after filtration through 0.22 μm; 4 – flow-through; 5-8 – wash fraction; 9-14 – fractions of purified protein. Recombinant proteins are marked with asterisks

As seen in the figure 6, lane 4, the cell lysate was not completely adsorbed onto resin. Washing the agarose with a buffer containing both 20 mM imidazole (figure 6, lane 7, 8) and 10 mM imidazole (figure 6, lane 5, 6) resulted in the loss of the target protein. The target protein eluted with a buffer containing both 300 mM imidazole (figure 6, lane 11-14) and 100 mM imidazole (figure 6, lane 9, 10) contained cell protein impurities. Purification of the protein under standard native conditions according to the recommendations of the resin manufacturer did not give satisfactory results. The protein purification protocol was optimized.

Hybrid conditions were selected for purification (see the Materials and Methods section), which made it possible to obtain a protein preparation with a high degree of purification without significant losses at intermediate stages (figure 7). The yield of recombinant protein L7/L12 was 13 mg per 1 liter of culture.

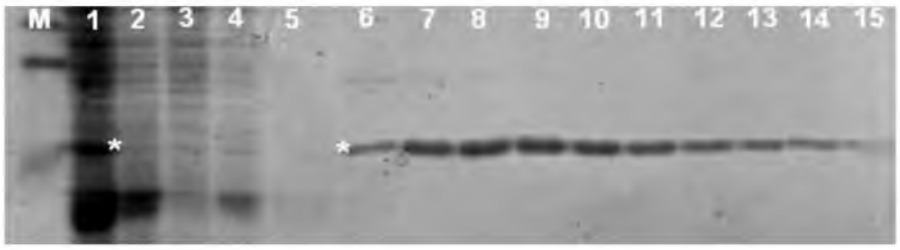


Figure 7 – Electrophoretic analysis of the recombinant protein L7/L12 during the purification process using optimal (hybrid) conditions. M – molecular weight marker; 1 – cell lysate after treatment with lysozyme; 2 – flow-through; 3-5 – washing fractions; 6-15 – fractions of purified protein. Recombinant proteins are marked with asterisks

SodC Protein Purification. Protein purification was performed under denaturing conditions (figure 8). When purifying the recombinant SodC protein, there were also problems with its adsorption to agarose. Only a small amount of protein was bound to the resin (figure 8, lane 2). Washing the resin led to the loss of the target protein (figure 8, lane 3-6), and the protein eluate contained a significant amount of impurities of cellular proteins (figure 8, lanes 7-10). An optimized purification protocol (see the Materials and Methods section) made it possible to obtain a SodC protein preparation with a high degree of purity (figure 9).

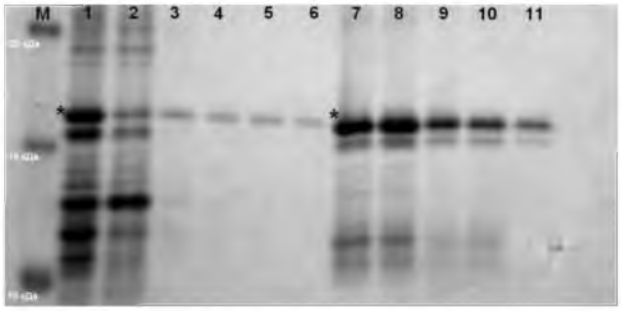


Figure 8 –
Electrophoretic analysis of recombinant SodC protein during purification under denaturing conditions.
M – molecular weight marker;
1 – cell lysate after treatment with lysozyme;
2 – flow-through; 3-6 – wash fraction;
7-12 – fractions of purified protein.
Recombinant proteins are marked with asterisks

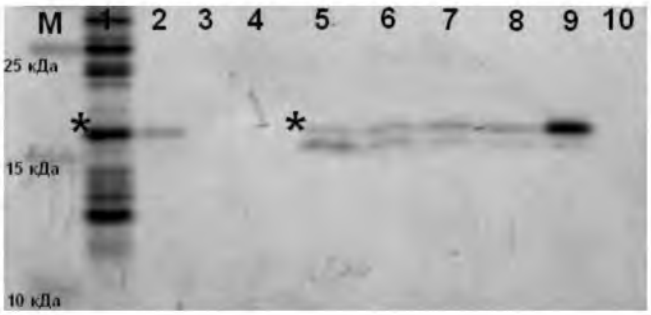


Figure 9 –
Electrophoretic analysis of recombinant SodC protein during purification under optimal denaturing conditions: M – molecular weight marker;
1 – cell lysate after treatment with lysozyme;
2 – flow-through; 3-4 – washing fractions;
5-10 – fractions of purified protein.
Recombinant proteins are marked with asterisks

As seen in the figure 9, a slight loss of the target protein was observed upon binding to agarose (lane 2). Loss of the target protein during resin washing was not detected (lanes 3-4). There were no impurities of cell protein in the eluate (lanes 5–9), which confirms the high purity of the obtained recombinant protein preparation. The yield of purified SodC protein was 10 mg per 1 liter of culture.

In addition, mouse specific sera for recombinant SodC and L7 / L12 proteins were obtained. For this, mice were immunized with triply recombinant proteins mixed with Montanide 01 gel (seppic, USA). Recombinant proteins induced the production of specific antibodies in animals. The antibody titer in ELISA for both SodC and L7 / L12 proteins was 1: 20480.

Purified recombinant proteins and their specific sera are suitable for use in the development of specific diagnostic and prophylactic agents against *Brucella* spp.

Conclusion. As a result of studies, purified preparations of recombinant proteins SodC and L7/L12 were obtained. Optimal protocols have been developed for the expression and purification of recombinant proteins in E.coli. The specificity of the obtained purified recombinant proteins was established using the western blot with sera from animals with brucellosis. Highly active specific sera for recombinant proteins were obtained, an antibody titer was 1:20480 for both the SodC and the L7/L12 proteins. Proteins and

serums for them will be further used in the development of specific diagnostic and prophylactic agents against *Brucella* spp.

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E. COLI-ДЕ *BRUCELLA SPP.* L7/L12 ЖӘНЕ SODC РЕКОМБИНАНТТЫ БЕЛОКТАРДЫ КОНСТРУИРЛЕУ, ЭКСПРЕССИЯЛАУ ЖӘНЕ ТАЗАЛАУ

Аннотация. Бруңеллез инфекцияның табиғи резервуарлары бар болғандықтан, денсаулық сақтаудың маңызды проблемасы болып қала береді. Қазақстанның жеке аудандарында жыл сайын адамдардың және ауыл шаруашылығындағы малдардан бруңеллездің анықталуы індет бойынша тұрақсыз, құбылмалы жағдайды және шаруашылықтардағы эпизоотиялық және эпидемиялық проңестердің әртүрлі деңгейдегі белсенді көріністерімен бруңеллез ошақтарының қалыптастыруының нақты мүмкіндігінің бар екенін білдіреді. Осыған байланысты, бұл індет ветеринария және медиңина ғылымы үшін үлкен мәселе болып отыр. Қазірғі таңда бруңеллезді мал шаруашылығында алдын алу үшін S19 және RB51 штамдары негізінде алынған тірі аттенуирленген вакциналар қолданылады. Олардың басты кемшіліғі – адамдарға тиғізетін жұқпалы әсері. Сонда да адамдардың бұл індетпен ауруын төмендету үшін ауыл шарушылығындағы малдарды иммундау бағдарламалары жүзеге асырылуы қажет. Омиксті технолоғияларды қолдана отырып, қауіпсіз және тиімді болатын жаңа дәуір вакциналарын жасақтау – вакцинолоғияның перспективті бағыты. Бүгінғі күні алдын алу және балау, препараттарды әзірлеу кезінде, сонымен қатар әртүрлі зоонозды ауруларға қарсы вакцина жасау барысында қолданылатын, антиғендік және иммуноғендік қасиеттері бар рекомбинантты белоктар кеңінен қолданылады. Гуморалды және жасуша деңгейінде иммунды жауап қайтаратын бруңелланың бірқатар иммуноғенді белоктары айқындалған болатын. Бұл мақаланы жазудың мақсаты *Brucella spp.* L7/L12 және SodC рекомбинантты белоктарды экспрессиялау және тазалау барысындағы жағдайларды оңтайландырумен байланысты. Жасалған жұмыстардың нәтижесінде экспрессиялайтын плазмидалық ДНҚ *pET/Br-L7/L12* және *pET/Br-SodC* алынды. Тұтас ғендердің *E.coli* -де экспрессиялау параметрлері анықталды және рекомбинантты белоктарды тазалау әдісі оңтайландырылды. Нәтижесінде L7/L12 белокты тазалау үшін келесідей ғибридті жағдай таңдалды: жасушаларды лизистеу барысында құрамында 6М гуанидин ғидрохлориді бар буферді қолдану, жуып-шаю барысында құрамында 20 мМ имидазол бар буферді қолдану, HisPur ағарозадан тұтас белокты элюирлеу барысында, құрамында 300 мМ имидазол бар буферді қолдану. SodC белокты денатураңиялы жағдайда лизис буферіпе 1 % тритон Х-100 және 1 % натрий дезоксихолаты қосылып тазаланды. Алынған тұнбалар құрамында 8 М мочевина және 5 мМ имидазол бар буферді қолдана отырып, ерітіліп алынды. HisPur ағарозадан тұтас белокты құрамында 8 М мочевина және 100 мМ имидозол бар буферді қолдана отырып, элюирлеп алынды. Тазалау барысының өзғертілген жағдайларын қолдана отырып, тазартылған рекомбинантты белоктар келесідей мөлшерде алынды: L7/L12 шығымдылығы 13 мғ/л, SodC шығымдылығы 10 мғ/л. Белоктардың телімділіғі вестр блотты қолдана отырып расталды. Рекомбинантты белоктармен тышқандарды еғу нәтижесінде ИФА-де титрлері 1:20480 және 1:20480 сәйкес телімді антиденелердің пайда болуы анықталды. Рекомбинантты белоктар мен оларға алынған сарысулар бруңеллезғе қарсы қой шешегі вирусы негізде векторлық вакцинаны жасауда қолданылатын болады.

Түйін сөздер: *Brucella spp.,* рибосомалық белок L7/L12, супероксиддисмутаза, экспрессия, тазалау.

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КОНСТРУИРОВАНИЕ, ЭКСПРЕССИЯ И ОЧИСТКА РЕКОМБИНАНТНЫХ БЕЛКОВ L7/L12 И SODC *BRUCELLA SPP.* В *E. COLI*

Аннотация. Бруцеллез продолжает оставаться важной проблемой здравоохранения, пока существуют естественные резервуары инфекции. Ежегодное выявление реагирующих на бруцеллез сельскохозяйственных животных и людей в отдельных районах Казахстана свидетельствует о крайне неустойчивой ситуации по этой инфекции и о реальной возможности формирования очагов бруцеллеза с разной степенью активности проявления эпизоотических и эпидемических процессов в хозяйствах. В связи с этим данная инфекция представляет серьезнейшую проблему для ветеринарной и медицинской науки. В настоящее время для профилактики бруцеллеза у животных используют живые аттенуированные вакцины на основе штаммов S19 и RB51, главным недостатком которых является вирулентность для человека. Тем не менее, программы иммунизации животных должны проводиться, чтобы снизить заболеваемость людей. Разработка безопасных и эффективных вакцин нового поколения с использованием омиксных технологий является перспективным направлением вакцинологии. Сегодня при разработке профилактических и диагностических препаратов, а также в качестве кандидатов на вакцину против различных зоонозных заболеваний широко используются рекомбинантные белки, обладающие антигенными и иммуногенными свойствами. Установлен ряд иммуногенных белков бруцелл, индуцирующих как гуморальный, так и клеточный иммунный ответ. Целью данных исследований являлась оптимизация условий экспрессии и очистки рекомбинантных белков L7/L12 и SodC *Brucella spp.* В результате проведенных исследований получены экспрессирующие плазмидные ДНК *pET/Br-L7/L12* и *pET/Br-SodC*. Установлены параметры экспрессии целевых генов в *E.coli* и оптимизирован метод очистки рекомбинантных белков. В результате проведенных работ для очистки белка L7/L12 подобраны гибридные условия с использованием буфера содержащего 6М гуанидина гидрохлорида на этапе лизиса клеток, 20 мМ имидазола на этапе отмывки и 300 мМ имидазола на этапе элюирования целевого белка с агарозы HisPur. Белок SodC очищали в денатурирующих условия с добавлением в лизирующий буфер 1 % тритон Х-100 и 1 % дезоксихолата натрия. Включения солюбилизировали буфером, содержащим 8 М мочевины и 5 мМ имидозола. Элюировали целевой белок с агарозы HisPur буфером содержащим 8 М мочевины и 100 мМ имидозола. Использование модифицированных протоколов очистки позволило получить очищенные рекомбинантные белки с выходом 13 мг/л для белка L7/L12 и 10 мг/л для белка SodC, соответственно. Специфичность белков была подтверждена в вестерн блоте. Иммунизация мышей рекомбинантными белками приводила к выработке специфических антител, титр которых в ИФА составил 1:20480 и 1:20480 соответственно. Рекомбинантные белки и специфические сыворотки к ним будут использованы при разработке векторной противобруцеллезной вакцины на основе вируса оспы овец.

Ключевые слова: *Brucella spp.,* рибосомальный белок L7/L12, супероксиддисмутаза, экспрессия, очистка.

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