Cloning, Sequencing and Expression of the *Brucella Melitensis* Novel Lomr Protein

Othman Jamal Nassrullah[1], Mohammed Omer Mohammad[2], Shwan Kamal Rachid[3]

[1]Assist. Prof., Infectious Diseases, Clinic and Medicine Department, College of Veterinary Medicine, Sulaimani University, [2] FICMS-FRCP of medicine. College of medicine Sulaimani University, [3]Prof., Molecular, Pharmaceutical and Biotechnology. Charmo University

Abstract

Aim: Brucellosis is a zoonotic infection transmitted from animal to human directly by the animal or indirectly from their products. It is worldwide distribution especially in the middle east including Iraq and Kurdistan Region. **Materials and methods:** The gene coding for the outer membrane protein assembly factor LomR of 18kDa, now designated of *Brucella melitensis* 16M was cloned and sequenced. Cloning of insert DNA from bacteria into pET-28$_+$ allowed the selection of a plasmid bearing a 5.5-kb NcoI fragment that seemed to contain the entire omp gene under control of its own promoter. **Results:** LomR was localized within a region between the NcoI and XhoI insert of approximately 396 bp. The reliability of the constructed plasmid was established by restriction enzyme mapping and sequencing. LomR was expressed after induction with IPTG in Escherichia coli BL21. Recombinant LomR was purified by chromatography through Ni-agarose. Sequencing of this region revealed an open reading frame of 390 bp encoding a protein of 132 amino acids and a predicted molecular mass of 20 KDa. **Conclusion:** The availability of recombinant LomR and the identification of the antigenic determinant recognized will allow the evaluation of their potential protective activity and their potential for the development of subunit vaccine against brucellosis.

Keywords: *(PCR, cloning, LomR, DNA, Gene, Protein, expression, Brucella melitensis)*

Introduction

Brucella types are facultative intracellular gram-negative bacterial pathogens that infect both phagocytic and nonphagocytic cells. *Brucella abortus* causes abortion and economic losses in dairy cattle and furthermore different zoonotic diseases in human. *Brucella melitensis* is the most pathogenic species for people and may cause premature births in sheep, goats, and cows. Immunization of sheep and goats against *B. melitensis* with live attenuated smooth *B. melitensis* Rev. 1, the strain most generally use for disease control [12], this bacterium needs to oppose oxygen consumption. Without treatment, brucellosis may get continuous, bringing about restricted disease of liver, spleen or brain. [11].

Brucella melitensis Rev.1 vaccine induces abortions when applied during pregnancy, is virulent for humans, and elicits antibodies to smooth lipopolysaccharide (LPS) of Brucella interfering in serodiagnosis. Moreover, Rev.1 is resistant to streptomycin, an antibiotic used to treat the disease. Effective brucellosis vaccines not interfering in the diagnosis of infected animals would represent a major breakthrough [8]

The sickness remains a general medical issue and results in serious monetary misfortunes regarding loss of fetus and loss of animals.

There is significant financial weight of brucellosis reflecting, either the expenses of accomplishing and keeping up illness free status, or the expense of infection regarding loss of profitability and control costs. Over numerous years Brucella taxonomists

Corresponding author:
Othman Jamal Nassrullah
Assist. Prof., Infectious Diseases, Clinic And Medicine Department, College of Veterinary Medicine, Sulaimani University

built up a characterization framework that perceived six established species dependent on unpretentious phenotypic and antigenic contrasts and differential host specificity. Accordingly generally *B. abortus* (cow), *B. melitensis* (caprine and ovine), *B. ovis* (ovine), *B. canis* (canine), *B. suis* (porcine, rangiferine, leporine) and *B. neotomae* (rat) are perceived. [4].

The live attenuated strain *B. melitensis* Rev.1 is viewed as the best immunization accessible for the prophylaxis of brucellosis in sheep [5].

During the last two decades, a number of *Brucella* antigens have been identified, such as Omp16, Omp19, Omp25, Omp31, SurA, Dnak, trigger factor (TF), ribosomal protein L7L12, bacterioferritin (BFR) P39, and lumazine synthase BLS [9]. These antigens were selected based on empirical screening approaches that are typically laborious and expensive and require strict safety precautions and particular lab facilities, as the relevant species of *Brucella* are classified as biosafety level 3 microorganisms. This insufficiency of the empirical methods represents a great need for a rational and comprehensive approach to discover potential antigen candidates that can be used to develop a safe and effective anti *Brucella* vaccine. In any case, its utilization is known to invigorate counter acting agent reactions in sheep serum by the current ordinary serological tests from those saw in B. melitensis-infectred sheep. These tests, of which the most ordinarily utilized are the Rose Bengal test, the seroagglutination test, and the complement fixation test, chiefly measure antibodies against the immunodominant smooth lipopolysaccharide (S-LPS) [7]

Hence, a noteworthy objective in immunological investigations of brucellosis has been the ID of protein antigens valuable for analysis and conceivably helpful for recognizing the immunological reactions of infected animals from those of animal inoculated with live attenuated strains. The aim of this work was to discover antigen candidates conserved among the three pathogenic species. In the present study we purified a protein that is a part of the Brucella melitensis membrane to try using it later as antigen for diagnosis and control concepts.

Materials and Method

Ethical statement: This study was performed in strict accordance to the ethical committee condition of the university of Sulaimani , college of veterinary medicine.

Bacterial strains

Bacterial strains E. Coli Xl blue and BL21 (DE3) a gift from (Genetic department, Tehran University of Medical Sciences) were used for expression of LomR recombinant protein. Bacterial strains were routinely grown at 37◦C in LB broth or agar

Cloning and DNA sequencing LomR gene:

Bacterial DNA from cultures of *B. meliteinsis* 16M full-grown all night was isolate using a Qiagen DNA taking out kit. The DNA was checked using agarose gel electrophoresis, and the purity and quantity were calculated using a spectrophotometer. The section encoding the LomR gene, consisting of 554 bp, was amplified and legated into pET-28a.DNA of recombinant phages pET-28a expressing was recovered following the protocol of Maniatis *et al*. for the rapid, small-scale isolation of DNA. Plasmid DNA was then incise with NcoI and XhoI restriction fragments were ligated into pET-28a (Promega) cut with NcoI. Competent *E. coli* DH5a cells (Promega) were transformed with recombinant plasmid DNA as described by Maniatis et al.Then spread on LB-kanamycin (50 mg ml) plate's one positive colony was further selected for restriction analysis of the insert containing the gene coding for LomR. Restriction analysis was done with the following enzymes of the multiple cloning site sequence of pET-28a NcoI, XhoI.

Expression and Purification of recombinant LomR protein.

The ligated product was then used to change the expression host E. coli BL21. The exponential-phase culture of the established LomR clone was induced with different IPTG concentrations of 0.5, 1.0, 1.5 and 2.0 mM, and checked for expression at hourly intervals up to 5 h. Induced cells, as well as uninduced cells exposed to the identical conditions, were lysed in 1× lysis buffer (PBS) and analysed by 10% PAGE, as described by Laemmli (1970) . In order to find out the location of the expressed recombinant protein, the bacterial cell suspension was sonicated for 10 min with a pulse interval of 8 s. The

sonicated extract was centrifuged at 14000 rpm for 30 min at 4 °C. The supernatant and cell pellet, with suitable controls and molecular mass markers, were analyzed by 12% SDS-PAGE. After verification of the solubility, the protein was purified by His-tag binding affinity to Ni-NTA agarose. Purification of the cell lysate was carried out using a ROTH (Germany) Ni-NTA spin column with a native purification protocol as specified by the maker, and mass purification was approved by gel filtration affinity column chromatography using Ni-NTA Super flow (ROTH). The purified protein was checked by SDS-PAGE followed by coomassie blue staining, and protein concentration was expected by the Bratford method using BSA as a standard.

Results:

Cloning and sequencing of DNA coding for rLomR protein

Genome DNA ready from B. melitensis was use as guide in the PCR by using Prime DNA polymerase. Outcome show that single band through accurate molecular weight has been augmented for LomR gene. Fig 1

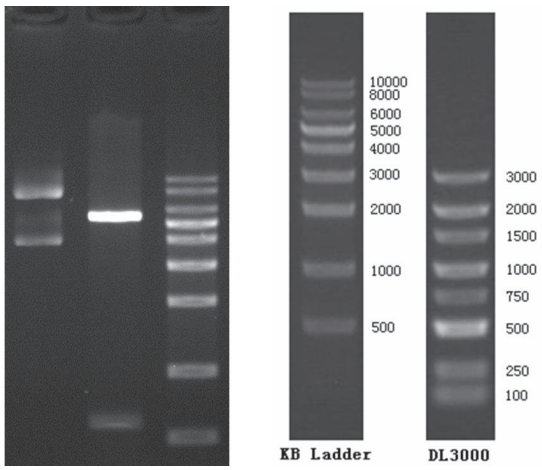

Fig. 1. Confirmation of cloning, expression and purification of LomR. Lane 1: pET-28a+ LomR, Lane2: pET-LomR digested with XhoI and Mlul , Lane 3: molecular marker. Digestion Conditions Digestion in water-bath, 37C for 40 minutes about 300ng plasmid digested 1% Agarose gel.

Cloning of the *B. melitensis* LomR gene

Viewing of the recombinant pET-28a plasmids were passed with restriction digestion and sequenced (Fig. 1). Insertions were excised from recombinant pET-28a with positive results and sub cloned into pET28a (+). Cloned plasmids were examined with restriction digestion XhoI and NcoI restriction enzyme and plasmid sequencing.

Expression of rLomR in *E. coli*

Expression of induced cells was checked, at hourly intervals up to 4 hours after induction with 1mM IPTG (Fig 2).

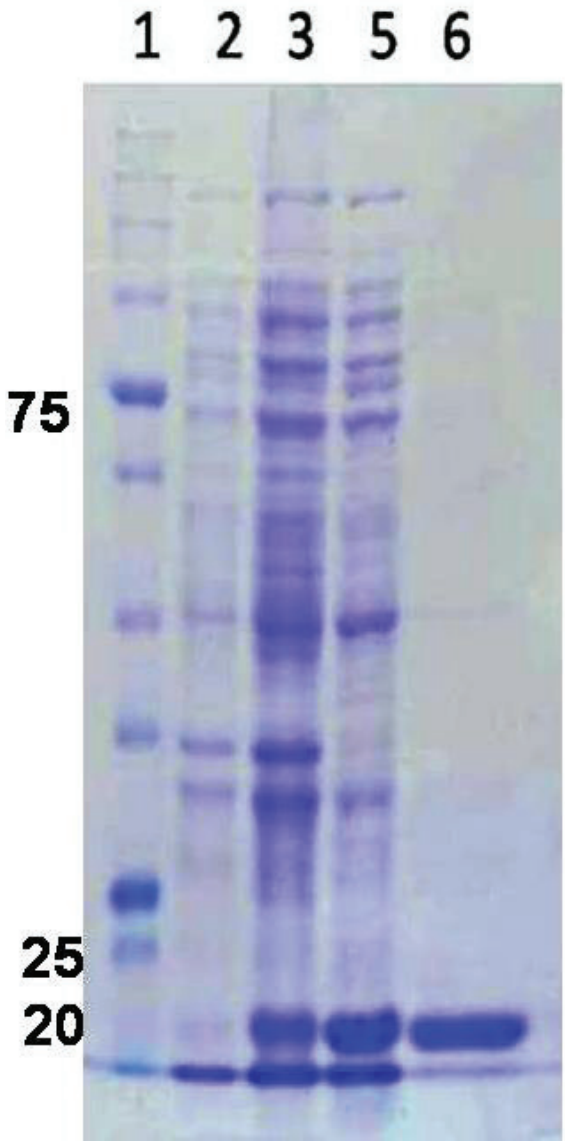

Fig 2. SDS-PAGE analysis of LomR purification. Lane 1: molecular weight marker, Lane 2:E.coli BL21 with pET-28a+ LomR before IPTG induction, Lane 3: Ecoli BL21 with pET-28a+ LomR after IPTG induction –Lane 4: eluted recombinant protein with elution buffer containing 500m M Imidazole.

Disscusion

Brucellosis is an extensive zoonotic disease that can communicate a disease to variety of farm animals and wildlife. This disease is widespread in many countries and it leads to cost-effective losses [2]. Rev1 and S19 are Brucella typical vaccines which used generally. They are live attenuated bacteria strains. These vaccines have some harm, such as weak inducible for immune system and have potential of human infection. Based on this data, the detection of immunogenic bacterial constituent is vital component of subunit vaccine and diagnostic development process. Surface proteins of the bacteria have been reported to take part in significant role through Brucella infection and inducing immune response. Commonly, successful vaccines against their bacteria mainly induce antibodies against surface composition. These surface proteins of the bacteria have been thought of as helpful antigens for progress of both diagnostic reagent and vaccine candidates [3] Antigen based diagnosis of brucellosis has not been basically used and so antibody based diagnosis is only efficient substitute that can be used in laboratories. The antibodies against different Brucella antigens can be detected in sera from little days of infection and up to 3 months.

Selection and production of new vaccine candidates are the primary practical steps toward introducing new vaccines. During the last two decades, a number of *Brucella* antigens have been identified, such as Omp16, Omp19, Omp25, Omp31, SurA, Dnak, trigger factor (TF), ribosomal protein L7L12, bacterioferritin (BFR) P39, and lumazine synthase BLS [10.]

The OMPs of Brucella spp. have been broadly characterized as immunogenic and protective Ags. Yet studies have been focused on the major OMPs [1].

immunization with purified recombinant LOmp16 or L-Omp19 protein plus IFA induced protection against B. abortus infection prompted us to study in further detail different aspects that we consider important at the moment of choosing an Ag for further commercial use as a vaccine. In this regard, the capacity to manufacture an Ag that is molecularly defined and pure is highly beneficial in terms of safety, effectiveness, and large-scale production. [10].

At present, native antigens like, complete cell extract and lipopolysaccharides have been used in serological assays. RBPT which is based on the stained complete cell heat killed acidified bacteria is used for early screening. Although RBPT has high sensitivity but low specificity because these antigens show cross reactivity with other bacteria like E. coli O: 159, Y. enterocolitica O: 9, Vibrio cholera, and Salmonella spp. [6].

The gene of LomR was PCR synthesized and cloned into the pET28a (+) expression system. The system provides assistance for best expression of Brucella melitensis 16M outer membrane proteins.

Conclusion

The outer membrane resembling protein LomR protein is a novel protein that expressed. These expressed recombinant proteins have been intended to be fused with 6-His tags at their N terminal. In this study, all expressed proteins were confirmed by SDS-PAGE. Purified recombinant proteins with Ni- NTA agarose resins arranged like truth folding proteins. Purified 21 KDa proteins candidate for diagnostic purpose. This recombinant protein will be examed later in vivo model to test their immune stimulation.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the college of veterinary medicine, Iraq and all experiments were carried out in accordance with approved guidelines.

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