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CLONING AND EXPRESSION OF RECOMBINANT BRUCELLA GROEL USING PHEN6 PLASMID

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ABSTRACT

Brucella has a great impact on health and economy in Syria, thus much effort is being placed on the development of the next generation of diagnostic kits and vaccines. These imply mainly the setup of high-yield production systems for some of the most important immunogenic proteins of this pathogen in order to replace the need for direct Brucella-antigens, including lipopolysaccharide, which is associated with serological cross-reactivity and biohazard risk. The heat shock protein of 60 kDa (HSP-60) or alternatively named GroEL, is an interesting Brucella- immunodominant antigen with great roles in the parasite life cycle, mainly adhesion and penetration during the infection of the macrophages.

In the present work, we developed an expression system to produce a soluble recombinant Brucella-GroEL in the periplasm of E. coli. Using B. abortus genomic DNA as template, GroEL gene was amplified by PCR with specific primers, which were designed to contain the two restriction sites XbaI and BstEII at 5' and 3' ends respectively. The insert fragment of 1643 pb was purified from gel, digested and then inserted into the expression vector pHEN6.

The new plasmid construct (pHEN6-GroEL) was able to express a soluble rGroEL in the periplasmic space of the transformated E. coli BL-21 codon plus RIL. One step purification process of this C-terminal

His6-tagged soluble protein, by affinity chromatography using Nickel charged resin, was monitored after SDS-PAGE separation, coomassie blue staining and immunoblotting with specific anti- His6 tag antibody. Brucella rGroEL has potential use in the development of diagnostic kits of brucellosis, both in laboratory and in field-based conditions.

Keywords: B. abortus; GroEL; pHEN6; cloning; protein expression.

INTRODUCTION

Brucellosis is a zoonotic disease caused by gram-negative Bacteria belonging to the genus Brucella which is a coccobacilli group classified within the phylum Proteobacteria **(Pappas, et al., 2005)**. Different species of Brucella mostly infect domestic livestock: cattle (B. abortus), sheep and goats (B. melitensis), pigs (B. suis) and dogs can also be infected with B. canis. These four species can infect humans by many ways **(Glynn & Lynn, 2008)**. The disease is characterized by abortion and reduced fertility in cattle and by chronic infections with symptoms such as undulant fever, arthritis and osteomyelitis in humans **(Pappas, et al., 2005)**. Thus, the development of a rapid and accurate diagnostic test for brucellosis in humans and animals remains an elusive target.

There are currently many of serological tests relying on the use of lipopolysaccharide (LPS) or whole-cell bacterial extracts as an antigen for ELISA or agglutination tests using colorimetry **(Pappas, et al., 2005)**. Beside their false positives due to serological cross-reactivity **(Alonso-Urmeneta, et al., 1998)**, the production of these antigens have many other problems including the difficulties associated with the biohazard risk since Brucella species are class III pathogens **(Pappas, et al., 2006).** In fact, these serum cross-reactivities are regularly observed between the LPSs of some other bacteria (i.e. Yersinia enterocolitica O:9, Vibrio cholerae, Salmonella typhimurium and Escherichia coli O157). An alternative method; the PCR has been used for the diagnosis of both primary infection and relapses, as well as for focal complications of the disease **(Al-Attas, et al., 2000; Hamdy & Amin, 2002 and Imaoka, et al., 2007)**. These approaches are often time consuming and poorly suited for use in general diagnostic laboratories **(Navarro, et al., 2004)**.

A possible strategy for circumventing the cross-reaction caused by anti-LPS antibodies is to base the diagnosis on selected Brucella

proteins. Such antigens were identified and characterized by proteomic method from various Brucella preparations **(Lin & Ficht, 1995 ; Teixeira-Gomes, et al., 1997 and Connolly, et al., 2006)**. The chaperonin GroEL, also known as heat shock protein of 60 kDa, was one of these immunogenic proteins, which is also suitable for serological assays **(Al Dahouk, et al., 2006)**. In fact, Brucella are facultative intracellular pathogens that infect phagocytic cells, such as macrophages, and multiply within **(Pappas, et al., 2005)**. Brucella synthe size GroEL protein in response to macrophage phagocytosis that helps denaturated protein under acid condition to refold, therefore enhance the ability of Brucella to survival **(Basharov, 2003)**. GroELs are major antigens involved in T-lymphocyte activation and induction of protective immunity against various intracellular pathogens **(Kaufmann, et al., 1991)**. Moreover, GroEL from Legionella pneumophila, Escherichia coli, Mycobacterium leprae, Mycobacterium tuberculosis and Yersinia enterocolitica induce either cell-mediated and humoral immune responses characterized by increased cytokine levels **(Retzlaff, et al., 1994)**. Furthermore, GroEL was found as an immune dominant antigen among many other proteins from the outer membrane of B. abortus and may play a critical role in virulence to both humans and bovines **(Connolly, et al., 2006)**. GroEL reacts with serum from human, cattle and sheep with naturally acquired brucellosis **(Amirmozafari, et al., 2008)**. (**Bae, et al., 2002)** evaluated the role of GroEL as a potential vaccine in mice. Thus, recombinant GroEL (rGroEL) may be an interesting candidate for the development of new Brucella serologic detection Kit.

MATERIALS AND METHODS

Bacterial strains, growth conditions and plasmid

Brucella strains were provided by Unite de Recherche en Biologie Moleculaire, Facultes Universitaires Notre-Dame de la Paix, Namur, Belgium. Prior to inactivation, B. melitensis strain 16M and B. abortus strain 2308 NalR were grown in Brucella broth medium (HIMEDIA Laboratories) supplemented with 5 % heat inactivated horse serum (PAN Biotech GmbH) for about 48 h in 37 ºC incubator. The inactivation step was performed by washing bacteria twice with cold phosphate buffered saline (PBS) followed by heat incubation at 60 ºC for 30 min. Inactivation was confirmed by inoculating 1 ml of bacterial suspension on Brucella agar solid media. E. coli strain Bl21-CodonPlus, which is

used in protein expression, was prepared to receive by electroporation the recombinant plasmid pHEN6 (kindly provided by Prof. Serge Muyldermans, Department of Cellular and Molecular Interactions, VIB, Brussel, Belgium). Generally, E. coli were grown in Luria Broth (LB) (Bio Basic INC) with the required antibiotic at 100 mg/ml (streptomycin or ampicillin; Applichem) at 37°C. Protein expression of positive clones was performed in Terrific Broth (TB) containing 100 mg/ml ampicillin, 2 mM $MgCl_2$ and 0.1 % glucose according to **(Arbabi Ghahroudi, et al., 1997)**.

Brucella Multiplex PCR

Multiplex PCR reactions were achieved directly on aliquot of heat killed bacteria. Before use, the killed organisms were rinsed once in distilled water and were then resuspended in distilled water at an optical (600 nm) density of 0.15 to 0.20 (approximately 10^9 cells/ml). Two µl of bacteria suspension were used in each PCR reaction. Brucella Multiplex PCR was performed using multiple pairs of primers able to amplify a specific genome locus, based on the work of **(Imaoka, et al., 2007)** with minor modifications.

Cloning of GroEL gene in pHEN6 plasmid

Brucella genomic DNA was extracted from heat killed bacteria according to standard CTAB/NaCl method **(Ausubel, et al., 2003)**. Using 100 ng of this DNA as template, the gene of GroEL was amplified by PCR with two specific primers (F-XbaI/R-BstEII), which were designed on the GroEL sequence of B. abortus (accession: YP_001932144) to amplify the full length of the gene and to added XbaI and BstEII (fermentas) restriction sites at the 3' and 5' ends respectively. For cloning purposes, AccuPrim™Taq Polymerase High fidelity Kit (Invitrogen) was used in PCR reaction. PCR high fidelity program consisted of 2 min of denaturation step at 94 °C followed by 35 cycles of short denaturation step at 94 °C for 30 sec, annealing at 55 °C for 30 sec and extension at 68 °C for 1.5 min. PCR amplified GroEL gene was phenol extracted and then ethanol-precipitated. GroEL and pHEN6 plasmid were digested with restriction enzymes and then ligated using T4 DNA ligase (Fermentas). Freshly prepared electro-competent E. coli BL21 codon plus RIL cells were transformed with the new plasmid construct pHEN6-GroEL by electroporation. Colony PCR screening for positive GroEL clones was performed using pHEN6-specific primers (RP/FP) (Table. 1). Plasmid

constructs were isolated from some positive clones by Plasmid Miniprep Kit (Qiagen) after being grown in LB/ampicillin medium. Successful cloning was confirmed for these plasmids ether by digestion with restriction enzymes or by sequencing using primers distributed along the inserted gene, giving an overlapping sequencing fragments covering the full-length of the cloned GroEL.

Table (1): Primers used for GroEL cloning

Protocol	Name	Oligo sequence	Length	TM (°C)	DNA fragment (bp)
GroEL PCR amplification	F(XbaI)	TCCGCATCTAGAGCTGCAAAAGACGTAAAATT	32	74,0	1662
	R(BstEII)	TATATAGGTCACCCCGAAGTCCATGCCGCCCATGC	35	83,8	
Sequencing Primers	F1	CACTTCGGAAGAAGTTGCCC	20	65,9	
	F2	GCGCCAAGAAGGTTTCGATC	20	68,0	
	R1	GGCAAGACGTTCCTGAAGCT	20	65,7	
	R2	TTGGCTTCTTCAACCGTGAT	20	64,5	
pHEN6 primers	RP	TCACACAGGAAACAGCATATGAC	22	49.4	1850 (+ colonies) 200 (- colonies)
	FP	CGCCAGGGTTTTCCCAGTCACGAC	24	66	

Different parameters (name, sequence, length and annealing temperature) of the primers, used for Brucella GroEL amplification and cloning into pHEN6 plasmid, are indicated in the table. The expected sizes of PCR amplified products are indicated as well for each pairs of primers. Underlined letters are the position of the restriction enzymes referred to in the names of some primers.

Expression and purification of soluble rGroEL

Large-scale production of rGroEL was performed in 250 ml shake flasks by growing the bacteria E. coli codon plus RIL in complete TB medium till an optical density of 0.6 to 0.9 was reached and then expression was induced with 1 mM IPTG (Isopropyl β-D-thiogaldctoside; Promega) for 16 h at 28 °C. After pelleting the cells, the periplasmic proteins were extracted by osmotic shock according to **(Skerra & Pluckthun, 1988).** Using fast protein liquid chromatography (FPLC) AKTAprime plus system (GE lifescience), this periplasmic extract was loaded on a 1 ml column of Nickel charged Nitrilotriacetic acid (NTA) superflow Sepharose (Qiagen). After washing, the bound proteins were eluted with a 250 mM imidazole buffer. The eluted fraction

was concentrated on Vivaspin concentrators with a molecular mass cutoff of 30 kDa (Vivascience) and loaded on a Superdex-75 10/30 gel filtration column (Pharmacia). The purity of the rGroEL was evaluated in a Coomassie-stained SDS-PAGE. The absorption at 280 nm and the extinction coefficient (7800) as calculated from the amino acid sequence of the Brucella rGroEL, were used to determine protein concentrations.

WB and coomassie blue staining of SDS-PAGE

SDS-PAGE was carried out using a Bio-Rad mini-Protean II system following the manufacturer's instructions. Gels were prepared using stacking gel 5 % and running gel 10 %. After electrophoresis, the gel was stained by coomassie blue for 2 h then distaining was done. For WB, proteins in acrylamide gel were blotted onto 0.45 µm nitro-cellulose membranes (BDH, Electran) using 1 X blotting buffer (800 ml SDW containing 14.4 g glycine and 3 g Tris, pH 8.3 and 200 ml methanol). After incubation in the blocking buffer (3 % skimed milk, 1 % BSA in 1 x PBS), blots were incubated with 1/2000 dilution of mouse anti- His6-HRP antibody (Roche) and detected by AEC (3-amino-9-ethylcarbazole) chromagen substrate in acetate buffer (pH 5) in presence of hydrogen peroxide.

Bioinformatics

GroEL amino acid sequences from different sources; B. melitensis (accession: NP_542026), B. abortus (accession: YP_001932144), B. ovis (accession: YP_001257264), Yersinia enterocolitica (accession: BAA03164), Arabidopsis thaliana (accession: BAB02911), Mus musculus (accession: NP_034607) and Homo sapiens (accession: NP_002147) were obtained from the database available at the National Centre for Biotechnology Informatics web site (http://www.ncbi.nlm.nih.gov/). Protein sequence homology searches, phylogeny tree prediction, primers design, plasmid constructs mapping and analysis of the Brucella GroEL amino acids were performed using Vector NTI software.

RESULTS AND DISCUSSION

Amplification and isolation of GroEl gene from Brucella genome

The entire sequence of the heat shock functional operon of B. abortus, containing groES and groEL genes with putative promoter and

terminator sequences, was early reported **(Gor & Mayfield, 1992)**. Using this sequence, a pair of specific primers (F-XbaI/R-BstEII) (Table 1) was designed in order to amplify the full-length gene of GroEL by PCR. For this aim, three different species of Brucella were used and their purity was confirmed by Multiplex Brucella PCR reaction, which was achieved directly on aliquot of the heat killed bacteria (Fig. 1A). These PCR reactions resulted clearly in a distinct profile for each strain in which the band of 1000 bp was used as control for the amplified bacterial genomic DNA and two repetitive bands at 302 pb and 393 bp referred to the genus Brucella. Beside these common bands, two specific bands at 794 bp and 498 bp described the two main species of Brucella; B. melitensis and B. abortus, respectively and both bands were absent when the PCR was performed on B. ovis (Fig. 1A).

Genomic DNA from the three Brucella strains were isolated and used as a template in PCR using GroEL specific primers (Fig. 1B). This resulted in the amplification of a single DNA fragment of 1662 bp from the three different species of Brucella (Fig. 1B). Obviously, Brucella GroEL polypeptide, which represents a relatively big protein of 587 amino acid residues and apparent 60 kDa molecular weight, is a highly conserved protein within Brucella genus comparing with bacterial GroEL from other genus, e.g. Yersinia, or from plant or animal kingdoms arriving to the Homo sapiens HSP-60. Furthermore, the heat shock response is highly conserved during evolution and presumably allows organisms to adapt to stressful environments **(Teixeira-Gomes, et al., 2000)**. This idea was clearly demonstrated by a phylogenetic tree which was established by comparing the amino acid sequence of GroEL from these creatures (Fig. 1C).

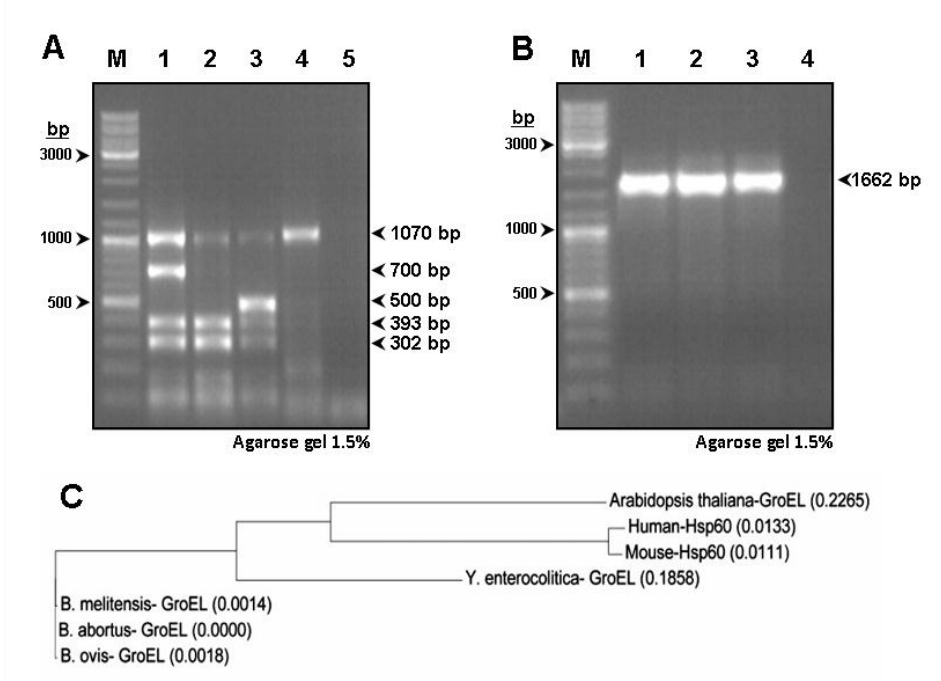

Fig. (1): Homology of GroEL gene in the genus Brucella.

PCR reactions were performed on 100 ng of genomic DNA extracted from B. melitensis (lane 1), B. ovis (lane 2), B. abortus (lane 3), Y. enterocolitica (lane 4) and in the absence of template DNA (lane 5) and PCR products were separated into 1.5 % agarose gel. (**A**) Showed the result of multiplex Brucella PCR performed to confirm the purity of Brucella strains. (**B**) The result of PCR amplification of the GroEL gene using specific primers (F-XbaI/R-BstEII). Side arrows indicate the expected positions and sizes (in bp) of the amplified bands as well as those of the molecular weight marker (M). (**C**) Phylogeny tree of the GroEL protein sequences demonstrating the high homology between different organisms belonging to distant genira.

GroEL gene of B. abortus localizes in the second chromosome in a complementary orientation, spanning 1641 bp actual long between ATG start and TAA stop codons (Fig. 2A). Full-length GroEL gene fragment was amplified by PCR using two long primers containing the restriction sites of XbaI and BstEII, respectively. The digestion of this DNA fragment resulted in a sticky ended fragment ready to be ligated in

the pHEN6 protein expression plasmid after being linearized with the same two restriction enzymes (Fig. 2B). This plasmid construct should enable the expression of C-terminal- His6 tagged GroEL in the periplasm of the transformed E. coli due to the pelB leader signal peptide added to the N-terminal of the protein. In accordance with this strategy, GroEL was identified in B. abortus cell envelope (CE) using immuno-proteomic techniques beside many major protein components including outer-membrane proteins (OMP) **(Connolly, et al., 2006)**.

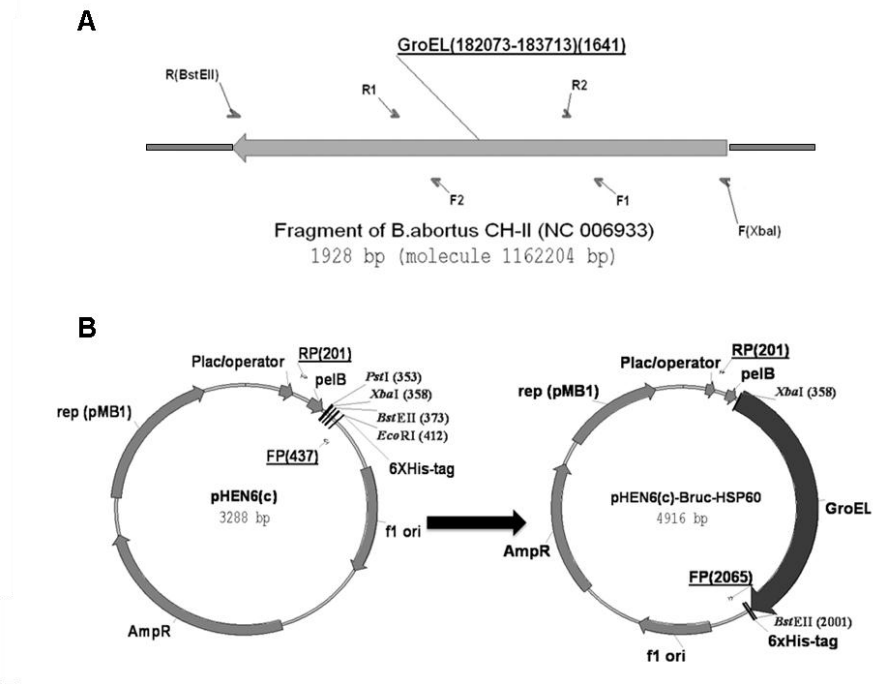

Fig. (2): Scheme of the GroEL gene and pHEN6 plasmid used in the cloning.

(**A**) Location and orientation of GroEL gene in B. abortus chromosome II. Positions of different primers (Table. 1) used in the cloning and sequencing are indicated as well. (**B**) Maps of the expression vector pHEN6 as well as the resulted plasmid construct (pHEN6-GroEL) where the position of the inserted GroEL gene is indicated. The most important elements of the plasmid are indicated. These include Plac promoter, His6 tag downstream the two restriction sites (XbaI and BstEII) used for GroEL gene ligation. We locate also the

pelB sequence coding for a leader signal in the N-terminal side of the rGroEL, which is necessary for periplasmic expression of the protein. Position of the two primers RP/FP used for PCR positive-colonies screening and for sequencing is shown.

Cloning of the GroEL in pHEN6

XbaI/BstEII digested pHEN6 as well as GroEL DNA insert were purified from gel and used in a ligation reaction in 1:3 ratio, respectively (Fig. 3A). Ligated products were used to transform the E. coli BL21 codon plus by electric shock and after short time for cells to recover. Positive colonies on the plates were screened by PCR using pHEN6-specific primers (RP/FP) (Fig. 2B). This approach enabled the distinguishing between two types of colonies; empty pHEN6-containing colonies which resulted in small amplified DNA fragment (200 bp) and pHEN6-GroEL-containing colonies which gave a big fragment of 1850 bp due to the presence of the insert gene within (Fig. 3B). Positive colonies were grown and used for plasmid mini-preparation and the right structure of the plasmid constructs were verified by digestion with the same restriction enzymes used in cloning (Fig. 3C). Digestion of pHEN6-GroEL with only one enzyme (BstEII) resulted in a DNA fragment (4916 bp), containing GroEL gene, longer than that obtained from empty plasmid (3255 bp) (Fig. 3C, lane 2 and 5). In addition, the inserted gene (1635 bp) could be extracted from the positive plasmid constructs and not from the control plasmid, after simultaneous digestion with both enzymes, beside a big fragment (3255 bp) of the linearized empty pHEN6 (Fig. 3C, lane 3 and 6). DNA sequences of pure plasmids prepared from positive colones were confirmed by sequencing for the absence of mutations and for the perfect matching with the open reading frame (ORF) of the upstream pelB leader signal.

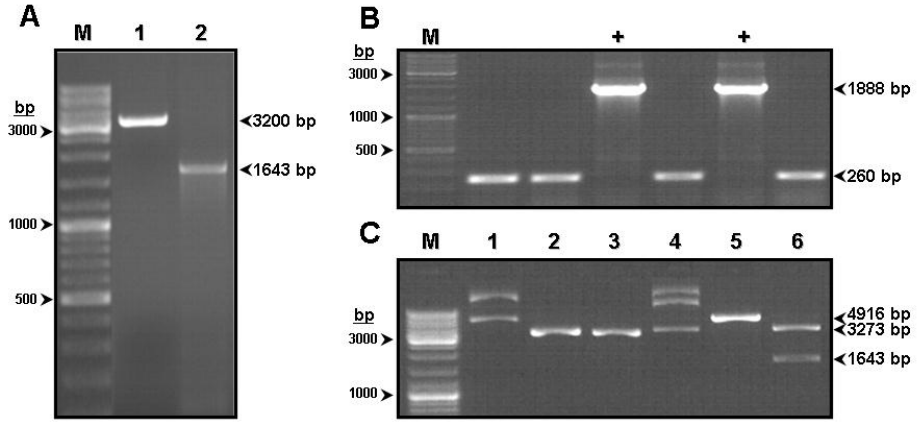

Fig. (3): Cloning of the GroEL into pHEN6 plasmid.

DNA fragments from the different steps of the cloning were separated into 1.5 % agarose gel where side arrows indicate the expected positions and sizes (in bp) of these fragments as well as the bands of the molecular weight marker (M). (**A**) DNA bands of pHEN6 plasmid (lane 1) and GroEL PCR amplified gene (lane 2) after being digested with XbaI/BstEII restriction enzymes before the ligation reaction. (**B**) Results of colony PCR screening performed on 6 randomly selected clones after E. coli BL21 codon plus RIL transformation with the ligation reaction products. Positive clones, which contain full-length GroEL gene, were indicated (+). (**C**) The pHEN6 (lanes 1, 2 and 3) as well as the new plasmid construct pHEN6-GroEL (lanes 4, 5 and 6) were isolated by mini-prep from their transformed colonies and then digested ether by BstEII alone (lanes 2 and 5) or in combination with XbaI (lanes 3 and 6).

Expression and purification rGroEL

Production of the Brucella rGroEL as soluble proteins was obtained after growing the transformed bacteria in liquid medium supplemented with antibiotic and protein expression was then induced by IPTG. In this system the expressed protein, carrying a His6 tag to facilitate purification, is transported into the periplasmic space of E. coli. BL21 codon plus RIL. Purification of the rGroEL from sucrose-osmotic-shock prepared periplasmic extract was done on immobilized-metal affinity chromatography, using Nickel-charged NTA column installed on AKTAprime system. The UV-detector, supplemented with this system,

enabled the real-time monitoring of the different steps of rGroEL purification (Fig. 4A). The protein expression and purification procedures of rGroEL were followed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) stained with coomassie blue reagent (Fig. 4B) or immune blotted then detected by anti-His6-HRP conjugated antibody (Fig. 4C). A remarkable expression of rGroEL could be observed after IPTG induction followed with 18 h of incubation at 18 °C. Although, the expressed protein was totally purified from bacteria periplasmic extract by column purification which yielded 90 % pure rGroEL. The yield of purified recombinant protein reached 10 mg/liter of bacteria culture.

(**A**) Diagram of purification procedure using Ni^{+}-NTA column installed on FPLC AKTAprime system. Continuous line represents the absorbance of the eluate, different purification steps are shown below and peaks of the flow-through sample and of purified GroEL are indicated. Dashed line represents conductivity of the eluate. Protein migration in SDS-PAGE (acrylamide 10 %) of the protein samples obtained after different steps of expression and purification. Total periplasmic extract before (lane 1) and after 4 h (lane 2) and 18 h (lane 3) of IPTG induction, flow-through sample from Ni-NTA column (lane 4) and purified rGroEL protein (lane 5). Detection of migrated proteins was done ether by coomassie blue staining (**B**) or by WB detection using mouse anti- His6-HRP conjugated antibody (1/2000) (**C**). The location of rGroEL in the gel is indicated and defined as 60 kDa by comparing to the protein molecular weight ladder in the first lane (M).

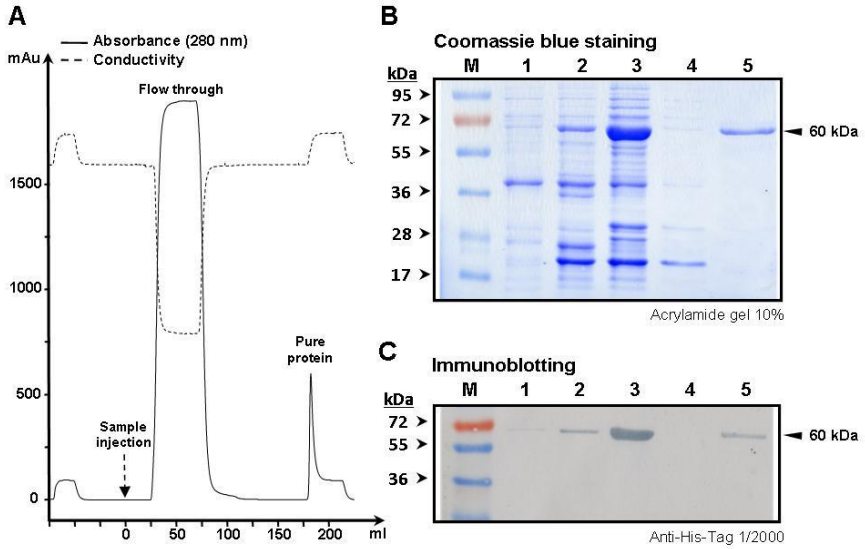

Fig. (4): The expression and purification of rGroEL

Several past studies of murine models have been carried out to test the ability of different proteins of Brucella to induce a protective immune response and/or to react specifically with the immune-products of this response; such proteins as Bacterioferritine (BFR) and the P39 **[Al Mariri et al., (2002)]**, HtrA **(Roop, et al., 1994)**, GroES **(Bae, et al., 2002)**, and L7/L12 **(Abtahi, et al., 2004)** and SP41 **(Castaneda-Roldan, et al., 2006)**. Brucella GroEL might be an important potentially protective molecule since it is implicated in antigen-specific T-lymphocyte-mediated activation of macrophages which leads usually to the elimination of intracellular pathogens **(Oliveira, et al., 1996)**. Therefore, **(Lin, et al., 1992)** attempted to evaluate the potential roles of GroEL as vaccine or as antigen for brucellosis serological tests.

In the current work, we established a high-level expression system of His6-tagged soluble Brucella GroEL in E. coli. Previously published works described the attempts to produce rGroEL proteins from many bacteria such as E. coli **(Kamireddi, et al., 1997)**, Vibrio **(Mizunoe, et al., 1999)**, Salmonella **(Paliwal, et al., 2008)** and Brucella **(Oliveira, et al., ,1996)**. But the most important problems in these attempts were the loss of the protein folding and its accumulation in the

inclusion bodies. This problem was partially resolved by fusing the GroEL in a complex protein with a large soluble protein like the maltose binding protein **(Lin, et al., 1992)**. The rate of expression using this system was relatively low and the resulted GroEL should not be in its optimal folded state. We have avoided many of these problems by using the pHEN6 plasmid, which is characterized with many useful properties in this context **(Arbabi Ghahroudi, et al., 1997)**. The pHEN6 allows the release of the proteins in the periplasmic space, instead of accumulation in the cytoplasm, due to the presence of a leader signal (pelB) attached to the N- terminal of the expressed protein. Although, the moderate Plac promoter of pHEN6 allows the calm expression of the protein enabling its correct folding rather than accumulating in the inclusion bodies, as seen with other expression systems with stronger promoters as T7 and T5 **(Burgess, 2009)**. Furthermore, recombinant proteins expressed by the pHEN6 system have a C- terminal His6 tag, which is necessary for protein purification and detection with anti-His6 antibody. Free N-terminal rGroEL should be functional as the reactive domain and the binding sites for the ATP and the GroES are all assured by this extremity **(Kuroda, et al., 2006)**. It would be of a great interest to proceed with functional in vitro studies of this Brucella rGroEl as a foldase enzyme after obtaining the rGroES using the same expression system. In addition, the development of serological ELISA test using this pure protein if does not replace existing screening methods; it could serve as a confirmation method when used, for instance, in parallel with other applied methods.

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تنسيل وتعبير بروتين البروسيلا GroEL المؤشب باستخدام البلاسميد pHEN6

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تؤثر بكتريا البروسيلا بصورة كبيرة على الصحة والثروة الاقتصادية في سوريا مما دفع إلى بذل المزيد من الجهد في محاولات تطوير جيل جديد من أدوات التشخيص واللقاحات. يقتضي هذا الأمر بشكل أساسي أن يتم إعداد أنظمة إنتاج عالية الكفاءة لبعض البروتينات المهمة استمناعياً بدلا من الاستخدام المباشر لمستضدات البروسيلا، مثل متعدد السكريد اللبيدي LPS الذي يعد من المكونات التي تسبب بعض التفاعلات المصلية الكاذبة. يعد بروتين الصدمة الحرارية (HSP-60)، وما يسمى أيضاً GroEL، هو واحد من البروتينات السائدة استمناعياً والذي يلعب دورا مهما في دورة حياة تطفل البروسيلا على البالعات الضخمة، حيث يسهم في عملية الالتصاق بهذه الخلايا واختراق أغشيتها خلال الإصابة.

هدفنا من هذه الدراسة إلى تطوير نظام تعبير بروتيني لإنتاج بروتين الصدمة الحرارية المؤشب للبروسيلا بالشكل المنحل والمفرز في منطقة البلاسما المحيطية لبكتريا Escherichia coli. استخدمنا في سبيل هذه الغاية الدنا الجينومي لبكتريا البروسيلا المجهضة Brucella abortus كقالب لتضخيم المورثة بوساطة تفاعل PCR باستخدام مُرئسات نوعية حاوية على موقعي أنزيمي التقييد XbaI وBstEII في النهايتين '3 و'5 على التوالي. نقيت شدفة الدنا 1643 pb الناتجة عن التضخيم من هلامة الأغاروز، وقُطعت ثم أُدخلت إلى بلاسميد التعبير البروتيني pHEN6. تميزت البنى البلاسميدية الناتجة (pHEN6-GroEL) بالقدرة على التعبير عن البروتين المؤشب المنحل في منطقة البلاسما المحيطية لبكتريا E. coli BL21 codon plus RIL، والذي تم تنقيته عن طريق ذيل متعدد الهستيدين الموجود في طرفه الكربوكسيلي باستخدام كروماتوغرافيا الألفة الشاردية، تم التحقق من نجاح عملية التعبير

البروتيني بعد الترحيل على هلامة SDS-PAGE إما بتلوين الهلامة بأزرق الكوماسي أو بالتبصيم المناعي باستخدام أضداد موجهة للذيل الهستيديني. يمكن مستقبلا استخدام بروتين GroEL المؤشب من البروسيلا في تطوير أدوات تشخيصية لداء البروسيلات في شروط عمل مخبرية وحقلية.