

PURIFICATION, CHARACTERIZATION AND EXPRESSION, OF RECOMBINANT OUTER MEMBRANE PROTEIN A (OMP A) OF *ACINETOBACTER BAUMANNII* LI311

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ABSTRACT

Acinetobacter baumannii is a known hospital acquired pathogenic bacterium that increasingly resists antibiotics treatment. In order to characterize and produce a soluble OmpA protein that can be used to develop *Acinetobacter* vaccine, polymerase chain reaction (PCR) was used to produce the *ompA* gene, of *A. baumannii* strain LI311, which was cloned into the histidin taged pET19b expression plasmid. Immobilized metal affinity chromatography (IMAC) was utilized to purify the recombinant protein, and amino acid sequences for OmpA protein homologs were attained from the National Center for Biotechnology Information (NCBI) protein resource then analyzed using the blast tool and Jalview program. Protein topology prediction was done using NCBI tools and PRED-TMBB2. Analysis of amino acid sequence of OmpA of *A. baumannii* strain LI311 showed that it has homologies to other clinical *Acinetobacter* species, including: *A. pittii*, *A.nosocomialis*, *A.seifertii*, *A. calcoaceticus*, and *A. ursingii* with identity percentages of 100%, 100%, 96%, 92%, and 91%. Protein topology prediction revealed two conserved domains belonging to OmpA family protein, which are beta-barrel domain outer membrane protein (OMP_b-brl) and OmpA-C-like domain, and it is a 10-β-stranded transmembrane Outer Membrane Protein with a signal peptide at residues 1–22A. A recombinant Histidine tagged- OmpA (39.31kDa) was successfully expressed and purified in this study. In conclusion, OmpA protein of *A.baumannii* strain LI31 is highly conserved across clinical species of *Acinetobacter*, and the soluble recombinant OmpA created in this study can be used to develop a putative vaccine that may prevent infections caused by the clinical species of *Acinetobacter*.

Keywords: *Acinetobacter baumannii*, *ompA* gene, expression, homology, putative vaccine

INTRODUCTION

The Gram-negative genus, *Acinetobacter*, involves a naturally varied group of aerobic bacteria, non-fastidious, oxidase negative, unable to motile, catalase-positive bacilli (Peleg et al., 2008). Although *Acinetobacter* members are generally depicted as ubiquitous nonpathogenic bacteria, strains of the *Acinetobacter baumannii-Acinetobacter calcoaceticus* become apparent as important opportunistic pathogenic bacteria to humans (Dijkshoorn et al., 2007; Perez et al., 2007). The capability of *A. baumannii* to have numerous virulence factors, such as iron gaining mechanisms, motility, and efflux force in unfavorable environmental circumstances helps this bacterium to develop infections. *A. baumannii* has the ability to survive and resist in hospital conditions, so it has become one of the highly thriving hospital acquired pathogenic bacterium that can stimulate crucial infections to humans (Howard et al., 2012; Montefour et al., 2008; Peleg et al., 2008). Moreover, this bacterium is implicated in outbreaks happening in rigorous hospital rooms (Bergogne-Berezin and Towner, 1996), and it is frequently a source of pneumonia, blood poisoning, and cystitis after hospitalization of people with further strict diseases (Coelho et al., 2004). The potential pathogenicity of *A. baumannii* includes the surfaces adherence capability, biofilms formation, antimicrobial resistance, and acquisition of genetic materials from distinct genera (Anstey et al., 2002; Howard et al., 2012). As resistance to multiple antibiotics among *A. baumannii* are increasing (Kuo et al., 2007; Qureshi et al., 2015), prevention and cure of diseases caused by this pathogen have become a major health care threat; therefore, novel approaches to manage and prevent *A. baumannii* infections are required. Although vaccination signifies the greatest strategy to prevent *A. baumannii* infections (Mishra et al., 2012; Pachon & McConnell, 2014), no effective vaccine has been developed for this pathogen to date. Antigens that induce immunity against proteins of the bacterial outer membrane are vital antigen candidates because of their ability to interact with the host as well as their position on the cell surface, which make them available for antibody neutralization (McConnell et al., 2011). The potential and viability of immunization have previously been validated through

active and passive immunization to the outer membrane protein A (OmpA) (Fattahian et al., 2011) (Luo et al., 2012) and a multifunctional trimeric autotransporter (Ata) antigens (Bentancor et al., 2012a). Nevertheless, the prevalence and solubility of these antigens represent major obstructions for achievement of a generally protecting vaccine.

The outer membrane proteins (OMPs) in Gram negative bacteria are distinctive classes of essential membrane proteins. These proteins have vital functions for the cell, such as adhesion, nutrients utilization, signaling, secretion of waste as well as serving as a virulence factor in pathogenic strains. OMPs are highly stable in membrane and capable of resisting very severe environments because their β-barrel domains have 8 to 26 strands. The large, expanded loops linking the strands on the extracellular region, while the small loops were found on the periplasmic area (Rollauer et al., 2015). The OMPs possess comparable composition and biological features even though they have diverse sequences (Chaturvedi and Mahalakshmi, 2017). The variety of OMPs sequence is much more at N terminal significantly than C terminal, and proper assembly and folding of OMPs is controlled by the conserved β strands (Gessmann et al., 2014). Nevertheless, the kinds of OMPs in *A. baumannii* have not been characterized evidently, up to now, and only some reports are exist, including Omp33–36, OmpW, and OmpA (Choi & Lee, 2019). Among them, OmpA is extremely abundant protein on *A. baumannii* surface, and it is implicated in bacterial growth, virulence, and drugs resistance. Due to the high immunogenic properties of OmpA, it has been previously identified as a putative antigen to develop vaccines as well as passive immunotherapy for diseases caused by this bacterium (Fajardo Bonin et al., 2014; Moriel et al., 2013). Interestingly, OmpA of strain LI31 of *A. baumannii* is found to be a homolog to OmpA of other clinical *Acinetobacter* species, including, *Acinetobacter pittii*, *Acinetobacter nosocomialis*, *Acinetobacter seifertii*, *Acinetobacter ursingii*, the possible causes of bacteremia (Kishii et al., 2016; Liu et al., 2017; Loubinoux et al., 2003), *Acinetobacter calcoaceticus* that causes burns and wounds infections (Pavlica and Tomanovic, 1989). A successful vaccine antigen to *A. baumannii* infections may be helpful to protect against *Acinetobacter* species; therefore, the current study aimed to:

1. Create a soluble recombinant OmpA protein that can be used as an antigen candidate in *Acinetobacter* vaccine through cloning the corresponding *ompA* open reading frame from *A.baumannii* strain LI31 into pET19b vector, expressing the histidine tagged OmpA protein, and purifying the fused OmpA using immobilized metal affinity chromatography (IMAC).
2. Analyze the sequence of OmpA amino acid sequence of *A.baumannii* strain LI31 for topology prediction and conservation .

MATERIAL AND METHODS

Amplification of *ompA* gene

DNA of *Acinetobacter baumannii* strain LI311(standard strain obtained from the central health lab in Baghdad)was elicited using bacterial RTP® extraction Kit (InVitek- Germany). Primers used for detection of *ompA* gene were designed using clone manager software based on the published sequence of the gene on NCBI (Accession number: EU332799). The sequence of the forward primer including *XhoI* restriction site was:- 5' **CTCGAGGGAATGAAATTGAG3'**. The sequence of the reverse primer with *BlnI* restriction site was: - 5' **GTGTATTTCGCGACAGCTGAGC3'**. The entire *ompA* code (1002 bps) was synthesized utilizing the PCR Kit, Phusion™ High-Fidelity from Thermo Fisher scientific. PCR testing was executed in a final amount of 50 µL of test combination which contains 0.5 µM for each primer, 25 µL of 2X Phusion Master Mix, 100 ng/ 50 µL of DNA, and nuclease liberated water. DNA was synthesized to be first denatured for 30 seconds on 98°C then for 10 seconds (30 rounds), annealed at 54°C intended for half a minute, elongated by 72°C for a period of 40 seconds, and last extended on 72°C for a period of 5 minutes. The product was visualized by agarose gel system using 1% gels supplemented with ethidium bromide(0.5 µg/ml). Gels were explicated via a ChemiDoc MP (Bio-Rad). Bands matching the predictable size of *ompA* gene with restriction sites (1018 bp) then extracted from the gel by the Thermo scientific Gene Extraction Kit.

Creation and validation of recombinant construct

The histidine tagged pET19b and PCR fragment were cut by Fast Digest restriction enzymes (*XhoI* and *BlnI*) (Thermo scientific). The cut PCR fragments were then cloned into the digested vector using T4 DNA ligase and ligase buffer. The ligated recombinant vectors were then moved into *E. coli* strain DH5a(Thermo Fisher Scientific) then cultivated on Luria-Bertani (LB) plates having hundred microgram per milliliter of ampicillin for selection. Clones were chosen randomly and cultivated on 37 °C in a test tube having five milliliters of LB broth and ampicillin (100 µg/ ml). Constructs were extracted from overnight cultures employing the Gene JET™ Plasmid Mini prep Kit (Thermo scientific). Recombinant vectors were validated by colony PCR, cutting by restriction enzymes, and DNA base Sequencing using a DNA automatic sequencer. For protein production, the created construct was moved to *E. coli* BL21 (DE3)(NEWENGLAND Biolabs).The creation and validation of our construct was done following the instructions of pET System Manual2 (Novagen).

Omp A protein induction and expression

One colony of the expression *E. coli* strain having pET19b-*ompA*, was inoculated in a tube having three milliliters of LB broth supplemented with ampicillin then put in a shaker incubator at 37 °C overnight. Samples then were sub-cultured at 1:100 in six milliliters of LB/ ampicillin broth then put in an incubator adjusted to 37 °C. A spectrophotometer was used to measure the (OD) at 600 nm, and when it was about 0.6-0.8 and before protein production, 500 µL of each sample was taken; then isopropyl-D-thiogalactopyranoside(IPTG) was mixed at 1 mM with the growing cultures. The induced bacterial cells were cultivated in a shaker incubator at 37 °C for a period of 3 hours; a post induction sample (500 µL) was possessed then centrifuged at 13 rpm, 4 °C for two minutes. Supernatants were removed and 125 µL of 1X laemmli buffer was mixed with the pellet. Pellets were vortexed and placed in a 90 °C hot block for five minutes prior to storage at - 20 °C. Protein samples were examined utilizing SDS-PAGE and western blotting(10% gels) using Mouse monoclonal IgG anti-His (Millipore) (NEB) primary antibodies and the Goat anti-mouse IgG secondary antibody (Millipore). The blots images were captured using a Bio-Rad (ChemiDoc MP).Protein induction and expression was done following the instructions of pET System Manual2 (Novagen).

Purification of the histidine-tagged OmpA

Immobilized metal affinity chromatography (IMAC) (Nickel-chelated) was utilized in order to purify the histidine-tagged OmpA from the expression *E. coli* strain as described in(**Chiang et al., 2015**). The fusion histidine-tagged OmpA, was validated using SDS-PAGE as well as western blot.

Sequencing alignments

Amino acid sequences for OmpA protein homologs were attained from (NCBI) protein resource then analyzed using the blast tool (NCBI). Homologs for Omp A protein from other pathogenic *Acinetobacter spp* were chosen [Accession numbers: ABY47586.1, WP_044100281.1,WP_107972446.1, WP_111034814.1,PMC94806.1, WP_016137162.1] and aligned using clustal Omega website. The color shades were given according to % identity coloring of Jalview.

Topology prediction

Protein topology prediction was done using NCBI tools and PRED-TMBB2(**Elofsson et al., 2016**)

RESULTS

Topology prediction of OmpA from *A.baumannii* strain LI31

Sequence analysis of OmpA from *A.baumannii* strain LI31 revealed two conserved domains belonging to OmpA family protein: OMP_b-brl and OmpA_C-like (Figure 1.A). These domains are highly conserved across *Acinetobacter spp*. Results from topology prediction revealed the OmpA peptide as a 10-βeta -stranded transmembrane outer membrane protein categorized as the Outer Membrane Protein beta-barrel domain Family(Figure1.B)

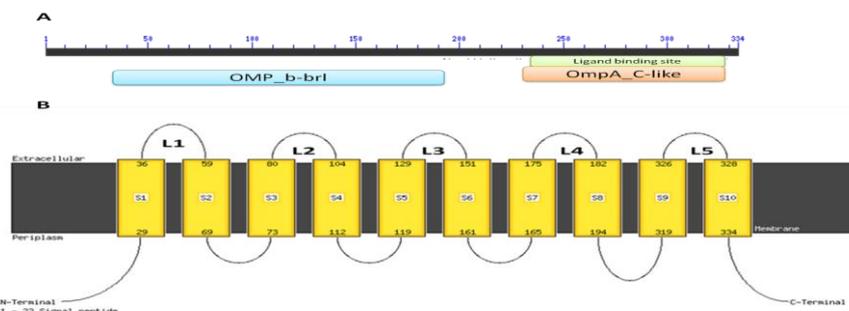


Figure 1 Topology prediction of OmpA of *A.baumannii* strain LI31. **A.** Conserved domains were identified based on the BLAST search tool of NCBI. **B.** External loops: L1–L5, transmembrane β-strands: B1–B10 based on PRED-TMBB2

Analysis of OmpA conservation within *Acinetobacter spp*

Representative alignments for Sequence of the Omp A of *A. baumannii* strain LI311 with homologous sequences of OmpA protein from other

Acinetobacterspp is shown in Figure2. Selected homologous sequences belong to *Acinetobacter pittii*, *Acinetobacter seifertii*, *Acinetobacter nosocomialis*, *Acinetobacter calcoaceticus*, *Acinetobacter ursingii* with identity percentages of 100%, 100%, 96%,92%, 91% respectively .

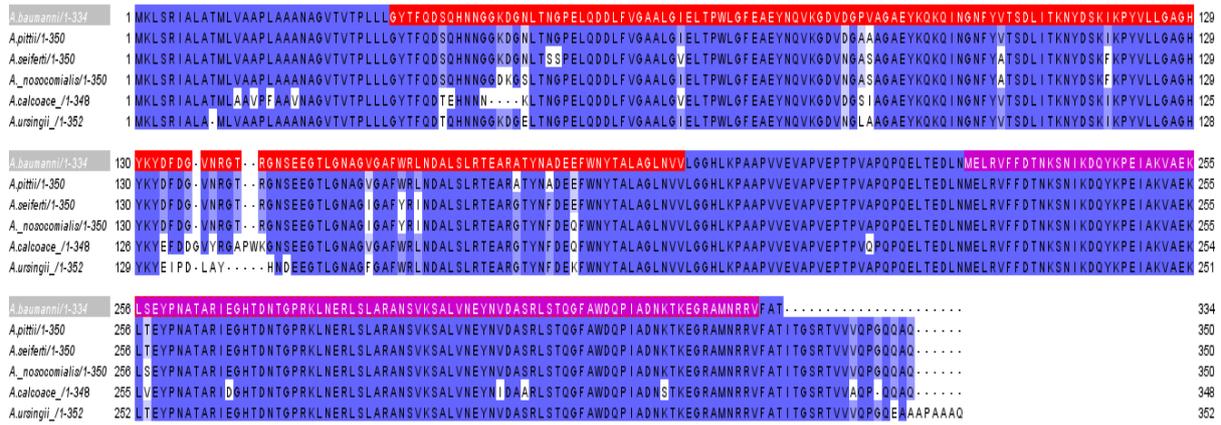


Figure 2 OmpA amino acid alignment within *Acinetobacter* spp. Alignment was performed using clustal , and the colors were given according to % identity coloring of Jalview. OMP_b-brl is denoted by red and OmpA_C-like is denoted by purple

Creation and validation of recombinant construct

The amplified *ompA* gene from *Acinetobacter baumannii* strain LI311 (1018 bp) was cut by *XhoI* and *BlnI* and ligated into the matching positions of pET19b plasmid digested with the same restriction enzymes. Clones were selected randomly from LB/ Ampicillin plates for PCR verification (Figure 3.A).The recombinant plasmid, pET19b-*ompA*, was also validated by restriction digestion using *XhoI* and *BlnI* and analyzed by agarose gel electrophoresis (Figure 3.B). Sequencing of DNA was utilized using a DNA automatic sequencer in order to ensure there is no mutation. The nucleotide sequence of *ompA* gene in the created construct of pET19b-*ompA* was consistent with *ompA* gen sequenc of *Acinetobacter baumannii* strain LI311 published in the GenBank (Accession: EU332799).

DISCUSSION

Acinetobacter baumannii is a significant microorganism that can affect humans health nowadays due to the brisk appearance and dissemination of strains capable of resisting antimicrobial agents with elevated mortality rates related to their infections(Wang-Lin et al., 2017). Despite that there is no authorized vaccine at present to *A.baumannii* , immunization approaches are promising as a feasible choice to control multi- resistant infections. One of the most important principles that should be taken for consideration when selecting an antigen from the proteome of a specified pathogen is that it has to be ubiquitous and conserved among diverse bacterium strains(Ahmad et al., 2016; Chen, 2015). OmpA of *A.baumannii* strain LI31, encodes the polypeptides of 334 amino acids, was selected as a putative vaccine in this study because the blast analysis of OmpA of *this bacterium* showed that this protein is not only completely conserved across various strains of *A.baumannii*, but it is also a homolog to OmpA of some clinical *Acinetobacter* species, among them, *A. pittii* , *A. nosocomialis* , *A. seifertii*, *A. calcooeticus*, and *A. ursingii* with identity percentages of 100%, 100%, 96%,92%, 91% respectively. In addition, the OmpA protein of *A.baumannii* possesses high immunogenic property making it as a putative antigen for vaccines development(Fajardo Bonin et al., 2014). Sequence analysis of OmpA from *A.baumannii* strain LI31 revealed two conserved domains belonging to OmpA family protein (domain architecture ID 10607194) . The first is OMP_b-brl, which is identified in a broad range of outer membrane proteins as a membrane bound beta. The second domain is OmpA_C-like , which is peptidoglycan binding domain like the carboxyl terminal domain of OmpA (Marchler-Bauer et al., 2017). These domains are highly conserved across *Acinetobacter* spp. The topology prediction revealed the OmpA peptide as a 10-β-stranded transmembrane outer membrane protein categorized as the Outer Membrane Protein beta-barrel domain Family). Residues 1–22 were considered as a signal peptide.

A. baumannii is the favored genus of *Acinetobacter* for scientists to produce vaccine because it is linked to the greatest rate of morbidity, lethality, and antibiotics resistance (ournier & Richet, 2006) . To present, confined research were done on *A. baumannii* to find effective immunization approaches. Among which, active and passive immunization against the biofilm associated protein (Bap) (Fattahian et al., 2011), OmpA(Luo et al., 2012) , poly-N-acetyl-β-(1-6)-glucosamine (PNAG)(Bentancor et al., 2012b) , Ata(Bentancor et al., 2012) ,and polysaccharide capsule (Russo et al., 2010) were tested. In addition,62 antigens were determined in the entire species of *A. baumannii* , which could be utilized as vaccin candidates; among them *ompA* was considers as one of these possible targets (Moriel et al., 2013).Furthermore, a previous study showed that active and passive immunization can be produced by recombinant OmpA protein after infecting diabetic mice by *A. baumannii* eventhough sera resistance was not accomplished (Luo et al., 2012) . OmpA protein is also demonstrated to be able prevent infections of *A. baumannii* in mice by trigaring signal pathways of INF-γ and IL-4(Lin et al., 2013) . In addition,in a recently published study, eukaryotic expression vector .pBudCe4.1, was used to clone and express *ompA* gene of *A. baumannii* as a DNA vaccine, but the researchers referred to the oncogenic possibility of the created recombinant molecules after an irregular combination with hosts genomes (Ansari et al., 2018).

CONCLUSION

In this report, a soluble recombinant OmpA protein of *A.baumannii* strain LI31, immensely conserved across clinical species of *Acinetobacter*, was successfully produced and purified. The outcome of our research can be employed to develop a vaccine candidate for *Acinetobacter* infections.

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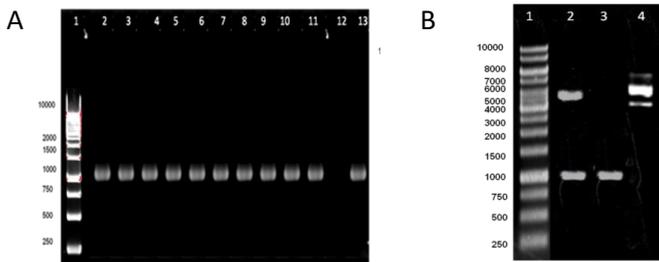


Figure 3 Agarose gel electrophoresis analysis and confirmation of the fused pET19b-*ompA* by A. colony PCR.Lane1: 10000bp DNA marker, Lanes 2–11: the target amplification of *ompA* gene (Predicted size is 1018bp), lane 12: negative control, and lane 13: positive control. B. Restriction digestion. Lane1: 10000 bp DNA marker, Lane 2: pET19b-*ompA* digested with *XhoI* and *BlnI*, Lane 3: *ompA* gene, Lane 4: uncut pET19b-*ompA*. Expected size of pET19b : 5671 bp, pET19b-*ompA* : 6673bp, *ompA* gene :1018bp

Expression and purification of Hist-tagged OmpA fusion protein

The Hist-tagged OmpA was successfully expressed using 1 mM of IPTG, and purification is done by Nickel-chelated immobilized metal affinity chromatography (Figure 4).

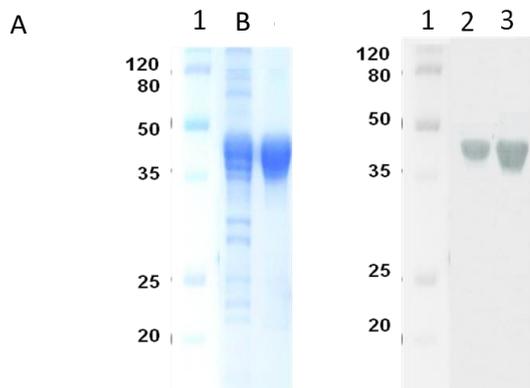


Figure 4 Sodium Dodecyl Sulfate -PAGE (A) and Western blotting (B) of purified His-OmpA. Lane1: molecular protein marker, Lane2: cell lysate after induction of His- OmpA by 1 mM IPTG, Lane3: purified His-OmpA. The expected molecular weight of His- OmpA is 39.31kDa)

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