

Research Paper

In silico Analysis of *Acinetobacter baumannii* Outer Membrane Protein BamA as a Potential Immunogen

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Abstract: *A. baumannii* is an emerging nosocomial pathogen associated with health care units. Its resistance to various antibiotics gives rise to the need of vaccine development against it. Outer membrane proteins are well described vaccine candidates. The outer membrane assembly complex protein of *A. baumannii*, known as BamA, is an essential component of β -Barrel Assembly Machine (BAM). Vaxign analysis showed that BamA has no trans-membrane helix, adhesion probability is 0.54, no similarity to human & mouse proteome and is highly conserved (>99%) in 114 strains of *A. baumannii* as well as in other virulent species of *Acinetobacter* (78 to 94%). Multiple sequence alignment (Multalin) of BamA protein sequences from different species of *Acinetobacter* revealed the conserved regions and led to the identification of T cell epitopes. A number of (15) common T cell epitopes in 8 species of *Acinetobacter*, antigenicity (predicted score of 0.682) and outer membrane localization predict that BamA offers appropriate epitopes for immunity development against all species of *Acinetobacter*. Further, docking of MHC class I binding epitopes to HLA*B4405 allele and docking of epitope-HLA*B4405 complex to DM1-T cell receptor showed stable binding with low energies.

Keywords: *Acinetobacter baumannii*, Vaccine, BamA, Epitopes, Docking.

1. Introduction

A. baumannii is an emerging opportunistic pathogen that can cause septicaemia, pneumonia, wound sepsis, meningitis and urinary tract infections. This Gram negative, strictly aerobic, catalase positive, oxidase negative and non-motile bacillus can adhere to the inanimate surfaces like the hospital equipment and can survive on these dry surfaces for long periods of time [1]. Its resistance to most of the commonly used antibiotics and global spread has led to high mortality [2]. Recent isolation of resistant strains of *A. baumannii* in India bearing bla_{NDM-1} gene has made it a matter of grave concern. A few attempts have been made to develop vaccine against *A. baumannii*. Outer membrane protein, OmpA, a 38 kDa protein, was reported to have the ability to elicit a T-cell response [3]. Immunization with inactivated whole cell protein has been tried by McConnel *et al* [4]. But multiple proteins present in whole cell vaccine are cause of safety concerns and moreover, actual proteins involved in the protection were not described. Outer membrane vesicles (OMV) have been reported for their ability to induce immunity in mice [5]. Outfoldings of the membrane having a lot of proteins showed good vaccine potential but problem was same as with the inactivated whole cell vaccine. Therefore, serious attempts for vaccine development to cope with this problem are required.

To identify novel vaccine candidates for bacterial pathogens, a bioinformatic analysis of candidate proteins can be done before *in vitro* and *in vivo* experiments. This approach, called Reverse Vaccinology [6], provided a breakthrough in vaccine development against *Neisseria meningitidis* serogroup B [7].

A significant fraction (~25%) of a bacterial proteome comprises of membrane proteins [8]. The regions of these membrane proteins that span the membrane include α -helices and β -strands. α -Membrane proteins are generally part of cytoplasmic membranes whereas β -membrane proteins belong exclusively to outer membranes of bacteria, hence are called Outer Membrane Proteins (OMPs). Genomes of gram negative bacteria consist ~2–3% of OMPs [9]. Because of experimental difficulty such as denaturation, the number of OMP structures available in the Protein Data Bank [10] is limited. OMPs have biomedical interest because they contribute to the pathogenicity of bacteria [11] and are involved in antibiotic resistance [12]. Also, outer membrane proteins are the well described vaccine candidates. OMPs of bacteria play a key role in adaptation to extreme changes in external environments. In case of marine food borne pathogens *Vibrio parahaemolyticus* and *V. alginolyticus*, OMPs have appeared as UV-C radiation stress proteins expressed when irradiated with UV-C [13]. Few putative secreted toxins of type I secretion system proteins are found associated with virulence of *Vibrio harveyi* [14]. An OMP, called outer inflammatory protein A (OipA), of *Helicobacter pylori* was found to have toxic effects on gastric epithelial cells and cause gastric ulcer [15]. Recently, there is a report on extraction and identification of the 44.5 kDa OMP from bovine *Fusobacterium necrophorum*. This OMP showed immunogenic activity verified with western blotting [16]. OMPs are well suited for diagnosis as well. A Surface Plasmon Resonance biosensor has been proposed to detect *Bacillus anthracis* by detecting a common toxin produced by all live *B. anthracis* bacteria [17]. In another report, it was suggested that PCR amplicon of Omp85 gene can be used as a specific genetic marker for quick diagnosis of bacterial meningitis [18].

In addition to these, BAM proteins are other essential OMPs. β -Barrel Assembly Machine (BAM) is a multi-protein complex in outer membrane of gram negative bacteria that is involved in the targeting and folding of β -barrel outer membrane proteins. A fundamental biological process of OMP assembly is dependent on Bam complex that was discovered in *E. coli* where it comprises of four lipoproteins: Bam B, C, D and E (formerly YfgL, NlpB, YfiO and SmpA, respectively) that bind to the N-terminal periplasmic domain of OM β -barrel BamA [19, 20]. BamA is a highly conserved protein of the Omp85 family of proteins. Periplasmic domain of BamA is a helical chain of five structurally homologous POTRA (polypeptide translocation associated) domains and BamA barrel along with its POTRA domains, designated P1 to P5, is essential for viability of cells [21]. BamA and BamD are essential proteins and they interact to facilitate OMP assembly. Depletion of BamA or BamD causes

OMP assembly to halt [22]. It is also reported that BamA and BamD are required for autotransporter (secreted proteins) biogenesis [23].

All these above facts strongly support that BamA is a crucial protein and could act as vaccine candidate against *A. baumannii*. So, in this study, we attempted to analyze BamA protein of *A. baumannii* ATCC 19606 as a potential vaccine candidate by using various online tools and programs.

2. Materials and Methods

Sequence Retrieval and Conservation Analysis

BamA protein sequence of *A. baumannii* ATCC 19606 with sequence ID D0C6H3 was downloaded from UniProt protein database. BLAST at UniProt was run against 213 sequences of *Acinetobacter* genus for BamA to perform the similarity search. Highly similar BamA proteins sequences of all the species of *Acinetobacter* were retrieved in FASTA format. Multalin was used to perform all multiple sequence alignments. Eight selected sequences of different species were used to perform alignments with Multalin (<http://multalin.toulouse.inra.fr/multalin/>).

Analysis of BamA as Potential Vaccine Candidate

Vaxign server to check BamA for various attributes fulfilling the criteria to be a vaccine candidate was used (<http://www.violinet.org/vaxign>). Vaxign includes a pipeline of softwares to predict possible vaccine targets based on the various vaccine design criteria such as antigen sub-cellular location, adhesion, trans-membrane helix, epitope binding to MHC class I and class II, and sequence similarities to human, mouse and/or pig proteins.

Epitope Prediction and Docking with HLA-B*4405

MHC class II (helper T lymphocyte epitopes) and MHC class I (cytotoxic T lymphocyte epitopes) binding peptides were predicted by propped (<http://www.imtech.res.in/raghava/propped/>) and propped1 (<http://www.imtech.res.in/raghava/propped1/>) respectively.

Docking was performed using ClusPro v.2 online server [20]. The results were clustered according to their binding energies. 3D structure of HLA-B*4405 (PDB: 3DX8) and DM1-TCR (PDB: 3DXA) was obtained from PDB database. Nine MHC class I binding epitope sequences and 3D structure of HLA-B*4405 were submitted to ClusPro v.2 for docking. The resulting complex was further docked to DM1-TCR as a ligand. The most populated clusters of docked complexes were selected on the basis of lowest binding energy scores and are given in Table 1.

3. Results and Discussion

Conservation of BamA among *Acinetobacter* Species and *A. baumannii* Strains

BamA is an essential component of Bam complex and highly conserved protein of OMP85 family. To check its conservation among *A. baumannii* strains, BamA protein sequence of *A. baumannii* ATCC 19606 was retrieved and BLASTp showed homologues of BamA present in *A. baumannii* strains and other *Acinetobacter* species. It was more than 99% identical in all the 114 strains of *A. baumannii* available in UniProt (data not shown). Also, high conservation (78 to 94%) of protein among the species of *Acinetobacter* was observed by multiple sequence alignment (Fig. 1).

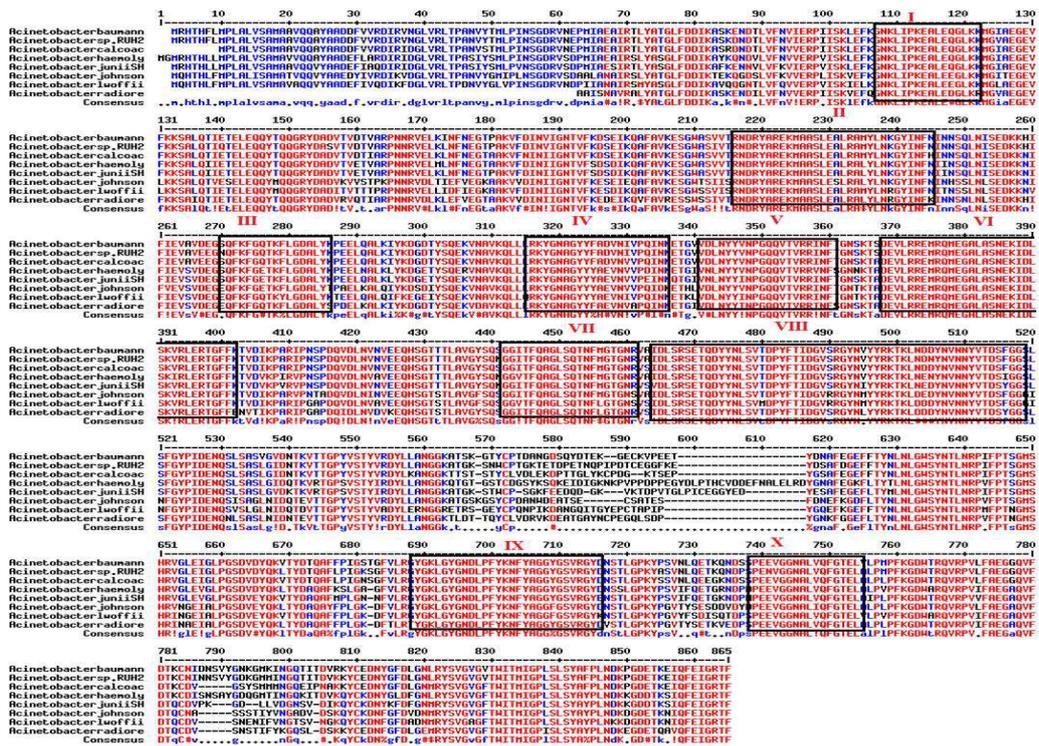


Fig. 1: Multalin sequence alignment of BamA proteins from different species of *Acinetobacter* showing highly conserved sequences (in box)

Region I to X shown in Fig. 1 are conserved in all mentioned species. No significant similarities were found with other genera which make it specific to genus *Acinetobacter*. Highly conserved nature of this protein makes it a promising broad range candidate for immunity development.

Analysis of BamA as a Potential Vaccine Candidate

A protein must have certain properties to be an ideal vaccine candidate. An outer membrane protein has strong tendency to elicit immune response in host. Protein should have less than two trans-membrane helices. Also, the presence of more than one trans-membrane helix often results in failure of recombinant protein isolation and purification. A protein with many T cell epitopes is preferred and it must not have similarity with host. To predict all of these features in BamA, Vaxign program was used. Vaxign computational pipeline includes following features: sub-cellular localization, topology (trans-membrane helices), adhesin probability, similarity to other pathogen sequences, similarity to host genome sequences (human or mouse), and MHC class I and II epitopes prediction. BamA was predicted as outer membrane protein with no trans-membrane helix. It had adhesin probability of 0.539, ability to bind to MHC class I and class II and was completely dissimilar to human, mouse and pig proteome. BamA fulfilled all the criteria to be a good vaccine candidate according to Vaxign.

T Cell Epitope Prediction

MHC class II and MHC class I binding epitopes were predicted by proPred (HTL) and proPred1 (CTL) respectively. Due to nearly complete similarity among the strains of *A. baumannii*, all epitopes were common. T cell epitopes common in 8 species of *Acinetobacter* are shown in Table 1. These epitopes can be used as to make an epitope based sub-unit vaccine for broad range of *Acinetobacter* species.

Table 1: Predicted epitopes in 1. *A. baumannii* ATCC 19606, 2. *Acinetobacter* sp. RUH2624, 3. *A. calcoaceticus* (strain PHEA2), 4. *A. haemolyticus* ATCC 19194, 5. *A. junii* SH205, 6. *A. radioresistens* DSM 6976, 7. *A. johnsonii* SH046 and 8. *A. lwoffii* SH145 binding (•) to MHC Class II and Class I

Peptides binding to MHC Class II	1	2	3	4	5	6	7	8
LIPKEALEQ	•	•	•	•	•	•	•	•
LRAMYLNKG	•	•	•	•	•		•	
MRQMEGALA	•	•	•	•	•	•	•	•
YRKTCLND	•	•	•	•	•	•	•	•
LRGYGKLG	•	•	•	•	•		•	•
ITMIGPLSL	•	•	•	•	•		•	•
Peptides binding to MHC Class I								
KMAASLEAL	•	•	•	•	•	•		•
LEALRAMYL	•	•	•	•	•	•		•
VGYSQSGGI	•		•			•	•	•
DPYFTIDGV	•	•	•	•	•	•	•	•
YPIDENQSL	•	•	•	•	•			
NLGWSYNTL	•	•	•	•	•	•	•	•
ALVQFGTEL	•	•	•	•	•	•	•	•
LSLSYAFPL	•	•	•	•	•	•	•	
IQFEIGRTF	•	•	•	•	•		•	•

The presence of a number of epitopes in BamA indicates that it could be a potential immunogen and a vaccine candidate.

Docking Studies

Nine epitopes of BamA binding to MHC I molecules were docked to HLA-B*4405 because of its potential involvement in T cell mediated responses among micropolymorphism [24]. These complexes were further docked to DM1-TCR to determine their potential binding affinities and binding conformations. The docked complexes were clustered according to minimum binding energy or score which is generated from an energy function of PIPER docking program. This function (E) is a sum of potential terms of shape complementarity (sum of attractive and repulsive contributions), electrostatics, desolvation contributions, and Decoys as reference states (DARS) [25].

The binding energies of epitopes-HLA-B*4405 and epitope-HLA-B*4405/TCR are shown in Table 2.

Table 2: Total binding energies of the 9 MHC class I epitopes

Epitope	Docked to HLA-B*4405		Epitope/HLA-B*4405 docked to DM1-TCR
	Energy (KJ/mol)	Interacting residues	Energy (KJ/mol)
KMAASLEAL	-490.5	GLU A:53, LEU a:32, ARG A:48, ARG A:239	-646.8
LEALRAMYL	-632.7	TYR B:63, ARG A:239, ARG A:48, THR A:31	-638.6
VGYSQSGGI	-521.6	HIS A:191, SER B:11, HIS A:188, ASP B:98	-638.2

DPYFTIDGV	-645.8	GLY A:237, TYR A:27, GLU A:212, THR A:233	-643.3
YPIDENQSL	-537.2	ARG B:12, ARG:48	-687.5
NLGWSYNTL	-657.4	ARG A:48, TYR A:27, TYR B:67, ARG B:12, GLU B:69	-697.8
ALVQFGTEL	-566.2	ARG B:12, ARG A:48, ASP B:53	-630.8
LSLSYAFPL	-780.9	ARG A:239, THR A:31, ARG A:48, TYR B67	-640.1
IQFEIGRTF	-697.0	TYR B:67, THR A:178, THR A:31, ARG A:48	-643.3

The residues of HLA-B*4405 having potential interactions with epitopes are shown in Table 2. ARG A:239, ARG A:48, ARG B:12, THR A:31 and TYR B:67 were among the most common residues of HLA-B*4405 showing significant interactions with the epitopes.

Epitope **NLGWSYNTL** was conserved in *Acinetobacter* genus. Therefore, the interactions occurring in the docked complex (Epitope NLGWSYNTL/HLA-B*4405) (Fig. 2) were analyzed.

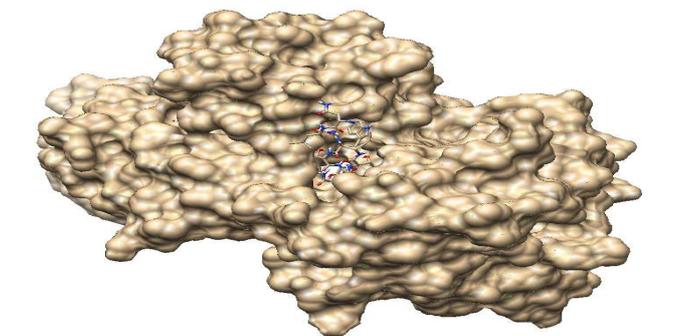


Fig. 2: Epitope NLGWSYNTL/HLA-B*4405 complex viewed by Chimera software. Epitope (in space fill form) is in groove of HLA molecule (in solid form)

Various interactions occurring in the complex are depicted in Fig. 3.

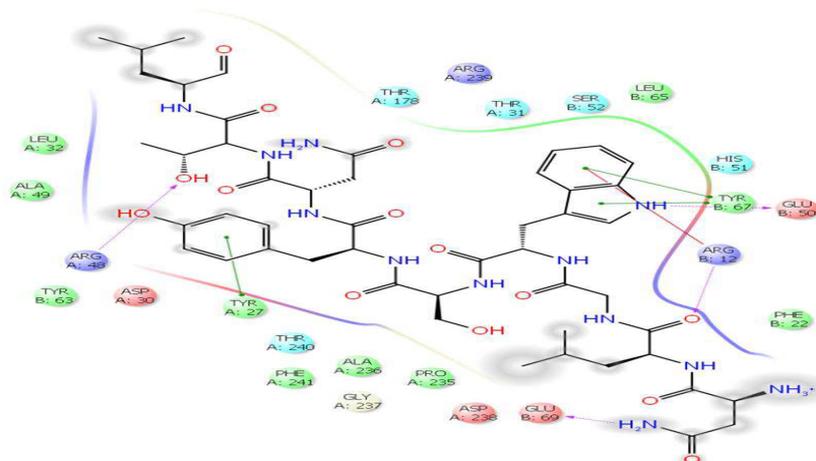


Fig. 3: Ligand interaction diagram made by Schrodinger showing various interactions occurring in the HLA/epitope complex

ARG A:48 was found to form a hydrogen bond with threonine (T) at a distance of 2.634 Å. GLU B:69 was also forming a hydrogen bond with asparagine (N) at a distance of 2.294 Å. π - π interaction was observed between TYR A:27 and phenol nucleus of tyrosine (Y). Another π - π stacking was observed between phenol nucleus of TYR B:67 and indole nucleus of tryptophan (W). All of the epitopes were found to accommodate in the groove between A and B chains of HLA-B*4405. Further docking of this HLA/epitope complex to DM1-TCR was performed and similar noticeable interactions were observed in all the cases.

These docking studies revealed favourable interactions between both the epitope-HLA-B*4405 and epitope/HLA-B*4405/DM1-TCR complexes with low binding energies.

4. Conclusions

Prediction of probable vaccine candidates from the proteome of a pathogen could provide potential leads to develop treatment of infections caused by it. Starting with the hypothesis that BamA could be a successful vaccine candidate as it is one of the most conserved protein in gram negative bacteria, here in *Acinetobacter* genus, *in silico* analysis supported the hypothesis. BamA is a part of the outer membrane protein assembly complex which is involved in the assembly and insertion of beta-barrel proteins into the outer membrane. Its localization in outer membrane and dissimilarity to human proteome warrants the analysis of its vaccine potential. Recognizing antigenic peptides in this membrane protein is an essential criterion for developing epitope driven vaccine against infections caused by *A. baumannii*. We predicted that BamA carries peptides (epitopes for both MHC class I and class II) which are conserved among the strains of *A. baumannii* and species of *Acinetobacter* genus and can be developed as cross protective subunit vaccine candidate. As *in silico* analysis, even with high sensitivity and specificity, is a prediction, this necessitates *in vitro* and *in vivo* evaluations to confirm immunogenicity.

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