IN-SILICO IMMUNOINFORMATIC APPROACH FOR VACCINE DESIGNING AGAINST *Edwardsiella piscicida*

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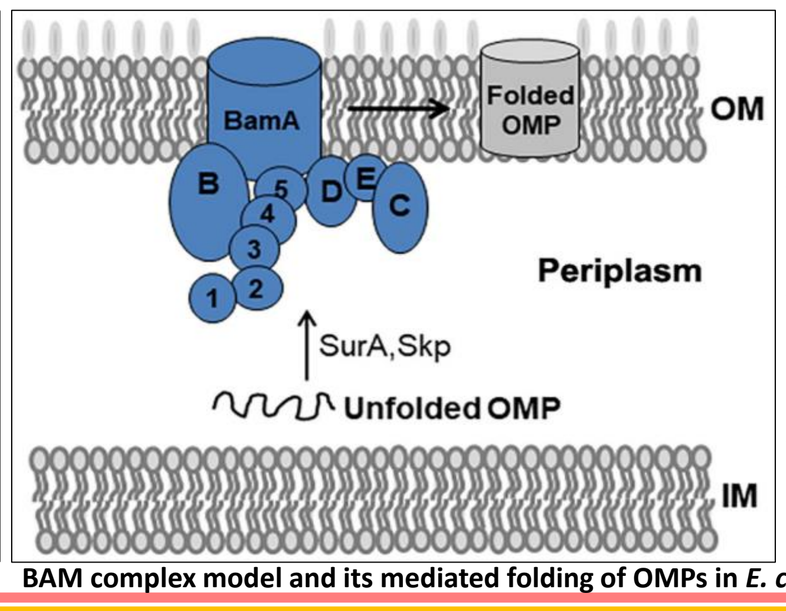
Abstract

In this study, proteomic sequence data of the previously generated *E. piscicida* extracellular vesicles were used for antigen selection. The outer membrane protein assembly factor BamA, which involve in assembly and insertion of β-barrel proteins to outer membrane (OM) was selected for predicting epitopes. First, the basic characteristics (potentiality of vaccine candidate, domains and characteristic motifs, physicochemical analysis) of the BamA sequence was performed using available *in-silico* open source software. BamA sequence contained polypeptide-transport-associated (POTRA) domain that hypothesized involving in beta strand formation in outer membrane proteins (OMPs) and have chaperon like activity. The antigenic sites of the BamA OMP was predicted using Kolaskar and Tongaonkar antigenicity tool with the accuracy of 75%. We observed 27 antigenic determinants with average antigenic propensity of 1.014 for the BamA protein (795 AA). The Emini surface accessibility prediction showed 18 epitopes. The CTL epitopes were predicted by NetCTL 1.2 server (integrated with TAP transport efficiency, MHC class I binding, and proteasomal C-terminal cleavage prediction) with selected human leukocyte antigen (HLA) alleles MHC supertype A1. Moreover, we identified 31 epitopes which having higher than prediction score threshold (0.75000) as CTL epitopes. By BCPREDS Server 1.0, fbcpred prediction showed 4 epitopes (14 AA) having prediction score of >0.95 whereas FBCPRED predicted 4 epitopes with the prediction score of 1. Using AllerTOP and VaxiJen the both non-allergenic and antigenic epitopes were determined. After further evaluation of epitope feasibility, the selected epitopes could be compile to design a multi-epitope vaccine candidate for boosting immune system and protect the fish from *E. piscicida* infection in future.

Background

- ❑ Vaccine is an effective treatment option against infectious pathogens due to the inherent drug resistance.
- ❑ OMPs considered to be effective protective antigens due to their capability of inducing host immune system and providing protection against bacterial challenge.
- ❑ BamA, an outer membrane β-barrel assembly protein has been identified previously in Gram –ve bacteria as potential vaccine candidate by *in silico* analysis.
- ❑ *Edwardsiella piscicida* (Gram negative bacteria) virulence factors are significantly influences bacterial survival and pathogenesis in the host.
- ❑ In this study, we focused to find epitopes from *E. piscicida* BamA protein, which could develop as safe and effective vaccine candidates in future.

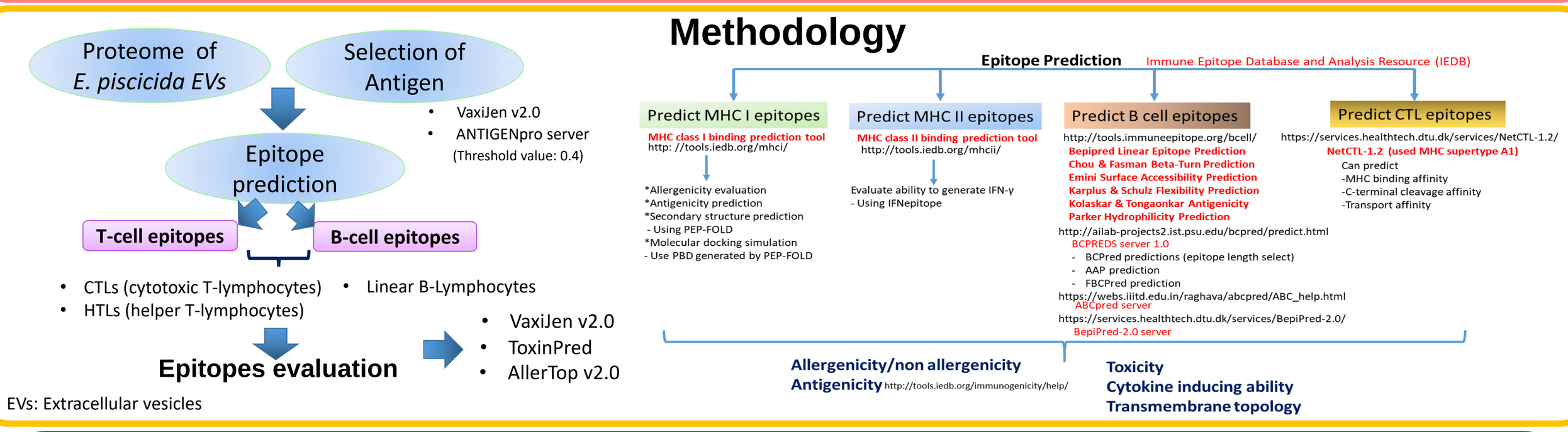


BAM complex model and its mediated folding of OMPs in *E. coli*.

- BAM complex consists of BamA, an OM β-barrel protein with five N-terminal POTRA domains, and the lipoproteins BamB–E.
- Nascent OMPs are translocated across the inner membrane (IM) into the periplasm.
- Chaperones (SurA and Skp) recognize unfolded OMPs in the periplasm and transport them to the BAM complex.
- BAM complex receives, folds, and inserts OMPs at the OM.

Li et al., 2020, Front. Microbiol.

Methodology



Results

➢ Physicochemical properties of the BamA protein

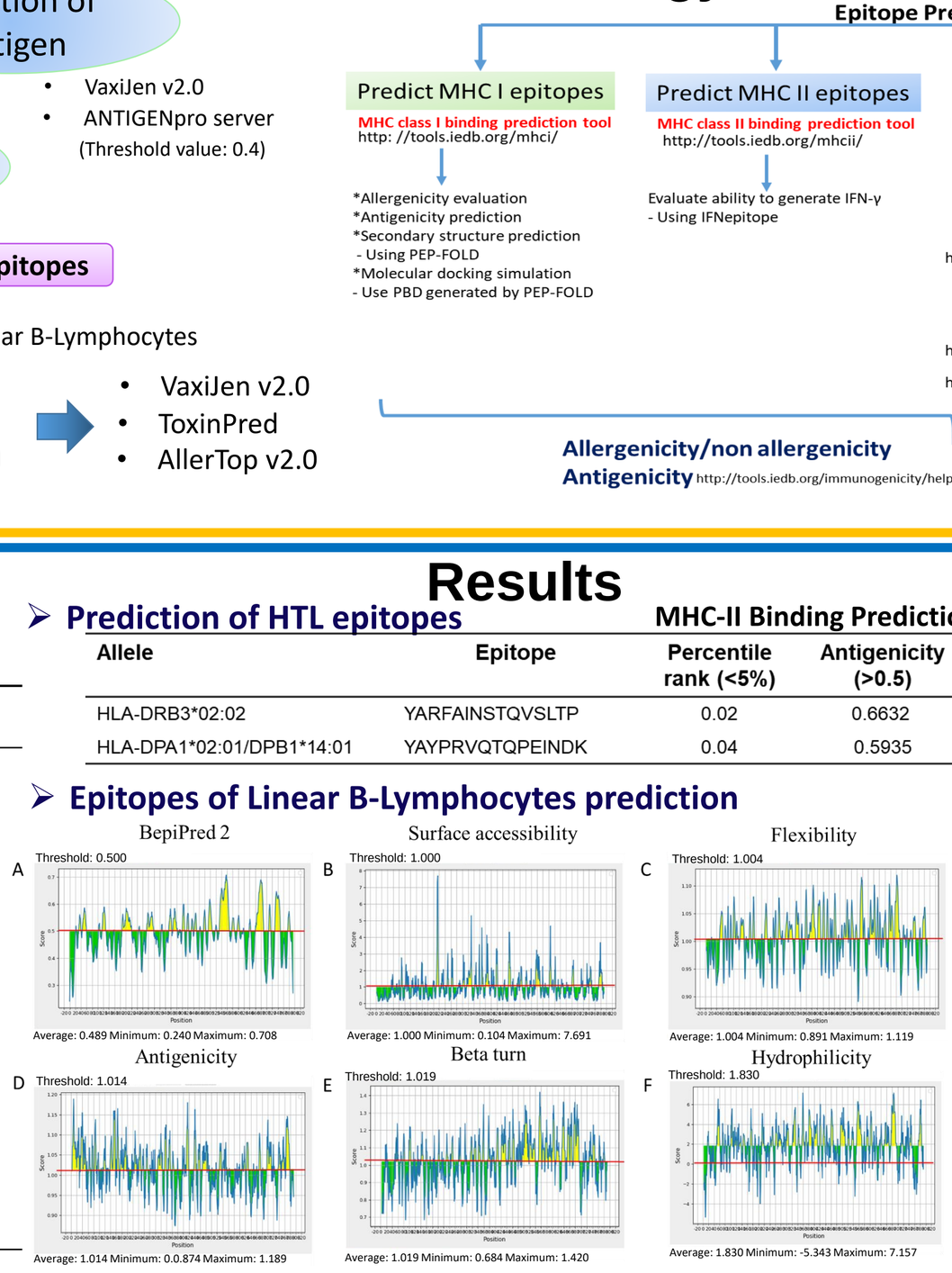
Physicochemical properties	outer membrane protein assembly factor BamA
Number of amino acids	795
Molecular weight	88305.70
Theoretical PI	5.47
Total number of negatively charged residues (Asp + Glu)	91
Total number of positively charged residues (Arg + Lys)	82
Molecular formula	$C_{3930}H_{6053}N_{1071}O_{1281}S_{16}$
Total number of atoms	12288
Extinction coefficient (Ext. coefficient) at 280 nm	131560 M/cm
Instability index (II)	24.56
Aliphatic index (AI)	75.76
Grand average hydropathicity (GRAVY)	-0.451

➢ Prediction of HTL epitopes

MHC-II Binding Prediction

Allele	Epitope	Percentile rank (<5%)	Antigenicity (>0.5)	Toxicity	Allergenicity	IL-4 inducing	IL-10 inducing
HLA-DRB3*02:02	YARFAINSTQVSLTP	0.02	0.6632	Negative	Negative	Inducer	Inducer
HLA-DPA1*02:01/DPB1*14:01	YAYPRVQTQPEINDK	0.04	0.5935	Negative	Negative	Inducer	Inducer

➢ Epitopes of Linear B-Lymphocytes prediction

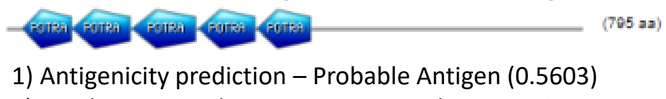


Propensity scale profiles for the *E. piscicida* BamA generated using (A) BepiPred 2, (B) surface accessibility, (C) flexibility, (D) antigenicity, (E) beta turn, and (F) hydrophilicity prediction. Regions with scores above the red line are more likely to contain linear B-cell epitopes.

➢ BCPREDS :Three linear B-cell epitope predictors

- BCPred, AAP, and FBCPred. Bold residues indicate epitope residues predicted by a least two methods.

➢ Selection of *E. piscicida BamA* protein



1) Antigenicity prediction – Probable Antigen (0.5603)
2) Localization prediction – Outer membrane protein
3) Signal peptide – Detected
4) N-terminal POTRA domains (5) detected

➢ Prediction of potential CTL epitopes

CTL epitopes chosen for the final vaccination

HLA Sub type	Epitope	C-Score	Antigenicity	Immunogenicity	Toxicity	Allergenicity
A1	YTWTAGWAY	0.9599	0.9637	0.39076	Negative	Negative
	VSLGGRLFY	0.6829	0.4583	0.17126	Negative	Negative
	LTDPYFTVN	0.3744	1.3593	0.15952	Negative	Negative
	KADDYTWTA	**0.7813**	**0.9306**	**0.27208**	**Negative**	**Negative**
	NVDVETQRV	0.9296	1.9552	0.11526	Negative	Negative
	QPQVAMWRY	0.9684	0.9126	0.09392	Negative	Negative
A2	KLAGDLEAL	0.8085	0.5214	0.16532	Negative	Negative
	KADDYTWTA	**0.7813**	**0.9306**	**0.27208**	**Negative**	**Negative**
	ELITPTPFV	0.7634	0.6127	0.16704	Negative	Negative

- **Predicted epitopes by BepiPred2**

Start	End	Peptide	Length	Antigenicity	Toxicity	Allergenicity
25	39	MPVRVGDTVSDEDLS	15	1.274	Negative	Negative
83	88	SVKDEM	6	1.118	Negative	Negative
96	107	SGVRVGEALDRT	12	0.6898	Negative	Negative
166	200	AFSSAELLGHFQLRDDVPWWNLMADRKYQKQKLAG	35	0.49	Negative	Negative
473	486	FKANDADLSDYTNS	14	0.683	Negative	Negative
512	548	HNDLSDMQPQVAMWRYLRSVGQNPSDSQRASYKADDY	37	0.5816	Negative	Negative
618	624	GFGGKEM	7	1.207	Negative	Negative
641	669	FQSNNIGPKAVYLNGDGSVDQSKTGDNDA	29	1.237	Negative	Negative
756	763	KKYEGDKA	8	0.9373	Negative	Negative

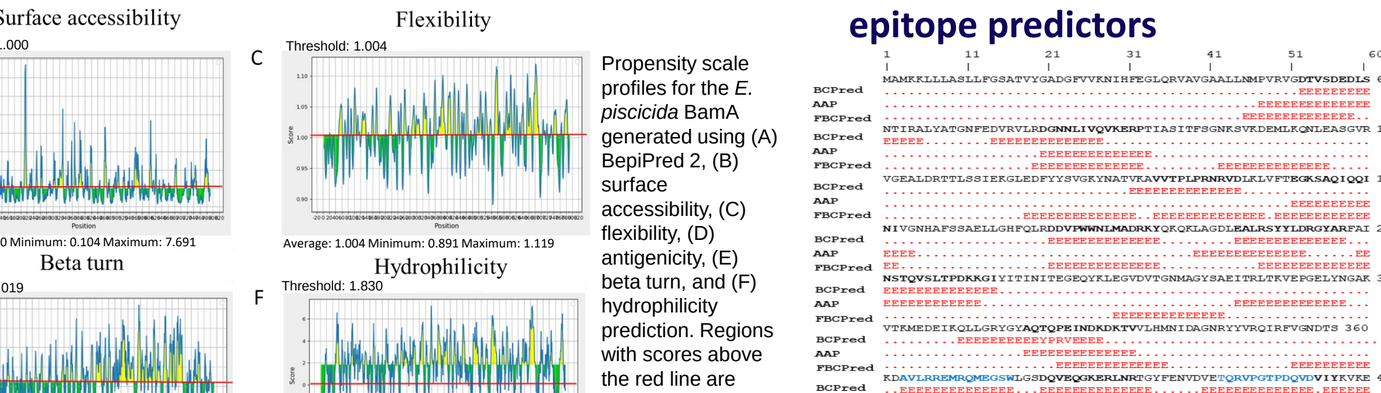
- **Predicted epitopes by BCPred**

Position	Peptide	Score	Antigenicity	Toxicity	Allergenicity
683	SKTGDNDAVGGNAM	0.994	2.4451	Negative	Negative
635	GYGNGFGGKEMPFY	0.998	1.3414	Negative	Negative
505	TNSSYGFDGTLGFP	0.947	0.4795	Negative	Negative
415	IYKVKERNTGSFNF	0.94	0.7655	Negative	Negative

- After obtaining the top 10 epitopes, we verified the antigenicity, toxicity, and allergenicity using VaxiJen v2.0, ToxinPred, and AllerTop v2.0 and short listed the number of epitopes from each category.

➢ Docking results for predicted epitopes *E. piscicida* BamA protein

- Three CTL epitopes of BamA were docked with MHC class I HLA-A0201, and docked structure of **KLAGDLEAL** -MHC, **KADDYTWTA**-MHC, and **ELITPTPFV**-MHC is shown below.



Docking energy score :-180.479 | **Docking energy score :-247.262** | Docking energy score :-231.143

Conclusions

- A range of computational techniques were used to find possible T- and B-cell epitopes in *E. piscicida* BamA protein. Nine CTL cell epitopes, two HTL cell epitopes, and thirteen B cell epitopes were selected using default parameters to construct the vaccine.
- In future, after further immunoinformatic screening and molecular docking analysis these epitope candidates were short listed for vaccine construct designing and physiochemical analysis and immunological studies.
- Developing a such multi-epitope vaccine construct could be utilized to tackle against antibiotic resistant *E. piscicida* infection in aquatic animals including fish.

Reference: Li, Y., Zhu, X., Zhang, J., Lin, Y., You, X., Chen, M. et al., (2020). Identification of a compound that inhibitis the growth of gram-negative bacteria by blocking BamA-BamD interaction, Front. Microb. 11, Article 1252.

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