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Potential Outer Membrane Protein Candidates for Vaccine Development Against the Pathogen *Vibrio anguillarum*: A Reverse Vaccinology Based Identification

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Abstract Reverse vaccinology is a widely used approach that has facilitated the rapid identification of vaccine candidates suitable in vaccine development for pathogens. *Vibrio anguillarum* is a major pathogen responsible for vibriosis in fish and shellfish leading to huge economic losses to the aquaculture industry. Although commercial vaccines are available for fish against this bacterium they have their own limitations. In this study, we used the reverse vaccinology strategy to screen and identify *V. anguillarum* outer membrane proteins (OMPs) that could serve as vaccine candidates. Our analysis identified 23 antigenic outer membrane proteins which were highly conserved (>98% identity) across serovars of this bacterium. Of the 23, two were identified as outer membrane lipoproteins. Among the OMPs identified 18 were novel to this study and conserved across several *Vibrio* spp. with an identity of 21–93%. While the least (>48%) identity was observed for *V. anguillarum* ferrichrome–iron transporter protein, the highest identity (>80%) was seen for outer membrane proteins OmpK, BamA, OmpU, Fatty acid transporter, and two hypothetical proteins. These potential vaccine targets identified could contribute to the development of effective vaccine not only against *V. anguillarum* but also across other *Vibrio* spp. In addition, several B-cell and T-cell epitopes were predicted for the novel OMPs in this study which could aid in narrowing down peptide selection in designing a suitable epitope-based vaccine.

Keywords *Vibrio anguillarum* · Outer membrane proteins · Reverse vaccinology · Vaccine candidates · Epitopes · MHC class proteins

Introduction

Vibrio anguillarum (*Listonella anguillarum)*, an important pathogen of the genus *Vibrio* is the leading cause of the disease 'vibriosis' in commercially important fish and shellfish culture [2, 24, 29]. Vibriosis, a fatal hemorrhagic septicaemic disease, is known to infect >50 species of fish and considered as a major economical threat to marine and freshwater aquaculture industry worldwide [14]. In aquaculture practice, control of vibriosis is mainly through the use of antibiotics. However, the rising incidence of resistance to commonly used antibiotics and also the transfer of resistance to other bacterial strains is considered as a major limitation and a global concern [30].

There is an increasing need for alternatives to antibiotics such as vaccines to control disease in aquaculture. However, the development of new vaccines depends on the identification of new potential vaccine antigens. In recent years, the availability of complete bacterial genomic sequences and various in silico tools has accelerated the identification of bacterial antigens through a computer-based approach called 'Reverse vaccinology' (RV). It involves the screening of the genome protein-coding sequences for antigenic proteins capable of eliciting an immune response in host organism [36]. The RV technique was first applied to design a vaccine against the serogroup B *Neisseria meningitides* [34]. Ever since, the technology has been successfully applied to

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several bacterial and viral pathogens such as *Acinetobacter baumannii* [7], *Bacillus anthracis* [1], *Campylobacter* [28], *Cryptosporidium hominis* [27], *Mycobacterium tuberculosis* [4], *Streptococcus pneumoniae* [45], and *Herpes simplex virus* [46].

In the RV approach for Gram-negative bacteria, special attention has been given to the exported outer membrane proteins (OMPs) due to their role in host-pathogen interaction. Approximately 2–3% of the genes encode for OMPs in Gram-negative bacteria [22] which are essential for maintaining the integrity and in the selective permeability of molecules across membranes [5]. In addition, few OMPs act as adhesins and take part in the pathogenicity of the bacterium. Furthermore, due to their surface localization, they are exposed targets to the host immune system [8] making them therefore ideal vaccine candidates.

Experimental evidence through work carried out by several researchers reveals specific Gram-negative bacterial OMPs to be useful as potent immunogenic molecules providing significant protection to fish when challenged with pathogenic bacteria [13, 21, 26, 35, 43]. Currently available commercial vaccines for *V. anguillarum* are inactivated products of the bacterium which do not provide cross protection to the several existing serotypes of this pathogen, thus limiting its use [41]. In *V. anguillarum*, exploiting OMPs as protective antigens against disease caused by the pathogen is very limited. Thus in this study, we identified novel outer membrane proteins using the RV strategy, candidates which could act as a potential source for the development of an effective vaccine against the pathogen *V. anguillarum*. We also report here OMPs conserved across Vibrio species which could be exploited as targets to develop a cross-protective vaccine capable of inciting protective immunity against pathogenic vibrios.

Materials and Methods

Sequence Retrieval and Identification of Antigenic Outer Membrane Proteins

The complete nucleotide (accession numbers CP002284.1 and CP002285) sequences of the O1 serotype *V. anguillarum* 775 strain were downloaded from the National Centre for Biotechnology Information (https://www.ncbi.nlm.nih.gov/) database. A total of 3867 protein sequences encoded by the two chromosomes were extracted in FASTA format for further analysis. In the RV approach, a protein vaccine candidate is identified based on defined desirable attributes such as protein subcellular localization, topology, adhesin/antigenicity probability, epitopes, and its binding to the major histocompatibility complex (MHC) class I and II molecules [46]. In this study, predicted protein sequences were analyzed for the presence of signal peptides using Signal P 4.1 server (http://www.cbs.dtu.dk/services/SignalP/) [33]. Prediction of transmembrane segments and the two-dimensional topology of OMPs were determined using the online server PRED-TMBB (http://bioinformatics.biol.uoa.gr/PRED-TMBB/) [3]. Lipoproteins in the outer membrane and their subcellular localization were predicted using LipoP 1.0 [20] and PSORTb ver. 3.0.2 [47] servers, respectively. The proteins identified as OMPs were evaluated for their adhesin and antigenicity probability. The Seapath ver. 1.0 program (CSIR-IGIB-Jalaja Technologies, Bangalore, India) was used to predict the adhesin probability of the protein. The Seapath program has an optimal sensitivity of 89% and specificity of 100% and can identify 97.4% of adhesins in a wide range of bacteria [9]. The probability of being an adhesin has a default cut-off of $\geq$0.7. Probability values >0.6 and <0.7 were considered as "twilight zone" for adhesin-like characteristics. The antigenicity of proteins was predicted using two different softwares: Vaxign (http://www.violinet.org/vaxign/) [17] and VaxiJen v2.0 (http://www.jenner.ac.uk/VaxiJen) [10]. Vaxign and VaxiJen programs have sensitivity and specificity corresponding values 0.494 and 0.853 and 0.554 and 0.909, respectively [19]. A threshold cut-off of 0.5 was used in both programs. The molecular weight of the OMPs was determined using the Sequence manipulation suite [42]. The EMBOSS tool 'Antigenic' (http://bioinfo.nhri.org.tw/gui/) [23, 31] was used in identifying the potential antigenic peptides in shortlisted outer membrane proteins. Minimum length of antigenic residues was set to 6 and minimum score to 1. The BLASTp program (https://blast.ncbi.nlm.nih.gov/Blastp) was used to find the conservation of identified OMPs among *V. anguillarum* serovars as well as those present in other *Vibrio* spp.

The antigenic OMPs in this study were subjected to the identification of B-cell and T-cell epitopes. Linear B-cell epitopes were predicted using the BCPreds server (http://ailab.ist.psu.edu/bcpred/predict.html). The server provides three methods based on different algorithms: BCPred and AAP (amino acid antigenicity) which is a fixed length epitope prediction method [6, 11] and FBCPred a flexible length prediction method [12]. In this study, B-cell epitopes were predicted using all three methods with epitope length as 12 and a specificity of 75%. Non-overlapping epitopes predicted by the three prediction methods were combined to derive at a consensus B-cell epitope sequence.

To identify T-cell epitopes Vaxitop, an internally developed program of Vaxign server that relies on statistical *P* value as the cut-off was used. To predict T-cell epitopes, the default *P* value of 0.05 was selected as it provides a high and balanced sensitivity and specificity [17]. Fish MHC sequences were retrieved from the IPD-MHC FISH database (https://www.ebi.ac.uk/ipd/mhc/group/FISH).

Results and Discussion

Prediction of Antigenic Outer Membrane Proteins

In this study, screening of the 3867 proteins encoded by *V. anguillarum* 775 revealed 265 to have a signal peptide sequence (~16–41 amino acids in length) in the N-terminal region. In Gram-negative bacteria, signal peptides play a key role in transport and subcellular localization of proteins to the inner membrane, the periplasm, the outer membrane, or to the extracellular environment [40]. Proteins localized in the outer membrane have a characteristic β-strand secondary structure that traverses the lipid layer in an anti-parallel manner to form a barrel-shaped structure. A Pred-TMBB analysis for the 265 proteins positive for the signal sequence, showed 90 proteins to form β-barrel proteins of which only 38 proteins were analyzed to be localized in the outer membrane. Proteins showing localization in regions other than the outer membrane were omitted and not subjected to further analysis. The predicted *V. anguillarum* OMP*s*, their residue numbers, molecular weight total transmembrane segments, and exposed antigenic peptides are provided in Table S1.

Outer membrane proteins that function as adhesins are preferred vaccine targets. The adhesin probability for the 38 OMPs ranged from 0.11 to 0.89. Among these, 23 OMPs (13 with probability values ≥0.7 and 10 showing values in the "twilight zone") were selected as adhesin proteins (Table 1).

Table 1 List of potential *V. anguillarum* vaccine candidates

Protein accession	Protein description	Length (amino acids[#])	Mol. wt. (kDa)	No. of TMs predicted	No. of exposed antigenic peptides	Adhesin probability	Antigenicity score	
							VaxiJen	Vaxign
AEH31724[a]	Hypothetical OMP	324	35.9	4	17	0.79	0.54	0.59
AEH31940	Ferrichrome-iron transporter OMP	664	73.9	22	12	0.66	0.64	0.52
AEH32461	OmpK-nucleoside-specific OMP	243	27.4	12	2	0.67	0.67	0.54
AEH32514	BamA/YaeT-outer membrane assembly factor protein	790	88.6	26	12	0.64	0.56	0.50
AEH32572[a]	LolB-outer membrane lipoprotein	191	21.4	4	7	0.68	0.67	0.55
AEH32793	OmpV-OMP	238	26.3	10	6	0.86	0.55	0.68
AEH32871	Hypothetical OMP	310	34.0	14	5	0.81	0.51	0.63
AEH32884	Hypothetical OMP	310	33.3	14	5	0.84	0.74	0.65
AEH33067	Periplasmic amino acid binding OMP	223	24.5	8	3	0.66	0.52	0.58
AEH33077	TonB-dependent iron transport OMP	695	76.8	22	9	0.61	0.68	0.53
AEH33152	Hypothetical OMP	141	15.0	6	2	0.72	0.89	0.52
AEH33621	Fatty acid transport OMP	398	43.2	14	9	0.80	0.59	0.62
AEH33622	Fatty acid transport OMP	399	43.3	14	6	0.89	0.59	0.70
AEH33699	Outer membrane porin protein	347	37.9	16	3	0.71	0.80	0.54
AEH33731	TetR-transcriptional regulator OMP	372	42.4	16	3	0.63	0.75	0.64
AEH34033	OmpU-OMP	309	33.4	14	4	0.62	0.81	0.50
AEH34348	Hypothetical OMP	235	26.6	12	5	0.64	0.82	0.52
AEH34505	Hypothetical OMP	167	18.3	6	3	0.62	0.75	0.51
AEH34566	TraF-conjugal transfer OMP	361	39.1	11	6	0.78	0.75	0.60
AEH34817	LamB-OMP	388	42.7	16	7	0.78	0.57	0.61
AEH35019	Hypothetical OMP	402	43.8	18	5	0.71	0.78	0.55
AEH35061	Long-chain fatty acid transport OMP	390	44.6	12	9	0.80	0.58	0.72
AEH35196	Cyclodextrin-specific OMP	322	37.3	14	2	0.74	0.56	0.54

OMP outer membrane proteins, *TMs* transmembrane regions

[#]Amino acids in proteins minus signal peptide

[a]Predicted lipoproteins

The VaxiJen and Vaxign servers identified 37 and 27 OMPs, respectively, to be antigenic having antigenicity probability scores >0.5 (Table S1). Based on the results of Seapath, VaxiJen, and Vaxign analysis, 23 OMPs that met the criteria of being an adhesin and having a threshold antigenic score >0.5 in both Vaxign and VaxiJen prediction were shortlisted as potential vaccine candidates. Table 1 lists the final selection of potential vaccine candidates together with their length, molecular weight, predicted transmembrane helices, exposed antigenic peptides predicted, adhesin, and antigenicity probability scores. Among the 23 outer membrane proteins two (AEH31724 and AEH32572) were identified as lipoproteins by the LipoP 1.0 server (Table 1). AEH31724 is a hypothetical OMP with 324 amino acids, while AEH32572 having a length of 191 amino acids has been annotated as LolB OMP. The β-transmembranes predicted for the two lipoproteins were 4 (Table 1). The lipoproteins were also predicted to be adhesins and antigenic. This suggests that the two lipoproteins are possible protective antigens worth experimental evaluation.

A literature search, for experimental work for the 21 *V. anguillarum* OMPs identified in this study, revealed only three OMPs (OmpK, OmpU, and LamB) to have been studied for their potential immunogenicity in fish. The Indian major carp *Labeo rohita* on vaccination with recombinant OmpK was reported to be well protected when challenged with *V. anguillarum* [16]. Similarly, vaccination with recombinant LamB protein showed cross protection in zebra fish injected with heterogenous *Vibrio* species [25]. *V. anguillarum* OmpU was shown to play a role in bile resistance and biofilm formation, considered important for bacterial survival and colonization in the fish gut [44].

To date, more than 20 serovars exist for *V. anguillarum*. Therefore, the identified OMPs in *V. anguillarum* 775 were also studied for their conservation across different serovars. A Pan-genome analysis of the shortlisted OMPs (Table 1), against *V. anguillarum* genomes available for 28 different serovars in the NCBI database, showed all OMPs to be highly conserved (>98%) across serovars isolated from different geographic locations (data not shown). In contrast, orthologous clustering of the identified outer membrane proteins across *Vibrio* spp. (*V. alginolyticus; V. campbellii; V. cholerae; V. fischeri; V. fluvialis; V. furnissii; V. harveyi; V. mimicus; V. owensii; V. parahaemolyticus; V. splendidus, and V. vulnificus*) revealed several of these proteins to be common with similarities ranging from 21 to 93% (Table S2). As seen from the table, at a 50% cut-off value, 90.5% of *V. anguillarum* OMPs were found to be highly conserved in *V. cholerae*. Among the outer membrane proteins, the Ferrichrome–iron transporter OMP (AEH31940), although conserved was seen to have a low identity (<48%) across *Vibrio* spp. and hence considered unique to *V. anguillarum*. Except in *V. cholerae* similar low conservation was also seen for the OMP porin protein (AEH33699). In contrast, few *V. anguillarum* outer membrane proteins considered immunogenic (Table 1) showed high conservation across different *vibrios* which included OMPs, OmpK (AEH32461), BamA/YaeT (AEH32541), Fatty acid transporters (AEH33621 and AEH33622), OmpU (AEH34033), and two hypothetical proteins (AEH33152 and AEH34348). While the protective efficacy of proteins OmpK and OmpU has already been established [16, 44], the remaining could be considered as novel vaccine candidates for which in vivo experiments could be performed to validate the immune and protective power of these antigens not only against disease caused by *V. anguillarum* but also against the disease caused by other vibrios.

Prediction of B-cell and T-cell Epitopes for Antigenic OMPs

Identification of epitopes in target antigens is one of the key steps in designing an epitope-based sub-unit vaccine. For an antigen to be considered ideal it should be capable of eliciting a humoral immune and cellular response by triggering the B-cells and T-cell selectively. Thus, an in silico approach was used to identify antigenic B-cell and T-cell epitopes present in the potential *V. anguillarum* OMPs identified as vaccine candidates in this study.

B-cell Epitope Identification

The cell surface B-cell epitopes of pathogens are antigenic determinants that interact with antibodies secreted by the B lymphocytes, eliciting a humoral immune response. Prediction of B-cell epitopes using the three predictors supported by the BCPred server identified several linear B-cell epitopes of length 12–15 (Table 2) for the antigenic OMPs identified in this study. Table 2 summarizes the number of B-cell epitope predicted for each OMP and their length. As seen from Table 2, the B-cell prediction methods failed to identify antibody binding epitopes in two of the OMPs, i.e., Fatty acid transport OMP (AEH33621) and long-chain fatty acid OMP (AEH35061). The B-cell peptides predicted for the remaining OMPs ranged from 1 to 7. The residue lengths for B-cell peptides in this study ranged from 12 to 15, which is in accordance to the experimental proven B-cell epitope length required to stimulate the immune cell to produce antibodies that bind the peptide [11]. B-cell epitopes representing bacterial OMP peptides have been used in the development of antimicrobial vaccines [18, 39]. Thus, the identified B-cell peptides of antigenic *V. anguillarum* OMPs could serve as a guide in assessing the B-cell-induced protective antibody response in vaccinated animals as well as in B-cell peptide vaccine design.

Table 2 Predicted B-cell epitopes for *V. anguillarum* outer membrane proteins

Protein accession	Antigen	Amino acid start position	Epitope	Epitope length
AEH31940	Ferrichrome-iron transporter OMP	243	FGEPDHDDFDKTQ	13
		287	SSAYQNAWQDSDPYT	15
		365	NPTYGNVPDLSS	12
AEH32461	OmpK-nucleoside-specific OMP	59	LSNPSSDKEGKEK	13
		179	GMDDKNTALKTSNG	14
AEH32514	BamA/Yae T-outer membrane assembly factor protein	566	AGNHQRAFYKMTV	13
		617	GYGKTDGNDNLF	12
		633	NYYAGGFTTLRGFG	14
		659	ATGCNGNNGGNNR	13
		674	SATDDSVGGNAV	12
		719	WDTEFDYKDGKL	12
AEH32793	Outer membrane protein	22	GAFGSTDLLKDQ	12
		162	NYYFGVKDKEAT	12
		213	SSDVANSPIVES	12
AEH32871	Hypothetical OMP	126	SITKSDEPNGWA	12
		285	TANTPEYKEDKN	12
AEH32884	Hypothetical OMP	63	TDESKESPYEGA	12
		288	IENGDAGKDQEE	12
AEH33067	Periplasmic amino acid binding OMP	127	NKPDANDLTTNL	12
		186	SGKANPNAQKYID	13
AEH33077	TonB-dependent iron transport OMP	176	NKTDSDIAHSSYK	13
		188	GQEQQNFADRKE	12
		301	GRPPYTPANADNQ	13
		356	SNTNTELNSDPA	12
		369	PNQVLVYTPDAT	12
		412	TDPGGSTTEPLV	12
		663	EYYRWNDIRGKT	12
AEH33152	Hypothetical OMP	120	MNYTLGDDDIGT	12
AEH33621	Fatty acid transport OMP	–	–	–
AEH33622	Fatty acid transport OMP	160	PAQTKPLPQGTT	12
		219	KESGKVVNDTGS	12
		363	ESRGYASDDAAQ	12
AEH33699	Outer membrane porin protein	321	TPADGDGKGNAD	12
AEH33731	TetR-Transcriptional regulator OMP	109	EQRGDNDTTLNLS	13
		155	DYKNNQNITQYQ	12
		199	SDIAPGSTLKDST	13
AEH34033	OmpU-OMP	62	DEGTADNKGDLD	12
		149	QESTSAITDNNA	12
		217	QDKAASKTDKTG	12
		288	DSDKVGKAKSED	12
AEH34348	Hypothetical OMP	60	LDKNGKENKRQA	12
AEH34505	Hypothetical OMP	81	SQATFTSTQVVD	12
AEH34566	TraF-conjugal transfer OMP	206	ADYDKSETNDNA	12
		324	AVTFGIGISPGD	12

Table 2 (continued)

Protein accession	Antigen	Amino acid start position	Epitope	Epitope length
AEH34817	LamB-OMP	58	NSTGRLGNEGNG	12
		98	EVGVPKAYAGGT	12
		144	GQGGGFYNLNLG	12
		165	SATGASPSDHPDG	13
		205	DDNSDPTAKKLN	12
		279	EDGAYKQYDRTN	12
AEH35019	Hypothetical OMP	30	TEFGKPDYKTAG	12
		203	EQANKDSKSDDG	12
		249	NFGGWSGGDDKQ	12
AEH35061	Long-chain fatty acid OMP	–	–	–
AEH35196	Cyclodextrin-specific OMP	242	GDNETNNASNSG	12
		298	LAGWEAKNESEL	12

T-cell Epitopes Identification

In vertebrates, the major histocompatibility complex (MHC) genes play an essential role in activating the adaptive immune response. The cell surface proteins encoded by the MHC genes bind peptide fragments derived from pathogens and present them to T-cells that activate a specific immune response. Therefore the prerequisite in vaccine development is the identification of T-cell epitopic regions within antigenic proteins which can recognize and bind to the MHCs [32]. At present, several in silico tools are available to predict epitope sequences for a protein. However, they are mainly trained to predict peptides based on binding to human and lower mammalian MHC alleles and not for fish MHC alleles. Therefore as a first step, we downloaded the MHC sequences available for salmonid fish in the IPD-MHC FISH database. Earlier studies have shown that MHC class I and class II are present in teleosts, many of which share genes that are evolutionarily related to the classical human MHC genes [15, 38]. A BLAST analysis in this study showed that among the several human MHC alleles, the fish MHC sequences had the closest identity (~35–38%) to human HLA-A (MHC class I) and HLA-DP and HLA-DQ (MHC class II) sequences. Hence prediction of T-cell epitopes for each antigenic OMP predicted in this study was performed using the Vaxitop program set against human MHC HLA-A2, HLA DPA1/DPB1, HLA DQA1/DQB1, and HLA DRA1/DRB1 alleles.

The Vaxitop analysis identified T-cell peptide fragments for antigenic OMPs in this which ranged from 1 to 8 (Table 3). Among OMPs, the predicted T-cell epitopes was highest for BamA (AEH32514) followed by LamB (AEH34817) with 8 and 7 peptides, respectively. The peptide length ranged from 9 to 20 residues and corresponded to exposed regions of the OMPs. The peptides binding to MHC class I and class II were seen to differ in their length. In peptides showing binding to class I and class II alleles, the MHC class I epitopes were observed to be shorter (underlined in Table 3) in comparison to the same peptide binding to MHC class II alleles. Overlapping peptides that correspond to the antigenic outer membrane proteins and their binding probabilities are presented in Table 3. Our study showed that none of the peptide fragments recognized the MHC class II allele HLA-DRA1/DRB1 (data not shown). As seen from Table 3, the peptides predicted had a greater binding frequency to HLA-A2 (MHC class 1) followed by HLA-DQ (MHC class II) and HLA-DP alleles. It was also observed that the peptides corresponding to the TetR-transcriptional regulator OMP (AEH33731), hypothetical OMP (AEH34505), and cyclodextrin-specific OMP had binding affinity only to MHC class II alleles and not to the MHC class I allele. In contrast, in few antigenic outer membrane proteins some of the peptide fragments were seen to recognize and bind to both the MHC class I and class II alleles (Table 3) which could be chosen for further narrowing down on epitope candidates during vaccine design. Further, among *V. anguillarum* OMPs, the Ferrichrome–iron transporter protein (AEH31940) exhibited low homology to same protein in other *Vibrio* spp. (Table S2). Hence, the immunodominant epitopes predicted for this protein could be exploited in designing an exclusive epitope vaccine against this bacterium. Similarly, few immunogenic outer membrane proteins showing homology across other *Vibrio* spp. such as BamA/YaeT (AEH32541) could be exploited to design and develop a single therapeutic vaccine effective across *Vibrio* spp. However, prior to designing an epitope-based vaccine, the peptides identified need to be checked and validated for their sharing to fish MHC proteins.

In conclusion, the reverse vaccinology strategy when applied to *V. anguillarum* proteins identified 23 OMPs

Table 3 Predicted T-cell epitopes for *V. anguillarum* outer membrane proteins and their binding probabilities to MHC class alleles

Protein accession	Protein description	Peptide sequence	MHC class I allele		MHC class II alleles			
			HLA-A2	*P* value	HLA-DP	*P* value	HLA-DQ	*P* value
AEH31940	Ferrichrome–iron transporter OMP	WLGNGTGSQVGV	+	0.020	−	−	+	0.010
		NAWQDSDPYTLARYTL	+	0.010	+	0.030	−	–
		SLNPTYGNVPDL	+	0.010	−	−	+	0.020
		SLAGCDFGTC	+	0.030	+	0.020	+	0.010
AEH32461	OmpK-nucleoside-specific OMP	TLMEWGGNSGVN	+	0.002	−	−	+	0.009
AEH32514	BamA/Yae T-outer membrane assembly factor protein	LNVDVAWWNFLSDD	+	0.020	+	0.038	+	0.082
		YAYQVRTIPEFN	+	0.022	−	−	+	0.012
		FIGSGNRVGI	+	0.013	−	−	+	0.038
		GINAMMNDYQKNI	+	0.030	−	−	+	0.015
		KIGNINEYVQV	+	0.035	−	−	+	0.001
		NLNRTIFPTAG	+	0.043	−	−	+	0.044
		NLFPFYENYYAGG	+	0.021	+	0.023	+	0.022
		TTLRGFGSNSAGP	+	0.038	−	−	+	0.006
AEH32793	OmpV-OMP	DYNNYYFGV	−	−	+	0.018	+	0.002
		AQRKAYHAG	−	−	+	0.010	+	0.003
		RLSSDVANSPIVESANQW	+	0.006	−	−	+	0.011
AEH32871	Hypothetical OMP	ATVAFNLDNDGVFG-VDRDY	+	0.007	+	0.002	+	0.009
		GDAALSEKAQKL	+	0.040	−	−	+	0.028
AEH32884	Hypothetical OMP	AYYDTFTESV	+	0.030	+	0.012	−	–
		DGFNVFQFRGQASSIA	+	0.030	+	0.061	+	0.019
AEH33067	Periplasmic amino acid binding OMP	MPWSRALDEV	+	0.035	−	−	+	0.025
		SYLENELSFI	+	0.016	+	0.046	−	–
		QKYIDAYNKGL	+	0.038	+	0.026	−	–
AEH33077	TonB-dependent iron transport OMP	PLNSVNPWNVVA	+	0.023	−	−	+	0.019
		ELPSATIVDI	+	0.038	+	0.043	+	0.025
AEH33152	Hypothetical OMP	–	–	–	–	–	–	–
AEH33621	Fatty acid transport OMP	GLSQKFQTTI	+	0.021	+	0.071	−	–
		TIPAGTALLNV	+	0.008	+	0.011	−	–
		VLPLPDIAEFSGFHKI	+	0.037	+	0.020	+	0.094
		NLDSSQSPIASILAG	+	0.030	−	−	+	0.011
		AGTVLTATTHADA	+	0.028	−	−	+	0.019
AEH33622	Fatty acid transport OMP	FSASHFGNQAMVK	+	0.047	−	−	+	0.009
		GSVMVKEEN	−	−	+	0.064	+	0.056
AEH33699	Outer membrane porin protein	VDWPHSNPGLGNVFDW	+	0.009	−	−	+	0.011
		NVFDWHNAIGAGYQDR	+	0.025	−	−	+	0.033
		DEAQAAYYL	−	−	+	0.010	+	0.029
AEH33731	TetR-transcriptional regulator OMP	NEYNTNNFN	−	−	+	0.013	+	0.004
		NITQYQNWD	−	−	+	0.036	+	0.061
AEH34033	OmpU-OMP	DNRYTYAGI	−	−	+	0.040	+	0.013
		GIGGNFGEVTYGKND	+	0.033	+	0.050	+	0.011
		GALGVITDF	−	−	+	0.030	+	0.01
		KAAYKIVVA	−	−	+	0.020	+	0.001
AEH34348	Hypothetical OMP	IEVPSPWNSQVELG	+	0.041		−	+	0.025
		SSTDIVFANTVE	+	0.022	+	0.002	+	0.032
		PAGLSKADS	−	−	+	0.036	+	0.028
AEH34505	Hypothetical OMP	GYSFTKGRF	−	−	+	0.026	+	0.031

Table 3 (continued)

Protein accession	Protein description	Peptide sequence	MHC class I allele		MHC class II alleles			
			HLA-A2	*P* value	HLA-DP	*P* value	HLA-DQ	*P* value
AEH34566	TraF-conjugal transfer OMP	LIAFGYSEFGLAL	+	0.021	–	–	+	0.006
		DTLDNAVTFGIGI	+	0.044	+	0.012	+	0.017
AEH34817	LamB-OMP	RLNGNSTGRL	+	0.008				
		TNLNDYFWMT	+	0.005	+	0.039	–	–
		QGGGFYNLNLGGI	+	0.032	–	–	+	0.005
		GIKFDASVV	–	–	+	0.045	+	0.042
		DRTNYNVLV	–	–	+	0.037	+	0.078
		IATNSSWKVTL	+	0.008	–	–	+	0.008
		KDGLFKAASNPDTVTI	+	0.040	–	–	+	0.034
AEH35019	Hypothetical OMP	RMVDGETVDTEF	+	0.038	+	0.047	–	–
		AAGILSGEFWKQ	+	0.035	+	0.030	+	0.028
		DAGAATTNPERTTL	+	0.023	–	–	+	0.040
		SSYDLYYYGV	+	0.037	+	0.004	–	–
AEH35061	Long-chain fatty acid OMP	KLTRHKGALAVF	+	0.030	+	0.022	–	–
		NLVGMTGETVALGWN	+	0.025	+	0.002	–	–
		TDWSSFKEL	–	–	+	0.080	+	0.021
AEH35196	Cyclodextrin-specific OMP	DIRKATLGY	–	–	+	0.030	+	0.040
		GEYEFANEV	–	–	+	0.003	+	0.003

Peptide sequences binding to MHC class I alleles are underlined; + indicates binding to MHC; – indicates lack of binding capacity. Lower the *P* value (= 0.05), higher the significance of binding probability

OMP outer membrane protein

including two lipoproteins as vaccine candidates. While three out of these OMPs (OmpK, LamB, OmpU) have been shown to elicit a protective immune response in fish, the remaining novel antigens could be further studied in the development of a vaccine against *V. anguillarum*. Few of the antigens identified also were seen to be conserved across vibrios which could be further exploited to develop a common vaccine against vibriosis in fish. The potential B-cell and T-cell epitopes identified would help to narrow down the selection of peptides and providing a framework for future designing of an effective epitope-based vaccine against vibriosis. Our future work would focus on validating the epitopes identified in this study through in vitro and in vivo studies and its application to vaccine design. MHC molecules are highly polymorphic and exhibit allelic diversity and there are >6000 alleles listed for class I and II molecules for higher vertebrates in IMGT/HLA database [32, 37]. However, in fish there is a paucity of data concerning MHC proteins and their allelic diversity. Hence, there is also a need for basic research on MHC proteins in fish of interest.

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