An *In Silico* Vaccine Designing Approach against Fish Pathogens *Flavobacterium columnare*

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**Abstract:** The development of peptide vaccines is a highly avant-garde approach to computer-aided vaccine design. Using various *in silico* approaches, an effort has been undertaken to identify potential peptides that could be used in developing a peptide vaccination. Research into vaccine design has advanced due to the creation of multiple sophisticated experiments that examine T-cell reactions to various vaccine candidates. This study identified potential peptides that bind specifically to Major Histocompatibility Complex (MHC) class I alleles in the outer membrane protein of fish pathogens. The results show that the majority of peptides bind effectively to the alpha 1 and alpha 2 grooves of the MHC class I complex. There were substantial hydrogen bonding connections between MHC and even the peptide domains.

**Keywords:** Vaccine design, T-cell, MHC class I, Peptides, Fish pathogens

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**Introduction**

The majority of fish are prone to different bacterial diseases. More environmental infections come into direct contact with fish. As a result, these infections can quickly bind to and damage fish tissues (Ellis, 2021). Huge economic loss in fisheries is mostly driven by pathogen and disease induced illnesses in fish tissue. The world’s greatest sector of livestock farming is aquaculture. As a result, there is a big need to find solutions to fight fish infections. The most common intracellular bacterial infection that causes columnaris in fish is identified as *Flavobacterium columnare*. Effective and efficient vaccines and strong preventative and preventive medications against bacterial fish illnesses are being sought for by fish farming scientists (Sudheesh et al., 2012). *F. columnare* is a widely distributed, motile Gram-negative rod. All around the world, it causes freshwater fish to contract the columnaris illness. Columnaris has a significant negative impact on a number of fish species. Salmonid species, carp, peaches, eels, tilapia, channel catfish, and goldfish are the most commonly impacted by columnaris (Abbott, 2007). Fish with columnaris have
brownish-yellow scars on their skin, gills, and fins. Each bacterial cell has its own gill filament. These factors raise the necessity for the creation of novel vaccinations to protect against columnaris (Plumb, 1999). Resistance to antibiotics among microorganisms is a result of the use of antibiotics to treat illnesses. There are already vaccinations available to treat columnaris. For live attenuated vaccinations, there is a risk of reversion (Mohanty et al., 2007).

This study employed immunoinformatic methods and software including databases to identify possible T-cell epitopes that might serve as efficient vaccine candidates. Which peptide from the pathogenic bacterial pathogen’s proteome will attach to the major histocompatibility complex (MHC) molecules is to be determined by the T-cell epitope (Stock et al., 2001). A key factor in influencing T-cell immunogenicity is the degree of MHC molecule binding to epitopes (Farmer et al., 2007). Using the proper immunoinformatics methods, this study have modelled peptide-MHC complexes and examined their interactions in this investigation.

Materials and Methods

Retrieval of protein sequences:

The choice of outer membrane protein for this investigation was made possible by the high immunogenicity and considerable antigenicity of these proteins. The UniProt database, a well structured protein sequence database with precise structural and functional information about proteins, is where outer membrane protein was found. From UniProt, the outer membrane proteins’ amino acid sequences in FASTA format was obtained (Fig. 1).

Forecasting of antigenic locations:

Using the Kolaskar and Tongaonkar antigenicity tool (http://tools.immuneepitope.org/tools/bcell/iedb input), antigenic sites were predicted (Park et al., 2012). The prediction of peptides employs a semiempirical method that is based on the physical, chemical, and physico-chemical parameters of the amino acids as well as the frequency of occurrence of residues in experimentally known epitopes. The threshold value is 1.000, and it can predict antigens with an effectiveness of approximately 75%.

CTL epitope prediction:

The NetCTL.1.2 server (http://www.cbs.dtu.dk/services/NetCTL) was used to predict CTL epitopes (Woo et al., 2011; Tekedar et al., 2012). CTLs attack invading pathogens, and they are often activated by antigen-presenting MHC molecules on their exterior. Prediction of TAP transportation effectiveness, MHC class I binding, plus proteasomal C-terminal cleavage are all included in the NetCTL 1.2 server (Hawke et al., 1992; Declercq et al., 2013). The organism’s FASTA sequence was used as input. Human
leukocyte antigen (HLA) alleles and peptide length were chosen and uploaded. As a result, T-cell epitopes were produced. To forecast MHC class I binding and proteasomal C-terminal cleavage, artificial neural networks are applied. In order to forecast TAP transport effectiveness, weight matrix is being used. Further VaxiJen 2.0 software was used to determine antigenic property of all listed epitope (Gianfrani et al., 2000; Zhu et al., 2012).

**Protein-peptide complex modelling and docking:**
Protein-peptide complexes were modelled using the MODPROPEP programme (Gillespie et al., 2000). The protein-peptide complex was modelled using the input peptide sequence and the crystal structure of the MHC alleles (HLA-B2705) from Protein Data Bank (PDB). High-resolution peptide docking was performed using the Rosetta FlexPepDock tool (http://flexpepdock.furmanlab.cs.huji.ac.il) (Lazarski et al., 2005; Sarkar et al., 2023). For simultaneous docking and de novo folding of peptides, FlexPepDock makes use of the Rosetta fragment library and a representation of the receptor and peptide structures. High-resolution models of the results are presented in the publication. FlexPepDock precisely refines the peptide structure, starting from up to 5.5 of its native conformation's root mean square deviation (RMSD). Additionally, it enables the full flexibility of the peptide and side chain flexibility of the receptor. By employing fragment-based sampling, the overall conformation of the peptide in a centroid mode is discovered. The Biovia Discovery studio was used to retrieve the residues involved in the hydrogen bond interaction between the peptide and the MHC protein (Sarkar et al., 2022).

**Results and Discussion**
The development of peptide vaccines depends critically on the prediction of antigenic sites in outer membrane proteins. Using the Kolaskar and Tongaonkar antigenicity tool, this study predicted the antigenic sites of the outer membrane protein of *F. columnare*.

The present study discovered 25 antigenic sites from *F. columnare*’s A0A2N9PCB4 (Table 1). Peptide having Cys(C) Isoleucine (I) Aspartic acid (D) Valine (V) Proline (P) Glycin (G) sequence showed maximum highest antigenic property 1.8790. This site is located in the CTL epitope in between 540 to 545. Rest all other probable CTL epitope which also can be exhibited as antigens are illustrated in Table 1.

Possible candidates for the development of peptide vaccines against various illnesses include CTL epitopes. Using the prediction tool in NetServer 1.2, the present study predicted CTL epitopes (Sarkar, 2021). Based on the peptides’ MHC binding affinity, proteasomal C-terminal cleavage, and transport affinity, these predictions were made. MHC supertype A1 was used for assessment. From the outer membrane protein of *F. columnare*, 24 peptide sequences were predicted to be CTL epitopes. Out of 24, only 11 were found to have antigenic property (Table 2).

Drug resistance among microorganisms is a result of the application of antibiotics to treat illnesses. There are already vaccinations available to treat columnaris. For live attenuated vaccinations, there is a risk of reversal. The pathogens’ peptide sequences are bound by the MHC molecule before being shown on the cell surface (Sarkar et al., 2022). The CTLs kill the infected cell after identifying the peptide-protein combination. The peptide is introduced into the MHC I molecule by a protein complex known as T cell activating protein (TAP), after which it is delivered to the cell surface (Sarkar, 2022). The class I MHC groove’s invariant pocket, which is present at one end, is where the peptide’s terminal amino group attaches. The class I MHC molecule’s consistent pocket is located at the opposite end of the groove, where the terminal carboxyl group of the peptide attaches. Hydrogen bond interactions and networks hold the peptide’s N- and C-termini in place (Koronakis et al., 1997).

The major objective of this research was to locate possible T-cell epitopes for peptide vaccine development. To do prediction analysis and
Table 1: Notes: Number of antigenic sites identified in the outer membrane protein of *F. columnare* is 25 with residue score of 1.170 (threshold = 1.000). Only the probable CTL epitope antigens are highlighted in bold.

<table>
<thead>
<tr>
<th>No.</th>
<th>Start</th>
<th>End</th>
<th>Peptide</th>
<th>Length</th>
<th>VaxiJen 2.00 prediction (Threshold = 0.4)</th>
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<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>36</td>
<td>LLTLFSLFLCAEQIAQSYIGFVPDNYSGVHGMS</td>
<td>33</td>
<td>Overall Prediction for the Protective Antigen = 0.5369 (Probable ANTIGEN).</td>
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<tr>
<td>2</td>
<td>39</td>
<td>47</td>
<td>NPAAIVGSP</td>
<td>9</td>
<td>Overall Prediction for the Protective Antigen = 0.1741 (Probable NON-ANTIGEN).</td>
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<tr>
<td>3</td>
<td>49</td>
<td>59</td>
<td>RLDLNLVSGSA</td>
<td>11</td>
<td>Overall Prediction for the Protective Antigen = 1.7312 (Probable ANTIGEN).</td>
</tr>
<tr>
<td>4</td>
<td>67</td>
<td>73</td>
<td>GVKITDL</td>
<td>7</td>
<td>Overall Prediction for the Protective Antigen = 1.7312 (Probable ANTIGEN).</td>
</tr>
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<td>5</td>
<td>80</td>
<td>87</td>
<td>IDLQAKKF</td>
<td>8</td>
<td>Overall Prediction for the Protective Antigen = 1.5175 (Probable ANTIGEN).</td>
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<tr>
<td>6</td>
<td>97</td>
<td>104</td>
<td>NADVLPNS</td>
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<td>164</td>
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<td>282</td>
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<td>11</td>
<td>302</td>
<td>310</td>
<td>INQVLVESAR</td>
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<td>12</td>
<td>315</td>
<td>320</td>
<td>KILTLF</td>
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<td>13</td>
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<td>337</td>
<td>KVMLPTA</td>
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<td>Overall Prediction for the Protective Antigen = -0.2289 (Probable NON-ANTIGEN).</td>
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<td>14</td>
<td>373</td>
<td>380</td>
<td>NTYILTPR</td>
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<td>Overall Prediction for the Protective Antigen = -0.2289 (Probable NON-ANTIGEN).</td>
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<tr>
<td>15</td>
<td>387</td>
<td>394</td>
<td>SFALPVNY</td>
<td>8</td>
<td>Overall Prediction for the Protective Antigen = 1.0057</td>
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</table>
Table 2: Antigenic properties of selected peptides. Note: NetCTL-1.2 predictions using MHC supertype A1. Threshold 0.750000

<table>
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<tr>
<th>Residue No</th>
<th>Peptide Sequence</th>
<th>Predicted MHC binding affinity</th>
<th>Rescale binding affinity</th>
<th>C-terminal cleavage affinity</th>
<th>Transport affinity</th>
<th>Prediction score</th>
<th>MHC ligands Prediction score should be &gt;0.750000</th>
<th>VaxiJen 2.00 prediction (Threshold = 0.4)</th>
<th>Overall Prediction for the Protective Antigen</th>
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<td>16 408 417</td>
<td>FGPLFLGSGS</td>
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<td>0.9669</td>
<td>2.843</td>
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<tr>
<td>17 430 441</td>
<td>ADVYFGLKVPIY</td>
<td>0.6433</td>
<td>2.7312</td>
<td>0.9615</td>
<td>3.044</td>
<td>3.0276</td>
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<td>0.9741</td>
<td>2.952</td>
<td>3.0167</td>
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<tr>
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<td>Sequence</td>
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<td>Score 3</td>
<td>Score 4</td>
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<tr>
<td>30</td>
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<td>2.923</td>
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<td>Overall Prediction for the Protective Antigen = 0.4571 (Probable ANTIGEN).</td>
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<tr>
<td>57</td>
<td>GSALLGNDY</td>
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<td>2.2042</td>
<td>0.8141</td>
<td>2.857</td>
<td>2.4691</td>
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<td>189</td>
<td>YLQGFANSY</td>
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<td>1.4134</td>
<td>0.9739</td>
<td>2.993</td>
<td>1.7091</td>
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<td>386</td>
<td>FSFALPVNY</td>
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<td>373</td>
<td>NTYILTPRY</td>
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<td>339</td>
<td>HTNIDWNFF</td>
<td>0.2993</td>
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<td>1.4049</td>
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<td>Overall Prediction for the Protective Antigen = 1.7521 (Probable ANTIGEN).</td>
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<td>389</td>
<td>ALPVNYMEY</td>
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<td>0.8781</td>
<td>0.9769</td>
<td>2.968</td>
<td>1.173</td>
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<td>Overall Prediction for the Protective Antigen = 1.7521 (Probable ANTIGEN).</td>
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<td>312</td>
<td>NLDKILTTLF</td>
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<td>625</td>
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<td>0.648</td>
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<td>22</td>
<td>YIGFVPDNY</td>
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<td>2.924</td>
<td>1.049</td>
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<td>YMEYRGLNV</td>
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<td>AMSALLKEY</td>
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<td>Overall Prediction for the Protective Antigen = 0.6252 (Probable ANTIGEN).</td>
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docking between the peptide and the protein, *in silico* immunoinformatics methods were employed. The limitations of conventional experimental methods are numerous. Using *in silico* immunoinformatics research, the full range of possible antigens was examined. Antigens that cannot be expressed in vitro because pathogen culture is not feasible can also be studied *in silico*. *In silico* vaccine candidates have been reported by several immunoresearch groups, and they have shown encouraging preclinical outcomes. The outer membrane protein of the pathogenic pathogen *F. columnare* contains potential T-cell epitopes that bind with MHC protein (Feriancikova et al., 2013). Antigenic domain forecasting aids in the discovery of possible outer membrane protein residues. There are nine *F. columnare* epitopes that have the potential to serve as peptide vaccine targets. YSGVHGMFY, FSFALPVNY, NTYILTPRY, HTNIDWNFF, ALPVNYMEY, TTSIANYTI, YMEYRGLNV and YKSNTENHY are the peptides in question (Table 2). Interaction between MHC bound complex with respective interaction residues and bonds involved are shown in Figures 2-9. A significant development in the field of *in silico* vaccine design is T-cell epitope forecasting. By utilising *in silico* epitope prediction methods, numerous research organisations that have been involved in the discovery of several vaccines targeting various viral diseases, including autoimmune and cancer, have achieved great outcomes (Sarkar, 2021). CTL epitope identification by this approach acts as a crucial tool in the process of creating vaccines because the *in silico* methodology simplifies the number of *in vitro* testing. A procedure used in numerous computer research alignments the MHC molecule’s existing sequences and identifies residues that facilitate peptide binding (Sarkar et al., 2022). Knowing this data and comprehending crystallography can help you comprehend the idea underlying peptide-MHC identification (Feriancikova et al., 2013).

*In silico* analysis improves the accuracy and identification of functional CTL epitopes (Zhu et al., 2012). The CTL epitopes of conserved hepatitis C virus (HCV) proteins were predicted by the researchers, while their *in vitro* MHC class I antigen complexation was evaluated. To confirm the protective HCV CTL epitopes, they further tested the immunogenicity of the discovered epitopes in living organisms. They verified *in silico* methods and came to the conclusion that it is possible to identify and restrict the pool of potential CTL epitope candidates for the creation of therapeutic vaccines (Park et al., 2012). For more precise CTL epitope forecasting, they have also recommended integrating a number of algorithms for MHC class I and proteasomal degradation site assessment. These results highlight the value of the *in silico* methods for
peptide vaccine production as well as its useful in vitro implementation. This study anticipate that using the forecasted CTL epitopes from present investigation for in vitro research might result in the production of peptide vaccines having biomedical applications.

**Conclusion**

An extremely innovative method of computer-aided vaccine design is the creation of peptide vaccines. An effort has been made to find possible peptides that could be employed in creating...
a peptide vaccination using various *in silico* methodologies. The development of numerous sophisticated experiments that test the T-cell responses against diverse vaccine candidates presently has aided in the advancement of vaccine-designing investigation. The outer membrane protein of fish pathogens contained putative peptides that bind effectively to MHC class I alleles. The findings indicate that the majority of the peptides bind efficiently to the MHC class I complex's grooves alpha1 and alpha 2. Significant hydrogen bonding linkages existed in
between MHC and even the peptide domains.

References


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