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In-silico Characterization and 3D Structure Prediction of MX Protein of *Lates Calcarifer (Barramundi)*: A Major Threat to Aqua Industry

Ruby Singh[1], K. Pani Prasad[2], Ajey Pathak[3] and Prachi Srivastava[1]*

[1]Amity Institute of Biotechnology, Amity University, Lucknow - 226028, Uttar Pradesh, India; psrivastava@amity.edu
[2]ICAR- Central Institute of Fisheries Education, Versova, Mumbai - 400061, Maharashtra, India
[3]National Bureau of Fish Genetic Resources (Indian Council of Agricultural Research), Canal Ring Road, P.O. Dilkusha, Lucknow - 226002, Uttar Pradesh, India

Abstract

Betanoda virus is one of the most important and emerging groups of viruses known to infect around 40 species found to be worldwide in distribution. The most common and virulent target of infection for this virus is *Lates Calcarifer* (*barramundi*). It is found that the expression of MX protein is found to be the more susceptible reason for this viral infection. Considering this current study including characterization to structure prediction revolves around the MX protein as a target. The progression of this study describes the amino acid sequence of MX protein was retrieved from UniProt database in Fasta format and further primary structure analysis and characterization including nature of amino acids, instability index reading, GRAVY, determination of phosphorylation as well as signal peptide cleavage sites was done with the help of various tools. Secondary structure prediction has proceeded through SOPMA server analysis revealed that MX protein has mixed secondary structure, i.e., mostly alpha-helix and beta-turn. The progression of this work prediction of a 3D structure along with functional site prediction of MX protein of Fish (*Lates Calcarifer*) is done through standard modeling tools. The 3D structure of this protein of *Lates Calcarifer* as documented in this study may provide a valuable aid for designing an inhibitor or better ligand against viral nervous necrosis disease and could play a vital role in drug design.

Keywords: Barramundi, Betanoda Virus, Ligand, MX Protein, Viral Nervous Necrosis

1. Introduction

Aquaculture is one of the fastest-growing industry which is increasing the economy of our nation and giving a positive impacting on agriculture as well. Aquatic culture helps fulfil the demand and supply of the food fish, ornamental fish, fish egg, etc[1]. Successful cultivation of fishes require a need to focus on fish health by keeping the aquatic environments free from viruses, bacteria, parasites. Among the many relevant issues dealing with Fish biology, microbial infection is one of the major issues[2]. Different kinds of viral infection are continuously creating a leading problem in cultivation of different types of fishes. Among different kind of viral infections, Betanoda viral infections are a leading problem of aquatic culture[3]. The major target of this virus is the Central nervous system and Retina of Fishes especially *Lates Calcarifer* (Barramundi or Asian Sea bass)[4]. The edible value of this Asian sea bass fish is very high, hence no doubt mortality rates reduces the rate of a culture of this fish and gives a

**Author for correspondence*

direct negative impact on the Indian economy too. The Mortality rate of fish stock is very high that it may reach up to 100% within 2–3 days of onset of betanodaviral infection[3]. There are many advanced molecular approaches, antimicrobial peptide actions, interferon therapy, etc. has been made to fight against this disease of *Lates Calcarifer*, but still, more confined results are waiting against the protective approaches of this disease[5].

Nodavirus increases the expression of MX protein in fish brain. MX protein in barramundi plays an important role in understanding the mechanism of this disease as this MX protein in fish(BB cell line) helps in the clearance of betanodaviral RdRp protein[6]. The BB cell line is the first cell line reported to be persistently infected with NNV and is an important system for studying the mechanism of NNV[7,8].

2. Materials and Methods

2.1 Retrieval of MX Protein Sequence

Protein sequence of MX (Barramundi MX cDNA) was retrieved in FASTA format from the UniProt protein database using the Uniprot ID- Q2VTP1 and then further used for *in-silico* characterization and 3D structural prediction[9].

2.2 *In-silico* Characterization and Secondary Structure Prediction

The amino acid composition of the corresponding protein sequence was calculated using the tool ProtParam from ExPasy (Expert protein analysis system)[10]. Percentage of hydrophobic and hydrophilic residues was calculated manually based on their nature from the results obtained after primary structure analysis. ThePhysico-chemicalparameters,i.e.theoreticalisoelectric point (pI), molecular weight, the total number of positive and negative residues, extinction coefficient, half-life, instability index, AI and GRAVY was computed using the Expasy's ProtParam prediction server[11]. Secondary structure prediction was done by using SOPMA server[12,13]. The NetPhos-2.0 server was used for studying potential phosphorylation sites of the protein[14]. Further Signal P-4.1 server was used to denote the presence and location of signal peptide cleavage sites in given sequences[15].

2.3 3D Structure Prediction of MX Protein

Homology modeling is a computational structure prediction technique of proteins and is used to identify the structure of the query protein sequence based on one or more known available protein structures. The prediction is based on the alignment which maps residues with the query sequence under the template sequence[16-19]. Quality of protein structure depends on the relationship between target and template sequence by using online bioinformatics tool BLASTP[20,9]. The functionality of Protein is attained in its tertiary conformation, hence the 3D structure is most important for any kind of proteomics studies. MX protein of *Lates calcarifer* is an important target to study its association with betanodaviral infection. In the current course of work, there is an attempt to predict the 3D structure of MX protein by using Discovery studio 4.2[21].

2.4 Validation of Predicted 3D Structure

Predicted 3D structure is validated using 3D profiles verify algorithm available in DS 4.2 which was depicted in the form of Ramachandran Plot [Figure 6].

>gi|56684631|gb|AAW22002.1|MXProtein[*LatesCalcarifer*]
MNTLNQQYEEKVRPCIDLIDSLRSLGVEKDLALPAIAVIGDQSSGKSSVLEALSGVALPRGSGIVTRCPL
ELKMKRKKEGEEWYGKISYQDHEEEIEDPAHVEKKIREAQDEMAGVGVGISDDLISLEIASPDVPDLTLI
DLPGIARVAVKGQPENIGEQIKRLIQKFIKRQETISLVVVPCNVDIATTEALKMAQEVDPDGERTLGILT
KPDLVDKGTEETVVDIVHNEVIHLKKGYMIVKCRGQKEITEKVSLTEAMEREKAFFQDHVHFHTLYNDGH
ATVPKLAEKLTLELVHHIERSLPRLEEQIEEKLAQTQAEMERYGNGPPTDAAERLVYLIDKVTAFTQDAI
NLAAGEELQCGDKLNVFSTLRREFGKWYSQLDHSGQNFNRRIDREVEEYEERYRGRELPGFINYKTFEVM
VKEQIKQLEEPAVKKLKDIGDAVRKAFVQLAHSSFIGFPNLMKTAKTKIEAIKQEKESTAESTLRTQFKM
EMIVYSQDRTYSSSLSDRKREEDEDELKCPKFNTKRSIVHCMDNHATLQELMLHLKSYYSIASQRLADQI
PMVIRYQMLQESAVQLQREMLQMLQDKENLEFLLKEDFDIGHKRAALQSRLNRLMKARAYLVEF

Figure 1. Retrieval of Protein Sequences

3. Results

3.1 Retrieval of MX Protein Sequence

FASTA format of MX protein sequence is shown in Figure 1, which is taken from the Uniprot ID- Q2VTP1[5].

3.2 *In-silico* Characterization and Secondary Structure Prediction

Primary structure analysis of target protein revealed that most of the amino acids are negatively charged, polar and hydrophilic. The total number of negatively charged residues (Asp + Glu): 106 and the total number of positively charged residues (Arg + Lys): 88 Table 1.

Extinction coefficient at 280nm is 36705/m/cm assuming all pairs of Cys residues form cysteine and Ext. the coefficient is 36330 after assuming all Cys residues are reduced, this value indicates that this protein can easily be analyzed by UV spectral methods and can be very helpful in docking studies[12]. The bio computed half-life is 30 hours. The target protein is found to be stable as instability index is computed to be 43.77. The total number of atoms is 10085 and the atomic composition is given in Table 2.

Table 1. Amino acid composition of MX protein of *Lates Calcarifer*.

Amino acid	No. of amino acid	Composition
Ala (A)	41	6.6%
Arg (R)	37	5.9%
Asn (N)	17	2.7%
Asp (D)	37	5.9%
Cys (C)	7	1.1%
Gln (Q)	35	5.6%
Glu (E)	69	11.1%
Gly (G)	31	5.0%
His (H)	16	2.6%
Ile (I)	41	6.6%
Leu (L)	63	10.1%
Lys (K)	51	8.2%
Met (M)	18	2.9%
Phe (F)	18	2.9%
Pro (P)	21	3.4%
Ser (S)	33	5.3%
Thr (T)	29	4.6%
Trp (W)	2	0.3%
Tyr (Y)	17	2.7%
Val (V)	41	6.6%
Pyl (O)	0	0.0%
Sec (U)	0	0.0%
(B)	0	0.0%
(Z)	0	0.0%
(X) Bottom of Form	0 Bottom of Form	0.0%

Table 2. Atomic composition.

Carbon	C	**3142**
Hydrogen	H	5078
Nitrogen	N	872
Oxygen	O	968
Sulfur	S	25

Secondary structure prediction was done by using SOPMA server and secondary structure analysis revealed that MX protein has mixed secondary structure, i.e., mostly alpha-helix and beta-turn Figure 2 which shows secondary structure analysis by SOPMA thus it suggests as a better target against viral nervous necrosis disease.

The aliphatic index determines the thermal stability of the protein-based on the presence of alanine, valine, and leucine. Lower thermal stability of protein indicates a more flexible structure but here it is high enough (AI = 90.63)[22].

Grand average of hydropathicity (GRAVY) value for a protein is calculated as the total of hydropathy values of all amino acids, divided by the number of residues in the sequence (GRAVY = −0.504), this value of GRAVY indicates that this protein could result in better interaction with water also[23]. Further study in terms of secondary structure was predicted with the help of program SOPMA and it infers that this protein mostly has alpha-helices along with some beta-turn, thus this protein is structural. Phosphorylation is the addition of phosphate group to a protein or some other organic compounds and determination of the phosphorylation site is the major initial step in the explanation of any regulatory mechanism. Bioinformatics approach is one of the major approaches in the determination of phosphorylation sites among the other two genetic and biochemical approaches[24].

Phosphorylation is an important feature and it is dependent on the presence of serine, threonine and tyrosine residues in eukaryotic proteins Figure 3 shows the predicted phosphorylation sites in sequence. In MX protein we obtained the number of SERINE = 33, THREONINE = 29 and TYROSINE = 17 Figure 3 and Table 1.

Cleavage sites are specific peptide sequences, where site-specific protease cleave or cut the protein. These specific sites can be used to cleave off an affinity

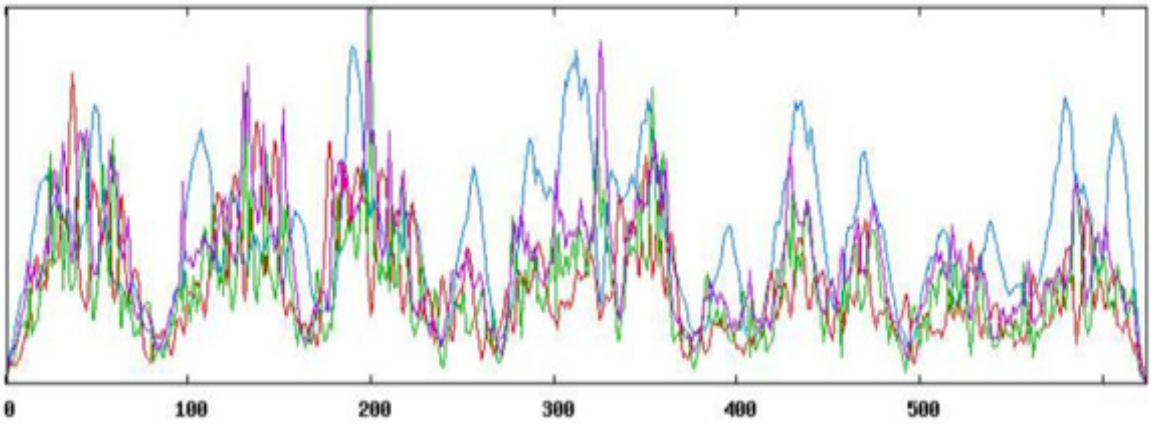


Figure 2. Result of SOPMA

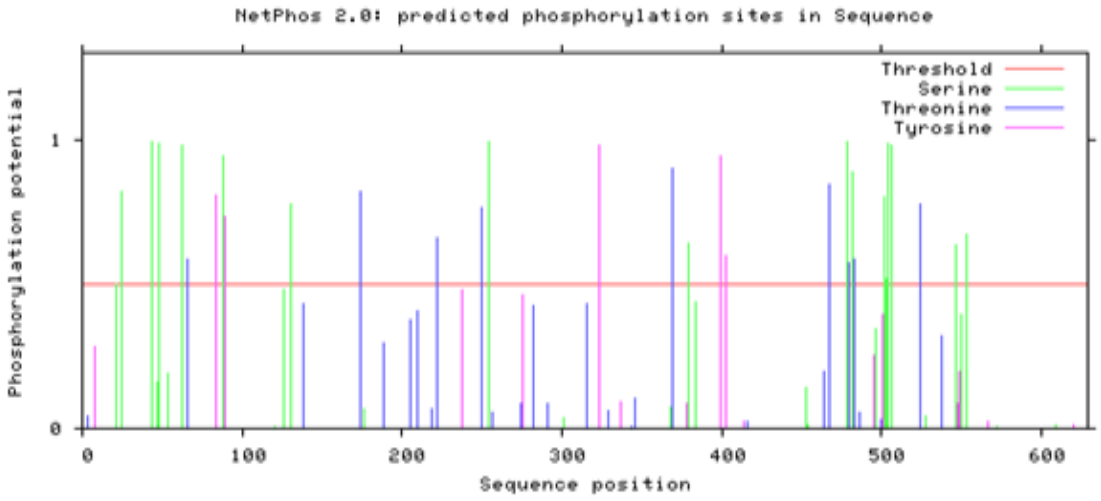


Figure 3. Result of NetPhos analysis

tag thereby restoring the natural protein sequence[25-27]. Figure 3 illustrates the signal peptide cleavage sites for MX through Signal P 4.1 server. Results are illustrated in terms of C (raw cleavage site score), S(signal peptide score) and Y (combined cleavage site scores)[9]. For MX protein C, S and Y scores are 0.1, 0.12, 0.1 respectively.

3.3 3D Structure Prediction of MX Protein

Biovia Discovery studio was used for the prediction of the 3D structure of query protein MX (accession number AAW22002.1). Based on the template protein 3ZYS(B) which is human dynamin, with a sequence identity of 57% and E value of 0.00 is selected. As we know that 3D structure is most important for the studies in relation with protein target, so this step is most important for finding drug against the virus[28]. The model with the DOPE (Discrete Optimization of Potential Energy) score –40190.90625 is selected as the best model[29]. This predicted structure reveals the detailed 3D structure of mx protein. MX 3D structure has eight alpha helices and seven beta pattern. Figure 5 (A) and (B) shows the 3D structure of MX Protein and it is shown by both Rasmol viewer and discovery studio. The helices are displayed in coiled shape in red, green, blue, yellow and sheets are displayed in red green blue, light blue and yellow.

3.4 Validation of the Predicted 3D Structure

Figure 6 (B) shows the verification of 3D structure by Ramachandran plot, which depicts that good quality of the model is expected to have over 90% in the most favoured region.

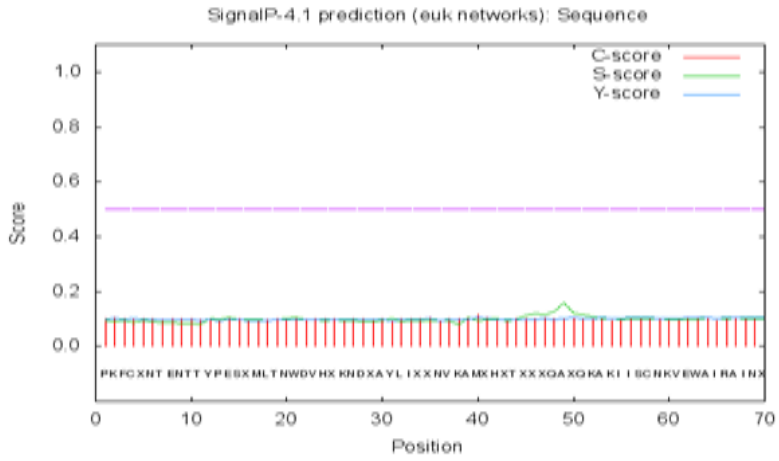
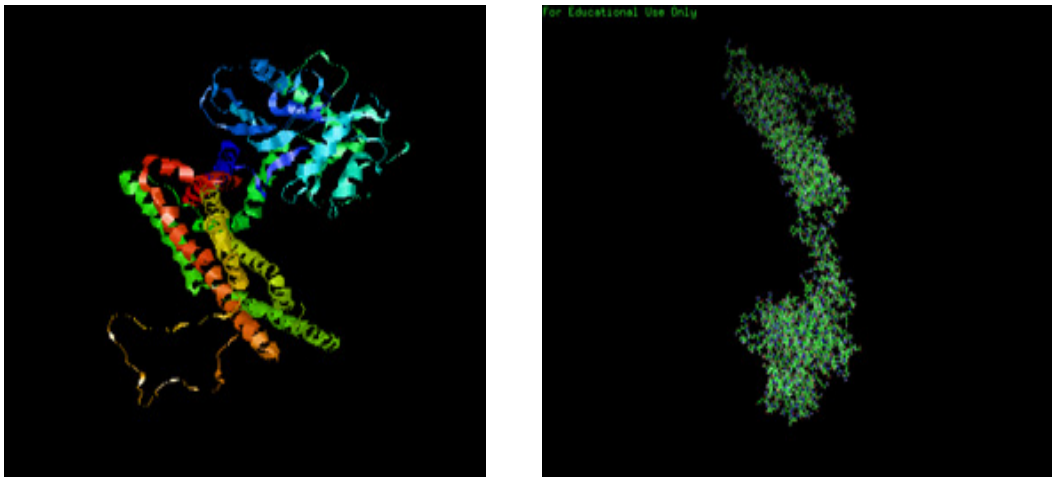
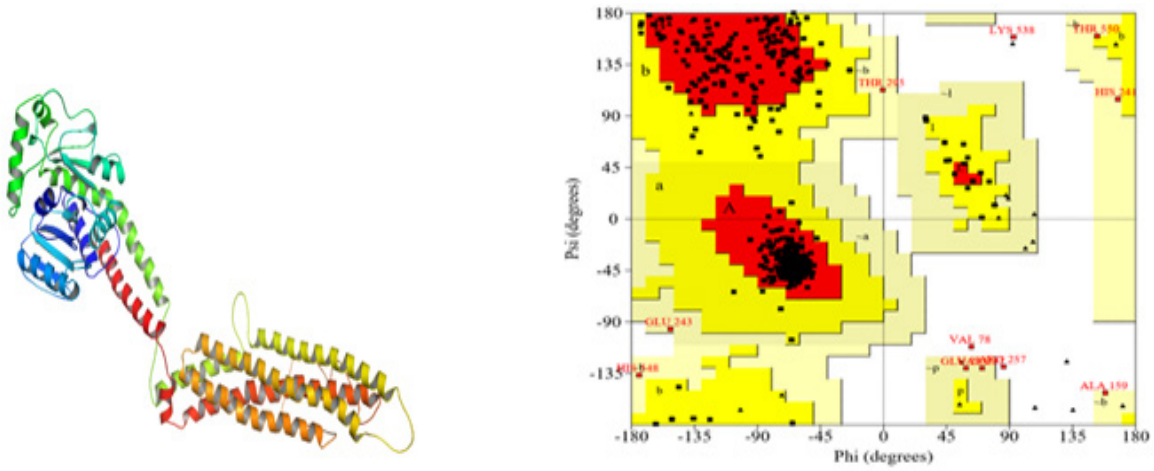


Figure 4. Result of Signal P analysis



(A) **(B)**

Figure 5. 3D structure of MX (A) Rasmol viewer (B) Discovery studio visualizer.



(A) **(B)**

Figure 6. (A) Modeled 3D structure of MX in the form of pattern and helices. (B) Ramachandran plot Showing 90% of Residues in favored regions.

4. Discussion and Conclusion

Primary structure analysis with the predicted 3D structure of MX protein reveals a very informative description of the nature of the protein. It is apt to discuss that proteins are the most important part of every functionality attain by living species. Viral infection is a major threat to the aquatic industry and specifically, Betanoda virus is a challenging group creating a disastrous effect on aquatic fauna[30-32]. Molecular models of this protein of *Lates Calcarifer* as documented in this study may provide a valuable aid for designing an inhibitor or better ligand against viral nervous necrosis disease and could play a significant role in prevention or cure for such fatal infections[33-35]. This may lead a relief to aquatic fauna from such microbial infection and hence may help raise the economic growth of nations.

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6. Conflict of Interest

The author declares no conflict of interest.

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