The Pharma Innovation Journal 2021; SP-10(4): 276-281

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; SP-10(4): 276-281

www.thepharmajournal.com
Received: 15-02-2021
Accepted: 19-03-2021

Rahul Singh
M.F.Sc., Department of Aquatic Animal Health Management, Kerala University of Fisheries and Ocean Studies, Panangad, Kochi, Kerala, India

Dr. Swapna P Antony
M.V. Sc, Assistant Professor, Department of Veterinary Physiology and Biochemistry, Veterinary College and Research Institute, Udumalpet, Tamil Nadu, India

Dr. Roshma Phlip
M.V. Sc, Post Graduate in Veterinary Pathology Field Veterinarian, Udumalpet, Tamil Nadu, India

Corresponding Author:
Rahul Singh
M.F.Sc., Department of Aquatic Animal Health Management, Kerala University of Fisheries and Ocean Studies, Panangad, Kochi, Kerala, India

Identification and molecular characterization of a histone derived antimicrobial peptide from *Catla catla*.

Rahul Singh, Dr. Swapna P Antony and Dr. Roshma Phlip

Abstract
A Cyprinids species *Catla catla*, being investigated as future aquaculture species in countries around the world. However they face a number of issues and limitations which will need to be overcome to ensure future stability and growth, one of which is disease outbreak. In the present study, gene based approach was employed for identifying AMP genesin freshwater fishes belonging to Family Cyprinidae. *Catla-catla*, were taken for identification and characterization of antimicrobial peptide genes present in these organisms. A novel Histone H2A derived AMP genes could be identified and characterized from *C. catla* from the current study. The AMP genes were the first report of these genes from this fish species. The AMP genes were named HIS-C1. An mRNA fragment of 124 bp encoding 41 amino acid corresponding to histone H2A gene could be amplified from *C. catla* (HIS-C1). Multiple alignment of these genes showed the presence of conserved regions in both nucleotide and amino acid sequences. Phylogenetic tree was constructed using histone H2A sequences and this also showed the basal placement of this AMP along with other histone identified from other fishes belonging to family Cyprinidae.

Keywords: AMPs, mRNA, *C. catla*

Introduction
Antimicrobial peptides (AMPs) are ancient and essential components of the fast acting innate immune system probably all classes of life. AMPs have been shown to possess activity against a wide range of microorganisims, including bacteria and fungi, and kill their targets multiple mechanisms mostly through membrane disruption. The present study was focused on identification of new AMP isoforms from Calta catla and its characterization at the molecular level. The present study was focused on identification of new AMP isoforms from fishes and its characterization at the molecular level. The identification of new AMPs in fresh water fishes especially those with culture importance will definitely bring interesting insight into its defense mechanisms as well as probable disease control in aquaculture systems.

Materials and Methods
Experimental animals
Healthy livespecimens of *Catla catla* were collected from College fish farm Kerala University of Fisheries & Ocean Studies, Kochi Kerala and were brought to the laboratory.

RNase control
Basic precautions
Some basic precautions were taken when working with RNA. These include:
Wearing gloves throughout experiments to prevent contamination from RNases found on most human hands. Changing gloves after touching skin, door knobs, and common surfaces. Having a dedicated set of pipettors that are used solely for RNA work. Using tips and tubes that are tested and guaranteed to be RNase-free. Using RNase-free chemicals and reagents and designating a "low-traffic" area of the lab that is away or shielded from air vents or open windows as an "RNase-free zone".
These common sense precautions helped to minimize RNase contamination problems.

RNase-free solutions, glassware and metalware
RNase free water and anticoagulant solutions were made RNase-free by treating with diethylpyrocarbonate (DEPC). Briefly, the solutions including anticoagulant and water, glasswares, metal wares and gloves were incubated with 0.1% DEPC at room temperature, overnight. This is followed by autoclaving the solution for 1 hr. to eliminate residual DEPC.

Blood collection
Blood was collected from the gills and intestine of *Catlla catla*, *Labeo rohita*, *Cyprinus carpio carpio* and *Carasius auratus* usingspecially designed capillary tubes (RNase-free) rinsed using pre-cooledanticoagulant solution (RNase free, 10% sodium citrate, pH 7.0). Haemolymph was suspended in TRI reagent (Sigma) for total RNA isolation.

RNA isolation
Total RNA was extracted from the haemocytes using TRI Reagent (Sigma) following manufacturer's protocol. Briefly, about 1 ml haemolymph was homogenized in 1 ml TRI Reagent. To ensure complete dissociation of the nucleoprotein complexes, samples were allowed to stand for 5 min at room temperature. Chloroform (0.2 ml per 1 ml TRI Reagent) was added to the homogenate, sample was covered tightly, vigorously shaken for 15 sec and allowed to stand for 2-15 min at room temperature. The resulting mixture was centrifuged at 12,000 x g for 15 min at 4 ^{0}C. Centrifugation separates the mixture into three phases: a red organic phase (containing protein), an interphase (containing DNA) and a colourless upper aqueous phase (containing RNA). The aqueous phase was transferred to a fresh tube and 0.5 ml isopropanol per ml TRI Reagent was added. The samples were allowed to stand for 5-10 min at room temperature and then centrifuged at 12000 x g for 10 min at 4 ^{0}C. The RNA precipitated out in the side and bottom of the tube was washed by adding 1 ml 75 % ethanol per 1 ml of TRI Reagent. The samples were vortexed and centrifuged at 7500 x g for 5 min at 4 ^{0}C. RNA pellets were dried for 5-10 min and dissolved in RNase free water by repeated pipetting with a micropipette at 55-60 ^{0}C for 10-15 min.

Determination of the quantity and quality of RNA
RNA was quantified and qualified by measuring optical density (O.D) at 260 and 280 nm in a UV spectrophotometer and visualizing RNA using electrophoresis. The ratio of absorbance at 260 nm and 280 nm is an indication of RNA quality. Only RNAs with absorbance ratios (A260: A280) greater than 1.8 were used for cDNA synthesis. For quantification of RNA, the O.D at 260 nm was taken and the concentration of RNA was calculated as follows:

1 O.D of RNA = 40 µg/ml
RNA concentration (µg/ml) = O.D at 260 nm x Dilution factor x 40

cDNA synthesis
Complementary DNA (cDNA) synthesis was performed using good quality RNA in a reaction by the enzyme reverse transcriptase. First stand cDNA was produced in a 20µl reaction volume containing 5µg total RNA, 1x RT buffer, 2µM dNTP, 2µM oligo d(T_{20}), 20 U of RNase inhibitor and 100 U of MMLV Reverse transcriptase. The reaction was carried out at 42^{o}C for 1 hrfollowed by an inactivation step at 85 ^{o}C for 15 min. the cDNA synthesized was stored at -20 ^{o}C until use.

PCR amplification
PCR amplification of cDNA were carried out using gene specific primer, desigedusing Gene Tool software based on consensus sequences of major AMP families present in freshwater fishes viz. Hepcidin, epinecidin, defensin, lateolabrax AMP, pleurocidin -1, pleurocidin-2 andhistone H2A derived AMPs. β-actin was used as the internal control to verify reverse transcription reaction. PCR amplification of1µl cDNA was performed in a 25µl reaction volume containing 1x standard Taq buffer (10mMTris-HCL, 50MKCL, pH 8.3), 200µM dNTPs, 0.4µM each primer and 1U Taq DNA polymerase. The thermal profile used was an initial denaturation at 94 ^{o}C for 2 min followed by 35 cycles of denaturation at 94 ^{o}C for 15 sec, and extension at 60 ^{o}C for 30 sec and a final extension at 68 ^{o}C for 10 min. for target genes. Annealing temperature varied for the different genes as given in (Table 2.). The PCR products were visualized by electrophoresis 1.5% agarose gel.

Table 1: Primers used for the study

Primer name	Target AMP Family	Annealing temp	Expected product size
Hepcidin	Hepcidin	60 ^{0}C	250 bp
Epinecidin	Epinecidin	60 ^{0}C	320 bp
Defensin	Defensin	60 ^{0}C	220 bp
Lateolabrax	Hepcidin	60 ^{0}C	250 bp
Pleurocidin -1	Pleurocidin	60 ^{0}C	250 bp
Pleurocidin -2	Pleurocidin	60 ^{0}C	250 bp
Histone H2A derived	Hiposin	60 ^{0}C	249 bp

Agarose gel electrophoresis
Electrophoresis was performed in 1.5% agarose gel prepared in 1 xTBE buffer (Tris-base -10.8 g, 0.5 M EDTA – 4ml, Boric acid – 5.5 g, double distilled water – 100 ml, pH – 8.0). To the melted agarose, 2µl ethidiumbromide (1mg/ml stock stored in dark) was added. After cooling to 45 ^{o}C, the agarose was poured on gel tray and was allowed to solidify. The gel tray with solidified agarose was then submerged in 1xTBE buffer, filled in a buffer tank. PCR product (10µl) was mixed with 2µl of 6x gel loading buffer (1% bromophenol blue-250 µl, 1% xylene cyanol-250µl, glycerol-300µl double distilled water-200µl) and loaded onto the well. Electrophoresis was done at a voltage of 3-5 volt/cm till the bromophenol blue dye front migrate to the middle of the gel. The gel was visualized on a UV transilluminator using the Gel Doc XR system and documented using Quantity One software (Bio-Rad Hercules, Ca).

Sequencing and sequence analysis
The plasmid DNA were sequenced using ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction kit using T7 and SP6 primers on an ABI Prism 377 DNA sequencer (Applied Biosystem) at SciGenom, Kochi, India. The nucleotide sequences obtained were analyzed using BioEdit and GeneToolsoftware. Open reading frame of the sequences were found out using GeneTool software. Gene translation and prediction of deduced protein were performed with ExPASy(http://www.au.ExPASy.org/) and GeneToolsoftware. Molecular weight and isoelectric point of the target AMP were predicted using ProtParam tool of ExPASyprogramme. Conserved domains/motifs/pattern search were also found out using Motif Scan and ScanProsite tool of ExPASyprogramme. Homology searches of the nucleotide and deduced amino acid sequences were performed using BLASTn and BLASTp tools (Altschul *et al.*, 1990) at the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/blast). The signal peptide was predicted by SignalP program (http://www.au.ExPASy.org/). Similar sequences were

retrieved from the GenBank database and multiple sequence alignment of the peptides was performed with ClustalW, BioEdit and GeneDoc computer programs.Amino acid sequences of the target genes were retrieved from the NCBI GenBank and phylogenetic tree was constructed by the Neighbor-Joining (NJ) method using MEGA version 5.05 (Tamura *et al.*, 2007). Bootstrap analysis was based on 1000 replicates. The nucleotide sequence and the deduced amino acid sequences were submitted to GenBank. The physico-chemical properties and amino acid composition of the synthetic peptide were predicted and confirmed using ProtParam-tool of ExPASy programs (http://www.au.expasy.org/). The hydrophobicity (<H>) and program (http://heliquest.ipmc.cnrs.fr).Data for secondary structure and spatial organization of the obtained amino acid sequence was generated using SWISS – MODEL, a homology based protein modelling server (Guex and Peitsch, 1997; Schwede *et al.*, 2003 and Arnold *et al.*, 2006). Using the data generated by SWISS-MODEL, 3-Dimensional structure of obtained amino acid sequence was predicted using PyMOL software. The secondary structure analysis of the peptides was also carried out using STRIDE (http://webclu.bio.wzw.tum.de/cgibin/ stride/stridecgi.py).

Results

Molecular characterization and phylogenetic analysis of a Histone derived antimicrobial peptide from the Major carp, *Catla catla* (HIS-C1)

Nucleotide sequence analysis of HIS-C1

The present study report Histone sequences from Major carp, *Catla catla*, hereinafter designated as HIS-C1. A partial mRNA transcript of 124 bp belonging to the Histone H2A family of AMPs encoding 41 amino acids could be amplified from the mRNA of*Catla catla* blood cells by RT-PCR. BLASTn analysis confirmed the identity of sequences as histone H2A. The nucleotide sequence encoding 41amino acids was translated using GeneTool software and ExPASy programmes. BLASTp analysis also confirmed the identity of the amino acid sequences as histone H2A. The nucleotide and deduced amino acid sequence of the amplicon are shown in (Figure 3.). Multiple-sequence alignment of the amino acid sequence of the peptide with previously reported histone H2A derived AMPs revealed that the first 41 amino acid sequence at the N-terminal of the deduced peptide showed similarity to histone derived AMPs i.e. Histone H2A in *Cyprinus carpio, Sinocyclocheilus rhinocerous, Sinocyclocheilus graham, Ctenopharyngodon idella* and *Mylopharyngodon piceus.*

```
gccaagcgcgtcggtgctggtgctccggtttatctggcggctgtgctcgagtatctaacc
 A  K  R  V  G  A  G  A  P  V  Y  L  A  A  V  L  E  Y  L  T
gctgagatcttgcaagttggctggaaacgccgcaagggacaacaagaagactcgcatcat
 A  E  I  L  Q  V  G  W  K  R  R  K  G  Q  Q  E  D  S  H  H
cccc
 P
```

Fig 1: Nucleotide and deduced amino acid sequence of histone H2A fromHIS-C1

Amino acid sequence analysis of HIS-C1

The deduced 41 amino acid sequence of HIS-C1 was found to be rich in amino acid residues Alanine (A) 14.6% and Glycine (G) 9.8% and Valine (V) 9.8% and Arginine (R) 7.3% Histidine (H) 4.9% and Glutamic (E) 7.3% and Glutamine (Q) 7.3% and Leucine (L) 9.8% the molecular weight is 4.54 kDa and a theoretical isoelectric point (pI) is 9.40 as predicted by PROTPARAM software. The hydropathicity is -0.454.

Basic Local Alignment Search Tool (BLAST)

BLAST analysis of the nucleotide sequence showed that it belonged to LOC107686736 superfamily, the family of Histone H2A, confirming the sequence to be an isoform of HIS-C1. BLAST analysis of the nucleotide and amino acid sequence revealed the relation of HIS-C1 gene to other isoforms of Histone H2A present in *Sinocyclocheilus anshuiensis, Cyprinus carpio, Mylopharyngodon piceus, Sinocyclocheilus rhinocerous, Ctenopharyngodon idella, S. grahami, Hypophthalmichthys nobilis,S. anshuiensi* and *Danio rerio*.

BLASTn analysis of HIS-C1

Analysis of the nucleotide sequence revealed that sequence shared 91 % similarity to aHistone H2A isoform of *Sinocyclocheilus anshuiensis* for aquery coverage of 100% followed by similarity to another isoform Histone H2A of *Cyprinus carpio.* The similarity was also found to Histone H2A isoform from H2A of *Mylopharyngodon piceus, Sinocyclocheilus rhinocerous,* and *Ctenopharyngodon idella.* However, for these isoforms the query coverage was found to be less than 90 %.

BLASTp analysis of HIS-C1

Analysis of the amino acid sequences of the Histone H2A, also confirmed the above results (Table 5a.). Multiple alignments of nucleotide and amino acid sequences of Histone with a high degree of similarity and with other known Histone H2A of fishes showed the presence of conserved sequences for these AMPs. Multiple alignments confirmed the results of the BLAST analysis, where maximum similarity was with Histone H2A isoforms of *Cyprinus carpio* (Fig. 1.). Sequencecomparison using BLAST algorithm showed that the deduced amino acid sequence of HIS-C1 shared significant identity with *Triacanthus sp.* having 96% and *Balanoglossus clavigerus* 96% with 100% Query cover.

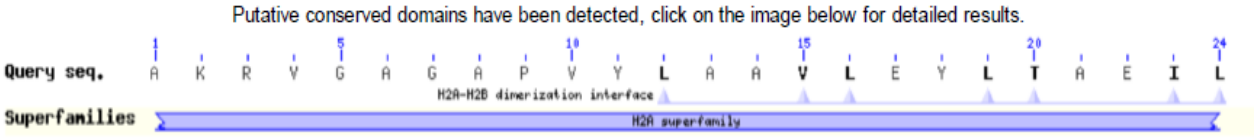
Multiple alignment of HIS-C1

Sequence comparison using BLAST algorithm showed that the deduced amino acid sequence of HIS-C1 shared significant identity with Histone H2A of *Triacanthus sp.* (96%) *Balanoglossus clavigerus* (96%) and *Labrus bergylta* (96%). Multiple alignment of HIS-C1 with other Histone H2A of cyprinids revealed the presence of conserve regions within the sequence and also revealed maximum identity to that of *Cyprinus carpio* and *Sinocyclocheilus anshuiensi.* The signal peptide region of HIS-C1 was not found to be highly conserved as in the case of other AMP families. HIS-C1 showed variation in at four, eighteen, thirty and thirty nine amino acid positions in their signal peptide region. Whereas, the mature peptide region of HIS-C1 shared greater similarities among each other, except at the 9rd 12th, 21th, 24th and 24th amino acid positions (Fig.1.).

Table 2a: Result of BLASTn analysis of HIS-C1 in *Catla catla*

Genbank Accession Number	Description	Query Coverage	E-Value	% Identity
XM_016483837.1	PREDICTED: *Sinocyclocheilus anshuiensis* histone H2A-like (LOC107686736), mRNA	100%	5e-39	91%
XM_019103060.1	PREDICTED: *Cyprinus carpio* histone H2A-like (LOC109088919), mRNA mRNA	96%	2e-38	92%
KJ704982.1	*Mylopharyngodon piceus* histone H2A mRNA, partial cds	100%	6e-38	90%
XM_016562367.1	PREDICTED: *Sinocyclocheilus rhinocerous* histone H2A-like (LOC107747778), mRNA	96%	7e-37	91%
KJ704983.1	*Ctenopharyngodon idella* histone H2A mRNA, partial cds	96%	7e-37	91%

Table 2b: Result of BLASTp analysis of HIS-C1 in *Catla catla*



Genbank Accession Number	Description	Max Score	Total Score	Query Coverage	% Identity
AEJ86581.1	histone H2A [*Triacanthus sp.* RP-2011]	71.5	71.5	100%	2e-13
CBM82479.1	histone H2A-XXIII protein [*Balanoglossus clavigerus*]	72.3	72.3	100%	2e-13
XP_002595665.1	hypothetical protein BRAFLDRAFT_64799 [*Branchiostoma floridae*]	72.3	72.3	100%	2e-13
XP_014134174.1	PREDICTED: LOW QUALITY PROTEIN: histone H2A, sperm [*Falco cherrug*]	72.3	72.3	100%	2e-13
XP_013802431.1	PREDICTED: histone H2A-like [*Apteryx australis mantelli*]	71.0	71.0	100%	4e-13

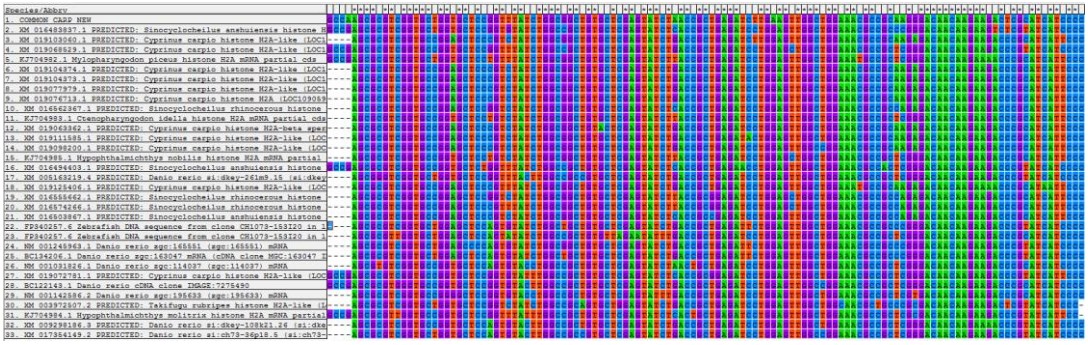


Fig 2: ClustalW multiple alignment of HIS-C1 with *Sinochceilus anshuiensis Cyprinus carpio, Mylopharyngodon piceus, Ctenopharyngodon idella,Hypophthalmichthys nobilisand Danio rerio* Histone H2A performed using MEGA 5.0 software.

Phylogenetic analysis of HIS-C1

The bootstrap distance tree calculated for the resulting Histone sequences confirmed that HIS-C1 possessed more similarity to that of other Histone H2A from *Hypophthalmichthys nobilis*, than to the Histone H2A of *Ctenopharyngodon idella*, *Mylopharyngodon piceus*, *Cyprinus carpio*, *Sinocyclocheilus anshuiensis* and *S. rhinocerous*Histone H2A (Fig. 2. ph. tree). Phylogenetic tree drawn based on known amino acid sequences of fish Histone H2A could be divided into two major groups, Group I again classified in three sub-groups which one of them include histone from Major carp *(Catla catla)* closely related with *Hypophthalmichthys nobilis*, *Ctenopharyngodon idella* and *Mylopharyngodon piceus* histone H2A can see in (Fig. 2.).

The phylogenetic relationship of the HIS-C1 sequence with all known Histone H2A with other fishes Histone-like peptides. The tree could be two major groups. Through the tree could be classified into major groups, but interestingly, it was found that in each group of Histone of fishes shared close similarity with other fishes Histone peptides.

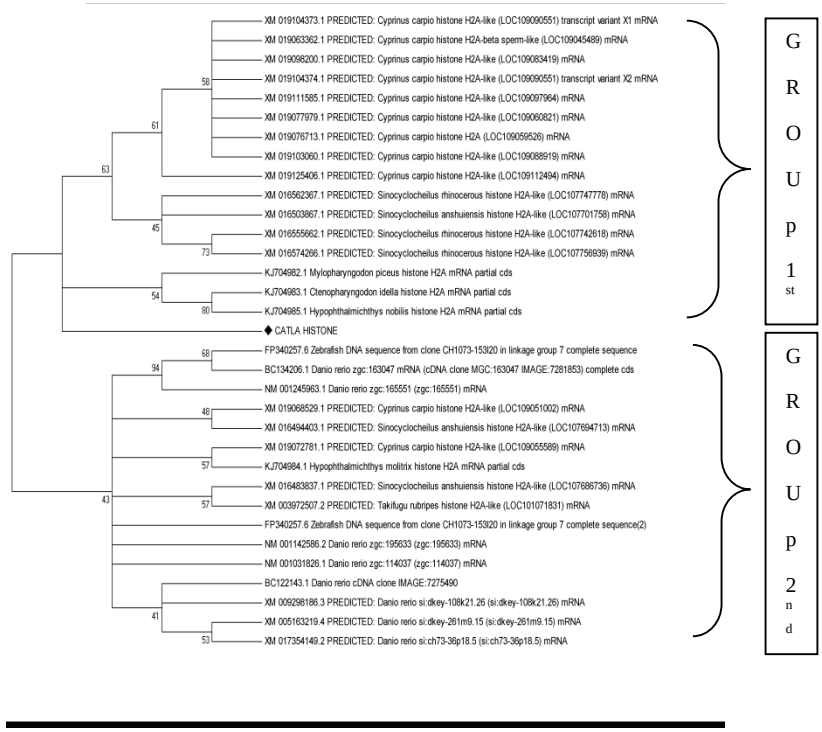


Fig 3: Bootstrapped Neighbour-Joining Tree obtained using MEGA version 5.0 illustrating relationships between the deduced amino acid sequences of the HIS-C1 with other Histones of cyprinids fishes. Values at the node indicate the percentage of times that the particular node occurred in 1000 and 100 trees generated by bootstrapping the original deduced protein sequences for the NJ and ML tree respectively.

Discussion

The *Catla catla* (HIS-C1) Histone H2A AMP gene possessed 124 nucleotides encoding 41 amino acids. The *C. catla* prepropeptide sequence aligment with selected vertebrate species showed highest similarity within the mature peptide region. To determine the relationship of the *Catla catla* Histone H2A with known vertebrate Histone genes, a phylogenetic approach was undertaken which included Histone predicted using the available genomes and that had been characterized in over 40 species of fish (Shike *et al.*, 2002; Masso-Silva & Dimond, 2014; Gong *et al.*, 2014). The phylogenetic tree clearly separated a number of the fish Histone into two distinct groups, whereas the Histone H2A of *C. catla* (HIS-C1), is placed into the group Ist alongside the *Hypophthalmichthys nobilis*, *Ctenopharyngodon idella*, *Mylopharyngodon piceus*, *Sinocyclocheilus rhinocerousan* and *Cyprinus carpio.*

This investigation characterizes important Histone peptides from Cyprinids fish *Catla catla.* AMPs are known to be a major component of a fishes innate immune system protecting them from pathogenic infections. Therefore, further studies into these molecules will be useful to help maintain or improve fish health within aquaculture (Falco *et al.*, 2009; Cabello *et al.*, 2013). As with other species, the fish peptides exhibit broad-spectrumantimicrobial activity, killing both fish and human pathogens. They are alsoimmunomodulatory, and their genes are highly responsive to microbes and innateimmuno-stimulatory molecules. Recent research has demonstrated that some of the uniqueproperties of fish peptides, including their ability to act even in very high saltconcentrations, make them good potential targets for development as therapeuticantimicrobials. Further, the stimulation of their gene expression by exogenous factorscould be useful in preventing pathogenic microbes in aquaculture.

Conclusion

Investigating novel AMP from fresh water organisms can provide new insight into the immune response of these organisms and a possibility of discovering new and effective drugs for potential application in medicine/aquaculture. The major objective of the study includes screening of freshwater fishes belonging to Family Cyprinidae for the presence of AMP genes, molecular characterization of antimicrobial peptides and its phylogenetic analysis. The present study was conducted at the Department of Marine Biology, Microbiology and Biochemistry, School of Marine Sciences, CUSAT, Kochi. Experimental organisms were collected from College Fish Farm, Kerala University of Fisheries and Ocean Studies, Kochi. Live samples were brought to the laboratory for performing further experiments. Gene based approach was employed for identifying AMP gene present in freshwater fishes belonging to Family Cyprinidae. Samples screened for the AMP screening include *Catla catla.* These experimental organisms were screened against Hepcidin, Piscidin, Pleurocidin-1, Pleurocidin-2, Defensin and Epinecidin primers designed specifically for amplifying AMP genes present in fishes. Among the four organisms screened, AMPs belonging to Histone H2A derived peptide family, could be detected in three Cyprinidis viz. *Carassius auratus*, *Cyprinus carpio carpio*, and *Catla catla.*

The results obtained are summarized as follows:

1. Histone H2A derived AMP derived AMP from the major carp, *Catla catla.*

a. The study presents first report of a histone H2A AMP to be identified from the Major carp, *C. catla.* An mRNA fragment of 124 bp encoding 41 amino acid corresponding to histone H2A gene could be amplified from the *C. catla.*

b. The peptides possessed a predicted molecular weight of 4.54 kDa and the theoretical isoelectric point (pI) is 9.40.

The peptide was found to be rich in amino acid residues Alanine (A) 14.6 % and Glycine (G) 9.8 % and Valine (V) 9.8 % and Arginine (R) 7.3 % and possessed a hydropathicity of -0.454.

c. Multiple alignment showed the presence of conserved regions in both nucleotide and amino acid sequences. In Phylogenetic tree constructed using histone H2A equences also showed the basal placement of this peptide along with other histone identified from Family Cyprinidae.

References

1. Arrighi RB, Nakamura C, Miyake J, Hurd H, Burgess JG. Design and activity of antimicrobial peptides against sporogonic-stage parasites causing murine malarias. Antimicrobial Agents and Chemotherapy. 2002;46(7):2104-2110.
2. Bao B, Peatman E, Li P, He C, Liu Z. Catfish hepcidin gene is expressed in a wide range of tissues and exhibits tissue-specific upregulation after bacterial infection. Developmental & Comparative Immunology 2005;29(11):939-950.
3. Barnes AC, Trewin B, Snape N, Kvennefors ECE, Baiano JC. Two hepcidin-like antimicrobial peptides in Barramundi Lates calcarifer exhibit differing tissue tropism and are induced in response to lipopolysaccharide. Fish & Shellfish Immunology. 2011;31(2):350-357.
4. Boman HG. Antibacterial peptides: key components needed in immunity. Cell, 1991;65(2):205-207.
5. Brogden KA. Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria?. Nature Reviews Microbiology. 2005;3(3):238-250.
6. Browne MJ, Feng CY, Booth V, Rise ML. Characterization and expression studies of Gaduscidin-1 and Gaduscidin-2; paralogous antimicrobial peptide-like transcripts from Atlantic cod (Gadus morhua). Developmental & Comparative Immunology 2011;35(3):399-408.