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# Identification and molecular characterization of a histone derived antimicrobial peptide from *Cyprinus carpio*

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**Abstract**
A Cyprinids family species including *Catla catla, Labeo rohita, Cirrhinus mrigala, Ctenopharyngodon idella, Hypopthalmichthys molitrix, H. nobilis, Cyprinus carpio* and *Carassius auratus* are 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. However, very little has been done to understand the immune system of fishes and very few immune genes have been characterized. Antimicrobial peptides (AMP) are naturally occurring low molecular weight polypeptides that play a major role in an organism's immune system and act effectively as a first line of defence. In the present study, gene based approach was employed for identifying AMP genes in freshwater fishe *C. carpio carpio* were taken for identification and characterization of antimicrobial peptide genes present in this organisms. A novel Histone H2A derived AMP genes could be identified and characterized from *C. carpio carpio* from the current study. The AMP genes were the first report of this gene from this fish specie. The AMP genes were named HIS-K1. An mRNA fragment 166 bp encoding 55 amino acid corresponding to histone H2A genes could be amplified from the koi carp, *C. carpio* (HIS-K1), multiple alignment of this gene 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. Examination of AMPs in significant fish families such as Cyprinidae, possessing aquaculture importance as food fish or ornamental fishcan help understand the role of these peptides in innate host defense of the fish. Furthermore, they can provide important information for the better design of novel therapeutic agents, both for microbial infections as well as other conditions. Unique for the field of fish AMPs is the potential application to aquaculture.

**Keywords:** AMPs, mRNA, *Cyprinus carpio carpio,* PRRs

## Introduction
In fishes, the innate immune system represents the first and primary line of defense against pathogens. It is considered as an evolutionarily conserved and fundamentally important defense mechanism. The innate immune system relies heavily on pattern recognition receptors (PRRs) to distinguish between self and non-self and recognize pathogens (Takeda and Akira, 2005; Rebl *et al.*, 2010). AMPs have been shown to possess activity against a wide range of microorganisms, including bacteria and fungi, and kill their targets by multiple mechanisms mostly through membrane disruption. These peptides are gene-encoded and either constitutively expressed or up-regulated during an infection. Additionally, transcriptional regulation of AMPs is often species specific and dependent on cell type (Hancock and Sahl, 2006) [6].

Fish are continually exposed to aquatic pathogens and secrete a wide range of AMPs as a primary defense mechanism. Teleost fish express a number of AMPs in mucus and blood as well as a variety of organs that are important for immune defenses including kidney, spleen, intestine, gills, reproductive organs and eyes. The increased expression of AMPs after bacterial induction highlights the important role these peptides play as a primary defense mechanism in fish innate immunity. Despite the many positive aspects of natural AMPs, many barriers still exist in their road to therapeutic application. Moreover, the antimicrobial activity of AMPs is antagonized to variable extents by cellular and physiological components (cationic salts) in mucus, blood, and tissue (Brogden *et al.*, 2003; Hancock and Sahl, 2006; Kruse and Kristensen, 2008) [6].

Consequently, most clinical trials to date have focused on AMPs for topical applications.

Therefore, the aim of the present study was to contribute to the knowledge on the functioning of the defense system of fresh water fish *Cyprinus carpio* in terms of AMPs. 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. The present study was undertaken with the following objectives: Screening of antimicrobial peptides in freshwater fishes belonging to Family Cyprinidae. Molecular characterization of the identified antimicrobial peptides. Phylogenetic analysis of the identified antimicrobial peptides.

## Materials and Methods

### Experimental animals

Healthy live specimens of *Cyprinus carpio* were collected from College fish farm Kerala University of Fisheries & Ocean Studies, Kochi, Kerala and were brought to the laboratory.

### RNase-free solutions, glassware and metal ware

RNase free water and anticoagulant solutions were made RNase-free by treating with diethylpyrocarbonate (DEPC) and were further autoclaved.

### Blood collection

Blood was collected from the gills and intestine of *Catlla catla*, *Labeo rohita*, *Cyprinus carpio carpio* and *Carasius auratus*. 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. And 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 incubate for 2-15 min at room temperature. The resulting mixture was centrifuged at 12,000 x g for 15 min at 4 $^0$C. 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 incubate 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. 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 and histone H2A derived AMPs. β-actin was used as the internal control to verify reverse transcription reaction. The thermal profile used was an initial denaturation at 94$^o$C for 2 min, and a final extension at 68 $^o$C for 10 min followed by 35 cycles. Annealing temperature varied for the different genes as given in (Table 1.). 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. The gel was visualized on a UV trans illuminator 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 Big Dye Terminator Cycle Sequencing Ready Reaction kit using T7 and SP6 primers on an ABI Prism 377 DNA sequencer (Applied Bio system) at SciGenom, Kochi, India. The nucleotide sequences obtained were analysed using Bio Edit and GeneToolsoftware. Open reading frame of the sequences were found out using Gene Tool software. Gene translation and prediction of deduced protein were performed with ExPASy (http://www.au.ExPASy.org/) and Gene Tool software. 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 Centre for Biotechnology Information

(NCBI) (http://www.ncbi.nlm.nih.gov/blast). The signal peptide was predicted by Signal P program (http://www.au.ExPASy.org/). Similar sequences were retrieved from the Gen Bank database and multiple sequence alignment of the peptides was performed with Clustal W, Bio Edit and Gene Doc computer programs. Amino acid sequences of the target genes were retrieved from the NCBI Gen Bank 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 Gen Bank. 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 Koi carp, *Cyprinus carpiocarpio* (HIS-K1)

### Nucleotide sequence analysis of HIS-K1

The present study report Histone sequences from Koi carp *Cyprinus carpiocarpio*, hereinafter designated as HIS-K1.A partial mRNA transcript of 166 bp belonging to the Histone H2A family of AMPs encoding55 Amino acids from the mRNA of *Cyprinus carpio carpio* blood cells by RT-PCR. BLASTn analysis confirmed the identity of sequences as histone H2A. The nucleotide sequence encoding 55 amino acids was translated using Gene Tool 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 1.).Multiple-sequence alignment of the amino acid sequence of the peptide with previously reported histone H2A derived AMPs revealed that the first 55 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.*

```
tgccgttccgcgtcagtcgtgttcgagacttctcctcaagggaactacgcccagggcgtc
 C  R  S  A  S  V  V  F  E  T  S  P  Q  G  N  Y  A  Q  G  V
ggtgccggagctcccgtctatctggcggctgtgctcgagtatctgaccgctgagatcctg
 G  A  G  A  P  V  Y  L  A  A  V  L  E  Y  L  T  A  E  I  L
gagttggctggaaacgccgcaagagacaacaagaagacccgcatca
 E  L  A  G  N  A  A  R  D  N  K  K  T  R  I
```

### Amino acid sequence analysis of HIS-K1

The deduced 55 amino acid sequence of HIS-K1 was found to be rich in amino acid residues Alanine (A) 18.2% and Glycine (G) 9.1% and Val (V) 9.1% and Arginine (R) 5.5% and Asn (N) 5.5% the molecular weight is 5.75 kDa and a theoretical isoelectric point (pI) is 6.30 as predicted by PROTPARAM software. The hydropathi city is 0.44.

### Analysis using Basic Local Alignment Search Tool (BLAST)

BLAST analysis of the nucleotide sequence showed that it belonged to LOC109088919 superfamily, the family of Histone H2A, confirming the sequence to be an isoform of HIS-K1. BLAST analysis of the nucleotide and amino acid sequence revealed the relation of HIS-K1 gene to other isoforms of Histone H2A present in *Cyprinus carpio, Sinocyclocheilus rhinocerous, S. grahami, S. anshuiensis, Danio rerio, Poecilia formosa, P. mexicana* (Table 3a. and 3b.).

### BLASTn analysis of HIS-K1

Analysis of the nucleotide sequence revealed that sequence shared 95% similarity to Histone H2A isoform of *Cyprinus carpio* for a query coverage of 100% followed by similarity to another isoform Histone H2A of *Sinocyclocheilus grahami.* The similarity was also found to Histone H2A isoform from H2A of *Sinocyclocheilus rhinocerous, Ctenopharyngodon idella* and *Mylopharyngodon piceus.* However, for these isoforms the query coverage was found to be less than 90% (Table 3a.).

### BLASTp analysis of HIS-K1

Analysis of the amino acid sequences of the Histone H2A, also confirmed the above results (Table 3b.). 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. 2.). Sequence comparison using BLAST algorithm showed that the deduced amino acid sequence of HIS-K1 shared significant identity with *Mus musculus* having 93% and *Labrus bergylta* 95% with 100% query coverage.

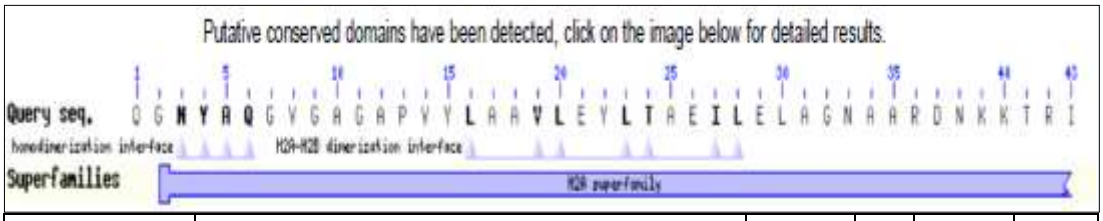
### Multiple alignment of HIS-K1

Sequence comparison using BLAST algorithm showed that the deduced amino acid sequence of HIS-K1 shared significant identity with Histone H2A of *Cyprinus carpio* (89-94%) *Sinocyclocheilus rhinocerous* (91-92%) and *S. grahami* (92-85 %). Multiple alignment of HIS-K1 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 rhinocerous.* The signal peptide region of HIS-K1 was not found to be highly conserved as in the case of other AMP families. HIS-K1 showed variation in at four, ten and fifteen amino acid positions in their signal peptide region. Whereas, the mature peptide region of HIS-K1 shared greater similarities among each other, except at the 8$^{rd}$ 10$^{th}$, 11$^{th}$, 12$^{th}$ and 14$^{th}$ amino acid positions (Fig. 2.).

**Table 3:** Result of BLASTn analysis of HIS-K1 in *Cyprinus carpio*

| Genbank Accession Number | Description | Query Coverage | E-value | % Identity |
|---|---|---|---|---|
| XM_019103060.1 | PREDICTED: *Cyprinus carpio* histone H2A-like (LOC109088919), mRNA | 100% | 6e-65 | 95% |
| XM_019077979.1 | PREDICTED: *Cyprinus carpio* histone H2A-like (LOC109060821), mRNA | 100% | 7e-64 | 94% |
| XM_016531615.1 | PREDICTED: *Sinocyclocheilus rhinocerous* histone H2A-like (LOC107723099), mRNA | 100% | 2e-58 | 92% |
| XM_016494487.1 | PREDICTED: *Sinocyclocheilus anshuiensis* histone H2A-like (LOC107694801), mRNA | 100% | 8e-57 | 91% |
| NM_001142586.2 | *Danio rerio* zgc:195633 (zgc:195633), mRNA | 100% | 1e-47 | 87% |

**Table 3b:** Result of BLASTp analysis of HIS-K1 in *Cyprinus carpio*




| Genbank Accession number | Description | Max score | Total Score | Query Coverage | % Identity |
|---|---|---|---|---|---|
| NP_783589.1 | histone cluster 1, H2aa [*Mus musculus*] | 83.6 | 83.6 | 100% | 1e-19 |
| AJP81889.1 | histone H2A [*Acipenser gueldenstaedtii*] | 82.4 | 82.4 | 100% | 1e-19 |
| XP_020511994.1 | histone H2A-like [*Labrus bergylta*] | 82.8 | 82.8 | 100% | 2e-19 |
| AEJ86580.2 | histone H2A [*Himantura sp*. RP-2011] | 80.9 | 80.9 | 100% | 3e-19 |
| XP_017546000.1 | PREDICTED: histone H2A-like [*Pygocentrus nattereri*] | 80.9 | 80.9 | 100% | 6e-19 |

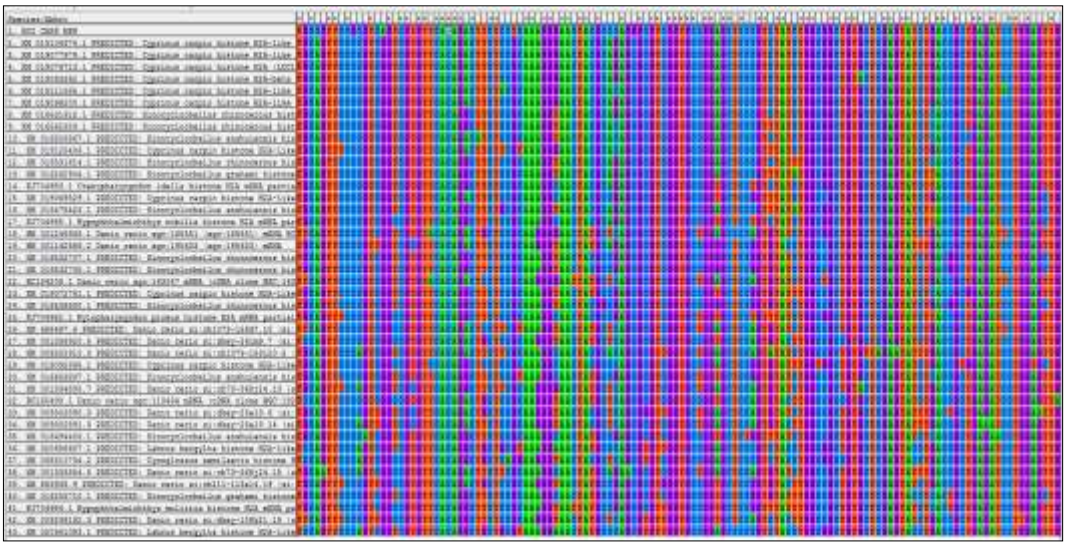


**Fig 2:** Clustal W multiple alignment of HIS-K1 with *Sinochceilus rhinocerous S. anshuiensis Ctenopharyngodon idella Hypophthalmichthys nobilis Danio rerio* Histone H2Aand *S. grahami* Histone H2A performed using MEGA 5.0 software.

**Phylogenetic analysis of HIS-K1**

The bootstrap distance tree calculated for the resulting Histone sequences confirmed that HIS-K1 possessed more similarity to that of other Histone H2A from *Cyprinus carpio*, than to the Histone H2A of *Sinocyclocheilus anshuiensis, S. rhinocerous and S. grahami* Histone H2A (Fig. 3. 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 17 sub-groups which one of them include histone from Koi carp *(Cyprinus carpio)* closely related with common carp*(Cyprinus carpio)* histone H2A can be seen in (Fig. 3).

The phylogenetic relationship of the HIS-K1 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.

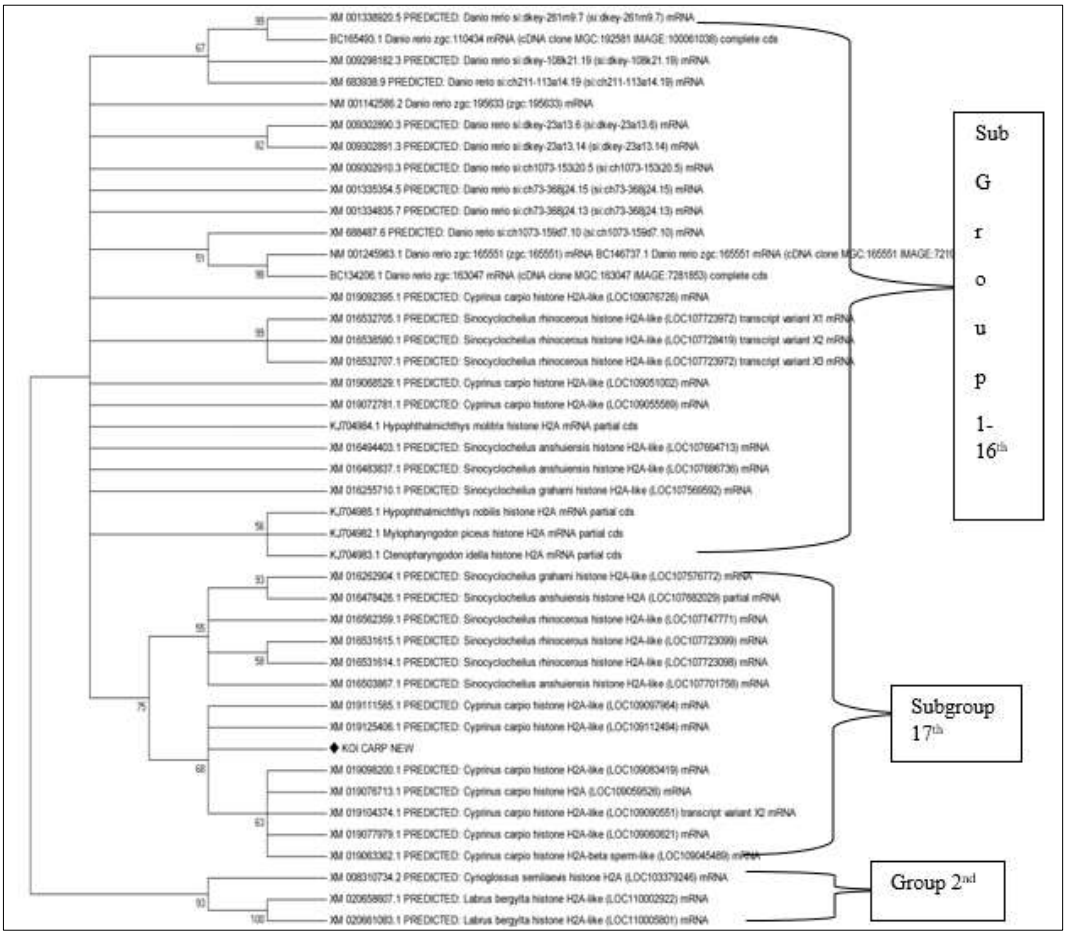



**Fig 3:** Bootstrapped Neighbor-Joining Tree obtained using MEGA version 5.0 illustrating relationships between the deduced amino acid sequence of the HIS-K1 with other Histones of cyprinid fishes. Values at the node indicate the percentage of times that the particular node occurred in 1000 and 100 trees generated by bootstrapping the original deduced protein sequences for the NJ and ML tree respectively

## Discussion

Histone proteins or derived fragments have antimicrobial activity in vertebrates ranging from fish to humans. Histone antimicrobial activity was first demonstrated in 1958 for histones A and B purified from calf thymus, which exhibited antibacterial activity against various Gram-positive and Gram-negative bacteria (Hirsch, 1958). It was not until the 1990s that other groups described such activity. In several fish, antimicrobial histone proteins have been detected in skin mucus or liver tissue: H2B-like proteins in catfish skin Robinette *et al.*, (1998), H2A in trout skin Femandes *et al.*, (2002), and H1 in the Atlantic salmon liver (Richard and Neil, 2001). Histone proteins are primarily involved in DNA packaging and regulation of DNA replication and transcription. These proteins form the basic building blocks of chromatin structure when the four core histone proteins, H2A, H2B, H3 and H4, come together as heterodimers to constitute the nucleosome. The core histone proteins are highly conserved between species. Histone H1 is the linker that condenses the nucleosomes and exhibits greater sequence variability. Many reports have shown that histone proteins or histone-derived peptides from various vertebrates possess antimicrobial activity. The antimicrobial properties of histones or histone-derived fragments have been described for various vertebrate species. Histone-derived AMPs have been identified in some fish species also, with broad-spectrum activity against both human and fish pathogens Noga *et al.*, (2011), including water molds Robinette *et al*, (1998), and a parasitic din flagellate (Noga *et al.*, 2001). However, there is only few previous report of an antimicrobial peptide derived from fishes belonging to family Cyprinidae. The present study deals with identification and characterization of a peptide from the histone H2A of fresh water cyprinids fish.

The *Cyprinus carpio* (HIS-K1), Histone H2A gene possessed 166 nucleotides encoding an 55 amino acids. The *Cyprinus carpio carpio* prepropeptide sequence alignment with selected vertebrate species showed highest similarity within the mature peptide region. To determine the relationship of the *Cyprinus carpio* 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 is placed into the Group Ialongside the common carp is a closely related species. However the grouping of the multiple Histone H2A is not a new discovery.

For any fish species that in being farmed, it is important to have an understanding of its immune system for the monitoring of its health or for the development of therapeutic approaches. This investigation characterizes important Histone peptides from *Cyprinus carpio.* 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).

**Summary& 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 *Cyprinus carpio carpio.* These experimental organism 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 *Cyprinus carpio carpio.* The results obtained are summarized as follows: Histone H2A derived AMP derived AMP from the Koi carp, *Cyprinus carpio carpio.* The study presents first report of a histone H2A AMP to be identified from the Koi carp, *Cyprinus carpio carpio.* An mRNA fragment of 166 bp encoding 55 amino acid corresponding to histone H2A gene could be amplified from the Koi carp. The peptides possessed a predicted molecular weight of 5.75 kDa and the theoretical isoelectric point (pI) is 6.30. The peptide was found to be rich in amino acid residues Alanine (A) 18.2 % and Glycine (G) 9.1 % and Arginine (R) 5.5 % and possessed a hydropathicity of -0.44. Multiple alignment showed the presence of conserved regions in both nucleotide and amino acid sequences. In Phylogenetic tree constructed using histone H2A sequences also showed the basal placement of this peptide along with other histone identified from Family Cyprinidae.

**Conclusion**

Characterization of AMPs from fish, on the structural, genetic and functional levels, will definitely provide a wealth of information. Examination of AMPs in significant fish families such as Cyprinidae, possessing aquaculture importance as food fish or ornamental fishcan help understand the role of these peptides in innate host defense of the fish. Studies on the similarities and differences with peptides from other fishes and non-fish species contribute to our understanding of the evolutionary relationships of innate host defense mechanisms among vertebrates. Furthermore, they can provide important information for the better design of novel therapeutic agents, both for microbial infections as well as other conditions. Unique for the field of fish AMPs is the potential application to aquaculture. Histone proteins and their derivative peptides play inevitable role in the defence of many organisms especially in fishes because they rely more on the rapid, nonspecific innate immune parameters than short-lived adaptive immune responses. The histone H2A derived AMPs identified and characterized in the present study possess all characteristic features of classical AMPs and has the potential to be developed into a novel therapeutic agent. Moreover they could be used as templates for the development of hybrid/stabilised AMPs. The present study inevitably provides a solid foundation for strong future work to better understand both the role of these peptides in host defense of the fish, as well as the development of these peptides and their derivatives as potential therapeutics.

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