*Available online at http://www.bretj.com*

**INTERNATIONAL JOURNAL OF INNOVATION RESEARCH**

**RESEARCH ARTICLE**

**International Journal of Innovation Research - Vol. 1, Issue, 1, pp.001 - 004, January, 2013**

# Antibacterial activity of ammonium precipitate extract of viralfish (*channa striatus*) skin mucus

**Loganathan, K., Prakash, M and Senthilraja, P**

Department of Zoology, Annamalai University, Annamalainagar, Tamilnadu (India).

**A R T I C L E I N F O**

***Article History:***

Received 10th December, 2012
Received in revised form 20th, December, 2012
Accepted 5th January, 2013
Published online 31th January, 2013

***Key words:***

Viralfish, *Channa striatus,* Antibacterial property, SDS-PAGE

**A B S T R A C T**

**Objectives**: The present study was carried out to assess the role of epidermal mucus of Viralfish, *Channa striatus* and their antibacterial activity on selected bacterial strains.

**Methods:** The fish mucus was collected by unanaesthesia and treated with ammonium precipitate. Isolated ammonium precipitate mucus extracts were subjected into the antibacterial activity. And also to determine the molecular weight of proteins by SDS-PAGE analysis.

**Results**: The highest resistance activity was found in *E.coli* from the mucus extract. In addition TLC technique presence of pink spots to indicates that proteins and amino groups. SDS-PAGE gel protein band at 14 kDa to 67 kDa was shown in clear; this peptides/protein is involved in the antibacterial activity against pathogenic bacterial strains.

**Conclusion:** The ammonium precipitate extracts of fish mucus have bioactive molecules (peptides/proteins) to exert strong antibacterial activity. Further detailed studies are required to understand the role and nature of the biomolecules found in the mucus.



## INTRODUCTION

Antimicrobial peptides (AMPs), also called host defense peptides, are an evolutionarily conserved component of the innate immune system and are found among all classes of life. AMPs are generally between 10 and 50 amino acids with a net positive charge of 2 to 8. These peptides exhibit a wide range of activity against numerous microbes including Gram-positive and Gram-negative bacteria, fungi, viruses, and parasites while with little or no toxicity to host cells [1]. In addition, certain peptides have the ability to enhance immunity by functioning as immunomodulators [2]. To date, about 1200 AMPs have been characterized, and a large number of them were isolated from mammals, amphibians, insects or other invertebrates. Although fish are by far the most abundant vertebrate in the world, relatively few AMPs have been isolated from fish presumably reflecting a simple lack of attention to this potentially rich source of AMPs.

The mucus layer on the fish surface performs a number of functions including disease resistance, respiration, ionic and osmotic regulation, locomotion, reproduction, communication, feeding and nest building [3, 4, 5]. The antimicrobial property of crude epidermal mucus against infectious pathogens was initially demonstrated in rainbow trout (*Oncorhynchus mykiss*) [6]. The removal of epidermal mucus in ayu (*Plecoglossus altivelis*) and turbot (*Scophthalmus maximus*) after challenging them with *Listonella anguillarum* resulted in increased mortality [7,8]. In carp (*Cyprinus carpio*), the loss of epidermal mucus increased susceptibility to bacterial infection [9].

Antimicrobial activity of epidermal mucus extracts against a broad range of microbial pathogens was observed by Hellio *et al.* [10]. These experiments supported the hypothesis that the epidermal mucus plays a protective function against microbial infection in fish. The mucus freshwater fishes have some pronounced activity against microbes, its useful for discover of new antibiotics. The antimicrobial function of epidermal mucus appears to result from itsmechanical and biochemical properties. The mucus layer on the surface of fish is continuously replaced, which possibly prevents stable colonization by parasites, bacteria and fungi [11]. In the present study was undertaken to identify the antibacterial protein from the skin mucus of Viralfish, *Channa striatus.*

*Corresponding author: +91
Email: dnaprakash@yahoomail.com

## MATERIALS AND METHODS

### *Mucus Collection*

The healthy *Channa striatus* was collected from Sirkali fish market, Nagai, Tamilnadu, India of an average weight 300 ± 5.67g. The fish were kept in large aerate concrete tank containing potable tap water (pH 7.5 ± 0.5) facilitated with water and air pumps. The tank were treated with disinfectant sodium hypochloride, with the concentration of 200 ppm for 1 hrs and washed three times with fresh tap water Bergsson *et al.*, [12] prior to the introduction of the fish in the water. The mucus was collected from the acclimatized healthy fish. The mucus was carefully scraped from the dorsal surface of the fish. Before collection of mucus any anesthetic chemicals are not given. Mucus was not collected in ventral side to avoid anal and sperm contamination.

### *Peptide Purification by Precipitation*

The collected mucus was centrifuged at 10,000 rpm at 4°C for 15 minutes in a refrigerated centrifuge and supernatant was precipitated by ammonium sulphate (75%) and stored at 4°C overnight. The precipitate peptide was collected by centrifuging at 15,000 rpm for 20 minutes at 4°C (REMI.) and the pellet was resuspented in an acetate buffer (50mM; pH 5.0), stored at 4°C for until use.

### *Thin layer chromatography*

Thin-Layer Chromatography profiling was done for the active fraction for separating, identifying and characterizing the unknown compound. Analysis thin layer chromatography mucus extract was performed on Butanol, Acetic acid and Water (B:A:W) in the ratio of 4:1:5. The 10µl of sample was spotted in the silica coated TLC plate and run in closed chamber previously equilibrated with solvent. Then the TLC plates were taken and solvent front was marked. The slides were air dried and sprayed with 0.1% ninhydrin, for detecting the compounds [13].

### *Antibacterial activity of viral fish mucus peptide*

In-vitro antibacterial assay was carried out by disc diffusion technique followed by [14]. The spectrum of antimicrobial activity was studied using 5 different strains of fish and human pathogenic bacteria. Whatman No.1 filter paper discs with 4 mm diameter were impregnated with known amount test sample of mucus extract and positive control contained a standard (Erythromycin) antibiotic disc. Negative controls not comprised (sterile disc only) with anything. The impregnated disc along with control (incorporated with solvent alone) was kept at the centre of Agar plates, seeded with test bacterial cultures

### *SDS-PAGE (protein profile)*

The fish mucus peptide samples were analyzed to SDS-PAGE to determine the molecular size of polypeptide of given samples. SDS-PAGE was done by following the procedure of Lammeli, [15].

## RESULTS AND DISCUSSION

Over the past few years many antibacterial peptides have been isolated from both plants and animals, from insects to mammals. These peptides are predicted to operate as a first-line host defense mechanism, acting against pathogenic bacteria, fungi, and other parasites. Generally nonspecific but rapidly active during a parasite invasion, they constitute host defense less costly than antibodies. Antimicrobial peptides (AMPs), also called host defense peptides, are an evolutionarily conserved component of the innate immune system and are found among all classes of life. AMPs are generally between 10 and 50 amino acids with a net positive charge of 2 to 8. These peptides exhibit a wide range of activity against numerous microbes including Gram-positive and Gram-negative bacteria, fungi, viruses, and parasites while with little or no toxicity to host cells [1].

**Fig 1:** TLC spot of mucus peptide

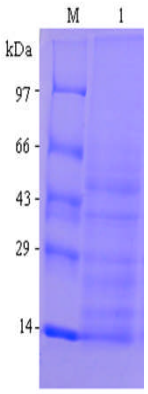


**Fig 2**: SDS-PAGE analyses of mucus peptide

Freshwater snake head Viralfish mucus peptide were subjected into the TLC, developing pink spot on a silica gel sheet presence of amino acids and peptide in the mucus sample. Visualized spot were kept in under UV spectrum at 254 nm (Fig 1). In antibacterial activity six bacterial strains are tested against Viralfish mucus extract *Escherichia coli*, *Aeromonas salmonicida*, *Staphylococcus aureus*, *Listonella anguillarum*, *vibrio anguillarum* and *Vibrio cholarae*. Among the six bacterial pathogens *E.coli* showed strong inhibitory activity against (18 mm) mucus

**Table 1** Antibacterial activity of fish mucus extract

| Sl. No | Bacterial pathogens | Zone of inhibition in mm | | Negative control |
|---|---|---|---|---|
| | | Test sample 100 µl/disc | Positive control 100 µl/disc | |
| 1 | *Escherichia coli* | 18 | 19 | - |
| 2 | *Aeromonas salmonicida* | 14.9 | 16 | - |
| 3 | *Staphylococcus aureus* | 6.8 | 7 | - |
| 4 | *Listonella anguillarum* | 14 | 15 | - |
| 5 | *Vibrio anguillarum* | 8.5 | 9 | - |
| **6** | ***Vibrio cholarae*** | **6** | **8** | - |

gel and the least activity was found in *vibrio cholarea.* The moderate activity was found in *Aeromonas salmonicida* (14.9 mm), *Listonella anguillarum* (14), *Vibrio anguillarum* (8.5 mm) and *Staphylococcus aureus* (6.8 mm) was observed against mucus extract (Table 1). The Viralfish skin mucus extract showed antibacterial activity was subjected to the SDS-PAGE to estimate the molecular weight of proteins as present in it. Ammonium precipitate peptide mucus sample showed eight protein bands from 14 kDa to 67 kDa. Only thick clear band are detected in the gel that represented as peptide/proteins of Viralfish mucus at 14 kDa (Fig 2).

Many antimicrobial compounds are hydrophobic and act by disrupting the membrane, which leads to the death of the target cell. The fractionated fish mucus to separate the water-soluble to hydrophobic compounds. Then, all fractions were reconstituted in planar lipid bilayer to determine their putative pore-forming activity. Fractions with water-soluble proteins did not induce the formation of ion channels whereas supernatants with hydrophobic proteins (supernatant with detergent) strongly induced the formation of ion channels in the bilayer of microbes [16]. The antimicrobial function of epidermal mucus appears to result from its mechanical and biochemical properties. The mucus layer on the surface of fish is continuously replaced, which possibly prevents stable colonization by parasites, bacteria and fungi [11]. Fish skin mucus has a potential role of prevention of bacterial and fungal pathogens [17]. The present study also showed the antibacterial potential of Viralfish skin mucus extract.

Disease control is a vital aspect in mass production of fishes. Outbreaks of diseases can seldom be attributed to a single causative factor. With the establishment of hatcheries for breeding of fishes especially on a commercial basis, the possibility of frequent disease outbreak becomes a threat. Mainly the bacterial, fungal and protozoan parasites diseases of fish are quite nature in culture. Once these diseases outbreaks, within very period the survival rate is reduced. In the present study, fresh water snake head Viralfish mucus extract that showed antibacterial activity was subjected to TLC to determine the presence of the amino acids and peptides. In the present investigations SDS-PAGE analyses of mucus sample showed 8 bands, the thick clear band at 14 kDa molecular weight protein was may be responsible for antibacterial activity. Similarly protein band at 13.6 and 13.9 kDa was isolated from the estuarine cat fish mucus [13]. Comparing the results, antibacterial protein have so far found in skin secretions or mucus, although most of them have been poorly characterized, hydrophobic proteins with 27 kDa and 31 kDa from *C. carpio*, 45 kDa protein from eel *A.anguilla*, 65 kDa protein from rainbow trout *O. mykiss* and a 49 kDa protein from tench *T. tinca*. These antimicrobial proteins are assumed to form ion channels in bacterial membrane and kill both gram positive and gram negative bacteria [16,18]. Further investigation to isolate the bioactive compound from the mucus of that fish and their molecular structure.

## Acknowledgement

We are obliged to Annamalai University authority for the support of the research work.

## Reference

1) Hancock REW, Scott MG. The role of antimicrobial peptides in animal defenses. *Proc. Natl.Acad. Sci. USA* 2000; *97*: 8856–8861.
2) Bowdish, DME, Davidson DJ, Scott MG, Hancock REW. Immunomodulatory activities of small host defense peptides. *Antimicrob Agents Chemother 2005; 49: 1727–1732.*
3) Negus VE. The functions of mucus. *Acta oto-laryngol 1963; 56, 204–214*.
4) Ingram GA. Substances involved in the natural resistance of fish to infection – a review. *J Fish Biol 1980; 16, 23–60.*
5) Shephard KL. Functions for fish mucus. *Rev Fish Biol Fish* 1994; 4:401-429.
6) Austin B, McIntosh D. natural antibacterial activity on the surface of rainbow trout (*Salmo gairdneri Richardson.*). J Fish Dis 1988; 11:275-277.
7) Kanno T, Nakai T, Muroga K. Mode of transmission of vibriosis among ayu *Plecoglossus altivelis. J Aquat Anim Health 1989; 1: 2–6.*
8) Fouz B, Devesa S, Gravningen K, Barja JL, Toranzo AE. Antibacterial action of the mucus of turbot. *Bull Eur Assoc Fish Pathol 1990; 10: 56–59.*
9) Lemaître C, Orange N, Saglio P, Saint N, Gagnon J, Molle G. Characterization and ion channel activities of novel antimicrobial proteins from the skin mucosa of carp (*Cyprinus carpio*). *Eur J Biochem 1996; 240,143–149.*
10) Hellio C, Pons AM, Beaupoil C, Bourgougon N, Gal YL. Antibacterial, antifungal and cytotoxic activites of extracts from fish epidermis and epidermal mucus. Int J Antimicrob Agent 2002; 20:214-219.
11) Pickering A. The distribution of mucus cells in the epidermis of the brown trout *Salmo trutta (L)* and the char *Salvelinus alpinus (L). J Fish Biol.1974; 6, 111–118.*
12) Bergsson G, Agerberth B, Jornvall H, Gudmundsson GH. Isolation and identification of antimicrobial components form epidermal mucus of Atlantic cod. (*Gadus morhua*). *FEBS Lett* 2005; 272: 4960-9.
13) Anbuchezhian R, Gobinath G, Ravichandran R. Antimicrobial peptide from the epidermal mucus of some estuarine catfishes. *World Appl Sci J 2011; 12(3):256-260.*
14) Bauer AW, Kirby, WMM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disc method. *Ame J Cli Pathol 1996; 45: 493-496.*
15) Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature 1970; 227:680–2.*

16) Ebran N, Julien S, Orange N, Saglio P, Lemaitre C, Molle G. Pore-forming properties and antibacterial activity of proteins extracted from mucus of fish. *Comp Biochem Physiol 1999; 122A:181-189.*
17) Loganathan K, Muniyan M, Arulprakash A, Gunasekaran G, Prakash M. studies on the role of mucus from *Clarias batrachus* (Linn.) against selected pathogens. *Int J Pharma Appl 2011; 2(3):202-206.*
18) Ebran N, Julien S, Orange N, Auperin B, Molle G. Isolation and characterization of novel glycoproteins from fish epidermal mucus: Correlation between their pore-forming properties and their antibacterial activities. *Biochim Biophys Acta 2000; 1467: 271–280.*

*******