International Journal of Fisheries and Aquatic Studies 2014; 2(2): 158-162

ISSN: 2347-5129
IJFAS 2014; 2(2): 158-162

www.fisheriesjournal.com
Received: 06-10-2014
Accepted: 31-10-2014

Md. Manjurul Islam
Department of Fisheries & Marine Bioscience, Jessore University of Science & Technology (JUST), Jessore-7408, Bangladesh.

Md. Mer Mosharraf Hossain
Department of Fisheries & Marine Bioscience, Jessore University of Science & Technology (JUST), Jessore-7408, Bangladesh.

Md. Sherajul Islam
Department of Fisheries & Marine Bioscience, Jessore University of Science & Technology (JUST), Jessore-7408, Bangladesh.

Shoumo Khondoker
Department of Fisheries & Marine Bioscience, Jessore University of Science & Technology (JUST), Jessore-7408, Bangladesh.

Mst. Aisha Khatun
Department of Fisheries & Marine Bioscience, Jessore University of Science & Technology (JUST), Jessore-7408, Bangladesh.

Correspondence:
Shoumo Khondoker
Department of Fisheries & Marine Bioscience, Jessore University of Science & Technology (JUST), Jessore-7408, Bangladesh.

Competitive antibacterial activity of two Indian major carps and two Chinese carps fish mucus against common pathogenic bacteria at aquaculture pond

Md. Manjurul Islam, Md. Mer Mosharraf Hossain, Md. Sherajul Islam, Shoumo Khondoker, Mst. Aisha Khatun

DOI: https://doi.org/10.22271/fish.2014.v2.i2c.238

Abstract
A search for antibacterial activity of carp fish mucus was performed for different protein concentrations, against four different bacterial strains *Aeromonas hydrophila*, *Aeromonas sorbia*, *Pseudomonas fluorescens* and *Vibrio anguillarum.* Protein concentrations (3.40 mg/ml) in catla mucus samples were found higher than the protein concentrations (3.12 mg/ml) of rohu mucus samples when tested by Bicinchoninic assay (BCA). Antibacterial activity was detected in mucus samples of four carp fish species, against the four bacterial strains. The results of this study indicate that the mucus of carp fish contain one or several components (protein, beneficial bacteria and other chemical component) with antibacterial activity. These bioactive substances may play an important role in the ability of mucus to defend carp fish against pathogenic bacteria or microorganisms. However, more investigation is necessary to confirm the antibacterial activity in carp fish mucus at different protein concentration and to purify and characterize the active components.
Keywords: Antibacterial activity, Protein concentration, Fish pathogens, Mucus.

1. Introduction
Advanced improvements and new formulations in the modern chemotherapeutic techniques have been used widely. But infectious diseases are still an alarming issue in case of public health in recent world [1]. So, still new methods of extruding pathogens along with the combination of existing methods are desired [2]. To promote this state, in recent times, research has been introduced to find out a potential method to prevent diseases worldwide. Several endeavors have been made to discover novel antimicrobial drugs from natural sources together with plant and animal product [3].
Natural chemicals have been a boon for shielding and treating the various indisposition of diverse origin [4]. Using these chemicals in human chemotherapeutics is a common practice. WHO stated that, out of 252 traditional medicines, 8.7% and 11.1% come from animals and plants originated sources respectively [5]. Fish body also exerts such by products like potentially valuable enzymes, proteins, minerals, flavours or pigments which have a key role in their innate defense mechanisms [6].
Fish lives in aqueous environment which itself is a source of microbial pathogens invading aquatic organisms. Even with a close contact with high concentrations of such pathogens, fish can still preserve a fit and vigorous system under normal condition. The fish produces mucus substances composing of biochemically diverse secretions from epidermal and epithelial cells which are key components of innate immunity [7, 8]. The mucus layer thought to act as a lubricant [9]. This layer wraps the surface of external body to lessen body friction against water and to protect from detrision injury [10] and as a mechanical barrier of the skin it hinders way in the majority of microorganisms into the body. Fish biologists suspected that mucus might be involved in ion regulation [11]. Increased rate of susceptibility to bacterial infection in carp (*Cyprinus carpio*) was evidenced with the loss of epidermal mucus [12]. A certain fatty acid compositional study of the flesh of the snakehead (*Channa striatus*) revealed unusually high arachidonic acids actively involved in initiating wound repair [13].

Antibacterial activity in mucus has been demonstrated in several fish species [14]. But studies are available mostly on cold water fishes. In the present study effort was made to study the antibacterial activity of two Indian major carps *Labeo rohita, Catla catla* and two Chinese carps *Hypophthalmichthys molitrix, Ctenopharyngodon idella* against selected bacteria (*Aeromonas hydrophila, Aeromonas sorbia, Pseudomonas fluorescens and Vibrio anguillarum.* These pathogens were selected because of their relevance in infections and high mortality in fish [15].

2. Materials and Methods

2.1 Collection of fish

The experimental fishes *Labeo rohita, Catla catla, Hypophthalmichthys molitrix* and *Ctenopharyngodon idella* irrespective of sex, healthy growing live fishes around 6 months old, weigh about 500 gms of each were purchased from nearby culture ponds located at Sadar, Jessore, Bangladesh. The purchased fish were acclimatized to laboratory conditions in well water and they were maintained for one week. During this period the fish were fed with conventional commercial feed once a day. Every day 50% of the water was changed. After one week of acclimatization the fish were used for mucus collection. Only healthy fish were chosen for mucus collection. Dead fish or fish with skin lesions were removed from the tank.

2.2 Collection and preparation of skin mucus extract

Mucus was carefully scraped from the dorsal body using a sterile spatula. Mucus was not collected in the ventral side to avoid intestinal and sperm contamination. Before collection of mucus sample any anesthetic chemicals were not applied. The collected fish mucus samples were stored at 4 ^{0}C for further use. The mucus samples were collected aseptically from the fish and thoroughly mixed with equal quantity sterilized physiological saline (0.85% NaCl) and centrifuged at 5000 rpm for 15 minute. The supernatant was kept at 4 ^{0}C for the antimicrobial studies.

2.3 Protein measurement by BCA kit

Protein concentrations of fish mucus were determined by BCA protein assay kit [16, 17] described by Mozumder [18]. This very assay is a detergent-compatible formulation based on Bicinchoninic acid for the colorimetric detection and quantization of total protein.

2.4 Inoculation of bacterial strains

Antibacterial activity of freshwater cultivable fish *L. rohita, C. catla, H. molitrix* and *C. idella* mucus was determined against four pathogenic bacteria *Aeromonas hydrophila, Aeromonas sorbia, Pseudomonas fluorescens* and *Vibrio anguillarum.* These pathogenic strains were obtained from the Microbiological Laboratory of University of Dhaka, Bangladesh.

In vitro antibacterial assay was carried out by disc diffusion technique [19]. Whatman No.1 filter paper discs with 4 mm diameter were impregnated with known amount (10 µl) of test sample of fish mucus and a standard antibiotic disc. At room temperature (32 ^{0}C) the bacterial plates were incubated for 24 h. The results were recorded by measuring the zones for growth inhibition surrounding the disc. Clear inhibition zones around the disc were expressed in terms of diameter of zone of inhibition and were calculated in mm by means of cm scale, recorded and the average were tabulated.

2.5 Determination of antimicrobial assay

The spectrum of antimicrobial activity was studied using the mentioned four different strains of pathogenic bacteria. One antibiotic agent Erythromycin was used for pathogenic bacteria.

3. Results

3.1 Antibacterial activity of the fish mucus

Antibacterial activity of the fish mucus of four freshwater fishes *L. rohita, C. idella, C. catla* and *H. molitrix* against four pathogenic bacteria are presented in the Table-1, Table 2, Table-3 and Table-4 in terms of zone of inhibition.

Table 1: Antibacterial activity of skin mucus from *L. rohita*

Sl. No.	Bacterial Pathogens	Zone of inhibition (mm in diameter)	+ ve control (in mm) (Erythromycin)
		Rohu (*L. rohita*)	
01	*A. hydrophila*	18	19
02	*A. sorbia*	30	26
03	*P. fluorescens*	26	22
04	*V. anguillarum*	28	30

Table 2: Antibacterial activity of skin mucus from *C. idella*

Sl. No.	Bacterial Pathogens	Zone of inhibition (mm in diameter)	+ ve control (in mm) (Erythromycin)
		Grass carp (*C. idella*)	
01	*A. hydrophila*	12	19
02	*A. sorbia*	09	26
03	*P. fluorescens*	19	22
04	*V. anguillarum*	16	30

The magnitude of zone of inhibition was *A. sorbia* > *V. anguillarum* > *P. fluorescens* > *A. hydrophila* for *L. rohita* as mentioned in Table-1. The mucus of *L. rohita* showed more effect in controlling the growth of gram-negative bacteria *A. sorbia* with an inhibition zone of 30 mm in diameter which is more than the other gram negative bacteria *V. anguillarum* (28 mm), *P. fluorescens* (26 mm) and A. *hydrophila* (18 mm). *L. rohita* mucus showed much more resistance than the positive control (Erythromycin) against *A. sorbia* and *P. fluorescens*.

Table 3: Antibacterial activity of skin mucus from *C. catla*

Sl. No.	Bacterial Pathogens	Zone of inhibition (mm in diameter)	+ ve control (in mm) (Erythromycin)
		Catla (*C. catla*)	
01	*A. hydrophila*	22	19
02	*A. sorbia*	28	26
03	*P. fluorescens*	26	22
04	*V. anguillarum*	23	30

Table 4: Antibacterial activity of skin mucus from *H. molitrix*

Sl. No.	Bacterial Pathogens	Zone of inhibition (mm in diameter) Silver Carp (*H. molitrix*)	+ ve control (Erythromycin)
01	*A. hydrophila*	17	19
02	*A. sorbia*	14	26
03	*P. fluorescens*	21	22
04	*V. anguillarum*	18	30

The maximum zone of inhibition was observed against *P. fluorescens* (19 mm) for *C. idella* and magnitude pattern is *V. anguillarum* > *A. hydrophila* > *A. sorbia* representing value 16 mm, 12 mm, 09 mm respectively. But *C. idella* mucus didn't demonstrate any greater resistance than positive control against four pathogenic bacteria.

The magnitude of zone of inhibition was *P. fluorescens* > *V. anguillarum* > *A. hydrophila* > *A. sorbia* in case of *H. molitrix* as stated in Table-4. The mucus of *H. molitrix* expressed more achievement in restraining *P. fluorescens* (21 mm). *V. anguillarum* (18 mm), *A. hydrophila* (17 mm) and *A. sorbia* (14 mm) showed less zone of inhibition than *P. fluorescens* but *H. molitrix* mucus didn't conveyed any fact of more zone of inhibition than positive control. The entire scenario of the above mentioned data are depicted in Figure-1.

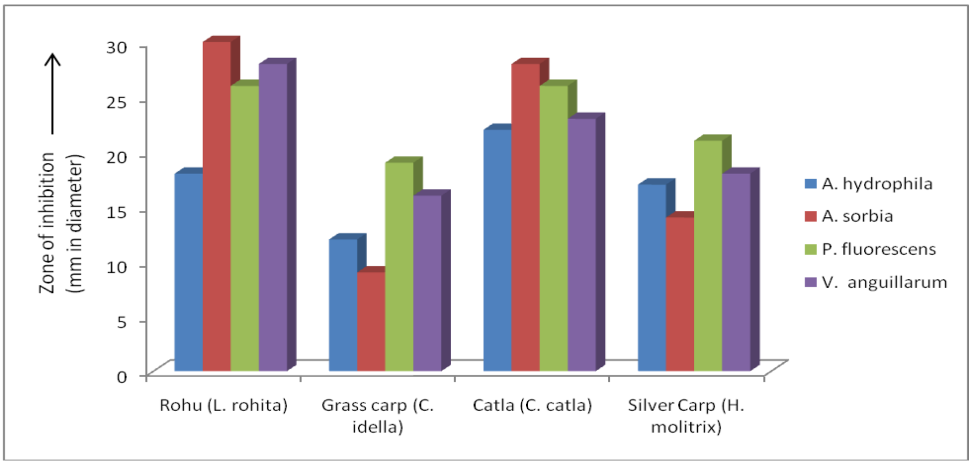

Fig 1: Antibacterial activity of *L. rohita* and *C. catla* is higher than *C. idella* and *H. molitrix* against *A. hydrophila*, *A. sorbia*, *P. fluorescens* and *V. anguillarum*

For *C. catla, A*. sorbia confirmed higher zone of inhibition (28 mm) whereas *A. hydrophila* evidenced lowest value of 22 mm diameter. *V. anguillarum* and *P. fluorescens* indicated 23 mm and 26 mm of zone of inhibition. In case of *C. catla* only *V. anguillarum* showed less zone of inhibition than positive control.

3.2 Protein concentration in mucus sample

The protein concentration of the skin mucus was measured by using BCA protein measurement kit collected from the Microbiological Laboratory of University of Dhaka. Protein concentrations of four carp fish mucus sample are tabulated in Table-5.

Table 5: Protein concentration of carp fish mucus sample

BCA method			
Mucus sample	**Weight of mucus sample (gm)**	**Protein conc. (mg/ml)**	**Mean protein conc. (mg/ml)**
H. molitrix	0.3	4.86 3.50	4.18
C. idella	0.3	5.49 4.19	4.84
L. rohita	0.2	3.56 3.24	3.40
C. catla	0.2	3.04 3.20	3.12

4. Discussion

In every human civilization, natural fauna have been used as medicinal resources for the cure and relief of a multitude of disease and illness [20]. Utilization of fish biomass offers a wide range of attractive methods for including and building protection against diseases [21]. Fish mucus is a multifunctional matter playing a crucial role in respiration, communication, feeding, ionic and osmotic regulation, resistance to disease, reproduction, excretion and nest building [8].The main site of interaction of the greater part of microbes is thought to be plasma membrane, where they cause pore formation or membrane lysis [22].

The mucus generating cells in epidermal and epithelial layers had been accounted to vary between fish species and consequently could influence the mucus composition. Mucus protein concentration of four carp mucus was conducted in the present study. Protein concentrations are measured through BCA kit method. The results show that all the samples possess protein concentration. However, protein concentrations in *C. idella* fish mucus samples are higher than the other three mucus samples. This study also reveals that mucus represents an important biological interface between carp fish and their aqueous environment. Two proteins (27 and 31 kDa) have been isolated which confer antibacterial properties in the skin mucus of carp [12]. More antibacterial activity at protein concentration ranges from 3.12-3.40 mg/ml is observed in two Indian major carp fishes (*Catla catla and Labeo rohita*) than exotic fishes (*Hypophthalmichthys molitrix* and *Ctenopharyngodon idella*) due to protein concentration ranges from 4.18-4.84 mg/ml.

Mucus holds some proteases (serine protease, cysteine protease, metalloprotease and trypsin like protease) having strong antibacterial activity [23]. This surface layer also contains numerous peptides which involve in the defense of invading microorganisms. Amphipathic α-helical peptides, such as dermaseptin, ceratotoxin and meganinin bind with anionic phospholipid rich membranes and dissolve them like detergents [24, 7]. A novel-25 residue antimicrobial peptide, pleurocidin was reported to discover and characterize by Alexander M. Cole *et al*. [25] from the epidermal mucus cells of winter flounder. Endogenous peptides play an important role in fish defense, possess broad spectrum of antimicrobial activity against bacteria. Lysozyme isolated from fish was an enzyme with bacteriostatic properties and was ubiquitous in its distribution among living organisms [26].

In the present study, dissimilarity in their antibacterial activity was observed among the fish mucus. This may be due to the variance in the relative levels of lysozyme alkaline phosphatase, cathepsin B and protease of the epidermal mucus of all fish species [27]. Fish lectins appear to play antibacterial or antifungal roles and in some instances seem to involve in egg-sperm fusion, polyspermy prevention and embryo development. Natural, non-Ig precipitins are found largely, but not exclusively in fish serum and precipitate with simple monosaccharides or long chain polysaccharides of certain stereochemistry and glycosidic linkages. Their functions remain unknown but C-reactive protein is induced following stress-induction and exposure to inflammatory agents [28].

The above findings support that, the mucus collected from *C. catla, H. molitrix, L. rohita and C. idella* showed resistance against the selected bacteria and Indian major carp *L. rohita* and *C. catla* mucus demonstrated much resistance to the microbes. The present work supports the view that fish mucus could be source of antimicrobial agent for human and fish pathogens. Further purification of the bioactive compounds is necessary in order to identify their chemical nature and to evaluate their potential as novel drug.

5. Conclusion

The results suggest that fish mucus have bactericidal properties and thus play important role in the protection of fish against the invasion of pathogens. Thus this work has been an excellent evidence to prove the medicinal value of the mucus collected from selected Indian major carps and Chinese carps. Further study will surely open new window of disease protection for promoting sustainable aquaculture.

6. Acknowledgement

Authors were thankful to Professor Dr. Md. Anisur Rahman, Chairman, Dept. of Fisheries & Marine Bioscience, Jessore University of Science & Technology (JUST), Jessore, Bangladesh for providing the lab facilities.

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