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STUDIES ON THE ROLE OF MUCUS FROM CLARIAS BATRACHUS (LINN) AGAINST SELECTED MICROBES

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ABSTRACT

The antimicrobial activity present in the skin mucus of cat fish (*Clarias batrachus*) was tested against five pathogenic bacteria and fungi. The mucus collected were tested against five pathogenic bacteria namely *Escherichia coli*, *Aeromonas hydrophila, Pseudomonas aeruginosa*, *Vibrio fischeri* and *Vibrio anguillarum* using Muller Hinton agar plates by disc diffusion method and five pathogenic fungi namely *Aspergillus niger*, *Aspergillus nidulans, Fusarium moniliforme, Candida albicans* and *Trichoderma koningi* using Potato Dextrose agar plates by disc diffusion method. The activity was measured in terms of zone of inhibition and expressed as millimeter (mm in diameter). The mucus collected from *Clarias batrachus* shows a strong inhibiting activity against the bacteria than the fungi.

Keywords: Antimicrobial activity, fish skin mucus, *Clarias* batrachus.

1. INTRODUCTION

Fish and its by–products contains potentially valuable and important proteins, enzymes, active compounds etc [1]. In apparently a direct contact with high concentrations of pathogens (bacteria and viruses) in their aquatic environment leads to the problem of infectious disease and have considerable effects on aquaculture. Diseases caused by bacteria and viruses have led to high mortality and reduces the economic income of fish form industries. It leads to a complex system of innate defense mechanism within the fish.

Due to the accumulation of domestic waste in the aquatic medium, the population of microbes is increased rapidly. In order to escape from such an environment, fishes produce (or) secretes some substance against invading microbes. Fishes are likely to rely on their innate immunity for their production against infectious diseases. The fish produces mucus substances which is a key component of innate immunity(2). Mucus secreted by fish play a major role in protection against infectious agents such as bacteria and fungi. However under such conditions the fish maintains a health state by defending itself against these potential invaders by a complex system of innate defense mechanisms(3,4).

The innate defense mechanism defense mechanisms of fish against infectious microbes include production of broad spectrum of antimicrobial substances acute – phase proteins. Non-classical complement activation, release of cytokine inflammation etc (5,6). The antimicrobial property of crude epidermal mucus against infectious pathogens was demonstrated in so many fishes (7,8,9). But their is no study on the cat fish *clarias batrachus*. Being a column feeder it lines with an environment having numerous decayed substances and muddy microbes the *clarias batrachus* produces more amount of mucus to escape from the infectious pathogens.

Hence, the present study was maied to study the anti-microbial potential of the mucus collected from the cat fish *clarias batrachus*.

2. MATERIALS AND METHODS

2.1 Collection of fish

The experimental fish *Clarias batrachus* irrespective of sex, healthy growing live fish approximately 6 months old weighed about

500 gms and 45 to 55 cm length of *Clarias batrachus* were collected from the nearby ponds located at Sirkali, Tamil Nadu, India.

2.2 Collection of mucus sample

The mucus samples were collected from the acclimatized healthy fish *Clarias batrachus*. The mucus was carefully scraped form the anterior to posterior on dorsal body using a sterile spatula. Before collection of mucus sample any anesthetic chemicals are not given. Mucus was not collected in the ventral side to avoid anal and sperm contamination. Before collection of mucus, fish were kept out of water in specimen tray for 1 hour. After one hour mucus was secreted on the epidermal surface of the body and was collected as a sample. The collected mucus samples were thoroughly mixed with equal quantity of sterilized physiological saline (0.85% Nacl) and centrifuged at 5000 rpm for 15 minutes. The supernatant was collected and stored at 4°C for the antimicrobial studies.

2.3 Microbial Strains used

Antimicrobial activity of fresh water fish *clarias batrachus* was determined against five bacterial strains viz, *Escherichia coli, Aeromonas hydrophila, Pseudomonas aeruginosa, Vibrio fischeri* and *Vibrio anguillarum* and five pathogenic fungi namely *Aspergillus niger, Aspergillus nidulans, Fusarium moniliforme, Candida albicans* and *Trichoderma koningi*. These pathogenic strains were obtained from the Division of Microbiology, Rajah Muthiah Medical College, Annamalai University, Annamalai Nagar.

2.4 Antimicrobial Assay

The spectrum of antimicrobial activity was studied using the above mentioned bacteria and fungi which are designated as human pathogens One antibiotic agent Erythromycin for pathogenic bacteria and Fluconazole for pathogenic fungi were used.

In-vitro antibacterial assay was carried out by the disc diffusion technique(10). Whatman No.1 filter paper discs with 4 mm diameter were impregnated with known amount of test sample skin mucus and positive control contained a standard antibiotic disc. Negative controls (sterile disc only) are also used. The impregnated discs along with control were kept on Agar plates, seeded with test bacterial cultures and fungal culture separately. At room temperature (37°C) the bacterial plates were incubated for 24 hrs. The fungal plates, were incubated at 30°C for 48 hrs to find out the antimicrobial activity. They were expressed in terms of diameter of zone of inhibition and was measured in mm using cm scale and recorded. In each strain triplicate were maintained, the average were taken and tabulated.

3. RESULTS

Antimicrobial effect of the mucus of fresh water fish namely *Clarias batrachus* were tested against, five pathogenic bacteria (three gram positive and two gram negative) and five pathogenic fungi. The activity was measured in terms of zone of inhibition and expressed in millimeter (mm).

3.1 Antibacterial activity of fish mucus

The magnitude of zone of inhibition was *Pseudomonas aeruginosa* > *Aeromonas hydrophila* > *Vibrio anguillarum* > *Vibrio fischeri* >*Escherichia coli*. The maximum zone of inhibition was observed against *Pseudomonas aeruginosa* which is about 25 mm in diameter. This was followed by *Aeromonas hydrophila* (22 mm), *Vibrio anguillarum* (21 mm), (*Vibrio fischeri* (20 mm) and *Escherichia coli* (18 mm) respectively.The mucus collected from *Clarius batrachus* shows more resistance to the *Pseudomonas aeruginosa* (25 mm) and least resistance to *Escherichia coli* (18 mm).

The collection of the fish sample of *Clarias batrachus* showed a significant activity with

regard to the Gram-positive as well as Gram-negative bacteria. The zone of inhibition values of mucus of *Clarias batrachus* were much more than the positive control (Streptomycin) tested and the inhibitory effect of mucus of *Clarias batrachus* against five bacterial strains are given in Table-1 and the graphical representation was shown in figure 1.

3.2 Antifungal activity of fish mucus

The magnitude of zone inhibition was *Aspergillus niger, Candida albicans* > *Fusarium moniliforme, Aspergillus nidulans* > *Trcihoderma koningi.* The maximum zone of inhibition was observed against both *Aspergillus niger* (fig-6) and *candida albicans* (15 mm), both of the inhibitory zone was measured about (15 mm). This was followed by *Aspergillus nidulans* and *Fusarium moniliforme,* and the inhibitory zone of both species were measured about (14 mm). But the *Trichoderma koningi*, shows least inhibitory zone of about (13 mm).

The collection of mucus from the fish *Clarias batrachus* showed a significant activity with regard to the fungal species. The zone of inhibition values of mucus collected from the fish *Clarias batrachus* against fungal growth were more than the *positive* control tested and the inhibitory effect of mucus of *Clarias batrachus* against five fungal strains are given in Table-2 and the graphical representation was shown in figure 2.

Sl. No	Bacterial Pathogens	Zone of inhibition (mm in diameter)		
		Test sample	+ve control	-ve control
1.	*Escherichia coli*	18	10	-
2.	*Aeromonas hydrophila*	22	12	-
3.	*Pseudomonas aeruginosa*	25	13	-
4.	*Vibrio fischeri*	20	15	-
5.	*Vibrio anguillarum*	21	15	-

Table-1. Antibacterial activity of the mucus of fish *Clarias batrachus*

Table-2. Antifungal activity of the mucus of fish *Clarias batrachus*

Sl. No	Fungal Pathogens	Zone of inhibition (mm in diameter)		
		Test sample	+ve control	-ve control
1.	*Aspergillus niger*	15	12	-
2.	*Aspergillus nidulans*	14	10	-
3.	*Fusarium moniliforme*	14	12	-
4.	*Candida albicans*	15	13	-
5.	*Trichoderma koningi*	13	9	-

Fig. 1. Antibacterial Potential of mucus from *Clarias batrachus*

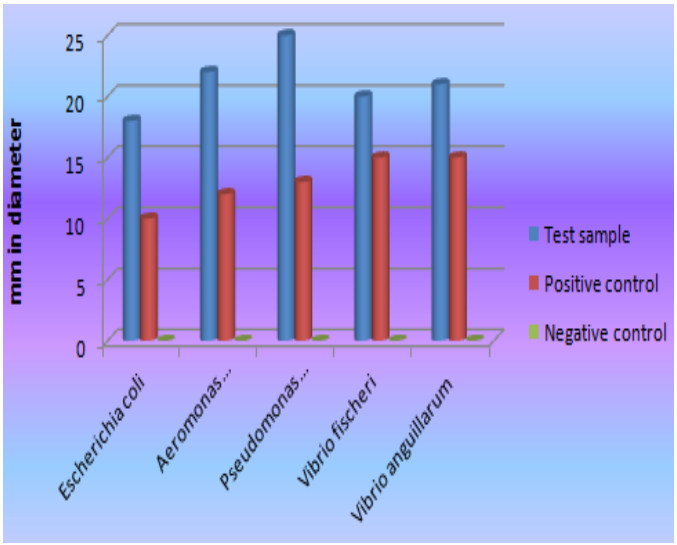
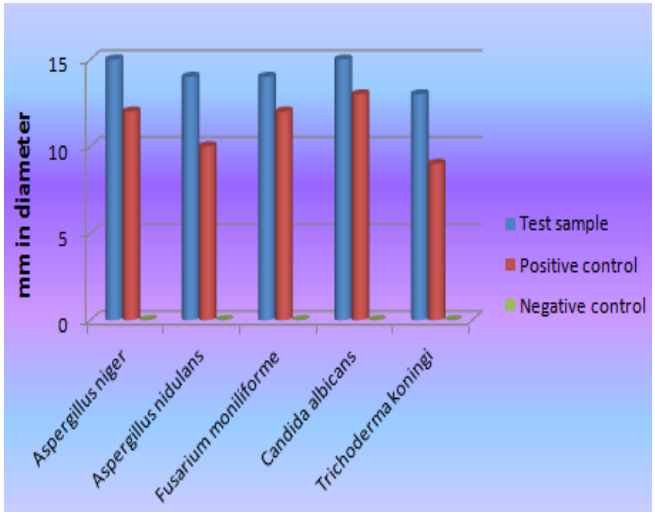


Fig 2. Antifungal Potential of mucus from *Clarias batrachus*



DISCUSSION

Fish are of great economic importance in aquaculture throughout the world. Intensive culture, the recent techniques for fish culture has increased the production but accompanied with the outbreak of many infectious diseases.

The main site of action of the majority of microbes is believed to be the plasma membrane, where they cause pore formation or membrane lysis(11).

Fish are in constant interaction with their aquatic environment, which contains a wide range of pathogenic and non-pathogenic microorganism. The epidermic and the epidermal mucus secretions act as biological barriers between fish and the potential pathogens of their environment (12).

Protection of fish against infectious diseases is a major challenge in aquaculture worldwide. However antibiotic use has raised concerns of antibiotic resistance development and antibiotic residues in fish. Virtually all fishes are covered with integumental mucus that is involved in many aspects of their biology ranging from disease resistance to rearing of young ones to shelter and locomotion. It was reported that epithelial tissue produces antimicrobial molecules which serve as the first line of host's defense against microbial invasion in a variety of vertebrates including humans (13).

The mucus producing cells in epidermal and epithelial layers had been reported to differ between fish species and therefore could influence the mucus composition. Furthermore, the biochemical substances of mucus have been showed to differ depending on the ecological and physiological conditions such as salinity, pH, handling stress and stages of growth and maturity (14,15). The variation in amount of mucus secretion between fish species had been observed to play a role in the susceptibility of the fish to infection. Pickering (16) reported that scale less fish produce higher levels of epidermal mucus for protection than fish with scales.

In agreement with the above studies in the present study also the fish secreted more amount of mucus which is having strong resistance to the invading microbes. The mucus collected from the fish shows strong activity against both the gram positive and gram negative bacteria.

The mucus of fishes contains a number of peptides which involve in the defence of invading microbes. Amphipathic α-helical peptides, such as dermaseptin, ceratotoxin and Magainin bind with anionic phsopholipid-rich membranes and dissolve them like detergents (17, 18). These peptides are known to exert action by binding to the surface of the microbial membranes and causing a lysis of the intracellular contents [19]. The present study is agreement with the above studies in showing the antimicrobial activity. The mucus collected from the fish *Clarias. batrachus* shows the strong antibacterial and antifungal activity against selected species.

Mucus contains several proteases (serine proteases, cysteine proteases, metalloproteases and trypsin like proteases) having strong antibacterial activity (20). Mucus represents an important biological interface between the animal and their aqueous environment. The mucus and epidermis are important in fish defense because they are the first sites of interaction between the host and potential pathogens. Within these layers are many enzymes and antimicrobial proteins, which are thought to be involved in innate immunity of the fish (21).

The antimicrobial substance present in the mucus may function either in the cytoplasm against intracellular pathogens or extracellularly through release to mucosal surfaces after infection-induced cell lysis or apoptosis. There are so many proteins present in the fish mucus which exerts strong

resistance to invading pathogens. Choncha (22) demonstrated that the lipoprotein A-I and A-II inhibits the growth of Gram-positive and negative bacteria including fish pathogens at micromolar contractions. These findings support out results that the mucus collected from the fish *Clarias batrachus* shows strong microbial resistance. Further purification of the active compounds is necessary in order to identify their chemical nature and to evaluate their potential as novel drug leads.

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