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Antimicrobial Activity of Skin Mucus of some Cultivable Fish Species

Singh A.S.[1], Talpade M.B[2]

Department of Zoology, Mithibai College, Vile Parle (W), Mumbai 400 056, India

Abstract: ***The present study antimicrobial activity of skin mucus of fish some cultivable fish species namely Catlacatla,Labeorohita, Hypophthalmichthys molitrix and Ctenopharyngodonidella against pathogenic bacterial strains viz. Staphylococcus epidermidis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi and fungal species viz Candida albicans, Aspergillus flavus and Aspergillus niger. Skin mucus of fish species were collected by skin scarping method. Antimicrobial activity of mucus extract was carried out by disk diffusion method and measured in terms of zone of inhibition in mm, where as antibiotic Ampicillin and griseofulvin were taken as standard. In the present study, skin of mucus of all the fish species showed antimicrobial activity against the tested microbial pathogens. Moreover, the mucus of fish possesses antimicrobial agents which could be used to formulate new drugs for the therapy of infectious diseases caused by pathogenic microorganisms.***

Keywords: Antimicrobial, skin fish mucus, disk diffusion method

1. Introduction

Fish live in a challenging environment facing so many problems. The microbes play a major role in affecting the fish health. They escape from such an environment by producing some substances on the dermal layer (Mucus). The epidermal mucus produced primarily by epidermal goblet or mucus cells are composed mainly of water and gel forming macromolecules including mucins and other glycoproteins (Shephard, 1993). The mucus substance secreted from the surface of fish performs a number of functions including disease resistance, respiration, ionic and osmotic regulation, locomotion, reproduction, communication, feeding and nest building (Ingram, 1980; Fletcher, 1978). Despite an intimate contact with high concentrations of pathogens (bacteria and viruses) in their environment, the fish can still maintain a healthy system under normal conditions. This could be attributed to a complex system of innate defense mechanisms within themselves, particularly the products of broad spectrum-antimicrobial compound. Many researchers have proved that the mucus substances are good resistant to invading pathogens (Ingram, 1980; Fletcher, 1978; Austin and Mcintosh, 1988; Fouz*et al.*, 1990).

The development of resistance by a pathogen to many of the commonly used antibiotics provides an impetus for further attempts to search for new antimicrobial agents, which overcome the problems of resistance and side effects. Action must be taken to reduce this problem such as controlling the use of antibiotics, carrying out research to investigate drugs from natural sources. Drugs that can either inhibit the growth of pathogen or kill them and have no or least toxicity to the host cell are considered for developing new antimicrobial drugs. It is well known that the global trade in animal based medicinal products accounts for billions of dollars per year (Kunin and Lawton, 1996). Unlike conventional antibiotics, which are synthesized enzymatically by microorganisms, are encoded by a distinct gene (AMP) and made from an mRNA template. The continuous use of antibiotics has resulted in multi resistant bacterial strains all over the world (Mainous and Pomeroy, 2001). Consequently, there is an urgent need to search for alternatives to synthetic antibiotics. In spite of modern improvements in chemotherapeutic techniques, infectious diseases are still an increasingly important public health issue (WHO, 2002). It has been estimated that in 2000, at least two million people died from diarrhoeal disease worldwide (WHO, 2002). Still there is a need for new methods of reducing or eliminating pathogens, possibly in combination with existing methods (Leistner, 1978). In the aquatic environment, fish are in constant interaction with a wide range of pathogenic and non-pathogenic microorganisms (Subramanian *et al.*, 2007).Hence it was decided to evaluate the bactericidal and fungicidal properties skin mucus of some fishes namely *C. catla, H. molitrix, Labeorohita and CtenopharyngodonIdella* against some bacterial and fungal pathogens

2. Materials and Methods

Collection of mucus

The healthy live fishes approximately 6 months old, weigh about 500 gms of each *C. catla, L. rohita, H. molitrix C. idella* were purchased from local fish farm in Mumbai, Maharashtra. Mucus was carefully scraped from the dorsal surface of the body using a sterile spatula. Mucus was not collected in the ventral side to avoid intestinal and sperm contamination. The collected fish mucus was stored at 4ºC for further use. Preparation of mucus sample for the antimicrobial The mucus samples were collected aseptically from the fish and thoroughly mixed with equal quantity of sterilized physiological saline (0.85% NaCl) and centrifuged at 5000 rpm for 15 min, the supernatant was used for the antimicrobial studies and kept at 4°C until use. A thin layer of molten agar (Muller Hinton Agar) was dispensed in petriplates of 10 × 10 cm and was labled properly. Triplicates were maintained for each strain. In the same way for fungal studies PDA medium was dispensed in petriplates for different strains of fungi in triplicates and the plates were marked.

Preparation of mucus sample for the antibacterial and antifungal studies: The mucus samples were collected

aseptically from the fish and thoroughly mixed with equal quantity of sterilized physiological saline (0.85% NaCl) and centrifuged at 5000 rpm for 15 min, the supernatant was used for the antimicrobial studies and kept at 4°C until use. A thin layer of molten agar (Muller Hinton Agar) was dispensed in petriplates of 10 × 10 cm and was labled properly. Triplicates were maintained for each strain. In the same way for fungal studies PDA medium was dispensed in petriplates for different strains of fungi in triplicates and the plates were marked.

Bacterial cultures: The standard pathogenic bacterial cultures were procured from IMTECH, Chandigarh, India and used in the present study (Table 1). The bacterial cultures were rejuvenated in Mueller- Hinton broth (Hi-media laboratories, Mumbai, India) at 37^0C for 18h and then stocked at 4^0C in Mueller-Hinton Agar. The inoculum size of the bacterial culture was standardized according to the National committee for Clinical Laboratory Standards (NCCLS, 2002) guideline. The pathogenic bacterial culture was inoculated into sterile Nutrient broth and incubated at 37^0C for 3h until the culture attained a turbidity of 0.5 McFarland units. The final inoculum size was standardized to 10^5 CFU/mL with the help of SPC and Nephlo-turbidometer.

Table 1: Bacterial cultures used in study (IMTECH, Chandigarh, India)

Bacterial Pathogens	MTCC Number
Staphylococcus epidermidis	435
Staphylococcus aureus	96
Escherichia coli	739
Pseudomonas aeruginosa	424
Salmonella typhi	733

Fungal cultures: The standard pathogenic fungal cultures were procured from IMTECH, Chandigarh, India and used in the present study (Table 2). The fungal culture rejuvenated in Sabouraud dextrose media (Hi-media laboratories, Mumbai, India) at 37^0C for 18h and then stocked at 4^0C in SDA. Subcultures were prepared from the stock for bioassay. A loopful of culture was inoculated in 10 ml of sterile Potato Dextrose broth and incubated at 37^0C for 24h. Turbidity of the culture was standardized to 10^5 CFU with the help of SPC and turbidometer.

Table 2: Fungal cultures used in study (IMTECH, Chandigarh, India)

Fungal Strain	MTCC Number
Candida albicans	183
Aspergillus niger	478
Aspergillus fumigatus	870

Antimicrobial activity: The microbial strains were collected from IMTECH, Chandigarh, India. In vitro antibacterial assay was carried out by disc diffusion technique (Bauer et al., 1996).Turbidity of inoculums was matched with McFarland turbidity standard (NCCLS, 2002, Dahikar, 2018). Inoculums were spread over the Nutrient agar plate using a sterile cotton swab in order to get a uniform microbial growth.For antifungal properties, 0.1 ml fungal suspension of 10^5 CFU ml^{-1} was uniformly spread on PDA plate to form lawn cultures. Whatman No.1 filter paper discs with 10 mm diameter were impregnated with known amount (10 µl) of test sample of fish mucus and a standard antibiotic disc. At room temperature (37ºC) the bacterial plates were incubated for 24 h. The fungal plates were incubated at 30ºC for 3 to 5 days for antifungal activity. The results were recorded by measuring the zones of growth inhibition surrounding the disc. Clear inhibition zones around the discs were expressed in terms of diameter of zone of inhibition and were measured in mm using cm scale, recorded and the average were tabulated.

3. Results and Discussion

Antimicrobial effect of the mucus of surface feeder and column feeder freshwater fishes namely, C. catla, H. molitrix (Surface feeder), L. rohita (Column feeder), C. idella were tested against, pathogenic bacteria viz, *K. pneumonia, V. cholerae, S. typhi, E. coli, P. aeruginosa* and five pathogenic fungi viz, *Mucor globosus, Rhizopus arrhizus, Candida albicans, Aspergillus flavus, Aspergillus niger*. The activity was measured in terms of zone of inhibition in mm. Antibacterial effect of mucus from surface feeders and column feeders. The inhibition effects of mucus of *C. catla, H. molitrix* against five pathogenic bacterial strains are given in Table 1 and the zone of inhibition by the mucus of *L. rohita, C. idella* are given in Table 2. The zone of inhibition values of mucus was compared with control (Ampicillin) and the observed values are tabulated in Tables 1 and 2, respectively.

Table 3: Antibacterial activity of Crude mucus extract of Catl*acatla, Labeorohita, Hypophthalmichthys molitrix and Ctenopharyngodonidella* against bacterial pathogens (Zone of inhibition of growth in mm, average of 3 readings)

Crude mucus extract		*S. epidermidis*	*S. aureus*	*E. coli*	*P. aeruginosa*	*S. typhi*	*C. albicans*	*A. niger*	*A. fumigatus*
Catlacatla,		22	32	27	21	21	17	19	17
Labeorohita,		22	32	24	17	17	20	17	16
Hypophthalmichthys molitrix		16	24	23	20	15	15	21	-
Ctenopharyngodonidella		21	18	20	23	14	16	23	19
Negative control		-	-	-	-	-	-	-	-
Positive control	Ampicillin (10mcg/disc)	16	25	24	11	16	-	-	-
	Griseofulvin (10mcg/disc)	-	-	-	-	-	18	22	25

The mucus of *C. catla* showed potent antibacterial effect against *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Escherichia coli*,while moderate against *Pseudomonas aeruginosa, Salmonella typhi*

The mucus of H. molitrix showed more effect in controlling the growth of *Escherichia coli* and *P. aeruginosa* with an inhibition zone of 23 and 20 mm in diameter. Moderate effect was observed in controlling the growth of *Salmonella typhi* with a zone of inhibition is 15 mm, Whereas the mucus

of *L. rohita* showed a strong effect in controlling the growth of *Staphylococcus aureus* with an inhibition zone of 32 mm diameter. *Staphylococcus epidermidis* and *Escherichia coli* showed a better effect in the mucus of *H. molitrix* with an inhibition zone of 23 mm in diameter. Among the five bacteria tested *S. typhi* showed less sensitivity to the mucus with an inhibition zone is 15 mm. The mucus of *C. idella* showed more effect in controlling the growth of *E. coli* with an inhibition zone of 20 mm diameter. The moderate effect was observed in controlling the growth of *S. typhi* (14 mm) and P. aeruginosa 23 mm) by the mucus of *C. idella*.

Skin mucus from *Catlacatla, Labeorohita, Hypophthalmichthys molitrix, CtenopharyngodonIdella* exhibited antifungal activity against phytopathogenic fungi like *C. albicans, A. nigerand A. fumigates* (Table 3). In the present study, variation in their antimicrobialactivity was observed among the fish mucus. This maybe due to the variation in the relative levels of lysozyme,alkaline phosphatase, cathepsin B and proteases of theepidermal mucus of all fish species (Subramanian et al.,2007).In future fish mucus may be exploited as biocontrolling agent for fungal diseases. The outermost surface of fish skin and gills are composed of epithelial cells. These outermost layers are covered with mucus layer composing of biochemically-diverse secretions from epidermal and epithelial cells. The mucus layer acts as a biological interface between fish and their aqueous environment. The skin mucous layer and epidermis are important in fish defence because they are the first sites of interaction between the host and potential pathogens. Over the past years, it has also been shown that mucus plays a role in the prevention of colonization by parasites, bacteria and fungi and the antibacterial role of mucus has been known for many years (Austin and Mcintosh, 1988). Fish mucus was found as a source of antimicrobial products (Hellio*et al.*, 2002). Now days there are number of synthetic drugs and food preservatives are available, which are considerably affects on environment and ecosystem due to their higher persistency and constant accumulation in the biological system. To overcome this, considerable investigations are being carried out to develop safer sourcea.

4. Conclusion

In the present study it was that the fish mucus has potent antimicrobial activity against some bacterial and fungal pathogens, So the study concluded that the mucusof fish possesses antimicrobial agents which could beused to formulate new drugs for the therapy of infectiousdiseases caused by pathogenic and opportunisticmicroorganisms. These properties of mucus suggest thatit may be beneficial in aquaculture and human health relatedapplications. Further studies are needed to isolatethe bioactive compounds (antimicrobial substances) fromthe mucus of these cultivable fish species and themechanism of antimicrobial action.

References

[1] Austin B, McIntosh D (1988). Natural antibacterial compounds on the surface of rainbow trout. J. Fish Dis., 11: 275-277
[2] Dahikar S.B. (2018). In Vitro Antimicrobial Activity of Fruit Extracts of *LagenariaSiceraria* (Mol.)Research Journal of Pharmacognosy and Phytochemistry, 10(2):183-186
[3] Fletcher T (1978). Defense mechanisms in fish. In: Malins D, Sargent J (Eds.). Biochemical and biophysical perspectives in marine biology. Academic Press, London. pp. 189-222
[4] Fouz B, Devesa S, Gravningen K, Barja JL, Tranzo AE (1990). Antibacterial action of the mucus of the turbot. Bull. Eur. Assoc. Fish Pathol., 10: 56-59.
[5] Hellio C, Pons AM, Beaupoil C, Bourgougnon N, Legal Y. (2002). Antibacterial, antifungal and cytotoxic activities of extract from fish epidermis and epidermal mucus. Int J. Antimicrob Agents, 20:214-219
[6] Ingram GA (1980). Substances involved in the natural resistance of fish to infection-a review. J. Fish Biol., 16: 23-60.
[7] Kunin WK, Lawton JH (1996). Does biodiversity matter? Evaluating the case for conserving species. In Gaston KJ (Ed) Biodiversity: A Biology of numbers and differences, Oxford: Blackwell Sci., 283-308.
[8] Mainous A, Pomeroy C (2001). Management of Antimicrobials in Infectious Disease. Humana Press, New York. p. 349.
[9] NCCLS (National Committee for Clinical Laboratory Standards), Performance Standards for antimicrobial susceptibility testing. 8th Informational Supplement. M100 S12. National Committee for Clinical Laboratory Standards, Villanova, Pa 2002.
[10] Shephard KL (1993). Mucus on the epidermis of fish and its influence on drug delivery. Adv. Drug. Deliv. Rev., 11: 403-417.
[11] Subramanian S, Mackinnon SL, Ross NW (2007). A comparative study on innate immune parameters in the epidermal mucus of various fish species. Comp. Biochem. Physiol., 148B: 256-263.
[12] WHO (2002). Food safety and food borne illness. World Health Organization Fact Sheet, Geneva. 237.