Vol II Issue IX March 2013

Impact Factor : 0.1870

ISSN No :2231-5063

Monthly Multidisciplinary Research Journal

Golden Research Thoughts

IMPACT FACTOR : 0.2105

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RNI MAHMUL/2011/38595 **ISSN No.2230-7850**

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Golden Research Thoughts
Volume 2, Issue. 9, March. 2013
ISSN:-2231-5063

Available online at www.aygrt.isrj.net
DOI : 10.9780/2231-5063/292013/1646

ORIGINAL ARTICLE

A STUDY ON THE ANTIFUNGAL PROPERTIES OF SKIN MUCUS FROM SELECTED FRESH WATER FISHES.

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Abstract:

Fish faces lot of diseases due to their aquatic environment, which contains a wide range of pathogenic and non pathogenic microorganisms. They may escape from such diseases by producing (or) secreting a jelly like substance called mucus, which act as a defensive barrier between the fish and potential pathogens. Several studies stated that the mucus secretion of fish consist of a number of antimicrobial peptides that provide a first line of defence against invading microbes (or) pathogens. Little is known about the antimicrobial properties of fresh water fish mucus. Hence the present attempt has been made to find out the potency of the mucus collected from seven different fresh water fishes such as Catla catla, Labeo rohita, Cirrhinus mrigala ,Cetenopharyngodon idella, Hypophthalmichthys molitrix, Mugil cephalus, and Channa punctatus against five different fungal strains viz., Mucor globosus, Rhizopus arrhizus, Candida albicans, Aspergillus flavus and Aspergillus niger. The result reveals that the mucus of different fish exert variation in their activity in controlling the growth of tested fungal strains.

KEY-WORDS:

Epidermal mucus, Zone of inhibition, antimicrobial activity.

INTRODUCTION

Modern improvement in chemotherapeutic techniques for infectious diseases are still an increasing important public health issue (WHO,2002). Hospitals worldwide have become literal breeding grounds for some of the most deadly bacteria. Many infections acquired in hospitals are caused by potentially fatal bacteria, such as Staphylococcus aureus, that survive for extended periods on medical devices such as intravenous lines and catheter tubes. It is now estimated that half of Staphylococcus aureus strains found at many medical institutions are resistant to antibiotics such as methicillin (Roder,1999). A series of natural compounds that exhibit antimicrobial activity and have been isolated in the past 20 years from many plant, insect and animal species (Zasloff, 2002).

Naturally occurring antifungal proteins or peptides, as well as synthetic derivative, have the potential to be very interesting clinical leads. Fungi are extremely diverse group of organisms, with about 2,50,000 species widely distributed in essentially every ecosystem (Ody, 1993).

During the past decades, an increase in the incidence of fungal diseases has been recognized mainly due to Candida spp. and filamentous fungi such as Aspergillus spp. (Mavor et al., 2005). To date, there are no licensed fungal vaccines and the use of antimycotics is the only option for the treatment of fungal infections, currently used antimycotics, however frequently have a limited activity spectrum are available only in intravenous formulations, favour resistance development and cause serious side-effects (Brakhage, 2005). Thus the search for new antifungal therapies is strongly stimulated and the use of antifungal peptides is a promising alternative.

Zootheraphy is the healing of human diseases by the use of therapeutics obtained or ultimately derived from animals. Evidence of zootheraphy has been found in archives of several civilizations of ancient Mesopotamia, mainly the Assyrian and the Babylonian. These contain descriptions of fish oil, bee wax and honey, manogoose blood, turtle shell, goat's skin, gazelle sinew and even sheep, deer and animal fat (Costa Neto,1999).

Animals are yet poorly explored source for medicines despite they are well known ingredients for many popular medicines some of them recognized by current and/or past pharmacopoeias around the world. Geckos, frogs and other various insects are used in many Asitic Materia medica; Spanish flies and leeches were listed for a long time in western pharmacopoeias and maggots has been listed in the US pharmacopoeia (Lev Efarin, 2003).

Kalita et al. (2005) collected the information about plant and animal based folk medicine used by people of Diabrugarh district, Assam for treatment of eleven different diseases. They collected information on utility of 19 plant species and four animal species. In modern society, zootherapy constitutes an important alternative among many other known therapies practiced world wide.

More recent investigations have revealed that the surface mucosa of some fish contains a range of AMPs (Bergsson et al., 2005). Haruan (Channa striatus) is a clear example as it is endowed with remarkable wound healing, anti inflammatory, antinociceptive, platelet aggregation, as well as mild antimicrobial and antifungal properties (Subramanian et al., 2009).

Fish are in constant interaction with their aquatic environment which contains a wide range of pathogenic and non-pathogenic microorganisms. The epidermis and the epidermal mucus secretions act as biological barriers between fish and the potential pathogens of their environment (Mat Jais, 2007). Previous demonstrations of the protective role of mucus from various fish species [Shephard,1993; Nagashima et al., 2001; Kuppulakshmi et al., 2008; Balasubramanian et al., 2012) suggest that the epidermal mucus act as a first line of defence against pathogens and therefore may offer a potential source of novel antimicrobial components. Although the epidermal mucus from several fish has been explored for antimicrobial components, to date little information is available on the antimicrobial activity of the fresh water fishes.

Studies on antifungal activity of epidermal mucus is restricted to a few fresh water fish species. So the fresh water cultivable and economically important wild species such as Catla catla, Labeo rohita, Cirrhinus mrigala ,Cetenopharyngodon idella, Hypophthalmichthys molitrix, Mugil cephalus, and Channa punctatus were taken for the present study.

The purpose of this study was to gain a better understanding of the antimicrobial functions of epidermal mucus in these fresh water indigenous and exotic fish species and to identify potential sources from above mentioned fishes.

MATERIALS AND METHODS

The healthy live fish with the weight approximately of 750 to 1000g Catla catla, Labeo rohita, Cetenopharyngodon idella, Hypophthalmichthys molitrix were purchased from near by fish farm in Pinnalur, Cuddalore district. Channa punctatus and Cirrhinus mrigala were purchased from near by fish pond in located at Puthur, Sirkali taluk, Nagappatinum district. Mugil cephalus were purchased from lower Anaikkat in Kollidam river, Thanjavur district.

Acclimatization

The collected fishes were acclimatized to laboratory condition in well water and they were maintained as such for four days. After four days these fishes were used for mucus collection.

Collection of mucus

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. The collected fish mucus were stored separately at 40 C for further use.

Preparation of mucus sample for fungal studies

The aseptically collected mucus samples from the fishes were thoroughly mixed with equal quantity of sterilized physiological saline (0.85%NaCl) and centrifuged at 5000 rpm for 15 minutes the supernatant was used for antimicrobial studies.

A thin layer of PDA(Potato Dextrose Agar)medium was dispensed in 90×45 mm petriplates for different strains of fungi in triplicates and the plates were marked.

Inoculation of fungal strains

The microbial strains were obtained from Raja Muthiah Medical College Annamali university, Annamali nagar, Cuddalore district, Tamil Nadu.

Invitro antifungal assay was carried out by disc diffusion technique (Sarmasik, 2002). Whatman No.1 filter paper disc with 4mm diameter were impregnated with known amount (10µl) of test sample of fish mucus and a standard antibiotic disc with Ketoconazole.

The fungal culture were incubated at 26±2oC for 3-5 days for antifungal activity. The results were recorded by measuring the zone of growth inhibition surrounding the disc. Clear inhibition zones around the disc were expressed in terms of diameter of zone of inhibition and were measured in mm using cm scale recorded and the average were tabulated.

RESULTS

Antifungal activity (Zone of inhibition) of the mucus of selected seven fresh water fish are tabulated in Table-1. Five fungal strains have been selected for the present study, they are Mucor globosus, Rhizopus arrhizus, Candida albicans, Aspergillus flavus and Aspergillus niger.

The mucus of the Catla catla showed a maximum effect in controlling the growth of Aspergillus flavus with an inhibition zone of 17mm diameter which is less than the control (19 mm). Next to this the Mucor globosus 16 mm and Rhizopus arrhizus 16 mm have more zone of inhibition. Whereas, the mucus of Catla catla showed less effective in controlling the growth of Candida albicans (14 mm) and Aspergillus niger (9 mm).

The mucus from Labeo rohita had more effect in controlling the growth of Aspergillus flavus (17 mm). It has very less zone of inhibition on the growth of Mucor globosus (14 mm) and Rhizopus arrhizus (14 mm). It showed better effect in controlling the growth of Candida albicans (15 mm) and Aspergillus niger (15 mm).

The mucus of Cirrhinus mrigala showed maximum zone of inhibition against Aspergillus flavus (9 mm) and Aspergillus niger (9 mm). It didn't show the zone of inhibition against Mucor globosus, Rhizopus arrhizus and Candida albicans.

The mucus of Ctenopharyngodon idella showed the highest activity against Aspergillus flavus with an inhibition zone of 16 mm. The better effects were observed in controlling the growth of Mucor globosus (15 mm), Rhizopus arrhizus (15 mm) and Candida albicans (13 mm). But it failed to control the growth of Aspergillus niger.

Likewise the mucus collected from Hypophthalmichthys molitrix showed highest effect in controlling the growth of Aspergillus flavus with an inhibition zone of 17 mm. On the contrary there was no effect in controlling the growth of Rhizopus arrhizus and Candida albicans. Moderate effects were observed in controlling the growth of the Mucor globosus (14 mm) and Aspergillus niger (8 mm).

Mucus from Mugil cephalus showed maximum zone of inhibition against Mucor globosus (12 mm), the moderate effects were observed in Candida albicans (10 mm), Rhizopus arrhizus (8 mm) and Aspergillus niger (7mm). Whereas the mucus of Mugil cephalus has less effect in controlling the growth of Aspergillus flavus (5 mm).

The mucus of Channa punctatus did not show any inhibition activity against selected pathogenic fungi.

The antifungal activity of control Ketoconazole showed a variety of activity against Mucor globosus (16 mm), Rhizopus arrhizus (15 mm), Candida albicans (17 mm), Aspergillus flavus (11 mm)and Aspergillus niger (11 mm).

Among these tested fishes the mucus of Labeo rohita showed highest inhibition zones against majority of fungal varieties. Next to that Catla catla showed higher inhibition zones against the tested fungi. These two are followed by the Ctenopharyngodon idella, Hypophthalmichthys molitrix and Mugil cephalus. On the contrary the Cirrhinus mrigala showed less activity in the two tested fungi.

Table -1. Antifungal properties of skin mucus from selected fresh water fishes.

Pathogens	*Catla catla*	*Labeo rohita*	*Cirrhinus mrigala*	*Ctenopharyngodon idella*	*Hypophthalmichthys molitrix*	*Mugil cephalus*	*Channa punctatus*	Ketoconazole
Mucor globosus	16mm	14mm	_	15mm	14mm	12mm	_	16mm
Rhizopus arrhizus	16mm	14mm	_	15mm	_	8mm	_	15mm
Candida albicans	14mm	15mm	_	13mm	_	10mm	_	17mm
Aspergillus flavus	17mm	17mm	9mm	16mm	17mm	5mm	_	19mm
Aspergilus niger	9mm	15mm	9mm	_	8mm	7mm	_	11mm

DISCUSSION

Fish posses strong immune system which act as the first line defence against a broad spectrum of pathogen (Bauer et al., 1996). The aquatic environment presents frequent and high levels of exposure to a huge number of pathogens.

Antimicrobial peptide also known as the host defence peptides, play major role in the innate immune system and protect against a wide variety of bacterial, fungal, viral and other pathogenic infections (Ruiz Herrera, 1992; Subramanian et al., 2008).

Both the indigenous and exotic fish species have the activity against the fungal pathogens, whereas some fungal pathogens were not controlled by the mucus of exotic fishes. But the mucus of indigenous fishes except the bottom dwellers (Cirrhinus mirgala and Channa punctatus) controlled all the tested pathogenic.

In our observation the fishes Catla catla, Labeo rohita, Mugil cephalus show the higher antifungal activity than that of exotic fish species such as Ctenopharyngodon idella, Hyphophthalmichthys molitrix but the Mugil cephalus show moderate antifungal activity in all the tested fungi.

Fungal cell walls are mostly composed of polysaccharide such as chitin and glucon (Ruiz Herrera, 1992). In addition fungal cell walls contain 3-20% protein, 1-10% lipids as major components. Spore cell walls exposed to the enzyme might be damaged by the enzyme through the oxidation of cell wall proteins and lipids (Griffin, 1994) and hence the spore cells might be disintegrated by the enzyme which will prevent the colony establishment. Antimicrobial peptides are preferentially more selective to proteolytic cell membrane.Under the natural conditions asexual spore serve as the primary inoculum for fungi. Disease incidence is directly related to the ability of spores to germinate (Griffin, 1994).

α-helical peptide, parasin has been isolated from the epithelial mucosa of cat fish Parsilurus asotus and apparently drives from histone H2A (Yang and Anderson, 1999). Both peptides are quite potent and show wide spectrum of antimicrobial activity against fungi. It has rod like helical structure and appears to penetrate and inactivate bacteria and fungi without causing membrane permeabilization (Parket al., 1998).

In the present study, variation in their antifungal activity was observed among the fishes. This may be due to the variation in the relative levels of lysozyme, alkaline phosphatase, cathepsins B and protease of the epidermal mucus of all fish species (Subramanian et al., 2007).

Fish epidermal mucus contains naturally occurring proteins (or) glycoproteins of non-immunoglobulin nature that react with a diverse array of environmental antigens and may concern an undefined degree of natural immunity. Those compounds exhibited fungal growth inhibitory activity via simple metal ions chelating mechanisms, which deprive microbes of essential inorganic ion sources (Alexander and Ingram, 1992).

In agreement with the above findings the present study demonstrated that the mucus collected from the selected fishes exhibited antifungal activity. Moreover the mucus of fish posses antimicrobial agents which could be used to formulate new drugs for the therapy of infectious disease caused by pathogenic and opportunistic microorganisms. These properties of mucus suggest that it may be beneficial human health related applications. Further studies are needed to isolate the bioactive compounds form the fish mucus and the mechanism of antifungal action.

Further the variation in antimicrobial activity might be due to the biochemical variation in the mucus of different species and depending on the ecological conditions (Mat Jais, 2007).The variation in the zone of inhibition among the different taxa of fish could be due to the multitude of different peptides produced by the mucus of fishes as described by Ashcroft et al. (Ashcroft et al., 2007) in the frog.

The absence of antifungal activity in the mucus of Channa punctatus and less activity in C. mirgala could be due to the (though they are the bottom dwellers) inactivation of enzymes by the incubation temperature and pH condition used in the antimicrobial study as described by Subramanian et al. (2008).

The suggested antifungal activity of mucus from fishes might be due to pore formation or disruption of fungal cell membrane in salt dependent and energy independent or formation of reactive oxygen species depletion or mitochondria or binding to a receptor on the fungal cell membrane (Jenssn et al., 2006). Rodrigues et al.(2009) mentioned that the antifungal activity of AMPs are due to the inhibition of germination of conidia or inhibition of chitin synthesis. In an unpublished data we observed that the mucus of Mugil cephalus posses higher content of lysine. Our finding was supported by Mor et al.(1994) who found that lysine rich linear peptides in the mucus of American frog Phyllomedusa sauvagii had fungicidal activity.

AKNOWLEDGEMENT

Authors thank the authorities of Annamalai University and the Head of the Department of Zoology for providing the facilities to carry out this study.

REFERENCES

Alexander JB, Ingram GA, 1992. Non cellular non specific defense mechanism of fish. Ann Rev Fish Dis., 2:249-279.

Ashcroft JW, Zalinger ZB, Bevier CR, Feket FA, 2007. Antimicrobial properties of two purified skin peptide from the mink frog(Rana septentrionalis) against bacteria from natural habitate. Com Bio Che Phys.,146(3) :325-330.

Balasubramanian S, Baby Rani P, Arulprakash A, Prakash M, Senthil Raja P and Gunasekaran G, 2012. Antimicrobial properties of skin mucus from four freshwater cultivable fishes (Catla catla, Hypophthalmichthys molitrix, Labeo rohita and Ctenopharyngodon idella). Afri J Micro Bio Res.,6(24):5110-5120.

Bauer AW, Kirby WM, Sherris JC, Turck M, 1996. Antibiotic susceptibility testing by a standardized single disc method. Am J Clin Pathol., 45:493-496.

Bergsson G, Agerberth B, Jörnvall H, Gudmundsson GH, 2005. Isolation and identification of antimicrobial components from the epidermal mucus of Atlantic cod (Gadus morhua). FEBS J., 272: 4960–4969.

Brakhage AA, 2005. Systemic fungal infection caused by Aspergillus species. Epidemiology, infection process and virulence determinants. Curr Drug Targe., 6:875-886.

Costa Neto EM, 1999. Healing with animals in Feira de Santana City, Bahia, Brazil. J Ethnopharmacol., 65(3):2225-2230.

Griffin DH, 1994. Molecular architecture. In:Fungal Physiology, 2nd Edn, Willy-Liss: New York.65-74.

Jenssn H, Gutteberg TJ, Lejon K, 2006. Modelling the antiherpse simplex virus activity of small cationic peptides using amino acid descritors. J Pept Res., 66 :48-56.

Kalita Dillip,Dutta Manashi, Islam Nazim Forid, 2005. Few plants and animal based folk medicines from Dibrugarh district, Assam. Indian J Tratitional Knowled.,4:181-185.

Kuppulakshmi C, Prakash M, Gunasekaran G, Manimegala G and Sarojini S, 2008. Antibacterial properties of fish mucus from Channa punctatus and Cirrihinus mrigala. Eur Rev Med Pharmacol Sci., 12:149-153.

Lev Efarin review; 2003. Traditonal healing with animals (zootheraphy). Medieval to present- day Levantine practice J Ethono Pharma., 85;107-118.

Mat Jais AM, 2007. Pharmacognosy and pharmacology of harun (Channa striatus)a medicinal fish with wound healing properties revision/review boltein Latinoamericano plants medicinal aromatics., 6(3):52-60.

Mavor AL, 2005. Thewes S,Hube B,systemic fungal infections caused by candida specis. Epidemiology , infection process and virulence attributes. Curr Drug Targets., 6:863-874.

Mor A, Hani k, Nicolas p, 1994. The vertebrate peptide antibiotics dermaeseptins have overlap structural features but target specific organisms J Bio Chem., 269;31635-31641.

Nagashima Y, Sendo A, Shimakura K, Kobayashi T, Kimura Fujii T, 2001. Antibacterial factors in skin mucus of rabbit fishes. J Fish Biol., 58: 1761–1765.

Ody P, 1993. The complete medicinal Herbal. Dorling Kindersley Limited. New York USA. 132-171.

Park IY, Park CB, Kim MS, Kim SC, 1998. Parasin I. An antimicrobial peptide derived from histone H2A in the catfish, (Parasilurus asotus). FEBS Lett.,437:258–62.

Roder BL, 1999. Clinical features of Staphylococcus aureus endocarditis. A 10-year experience in Denmark Arc Inter Med.,159:462–469.
Rodrigues E, Dobroff S, Taborda P, Travassos R, 2009. Antifungal and antitumor models of bioactive protective peptides. Anaisda academia brasileria deciencias;81(3):503-520.
Ruiz Herrera J, Chemical composition of the fungal cell wall. In: Fungal Cell Wall. Structure, Synthesis and Assembly. CRC press London 1992;5-40.
Sarmasik A, 2002. Antimicrobial peptides: a potential therapeutic alternative for the treatment of fish diseases. Turk J Biol., 26: 201–207.
Shephard KL , 1993. Mucus on the epidermis of fish and its influence on drug delivery. Adv Drug Deliv Rev.,11:403–417.
Subramanian S, MacKinnon SL, Ross NW, 2007. A comparative study on innate immune parameters in epidermal mucus of various fish species. Comp Bioche Phys.,148B:256-263.
Subramanian S, Ross NW, MacKinnon SL, 2008. Comparison of the biochemical composition of normal epidermal mucus and extruded slime of hagfish (Myxine glutinosa L.). Fish Shellfish Immu.,25:625–632.
Subramanian, S., Ross, N.W., MacKinnon, S.L, 2009. Myxinidin, a novel antimicrobial peptide from the epidermal mucus of hagfish, Myxine glutinosa. L Marin Biotech., 11: 748–757.
WHO, 2002. Food safety and food borne illness. World Health Organisation fact sheet 237. Geneva. Yang Y, Anderson EJ, 1999. Antimicrobial activity of a porcine myeloperoxidase against plant pathogenic bacteria and fungi. J App Micro Biol., 86:211-220.
Zasloff M, 2002. Antimicrobial peptides of multicellular organisms. Natu.,415: 389–395.

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