International Research Journal of Microbiology (IRJM) (ISSN: 2141-5463) Vol. 5(2) pp. 22-27, March, 2014
DOI: http:/dx.doi.org/10.14303/irjm.2014.013
Available online http://www.interesjournals.org/IRJM
Copyright © 2014 International Research Journals

Full Length Research Paper

Antibacterial studies of fish mucus from two marketed air-breathing fishes – *Channa striatus* and *Heteropneustes fossilis*

M. A. Haniffa*, S. Viswanathan, D. Jancy, K. Poomari, S. Manikandan

*Centre for Aquaculture Research and Extension, St.Xavier's College, Palayamkottai, Tamil Nadu (India) Post Graduate Department of Microbiology, Sri Paramakalyani College, Alwarkurichi, Tamilnadu (India).

*Corresponding authors e-mail: drsvmicro@gmail.com

Abstract

The purpose behind the current investigation was to understand the role of antibacterial activity of mucus in marketed air breathing fishes *viz*: against the selected human and fish pathogenic bacteria. In the current study, efforts have been made to screen the antimicrobial efficacy of the mucus harvested from two marketed air-breathing fishes namely *Channa striatus and Heteropneustes fossilis.* The antimicrobial effect of mucus was tested at 30ul concentration by well diffusion method *against ten bacteria such as Bacillus subtilis, Micrococcus luteus, Staphylococcus aureus, Streptococcus pyogens* (Gram Positive), *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella typhimurium and Vibrio cholera* (Gram Negative) and *Mycobacterium smegmatis* (Acid-fast bacilli). The activity was measured in terms of zone of the inhibition in mm. Ciprofloxacin was used as a positive control. The antibacterial effect was noted in the mucus collected from both the fishes. Even though the effect of mucus was found to be lesser when compared to the antibiotic – ciprofloxacin, considerable effect against all the bacteria was noted irrespective of their type and species. Higher antibacterial zones were noted against Gram positive bacteria than Gram negative and acid fast bacilli. Maximum zonation was noted against *Staphylococcus* sp and *Streptococcus* sp (12 mm) with *C.striatus* mucus. Except *M.smegmatis*, the effect of mucus from *C.striatus* for presented a better activity than that of *H.fossilis*. The findings of the current investigation candidly revealed that the mucus of air breathing fish may be a potential source of antibacterial agent towards the management of bacterial ailments among fish and human.

Keywords: Air breathing fish, Channa striatus, Heteropneustes fossilis, mucus, Antibacterial activity.

INTRODUCTION

Modern chemotherapeutic techniques have become highly reliable due to the advanced improvements and newer formulations (Ong yeong wei et al., 2013). Extracts and preparation made from the animal origin have been a great healing tool in folk and modern medicine (Kuppulakshmi et al., 2008).

Natural chemicals have been a boon for protecting and treating the various ailments of a diverse origin. Usage of natural chemicals is ancient practice is human civilizaiton. Exploration of natural compounds from different sources is a continuous task to improve and enrich their own lives (Agosta, 1996). Inspite of modern improvements in chemotherapeutic techniques, infectious diseases are still major issue of importance in public health issue (World resources' institute, 2000-2001).

Now-a-days 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 combat infections and overcome the problems of resistance with no side effects. Accelerated of action must be taken to reduce this problem controlling the use of antibiotics by carrying out research to investigate drugs from natural sources and also drugs that can either inhibit the growth of pathogen or kill them with least/nil toxicity . Several human ailments are treated using animal borne drugs since the demand for animal based medicines is ascending every year significantly (Kuppulakshmi, 2008).

The global trade in animal based medicinal products accounts for billions of dollars per year (kunin and Lawton, 1996). According to WHO, out of 252 traditional medicines, 8.7 % come from animals (Marques, 1997).

Mucus layer is a biological interface between fish and their aqueous environment that consists of biochemical diverse secretions from epidermal and epithelial cells (Pickering, 1974, Ellis, 1999). Several roles for this sticky layer have been suggested. This layer acts as a lubricant (Rosen and Cornford, 1971) has and is mechanical protective function (Cameron and Endean, 1971) involved in osmoregulation and locomotion to playing a possible immunological role (Fletcher, 1978) controls the intra-specific chemical communication (Saglio and Blance, 1989). Over the past years, it has also been shown that mucus plays a pivotal role in the prevention of colonization by parasites, bacteria and fingai (Bragadeeswaran, 2011). Many researchers have screened the antibacterial effect of mucus against the marine microbial strains. It has been reported that epithelial tissues produce antimicrobial molecules which serve as the first line of a host's defence against microbial invasion in a variety of vertebrates including humans (Ganz, 1999).

Antimicrobial drug resistance is a global issue dues to its indiscriminate use of antimicrobials in the treatment of infectious diseases. Development of alternative therapy is the need of the hour. New approaches involving animal and plant borne medicines should be tailored to avoid complications. Animals are an excellent source of novel antimicrobial agents. Antibacterial activity in mucus has been demonstrated in several fish species.

Many publications are available in explaining the antibacterial effect of saliva of fishes but there are no investigations regarding the marketed air breathing fishes. In the current investigation, an attempt was made to screen the antibacterial activity of the two marketed air breathing fishes *C. striatus* and *H. fossilis.*

MATERIALS AND METHODS

Marketed live fishes weighing about 500g and 300g of approximately 6 months and 4 months old *C.striatus* and *H. Fossilis* respectively were procured from Tenkasi Fish Market, Tiruneveli District, Tamil Nadu, India. The purchased fishes were climatized to laboratory conditions in bore well water and they were maintained for 7 days. After 7 days these fishes were used for mucus collection.

Collection of mucus from fish

Mucus was carefully scraped from the dorsal side of the body using a sterile spatula. Mucus was not collected from the ventral side to avoid intestinal and sperm contamination. The collected fish mucus was stored at 4℃ for further use. 5ml of the mucus samples were collected aseptically from the fish and thoroughly mixed with equal quantity of sterilized physiological saline (0.85% NaCl) and the mixture was centrifuged at 5000 rpm for 15 minutes. The supernatant was collected and stored at 4℃ for studying the antimicrobial activity.

In vitro antimicrobial evaluation

In vitro antimicrobial evaluation of fish mucus of *C. striatus* and *H.fossils* were carried out against ten bacterial strains- *B.subtilis*, *M.luteus, S. aureus, S. pyogenes* (Gram positive) and *E. coli, P. vulgaris, P. aeruginosa, S. typhimurium V.chlorea*(Gram negative) and *M. smegmatis* (Acid-fast bacteria). All the ten bacterial strains were freshly cultured (18-24 hours) from the mother culture preserved at the Post Graduate Department of Microbiology, Sri Paramakalyani College, Alwarkurichi.

Determination of antimicrobial assay

Antimicrobial activity was measured using the standard method of well diffusion on Muller Hinton agar plates. 0.1 ml of each bacterial culture was spread plated on Muller Hinton agar and incubated for 24 hrs at 37℃. The concentration of bacterial suspensions was adjusted to 10^8 colony forming units (10^8 cfu/ml) in Muller Hinton Agar. Wells holding the capacity of 30 ul were cut using a sterile cork borer. Sterile distilled water and antibiotics – Ciprofloxacin were used as negative and positive controls respectively.

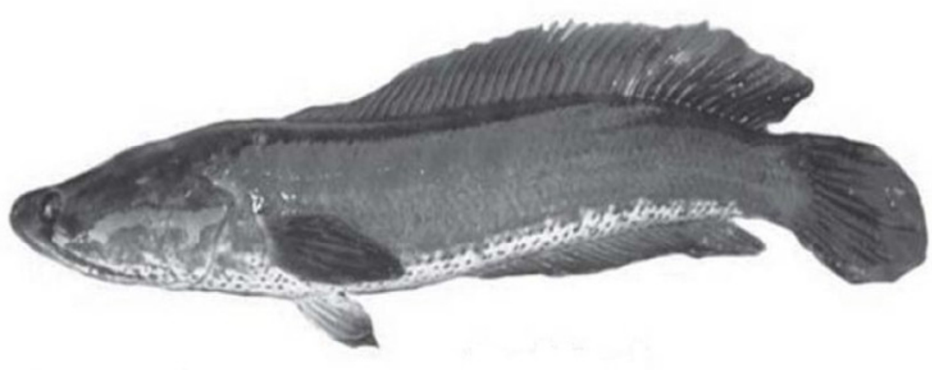
RESULT AND DISCUSSION

The antibacterial activity of mucus from *C.striatus* and *H.fossilis* analysed are presented in Table 1. The mucus collected from both marketed fresh water fishes showed a strong inhibition in the growth for the tested bacteria. Inhibitory effect was noted for the tested mucus against all the test strains irrespective of their type. Maximum zone of inhibition was observed against Gram positive bacteria – *S. aureus* followed by *S.pyogenes* (Table 1).

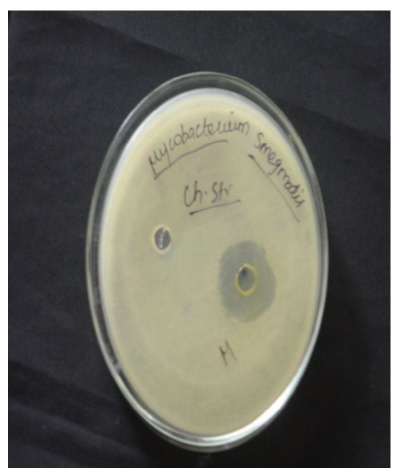
On the contrary, least inhibition was observed against *E.coli* followed by *P. aeruginosa*. Mucus collected from both fishes presented a better antibacterial effect over

Heteropneustes Fossillis

Channa Striatus



ANTIBACTERIAL ACTIVITY OF FISH MUCUS



Gram positive bacteria when compared to Gram negative and Acid – fast bacilli (Figure 1).

The antibacterial effect of the tested mucus from the two fishes were found to be more or less same. The comparative antibacterial effect of the mucus of the two marketed air breathing fishes *C.striatus* and *H. fossilis* with standard Drug Ciprofloxacin are also shown in Figure 1.

Across the world, drugs without side effect and more effectiveness with reliability are searched from natural sources to avoid several complications. The ill effect of man made drugs and antibiotics affect the ecology considerably due their persistence and bioaccumulation (Kuppulakshmi, 2008). To surmount this, intensive research is on to find out a safe source. Animals have been used as medicinal resources for the treatment and

Table 1. Antibacterial effect of the mucus from *Channa straitus* and *Heteropneustes fossilis (mm)*.

Type	Microorganisms	*C.striatus* 30µ1/well	*H. fossilis* 30µ1/well	Ciprofloxacin 30µg/well
Gram Positive Bacteria	*B. subtilis*	10	9	14
	M.luteus	10	8	13
	S.aureus	12	11	13
	S. pyogenes	12	9	14
Gram Negative Bacteria	*E. coli*	6	4	12
	P. vulgaris	10	5	15
	P.aeruginosa	7	6	16
	S.typhimurium	8	7	14
	V. cholera	8	6	12
Acid Fast Bacilli	*M. smegmatis*	9	10	13

All the values were the mean of three experiments. The values given are the diameter of zone of inhibition (mm).

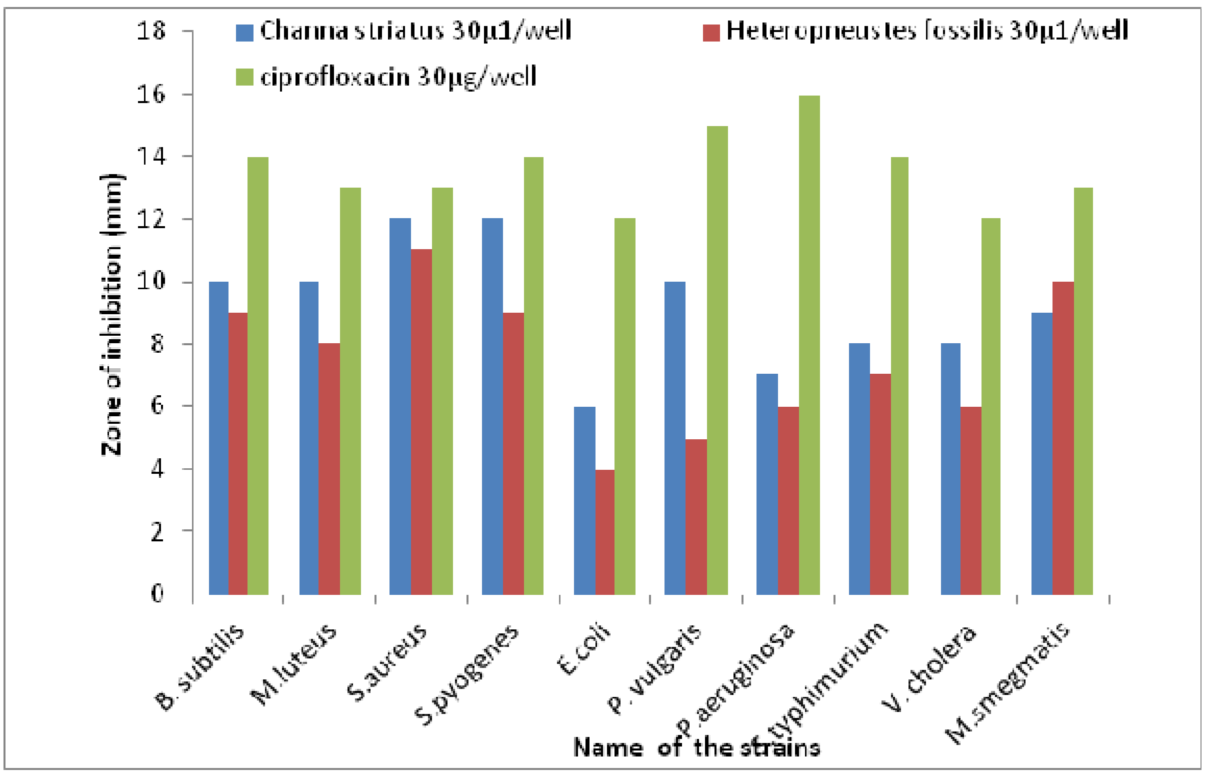


Figure 1. A omparative antibacterial effect of the mucus with the standard drug – ciprofloxacin

relief of a myriad of illnesses and diseases in practically every human culture (Eraldo, costa-neto, 2005)

Fish mucus is enticing the scientific community due to its antibacterial effect. The accumulation of domestic wastes in the aquatic environs have led to the population of microbes being increased rapidly. In order to escape from such an environment, fishes produce secretes some substance against the 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(Black stock and Pickering, 1982). Mucus secreted by fish play a major role in protection against major 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(Cancre ,1999, Choncha , 2004). The innate 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 (Durandp and Lagoin 1988, Ellis1974).The antimicrobial property of crude epidermal mucus against infectious pathogens has been demonstrated in so many fishes (Fagan ,2003, Faid, 1997). In the current study the mucus collected from C.*striatus* and H.*fossilis* showed a strong inhibiting effect on the varied bacteria types. The results also revealed that the antibacterial effect of fish mucus was also a source of bioactive potential that could be exploited. The inhibitory effect of the fish secretion may be due to the presence of antibacterial compounds. Many researchers claim that the killing effect of fish mucus is due to the presence of pore forming compounds including glycoprotein (Ebran, 1999, Tirupathi, 2011). The results of the present work show the wide spectrum activity of mucus collected from the selected two fishes. Mucus contains several proteases (serine proteases, cysteine proteases, metalloproteases and trypsin like proteases) having strong antibacterial activity (Shai, 1995). Mucus represents an important biological interface between the animal and their aqueous environment. The mucus and epidermis are important in fish defense mechanisms because they are the first site 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(Shephard, 1993) . The strong 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 andShephard, 1993) demonstrated that the lipoprotein A-I and A-II inhibited the growth of Gram-positive and negative bacteria including fish pathogens at micromolar contractions. These findings support out results where in that the mucus collected from the fish *C.striatus* and *H.fossilis* showed strong microbial resistance. The present work supports the view that fish mucus could be source of antimicrobial agent for human and fish pathogens (Ong yeong wei et al., 2013).
Further purification of the bioactive compounds are necessary in order to identify their chemical nature and to evaluate their potential as novel drug leads.

CONCLUSION

The present work has been an excellent evidence to prove the medicinal value of the mucus collected from selected air breathing fishes. Deep investigation will surely throw useful information for the discovery new drugs helping for sustainable aquaculture.

ACKNOWLEDGEMENTS

Authors would like to thank, The principal and faculty members of P.G Department of microbiology, Sri Paramakalyani college, Alwarkurichi, for providing the facilities to carry out the experiment.

REFERENCES

Agosta W (1996). Bombardier beetles and fever trees, a close-up look at chemical warfare and signals in animals and plants, New York. Addison-Wesely Publishing Company. 224.

Black stock N, Pickering AD (1982). Changes in the concentration and histochemistry of epidermal mucus cells during the alevin and fry stages of the brown trout (Salmo trutta) J. Zool Sci. 197:463-471.

Bragadeeswaran S, Priyadharshini S,Prabhu K, Sophia ran SR (2011).

Antimial and hemolytic activity of fish epidermal mucus Cynoglossus arel and Arius caelatus Asian Pacific Journal of Tropical Medicine. 305-309.

Cameron A, Endean R (1973). Epidermal secretions and evolution of venom glands in fishes Toxicon. 11:401-410.

Cancre I (1999). Secretagogues and growth factors in fish and crustacean protein hydrolysates. Mar Biotechnol. 1(5):489-494.

Choncha MI (2004). Appolipoprotein A-I and A-II are potentially important effectors of innate immunity of the teleost fish Cyprinus carpio. Eur J Biochem. 271:2984-2990.

Durand P, Lagoin Y (1988) . Valorisation des sous-produits de la peche. Bull Inst Peches Marit. 330: 5-19.

Ebran N, Julien S, Orange N, Auperin B, Molle G (2000). Isolation and characterization of novel glycoproteins from fish epidermal mucus: correlation between their pore forming properties and their antibacterial activities. Biochim Biophys Acta. 1467: 271-280.

Ebran N, Julien S, Orange N, Saglio P, Lemaitre C, Molle G (1999). Pore-forming properties and antibacterial activity of proteins extracted from epidermal mucus of fish Comp Biochem Physiol. 122:181-189.

Ellis A (1999). Immunity to bacteria in fish. Fish ShellfishImmunol. 9: 291-308.

Ellis AE (1974). Non-specific defense mechanisms in fish and their role in disease processes. Dev Biol Std. 49: 337-352

(E Eraldo costa-neto,(2005) Animal-based me medicines. Biological perspection and the sustainable use of zootherapeutic resources. An Acad Bras Cienc. 77: 33-43.

Fagan MS (2003). A biochemical study of mucus lysozyme, proteins and plasma thyroxine of atlantic salmon (Salmo salar) during smoltification J. Aqu. 222:287-300.

Faid M (1994). A biotechnological process for the valorization of fish waste J Bioresource Technol. 49: 237-241.

Faid M (1997). Biotransformation of fish wastes into a stable feed ingredient Food Chem. 113-18.

Fletcher T (1978). Defense mechanisms in fish. In: D. Malinsand J. Sargent (eds). Biochemical and Biophysical`Perspectives in Marine Biology. London Academic Press. 189-222.

Ganz T (1999). Defensins and host defense Science. 266: 420-421.

Shephard K.L (1994). Functions for fish mucus Rev Fish Biol Fisher. 4: 401-429.

Kunin WK, Lawton JH (1996). Does biodiversity matter? Evaluating the case for conserving species. In:Gaston KJ (Ed), Biodiversity: a biology of numbersand differences, Oxford: Blackwell Science. 283-308.

Kuppulakshmi C, Prakash M, Gunasekaran G, Manimegalai G, Sarojini S (2008). Antibacterial properties of fish mucus from Channa punctatus and Cirrhinus mrigala. European Review for Medical and Pharmacological Sciences. 12:149-153.
Lemaitre C, Orange N, Saglio P, Saint N, Gagnon T,Molle G (1996). Characterization and ion channel activities of novel antimicrobial proteins from the skin mucosa of carp (Cyprinus carpio) Eur J Biochem. 240:143-149.
Manivannan K,karthikai devi G, Anantharaman P, Balasubramanian T (2011). Antimicrobial potential of selected brown seaweeds from Vedalai coastal waters, Gulf of Mannar Asian Pac J Trop Biomed. 1(2):114-120.
Marques JGW (1997). Fauna medicinal: recurso do ambienteou ameaca a biodiversidade? Mutum.1-4.
Ong yeong wei R, Xavier K Marimuthu (2013). Screening of antibacterial activity of mucus extract of Snakehead fish, Channa striatus (Bloch). European Review for Medical and Pharmacological Sciences. 14:675-681.
Pickering AD (1974). The distribution of mucus cells inthe epidermis of the brown trout Salmo trutta (L)and the char Salvelinus alpinus (L) J Fish Biol. 6: 111-118.
Rosen M, Cornford N (1971). Fluid friction of fish slimes Nature. 234: 49-51.
Saglio P, Blance J (1989). Intraspecific chemo-communication in immature goldfish, Carassius auratus(L): attraction in olfactometer to free amino acidfractions from skin extracts. Biol Behav. 14: 132-147.
Sasidharan S, Prema P,Yoga LL (2011) . Antimicrobial drug resistance of Staphylococcus aureus in dairy products Asian Pac J Trop Biomed. 1(2): 1.
SHAI Y (1995). Trends Biochem Sci.20: 460-464.
Shephard KL (1993). Mucus on the epidermis of fish and its influence on drug delivery. Adv Drug Deliv Rev. 11: 403-417.
Tirupathi RG, Suresh BK, Kumar JU, Sujana P, Raoa AV, Sreedhar AS (2011). Anti-microbial principles of selected remedial plants from Southern india. Asian Pac J Trop Biomed. 1(4):238-305.
World resourses institute. Resources Report 2000-2001. People and ecosystems: the fraying web of life. Washington D.C. World Resources Institute.2000;389.

How to cite this article: Haniffa M.A., Viswanathan S., Jancy D., Poomari K., Manikandan S. (2014). Antibacterial studies of fish mucus from two marketed air-breathing fishes – *Channa striatus* and *Heteropneustes fossilis*. Int. Res. J. Microbiol. 5(2):22-27