Antimicrobial action of casing secretion of a new marine cat fish.

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ABSTRACT:

fish pathogenic bacterial strains viz. The aqueous environment, inhabited by fish often contains different types of pathogens. Mucus layer provides physical barrier which acts as first line of defense against attachment and penetration through potentially harmful agents and thus plays an important role in the protection of the fish.

INTRODUCTION:

Fish lives in microbe-rich environment and are vulnerable to invasion by pathogenic (or) opportunistic microorganisms. Fishes which directly come into contact with high concentrations of pathogens in their aquatic environment leads to the problems of infectious diseases (Loganatahan, 2013). Mucus substance is secreted from epidermal cells and it's a biological interface between fish and its environment consisting of potentially rich proteins, minerals, enzymes, pigments, inorganic salts, immune globulins (Whyte 2007). Based on some reports fish mucus possesses wound healing, antinociceptive, platelet aggregation, anti inflammatory, anti fungal, anti bacterial properties.

The distribution of lysozyme, proteases and alkaline phosphatase and their role in defense have been reported from a review mucus secretion of viz., Coho Salmon, Rainbow truot, arcitic char, Brook trout, Koi carp, Striped bass, Haddock, Atlantic cod and Hag fish (Fast et al., 2002, Subramanian 2007, Palaksha 2008). Zootherapy is the alternative to the healing of diseases by using therapeutics derived from animals (Anil. Tyor 2016; Haniffa 2014).

According to WHO 8.7 % of medicines are prepared from animals, out of 252 traditional medicines (Marques 1997). In the past thirty years many new antibiotics have been produced by pharmacological industries, but micro-organisms developed resistance to these drugs due to multi drug resistance efficiency of micro-organisms.

Fishes are in constant interaction with their aquatic environment, which contains a wide range of pathogenic and non-pathogenic microorganisms. The epidermis and its mucus secretions act as biological barriers between fish and the potential pathogens of their environment.

Skin mucus secretion of H. fossilis exhibited strong antibacterial activity for all selected microbes.According to our observation E.coli has shown high ZOI (Zone of inhibition) and K.pneumonia showed least ZOI. S.aureus and P.vulgaris have shown medium inhibition zones. The measurements of inhibition zones obtained were viz., Escherichia coli (30.78) and Klebsiella pneumonia (20.35) and Staphylococcus aereus (23.35) and Proteus vulgaris (25.66).

Antibacterial activity was conducted in the serum and mucus of rainbow trout (Oncorhynchus mykiss) and found a number of antibacterial factors increase in concentration following immunization and that these probably played a role in protection against microbial disease (Rainger & Rowley, 1993). Ebaran et al.,1999a), identified a strong antibacterial activity well correlated with pore-informing properties against several bacterial strains and these suggested that fish secrete antibacterial proteins able to permeabilize the membrane of the target cell and thus act as a defense barrier (Haug et al., 2002), detected antibractial activity in four marine crustacean decapods. mainly in the haemolymph and haemocyte extracts. L.anguillarum and C.glutamicum were found to be the most sensitive micro-organisms.

However,(Hellio et al., 2002), studed antibacterial, antifungal and cytotoxic activites of extracts from fish epidermis and epidermal mucus and found antifungal and antibacterial activities in the fish mucus. Two novel antibacterial muramidases were purified to homogeneity from skin of rainbow trout (Oncorhynchus mykiss) and these two muramidases probably contribute to epithelial defence of the fish against microbes, either alone or in synergism with antibacterial peptides. It was concluded that the mucus layer represents a hindrance with anguillarum, while A.salmonicida seems to be able to penetrate through it (Svendsen and Bogwald, 1997).

Fish mucus is a key component of innate immunity, because of the accumulation of domestic wastes in the aquatic environment has lead the population of microbes being increased rapidly. In order to escape from infectious diseases, mucous secretion of fish play an important role in the prevention of colonization of particles of bacteria and fungi (Black stock 1982; Yan et al., 2010). Protection of fish against infectious diseases is a major challenge in aquaculture worldwide.

Lysozyme (Muramidase) is a ubiquitous antibacterial enzyme identified in a wide range of organism including fish. Acid phosphatase and alakaline phosphatase increased activities were observed during skin regeneration in the cat fish H. fossilis.

The action these antibacterial peptides is non-specific and rapid; they kill bacteria by a pore formation in cell membranes followed by disruption and solublization (Andreu 1999, Anil Tyor 2016). Mucus contain pore forming compound glycoprotein (Tirupati 2011), proteases (Shai 1995), lipo-protein A-I and A-II the source of antimicrobial agents for human & fish pathogens (Shepherd 1993, Ong ye ong wei et al., 2013). Proteases in skin mucus secretion are involved in the natural resistance of fish to infection and can kill bacteria by cleaving their proteins (Ingram 1980, Ross et al., 2000). Palaksha et al., 2008 reported that the presence of 15 enzymes including lysozyme, protease, alkaline phosphate and esterase in skin mucus of Olive flounder (Paralicthyus olivaceus).

A strong substance present in the mucus may function either in the cytoplasm against intracellular pathogens (or) extracellular through mucosal surface after infection induced cell lysis (or) apoptosis (Haniffa et al., 2014).

U. Kumari (2011) reported that Channa and Rita has been showed no ZOI, but Anil (2016) reported antibacterial activity of crude mucus and aqueous mucus extract of H. nobilis against microbial strains and maximum ZOI was exhibited by S. epidermidis followed by E. coli (32.83;32.66mm), B. cereus (29.25), P. aeruginosa 927.620, S. aureus (26.33) and K. pneumonia (23.58).

The development of resistance by a pathogen to many of the commonly used antibiotics provide an impetus for further attempts to search for new antimicrobial agents which combat infections and overcome the problems of resistance with no side effects (Patil et al., 2015).

Skin mucus secretion of H. fossilis exhibited strong antibacterial activity for all selected microbes. According to our observation E. coli has shown high ZOI (Zone of inhibition in mm) and K. pneumonia showed least ZOI. S. aureus and P. vulgaris have shown medium inhibition zones. The measurements of inhibitions zones obtained were viz., Escherichia coli (30.78) and Klebsiella pneumonia (20.35) and Staphylococcus aereus (23.35) and Proteus vulgaris (25.66).

The present findings suggest that the epidermal mucus secretion of H. fossilis is a good source of antimicrobial compounds. The epidermal mucus secretion of H. fossilis showed a different zone of inhibition against different bacterial strains.

Further, research has to be focussed on purification and characterization of specific antimicrobial components of epidermal mucus so that it may be utilized as potent anti microbe.

METHODOLOGY:

Live fishes (H. fossilis) weighing about 50 ± 10g of approximately were procured from local pond Nagaram Village, Warangal District, Telangana, India. The fishes were acclimatized to laboratory conditions in bore well water and they were maintained for 7 days.

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°C 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°C for studying the antimicrobial activity.

In vitro antimicrobial evaluation In vitro antimicrobial evaluation of fish mucus of H. fossils were carried out against four bacterial strains Gram – ve bacteria Escherchia coli and Gram + ve bacteria Klebsiella pneumonia; Staphylococcus aereus and Proteus vulgaris are used. All the bacterial strains were freshly cultured (18-24 hours) from the mother culture preserved at the Post Graduate Department of Microbiology, Vaagdevi College Kishanpura, Hanamkonda, TS, India.

Determination of antimicrobial assay Antimicrobial activity was measured using the standard method of well diffusion on Asthana Hawkers agar medium plates. 0.1 ml of each bacterial culture was spread and plated on Asthana Hawkers agar medium and incubated for 24 hrs at 37°C. The concentration of bacterial suspensions was adjusted to 108 colony forming units (108 cfu/ml).

Antifungal activity For antifungal activity two fungal strains (Pencillium, and Aspergillus) were used. Fungal spore suspension was swabbed on the surface of the solidified Asthana Hawkers agar medium. 200µl of each extract was added into the wells and plates were incubated at 280 C for 6 days. The Inhibition zones were measured and tabulated.

RESULTS

In our present investigation antimicrobial activities of skin mucus secretion of H. fossilis exhibited strong antibacterial activity against all selected microbes. According to our investigation E. aureus and P. vulgaris have shown medium inhibition zones.

The measurements of inhibitions zones like viz., Escherichia coli (30.78) and Klebsiella pneumonia (20.35) and Staphylococcus aereus (23.35) and Proteus vulgaris (25.66) are presented Table. and Figure. The present findings suggest that the epidermal mucas secretion of H.fossilis is a good source of antimicrobial compounds.This anti microbial activity might be due to antimicrobial proteins present in

The epidermal mucus secretion of H.fossilis showed a different zone of inhibition against different bractirial strains. Thus indicating antimicrobial activity of skin mucus of H.fossilis.

DISCUSION

Fishes are in constant interaction with their aquatic environment, which contains a wide range of pathogenic and non-pathogenic microorganisms. The epidermis and its mucus secretions act as biological barriers between fish and the potential pathogens of their environment.

Fish mucus is a key component of innate immunity, because of the accumulation of domestic wastes in the aquatic environment has leaded the population of microbes being increased rapidly. In order to escape from infectious diseases, mucous secretion of fish plays an important role in the prevention of colonization of particles of bacteria and fungi (Black stock 1982; Yan et al., 2010). Protection of fish against infectious diseases is a major challenge in aquaculture worldwide. Acid phosphatase and alakaline phosphatase increased activities were observed during skin regeneration in the cat fish H. fossilis.

The action these antibacterial peptides is non-specific and rapid; they kill bacteria by a pore formation in cell membranes followed by disruption and solublization (Andreu 1999, Anil Tyor 2016). Mucus contain pore forming compound glycoprotein (Tirupati 2011), proteases (Shai 1995), lipo-protein A-I and A-II the source of antimicrobial agents for human & fish pathogens (Shepherd 1993, Ong ye ong wei et al., 2013). Proteases in skin mucus secretion are involved in the natural resistance of fish to infection and can kill bacteria by cleaving their proteins (Ingram 1980, Ross et al., 2000).

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The development of resistance by a pathogen to many of the commonly used antibiotics provide an impetus for further attempts to search for new antimicrobial agents which combat infections and overcome the problems of resistance with no side effects (Patil et al., 2015).

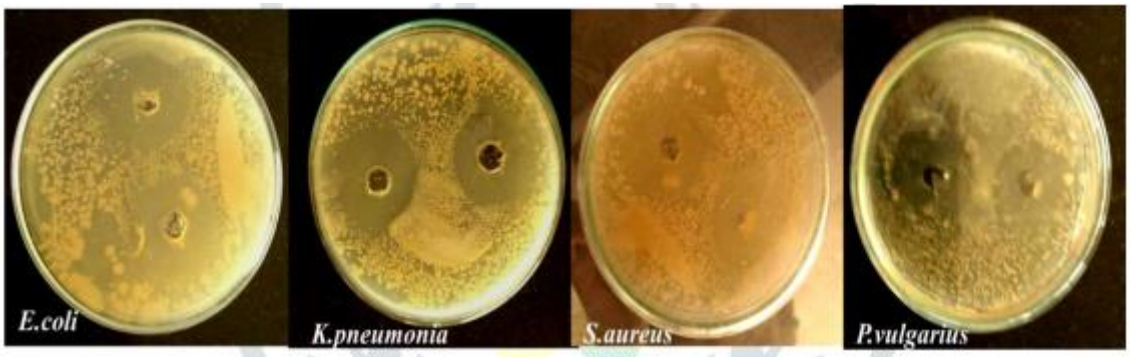
The present findings suggest that the epidermal mucus secretion of H. fossilis is a good source of antimicrobial compounds.

The epidermal mucus secretion of H. fossilis showed a different zone of inhibition against different bacterial strains. Further, a detailed investigation is required for purification and characterization of specific antimicrobial components of epidermal mucus so that it may be utilized as potent anti microbe.

Anti bacterial activity of Skin mucus secretion of H. fossilis

Microorganism Organism	**ZOI of Mucus Secretion**
Escherichia coli	30.78±0.57
Klebsilla pneumonia	20.35±0.62
Staphylococcus aerius	23.63±0.88
Protious vulgaris	25.66±0.60

Anti bacterial activity of Skin mucus secretion of H. fossilis



MIC = Minimal Inhibitory Concentration, The Lowest Concentration at which no growth A) B) C) D) E) F) G) H) was detectable

Discussion:

The present studies revealed that all the three carp species (H. nobilis, C. idella and C. carpio) investigated secreted huge amount of mucus and the volume of secretion varied among the three. These results are similar to studies of Nigam et al. (2012) for different fish species viz. Cirrhinus mrigala, Labeo rohita, Catla catla, Rita rita, and Channa punctata. These variations in mucus secretion may be attributed to the different ecological and physiological conditions and also to different mucus producing cells situated in epidermis and epidermal layers of different fish species.

It is now well established that fish skin mucus acts as mechanical barrier to fishes by lying at interface between them and surrounding pathogens (Reverter et al. 2017). In addition to trapping and sloughing of infectious pathogens, the skin mucus is the reservoir of antimicrobial components which acts in different ways and is gifted with innate antibacterial ability as reported by Nagashima et al. (2001). Furthermore, predominantly proteinaceous nature of the skin mucus secretions of different fish species viz. Anguilla japonica, Arius maculates, and Channa striatus has been reported by many authors such as Chong et al. (2005) and Manivasagan et al. (2009) etc.

This high protein content could be associated with mucosal innate immunity because most of bactericidal components identified in fish mucus, are also proteinous in nature. Arulvasu et al. (2012), demonstrated that crude and partially purified epidermal mucus of Tachysurus dussumieri have 0.48 ± 0.02 mg ml1 and 0.82 ± 0.05 mg ml-1 protein content. Similarly, Rao et al. (2015) have also investigated the protein as major constituent among different skin mucus extracts (crude, acidic, and organic) of C. micropeltes, C. striatus, Oreochromis niloticus, and Mystus nemurus.

Protein quantification results revealed that crude mucus extracts of all fish species contained a high amount of proteins ranging from 432.90 ± 28.20 to 579.90 ± 32.30 lg ml-1 when compared with other extracts. Our results are also in agreement with above studies. However, in present study, protein content ranges from 100.79 ± 0.03 to 305.00 ± 1.64 mg ml-1 for HFM and 159.78 ± 2.19 mg ml-1 to 378.00 ± 3.45 mg ml-1 for CFM.

The presence of comparatively high proteins content in our study might be due to the species difference and environmental factors such as water quality (DO, CO2, ammonia, pH) and the presence of contaminants. Our results also revealed that protein content not only vary with in the species (between HFM and CFM extracts), but also among species in both extracts (HFM and CFM). Also CFM extracts of all selected fish species.

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