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Antimicrobial Peptide from the Epidermal Mucus of Some Estuarine Cat Fishes

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Abstract: Antimicrobial proteins and peptides play key roles in innate immunity and they had been observed from a wide variety of organisms in the last few years. The present study was undertaken to characterize antimicrobial peptides from the epidermal mucus of cat fishes collected from the Parangipettai coastal environment. Antimicrobial properties of the cat fishes were tested against ten pathogenic bacteria and ten pathogenic fungi. The results of the present investigation reported that the mucus of the cat fishes having remarkable antimicrobial activity. In particularly, *Mystus gulio* had much activity than the *Arius maculatus*. The molecular masses of the peptides were 13.6 kDa and 13.9 kDa as determined by SDS- polyacrylamide gel electrophoresis. This peptide had shown very effective activity against various human pathogens. The present findings suggest that cat fish's mucus having very good antimicrobial activity against pathogenic microbes.

Key words: Cat fish · Mucus · Antimicrobial property · Antimicrobial peptides · SDS-PAGE · Protein

INTRODUCTION

The epithelical surfaces of fish, such as the skin, gill and alimentary tract, first provide with potential pathogens. Fish live in a microbe-rich environment and are vulnerable to invasion by pathogenic or opportunistic micro-organisms. Over the past years, it has also been shown that mucus plays a role in the prevention of colonization by parasites, bacteria and fungi [1- 6]. Use of fish skin or mucus for research on biologically active compounds could be an interesting exercise. Most of these antimicrobial macromolecules have been isolated from fish skin that constitutes a first line barrier against microbial invasion. The mucus layer covering the integument of fish has mechanical protective functions. The increasing resistance of bacteria to the available antimicrobial drugs has resulted in extensive studies of antimicrobial peptides as therapeutic agents [7]. Fish epidermal mucus AMPs has shown a broad spectrum of activity that is 12-100 times more potent than of amphibian AMPs against various fish and human pathogens [8].

We are still in great need of safer, cheaper and effective drugs. The mucus of some estuarine cat fishes have shown pronounced activities, useful in biomedicinal area. The potential of estuarine cat fishes as a source of biological products is largely unexplored in India. Hence, a broad based screening of estuarine cat fishes for bioactive compounds is necessary.

MATERIALS AND METHODS

Skin Mucus Collection and Preservation: Mucus was collected as described by Ross *et al.* [9] with slight modifications. Mucus was obtained from 15 fish representing each of the species. Before collecting epidermal mucus, fish were kept a week in the laboratory in running water and then electrocuted. Mucus was carefully scraped from the dorsal body surface using a plastic spatula. Ventral skin mucus was not collected to avoid intestinal and spermal contaminations. The skin mucus harvest was immediately frozen to prevent any external bacterial contamination, then lyophilized and stored at-20°C.

Purification of Epidermal Mucus: Aqueous extracts, the pooled mucus (150 mL) from each species was freeze dried, resuspended in 100 mM (w/v) ammonium bicarbonate at 1 mg/mL and centrifuged at 9500 ×g for 10 min at 4°C. The mucus retentate was freeze dried and resuspended in water for testing antimicrobial activity.

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Protein Content: Mucus, were collected by dissect the cat fish animal with a fine sterile scalpel. The amount of protein was measured by spectrometry according to the method of biuret using a calibration curve.

SDS-PAGE: The supernatants were analyzed by SDS-PAGE 15%, topped by 7% stacking gel according to Laemmli [10].

Thin-layer Chromatography (TLC): Aquatic extracts were applied to the thin-layer chromatographic plate (Merck) with a capillary tube and placed in a chamber containing methanol:chloroform (5:95) as developing solvent. After development, compounds were visualized as purplish pink spots on spraying with 5% methanolic sulfuric acid as detecting agent followed by heating at 100°C till the spots were visible.

Antimicrobial Assay: The potential of antimicrobial activity of skin secretion of cat fishes was screened against ten bacterial pathogens viz. Klebsiella oxytoca, K. pneumonia, Vibrio cholerae, Proteus mirabilis, Salmonella typhi, Escherisea coli, Staphylococcus aureus, Streptococcus sp. Bacillus sp. Pseudomonas aureginosa and ten fungal pathogens viz. Mucor sp. Rhizopus. sp. Pencillium sp. Trichophyton mentagrophytes, T. rubrum, Epidermophyton floccossum, Candida albicans, Alternaria alternaria, A. flavus, A. niger

RESULTS

Antibacterial Spectrum of the Skin Secretion: Figure 1 shows the antibacterial spectrum of the skin secretion by disc diffusion assay on agar plates. The skin mucus sample pooled from two specimens are inhibited the growth of five species of Gram negative bacteria (*E.coli*, *P. mirabilis*, *K. pneumonia, P. aeruginosa* and *K. oxytoca*). Among them *E.coli*, *P. mirabilis* and *K. pneumonia* were highly sensitive to the skin secretion. The antibacterial activity of the skin secretion appeared to vary among the individuals. The spectrum of antibacterial activity in the mucus of *A. maculatus* was observed maximum (8 mm) against *E.coli* and minimum (6.5 mm) was notified against *K. pneumonea*. *M. gulio* shown maximum (11 mm) activity against *P. aeruginosa* and minimum (10 mm) was observed against *K. oxyoca.*

Antifungal Activity: Figure 2 shown antifungal activity of the skin secretion by disc diffusion assay on agar plates. Antifungal activity of the cat fish mucus and positive control fungal agents Fluconazole (C) was investigated against all the ten pathogenic strains. The cat fish mucus has shown antifungal activity against the number of pathogenic fungal strains. The maximum antifungal activity was observed against *C. albicans* (12mm) and minimum was against *Mucor* sp. (6.5mm) in the mucus of

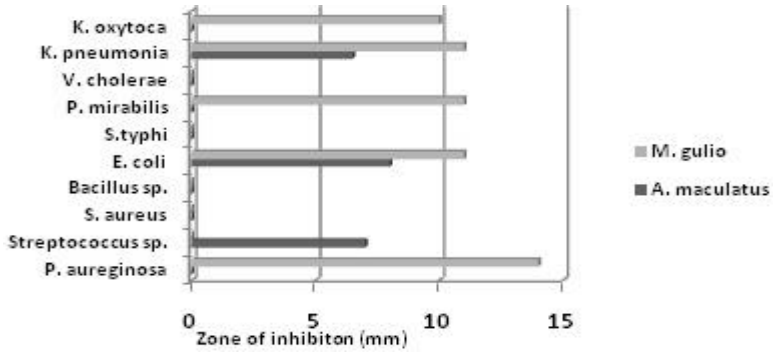

Fig. 1: Antibacterial activity of cat fish *A. maculatus* and M. gulio

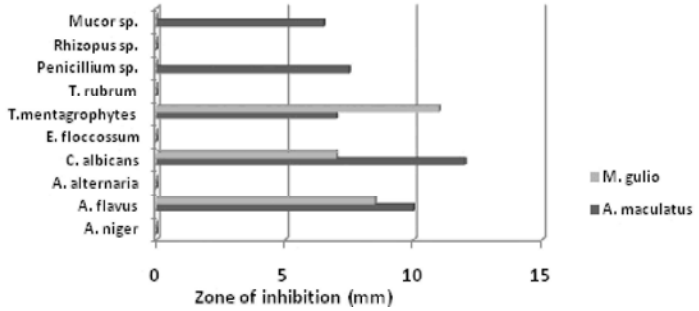

Fig. 2: Antibacterial activity of cat fish *A. maculatus* and *M. gulio*

Fig. 3: Thin Layer chromatography

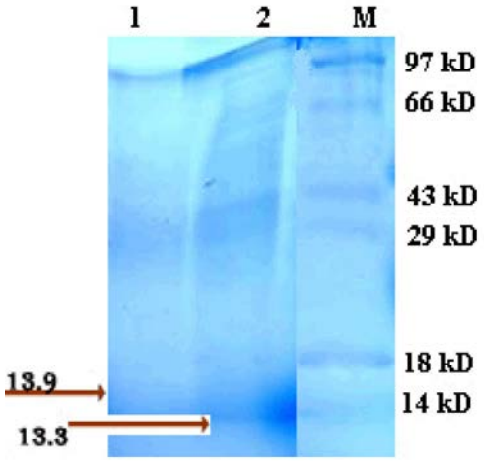

Fig. 4: Molecular weight of peptides by SDS-PAGE

A. maculates. The highest (11 mm) antifungal activity of *M. gulio* noticed against *T. mentagrophytes* and the lowest (8.5) was against *A. flavus*.

Protein: The active fish mucus samples were centrifuged and a supernatant part is aspirated through micropipette. 0.5ml of Separated supernatant part is subjected to protein quantification by Biuret method. The amount of protein present in the mucus of *A. maculatus* is 11.0% and *M. gulio* contains 9.0%.

Thin Layer Chromatography: Thin-Layer chromatography profiling was done for the samples mucus extract in solvent system of Butanol, Acetic acid and Water (B: A: W) in proportions of 5:1:4. The plates when developed in both the solvent systems showed light pink spots, when the TLC plate is sprayed with ninhydrin. The plate with fractions developed in BAW as the solvent system and sprayed with ninhydrin, showing pink spots indicating the presence of amino acids and peptides (Figure 3).

Determination of Molecular Weight by Using SDS-PAGE: The cat fish skin mucus samples showed antimicrobial activity was subjected to SDS-PAGE to estimate the molecular weight of proteins present in it. Different standard were used to determine the molecular weight of mucus proteins. The stained gel revealed that the mucus contained a simple population of proteins. Only one clear band in each of the mucus was detected in the gel that represented peptide of 13.3 kDa and 13.9 kDa (Fig. 4).

DISCUSSION

The biological interface between fish and their aqueous environment consists of a mucus layer composed of biochemical diverse secretions from epidermal and epithelial cells. Fish are in constant interaction with their aquatic environment, which contains a wide range of pathogenic and nonpathogenic microorganisms. The epidermis and the epidermal mucus secretions act as biological barriers between fish and the potential pathogens of their environment [11]. Previous demonstrations of the protective role of mucus and its components in various fish species [2, 4, 12-15] suggests that the epidermal mucus acts as a first line of defense 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 cat fish species.

The antimicrobial property of epidermal mucus against infectious pathogens has been demonstrated previously in rainbow trout (*O.mykiss*) [2], ayu (*Plecoglossu altivelis*), turbot (*Scopthalamus maximus*) and carp (*Cyprinus carpio*) [16, 4, 6]. Increased expression of one or more of the above- mentioned antimicrobial components in fish epidermal mucus has been observed following microbial stress [17, 18], thus supporting the role of epidermal mucus in protecting fish from infectious pathogens. The present study also had shown the antimicrobial potential of cat fish mucus. The maximum antibacterial activity was observed against *P. aeruginosa* (11mm) in the mucus of *M. gulio* and the minimum was against the *K. pneumonia* (6.5 mm) in *A. maculates* mucus. The antifungal activity was observed

maximum against *C. albicans (*12 mm) and minimum 6.1 mm was noticed against the *Penicillium* in *A. maculatus* mucus. Most of reported antimicrobial peptides typically have strong antimicrobial activity against a wide range of Gram-positive bacteria but very weak or no activity against Gram-negative bacteria, like mytimycin [19]. However, the above study anmals has higher antimicrobial activity against both Gram-negative bacteria and Gram-positive bacteria. Several antimicrobial compounds have been isolated from both plants and animals over the last decades. Over the past few years many antimicrobial protein have been isolated from both plants and animals, from insects to mammals. These proteins are predicted to operate as a first-line host defense mechanism, acting against pathogenic bacteria, fungi and other parasites. Generally nonspecific but rapidly active during a parasite invasion, they constitute host defense less costly than antibodies.

Fish mucus is believed to play an important role in the prevention of colonization by parasites, bacteria and fungi and thus acts as a chemical defense barrier. Several host defense peptides were found in marine animals, like pardaxin [20] isolated from Moses sole fish *Pardachirus marmoratus* and pleurocidin [21], an antibacterial peptide found in the skin secretion of winter flounder.

Despite the large number of antimicrobial peptides purified from varied animal sources [22], relatively few have been isolated from epithelial surfaces of aquatic animals. Pleurocidin was one of the first antimicrobial peptides isolated from a teleost. It is a 25-amino acid peptide with a broad-spectrum of activity and is expressed by the mucous cells of flounder skin [21, 23]. In the present study, crude extract of cat fish mucus that showed antimicrobial activity was subjected to TLC to determine the presents of the peptides and amino groups. In the present investigation mucus was subjected to SDS-PAGE to estimate the number and molecular weight of proteins present. The peptide of 13.6 kDa and13.9 kDa had been found from the cat fish mucus. AMPs previously isolated from the mucus of other fish species such as winter flounder (2,711.0 Da), catfish (2,000.4 Da) and Atlantic halibut (5,459.0 Da) [21, 8, 24].

The observed variation in antimicrobial activity among fish species examined is thought to be due to the diverse composition of the secreted mucus. The mucus producing cells in epidermal and epithelial layer had been reported to differ between fish species [11]. The results of the present investigation reported that the mucus of the cat fishes of the present study having remarkable antimicrobial activity. In particularly, *M. gulio* had much antibacterial activity than the *A. maculatus* . The mucus of *A. maculatus* had shown maximum antifungal activity than *M. gulio.* Further investigations in active compounds present in the above mucus samples will lead to find out new natural compounds against various diseases. This study will be base for further in-depth investigation and structural elucidation.

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