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Full Length Research Paper

Studies on the proteinaceous gel secretion from the skin of the catfish, *Arius maculatus* (Thunberg, 1792)

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The Catfish *Arius maculatus* (Thunberg, 1792) causes injury to the fisherman while handling the fish and it was proven that the skin mucus of the fish have several properties including the toxicity. In the present study, the biochemical property of the catfish skin mucus was characterized and it was found that the protein content of the soluble and insoluble fractions were 9.34 and 12.64 µg/g, respectively. The total lipid was 0.005 µg/g and the total carbohydrate was 0.08 µg/g. Of the total 17 amino acids recorded, cysteine availability was very low; 0.01 and 0.02 mole % in the soluble and insoluble fractions, respectively. Leucine was available in high quantity (9.8 mole %) in the soluble fraction and aspartic acid (9.0 mole %) was high in the insoluble fraction. On SDS-PAGE analysis, seven and six bands with a distinct band at 35 KDa in the soluble and insoluble fractions, respectively, were observed. On haemolytic activity, lysis was observed by 50 µg/ml of insoluble fraction and 25 µg/ml of soluble fraction. Both soluble and insoluble fractions showed maximum and minimum activities against *Escherichia coli* and *Pseudomonas aueruginosa,* respectively.

Key words: Catfish, *Arius maculates*, biochemical composition, haemolytic activity, antibacterial activity.

INTRODUCTION

The catfish, *Arius maculatus* is a common inhabitant of the inshore of India, Sri lanka and Pakistani waters and estuaries. The catfish has a thick skin that may be covered with a gel elaborated by epidermal proteinaceous cell. Such cells are commonly the site of the synthesis of "ichthyocrinotoxins" produced by fishes having no clearly defined venom apparatus (Cameron and Endean, 1971). The authors also suggested that an evolutionary development of venom glands with the proteinaceous epidermal cells as a starting point. It is therefore of interest to investigate the properties of the proteinaceous skin cell secretions of the catfish, *A. maculatus.*

The predominantly proteinaceous material is about 80% of insoluble polymeric form. The soluble fraction contains proteins which appear to be precursors for formation of insoluble gel and also proteins with enzymes properties often associated with animal venoms. These include several lytic activities and factors promoting red blood cell agglutination and clotting of plasma (Al-Hassan et al., 1982).

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Proteinaceous skin cell secretion of the catfishes possesses antimicrobial peptide, as part of their defence system. Antimicrobial peptides, which are widespread in nature and are among the earliest developed elements of innate immunity, are important components of the natural defense of living organisms against microorganisms (Park et al., 1998). Most of the antimicrobial peptides target cells rapidly, specifically and have unusual broad activity spectra. They may also have other function such as promotion of wound healing, stimulation of monocyte chemotaxis and inhibition of cytokine response. Of all these background characteristics of the proteinaceous gel, the present study was carried out to determine the biochemical composition (protein, carbohydrate, lipid and amino acids), haemolytic activity, antibacterial activity and SDS-PAGE analysis of the proteinaceous gel of catfish, *A. maculatus* (Thunberg, 1792).

MATERIALS AND METHODS

Collection of specimens

Fishes were caught on baited lines from Vellar estuary in Parangipettai (Lat; 11°46' Long; 79°46') (Southeast coast of India). The specimen was identified by FAO identification sheets 1983.

Table 1. Amino acid composition of gel proteins.

S/N	Amino acid	Soluble gel (mole %)	Insoluble gel (mole %)
1	Aspartic acid	9.3	9.0
2	Threonine	4.3	4.1
3	Serine	5.8	5.0
4	Glutamic acid	9.0	8.2
5	Proline	2.3	2.4
6	Glycine	6.0	6.2
7	Alanine	6.6	6.9
8	Cysteine	0.01	0.02
9	Valine	3.5	3.9
10	Methionine	0.2	0.1
11	Isoleucine	4.4	4.9
12	Leucine	9.8	6.1
13	Tyrosine	2.2	2.3
14	Phenylalanine	3.1	3.5
15	Histidine	0.3	1.0
16	Lysine	7.8	7.5
17	Arginine	2.8	2.4

Collection and processing of proteinaceous gel

The proteinaceous gel covering the skin was collected by the method of Al-Hassan et al. (1982). The fish was gently scraped with sterile blade. The materials was placed in tubes and frozen till use. The frozen material was subsequently thawed in the laboratory while maintaining temperature below 4°C using ice cubes. The thawed material was diluted about 50% with 0.9% saline, homogenized and centrifuged at 10000 x g for 10 min. The clear soluble supernatant and insoluble precipitate were separated and freeze dried.

Biochemical composition

The biochemical composition of the samples was analyzed by following appropriate methods. Protein was estimated by the method of Lowry et al. (1951), total lipid was estimated by the method of Folch et al. (1956) and the total carbohydrate was estimated by the method of Dubois et al. (1956). The amino acid composition of the supernatant and insoluble precipitate was estimated following the method of Yamamoto et al. (1994).

SDS–PAGE

One dimensional sodium dodecyl sulphate (SDS) poly acrylamide gel electrophoresis (PAGE) was carried out to estimate the molecular weight of the components of the proteinaceous gel (Laemmli, 1970).

Antibacterial activity

Antimicrobial activity of the samples was determined by the radial diffusion assay described earlier (Lehrer et al., 1991). A 20 ml culture of target cells in mid- logarithmic phase was washed with 10 mM sodium phosphate, pH 7.4, and resuspended in 10 ml of the same buffer. A cell suspension containing 1×10^6 bacterial colony forming units (CFUs) was added to 6 ml of under-layer agar (10 mM sodium phosphate, 1% (w/v) TSB, 1% (w/v) agarose, pH 6.5) and the mixture was poured into a Petridish. Samples were lyophilised and resuspended in 4 µl 0.01% acetic acid, and then added to the 3 mm wells that were made on the solidified under-layer agar. After incubation for 3 h at 37°C, the under layer was covered with a nutrient–rich top-agar overlay (6% (w/v) TSB, 1% (w/v) agarose) and incubated overnight at 25°C. Antimicrobial activity was determined by observing the zone of suppression of bacterial growth around the 3 mm diameter wells. The zone of inhibition was measured in millimetre.

Haemolytic activity

Freshly packed sheep erythrocytes (3 ml) were washed with phosphate-buffered saline (PBS), pH 7.4, until the colour of the supernatant turned clear. The washed erythrocytes were then diluted to a final volume of 20 ml with the same buffer. To 190 µl of the cell suspension in microfuge tubes, mucus samples (10 µl), serially diluted in PBS, were added. Following gentle mixing, the tubes were incubated for 30 min at 37°C and then centrifuged at 4000 rpm for 5min. 100 µl of supernatant was taken, diluted to 1 ml with PBS, and absorbance was read at 567 nm. The relative optical density, as compared with that of the cell suspension treated with 0.2% Triton X-100, was defined as percent hemolysis (Park et al., 1997).

RESULTS

Biochemical composition

The protein content was 12.64 µg/g in the insoluble and 9.34 µg/g in the soluble proteinaceous gel of the catfish. The total lipid content was 0.005 µg/g and the total carbohydrate content of was 0.08 µg/g. Of the 17 amino acids recorded, 9 were essential 8 were non-essential amino acids. Cysteine recorded minimum quantity of 0.01 and 0.02 mole % in both soluble and insoluble fractions,

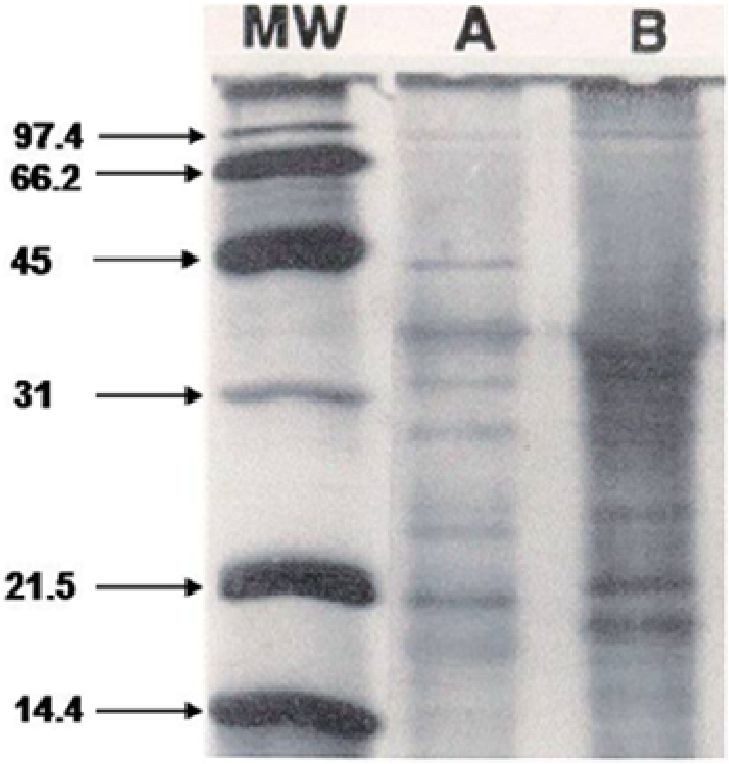

Figure 1. SDS-PAGE: Proteinaceous gel secretion of catfish (soluble and insoluble). MW = Molecular weight marker, A = soluble protein and B = insoluble protein.

respectively. Leucine recorded maximum amount (9.8 mole %) in the soluble fractions and aspartic acid was maximum in the insoluble fraction (9.0 mole %) (Table 1).

SDS-PAGE analysis

On SDS-PAGE analysis (Figure 1), the insoluble portion of the proteinaceous gel showed six bands with a range of 18.4 to 97.4 KDa, and with a distinct band at 35 KDa. The soluble portion of the proteinaceous gel showed seven bands with a range of 15.4 to 45 KDa, and a distinct band at 35 KDa.

Hemolytic activity

Both the soluble fraction and insoluble fraction of the proteinaceous gel showed strong hemolytic activity. The insoluble fractions showed high lysis activity than the soluble fraction (Figure 2). The cell lysis decreased with respect to dilution of the extract. The insoluble protein sample showed haemolytic activity from 50 µg and extended up to 100 µg. The soluble protein sample showed haemolytic activity from 25 µg and percentage of haemolysis increased with increase in concentration (Table 2).

Antibacterial activity

Both the soluble and insoluble fractions were tested against pathogenic Gram negative bacteria. The soluble and insoluble fraction showed activity against all the tested bacteria (Figure 2). Both soluble and insoluble fractions showed maximum activity against *Escherichia coli* and minimum against *Pseudomonas aeroginosa.* The results were tabulated in Table 3.

DISCUSSION

Fishes have managed to survive in a milieu of pathogenic organisms (Pickering, 1974). The primary contact of fish with their environment happens through a mucus layer that covers their entire body. Research has demonstrated that the mucus layer is composed of biochemically diverse secretions from epidermal goblet and epithelial cells (Hancock and Lehrer, 1998). The catfish has proteinaceous secretory cells in its epidermis (Al-Hassan et al., 1982). The biochemical composition of the crude mucus of the catfish shows protein as the major component. The concentration of protein in the insoluble and soluble fractions are 12.6 and 9.34 µg/g, respectively. It shows that the protein concentration in insoluble sample is higher than that of soluble portion of the mucus, and

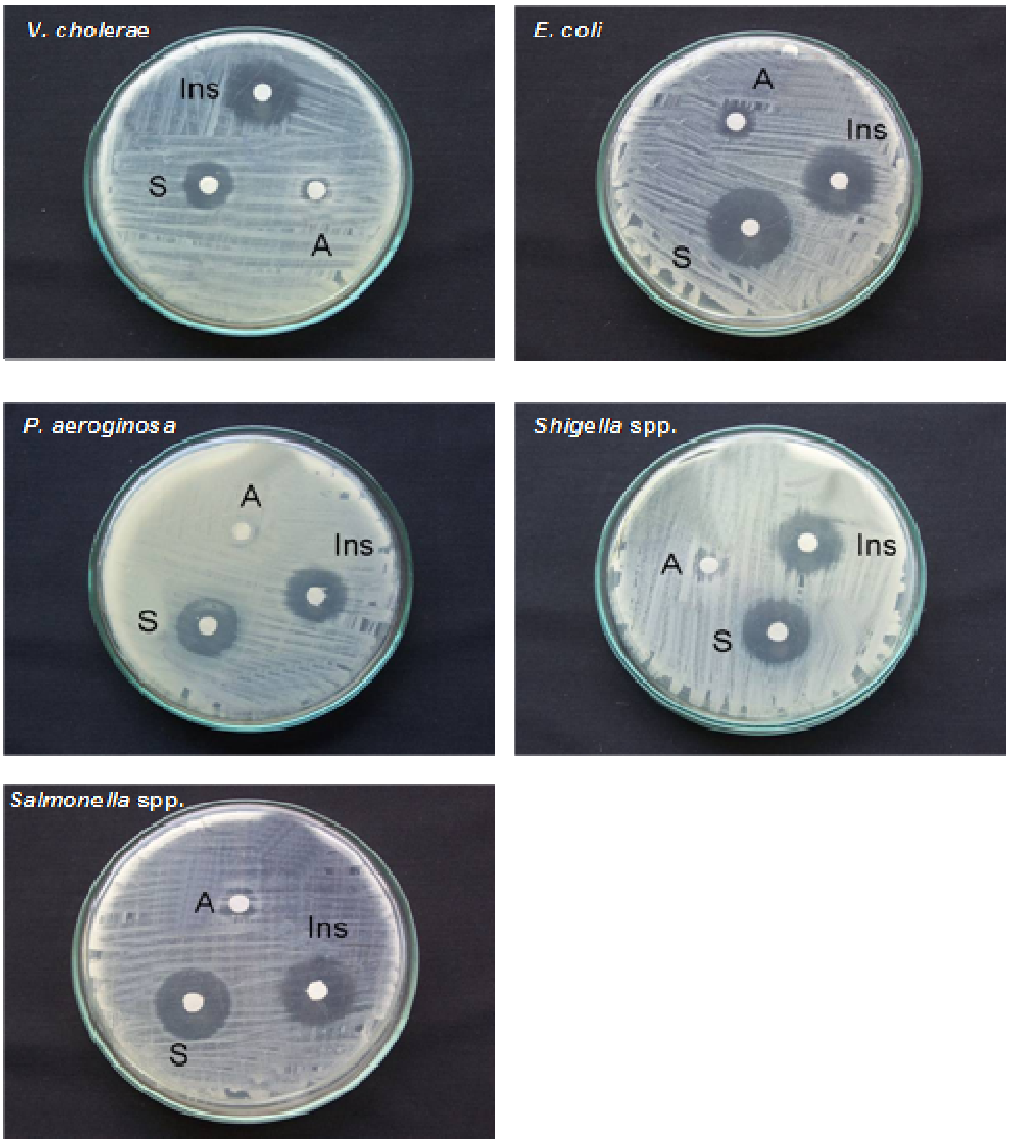

Figure 2. Antibacterial activity of proteinaceous gel secretion extracts against *Vibirio cholerae, Escherichia coli, Pseudomonas aeroginosa, Shigella* spp., *Salmonella* spp. A = Antibiotic; S = soluble; and Ins = insoluble.

Table 2. Hemolysis by the soluble fraction and insoluble fraction of gel of *A. maculates.*

Conc. (µg/ml)	Hemolysis of sheep blood (%)	
	Soluble fraction	Insoluble fraction
10	0.00	0.00
25	0.10	0.00
50	0.18	0.9
100	0.29	0.17

this supported by Al-Hassan et al. (1982), who reported that the proteins form gel which is insoluble to aqueous solvents. The total carbohydrate of the mucus, accounting for both soluble and insoluble fraction is 0.08 µg/g in *A. maculates* which is lower than that of the dry weight of the gel in *Arius thallasinus* (Al-Hassan et al., 1982). The

Table 3. Zone of inhibition of bacterial growth by insoluble and soluble fractions of the catfish skin mucus

Bacterial strains	Zone of inhibition (mm)	
	Soluble fraction	Insoluble fraction
Vibirio cholerae	7	6
Escherichia coli	10	8
Pseudomonas aeroginosa	6	5
Shigella spp.	8	6
Salamonella spp	7	6

total lipid composition of the soluble and insoluble fractions is 0.005 µg/g. The lipid present in the gel secretion includes phospholipids, neutral lipids and glycolipids in *A. thalassinus*, (Al-Hassan et al., 1986). The insoluble portion of the gel formed six bands and the soluble portion showed seven bands. The soluble protein portion of the *A. thallasinus* have fourteen components (Al-Hassan et al., 1982).

The gels showed broad antibacterial activity suggesting that it may play an important role in non specific immunity in catfish (Richards et al., 2001). The catfish haemolytic factor was not ichthyotoxic when tested against small fish and did not cause lethality when administered intravenously to rabbits (Alnaqeeb et al., 1989). Thus the skin mucus secretion of the catfish which is proteinaceous in nature also showed potent bioactivity.

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