

## Antibacterial Activity of Skin and Intestinal Mucus of Five Different Freshwater Fish Species Viz., *Channa striatus*, *C. micropeltes*, *C. marulius*, *C. Punctatus* and *C. gachua*

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**ABSTRACT** In the present investigation, efforts have been made to investigate out the antibacterial activity of skin mucus and intestinal mucus of five different *Channa sp* viz; *Channa striatus*, *C. micropeltes*, *C. marulius*, *C. punctatus* and *C. gachua*. The mucus collected were tested against five pathogenic bacteria namely *Aeromonas hydrophila*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Vibrio fischeri* and *V. anguillarum* using Muller Hinton agar plates by using disc diffusion method. All the five *Channa sp* showed a better antibacterial activity and among the five species *C. striatus* showed a broad spectrum of antibacterial activity of skin mucus against *A. hydrophila* ( $19.5 \pm 2\text{mm}$ ), *P. aeruginosa* ( $28 \pm 2.9\text{mm}$ ) and *V. anguillarum* ( $24.5 \pm 2.4\text{mm}$ ) and intestinal mucus against *A. hydrophila* ( $16 \pm 1.4\text{mm}$ ), *P. aeruginosa* ( $29 \pm 3.2\text{mm}$ ), *V. anguillarum* ( $18.3 \pm 1.5\text{mm}$ ) and *V. fischeri* ( $21.5 \pm 1.8$ ). Among the five pathogens tested the maximum antibacterial activity was observed for the skin mucus against *V. fischeri* and the intestinal mucus against *P. aeruginosa*.

**(Keywords:** *Channa sp*, *Aeromonas hydrophila*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Vibrio fischeri* & *V.anguillarum*.)

### INTRODUCTION

Animals in the aquatic environment consist of bacterial flora which is a reflection of the microflora present in the environment [1]. The most critical aspect of the environment for fish welfare is defined by their intimate contact with the medium through gills and skin [2]. The skin acts as a mechanical barrier by preventing the entry of microbes into the body [3]. Potentially valuable proteins and enzymes are present in the fish by products [4] and these compounds and the antagonistic bacteria help prevention of invading bacterial diseases. Virtually all fishes are covered with integumental mucus that is involved in many aspects of their biology, ranging from disease resistance to rearing of youngones to shelter and locomotion. Quite extensive work has been done on the skin of various teleosts during the past decades but only few studies have been carried out with regard to fish skin. It was reported that epithelial tissues produce antimicrobial molecules which serve as the first line of a host's defense against microbial invasion in a variety of vertebrates including humans [5]. Since the microorganisms have developed resistance to many antibiotics there is a need to develop an alternative antimicrobial drug. Antibacterial activity of mucus has been

demonstrated in various fish species [6] but it varies from species to species [7].

Murels commonly called snakeheads belonging to the genus *Channa* are highly priced among the freshwater fishes for their quality, taste, flavour and nutritive, recuperative and medicinal properties [8,9]. The present study was undertaken to determine the antibacterial activity of skin mucus and intestinal mucus of five different *Channa sp* (*C. striatus*, *C. micropeltes*, *C. marulius*, *C. punctatus* and *C. gachua*) against five pathogens viz; *Aeromonas hydrophila*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Vibrio fischeri* and *V. anguillarum*.

### MATERIAL AND METHODS

#### Collection of Fish

Healthy fish from each *Channa sp* approximately of 500 - 700g (*C. micropeltes* & *C. marulius*) were purchased from Kerala, 500 - 600g (*C. striatus*), 200 - 250g (*C. punctatus*) and 30 - 40g (*C. gachua*) were collected from fish market Tirunelveli, Tamilnadu ( $8.41^{\circ}\text{N}$ ,  $77.44^{\circ}\text{E}$ ), India. They were transported to Centre for Aquaculture Research and Extension (CARE) Aquafarm and reared separately in cement tanks (3m x 3m x 1m) for a period of ten days.

### Collection of mucus

The entire five different *Channa sp* were kept in sterilized water in fibre tanks (1000 l capacity) for an hour prior to mucus collection. The disease free fish samples were taken and the skin mucus (0.5 ml) was collected from the whole body. The intestinal mucus (0.2 ml) was collected by sacrificing the fish samples and then the mucus was collected by separating the intestine from the internal organs [10]. Equal amount of sterile physiological saline (0.85 % NaCl) was added to the mucus and centrifuged at 5000 rpm for 15 min. The supernatant was collected and stored at 4<sup>o</sup> C for further use.

### Bacterial strains

The *in vitro* antimicrobial evaluation of mucus of five different *Channa sp* was carried out against five bacteria viz; *A. hydrophila*, *P. aeruginosa*, *E. coli*, *V. fischeri* and *V. anguillarum*. All the five bacterial strains were freshly cultured (18 - 24 hr) from the mother culture preserved in CARE microbiology laboratory.

### Antimicrobial activity

Antimicrobial activity was determined by using disc diffusion method. 0.1ml of each culture was swabbed separately on priorly prepared sterile Muller Hinton agar plates. Sterilized No.1 Whatmann filter paper discs were loaded with 100µl of mucus supernatant separately for each fish sample and dried. The dried discs were impregnated on the pathogen inoculated Muller Hinton agar plates and triplicates were maintained. Then the plates were incubated at 37<sup>o</sup> C for 24 hr. Clear inhibition zones around the discs indicated the antibacterial activity [11].

## RESULTS AND DISCUSSIONS

The skin and intestinal mucus of *Channa sp* showed a strong inhibition against the selected pathogens. The skin mucus of *C. punctatus* and *C. marulius* exhibited a maximum zone of inhibition against *V. fischeri* (29 ± 3mm) and *E. coli* (24 ± 2.5mm) respectively followed by *C. striatus* against *A. hydrophila* (19.5 ± 2mm), *P. aeruginosa* (28 ± 2.9mm) and *V. anguillarum* (24.5 ± 2.4mm). Whereas the minimum zone of inhibition was observed in the skin mucus of *C. gachua* against *A. hydrophila* (10 ± 1.5mm) and *E. coli* (18 ± 1.5mm). In the case of intestinal mucus *C. striatus* elucidated a maximum inhibition against *A. hydrophila* (16 ± 1.4mm), *P. aeruginosa* (29 ± 3.2mm), *V. anguillarum* (18.3 ± 1.5mm) and *V. fischeri* (21.5 ± 1.8) followed by *C. marulius* against *E. coli* (20.3 ± 2mm). In contrast the least zone of inhibition was shown by *C. gachua* against *A. hydrophila* (7.5 ± 1mm). The antibacterial activity of

skin and intestinal mucus of the entire five *Channa sp* was found to be less against *A. hydrophila* when compared to the other pathogens. The maximum antibacterial activity was observed in the skin mucus of all the five *Channa sp* against *V. fischeri* and for the intestinal mucus against *P. aeruginosa*.

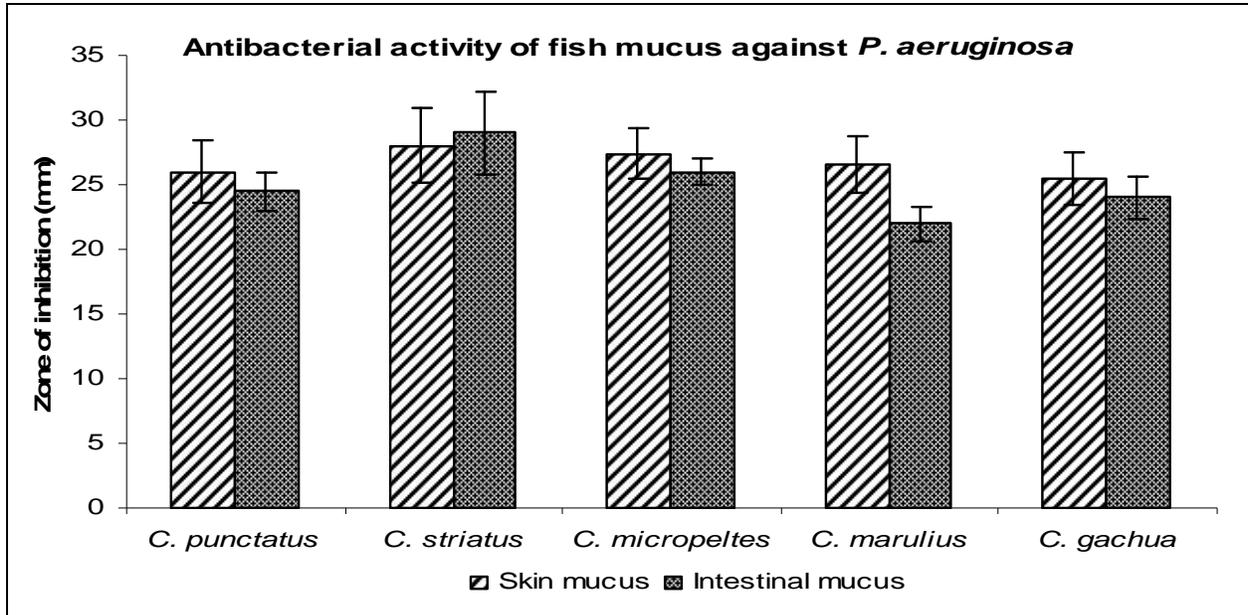
The mucus membrane present in fish skin and lining of the alimentary canal, respiratory and urogenital tracts prevents the entry of foreign microbes into the body. In addition the natural flora present on the surface of epithelial cells of mucosal surfaces of the host competes with the pathogenic bacteria [12] and prevents colonization of bacteria, fungi and parasites [6]. Mucus layer is composed of biochemical secretions from epidermal and epithelial cells [13] and plays a multirole viz; osmoregulation, reduction of friction and disease resistance [14]. The extracts of skin mucus of murels have antiseptic [15] and anti-inflammatory properties [16]. The presence of high amount of arachidonic acid and essential aminoacids in *C. striatus* helps in wound healing and promotes tissue growth [17]. Mat Jais et al., [18] have reported that ethanol added crude extract of *C. striatus* exhibited antifungal activity. In our investigation *C. striatus* showed a broad spectrum of antibacterial activity against *A. hydrophila*, *P. aeruginosa*, *V. anguillarum* and *V. fischeri*. In the present study the skin mucus of *C. punctatus* exhibited a minimum zone against *E. coli* (11 mm) and *P. aeruginosa* (20 mm). Similarly *C. gachua* exhibited a least zone of inhibition against *E. coli* (18 ± 1.5 mm). A better zone of inhibition was observed against *E. coli* (22 ± 2 mm) and *P. aeruginosa* (26 ± 2.4 mm) by skin mucus of *C. punctatus*. Similar findings have been reported by Kuppulakshmi *et al.*, [11] for the antibacterial activity of skin mucus of *C. punctatus* against ten bacterial strains.

Birkemo et.al., [19] have reported that hipposin a histone derived AMP (antimicropeptide) present in the skin mucus of Atlantic halibut possesses strong antimicrobial activity against several Gram positive and Gram negative bacteria. Previous authors reported maximum inhibition by *C. punctatus* and *Cirrhinus mrigala* against *V. cholerae* [11]. Similarly in our study the skin and intestinal mucus of all the five different *Channa sp* showed a significant inhibition against *Vibrio sp* (*V. fischeri* and *V. anguillarum*). Fish tissues and body contain naturally occurring proteins and glycoproteins of non immunoglobulin nature (transferrins, metallothionein) that react with environmental antigens and confer an undefined natural immunity to fish. Hence several endogenous peptides with antimicrobial activity have been purified from fish

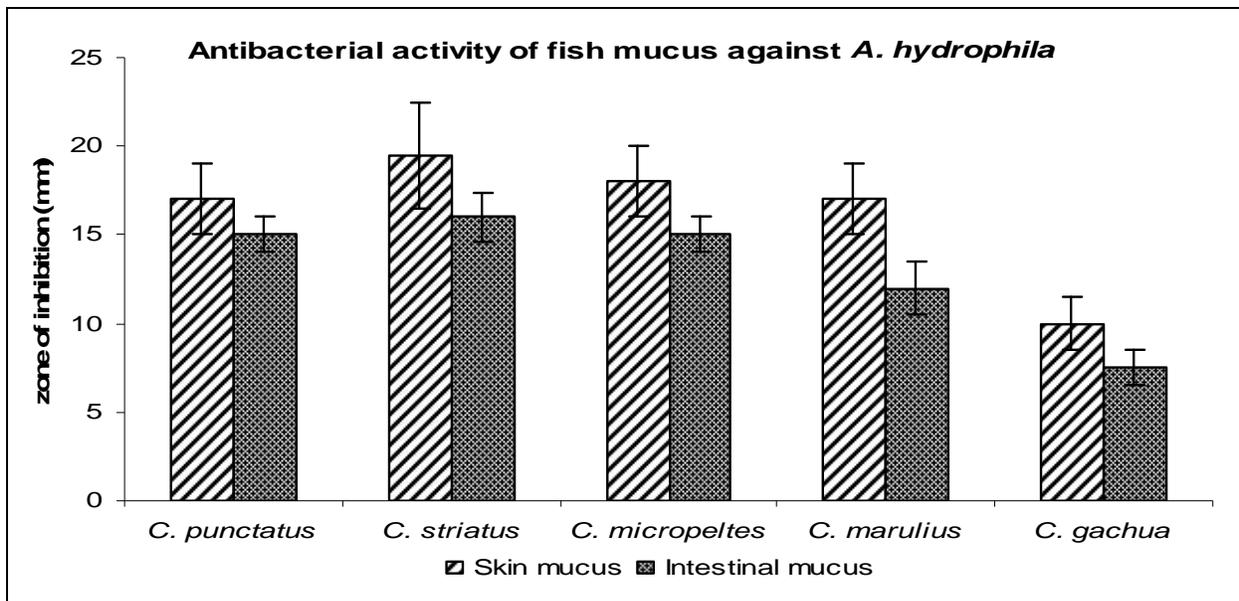
especially from the skin and intestinal mucus [20]. The metal ion chelating mechanism exhibited by these compounds deprives the microbes of essential inorganic ion sources and thus inhibiting the microorganisms. Fish also contain lectins which has antifungal and antibacterial activities [21].

In the present study the skin and intestinal mucus of the entire five *Channa sp* showed better antibacterial activity and in particular *C. striatus* showed a broad

spectrum of antibacterial activity inhibiting the growth of all the five pathogens tested. These results suggest that fish mucus has antibacterial proteins that make the membrane to act as a barrier. Moreover the mucus of fish possesses antimicrobial agents which could be formulated in new drugs for the therapy of infectious diseases caused by pathogenic organisms. This study has to be subjected to further evaluation to analyze the chemical composition of mucus to reveal its mode of action against microorganisms.



**Figure 1.** Antibacterial activity of skin mucus and intestinal mucus of five different *Channa* species against *P. Aeruginosa*.



**Figure 2.** Antibacterial activity of skin mucus and intestinal mucus of five different *Channa* species against *A. Hydrophila*.

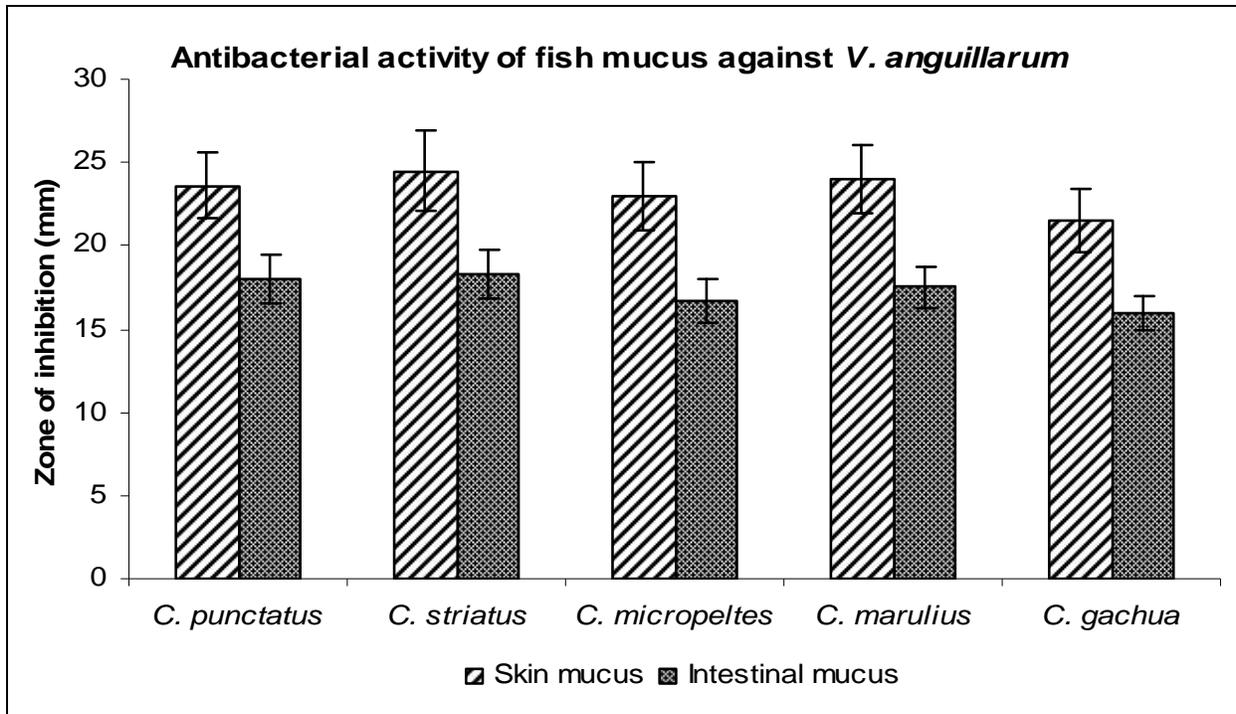


Figure 3. Antibacterial activity of skin mucus and intestinal mucus of five different *Channa* species against *V. anguillarum*.

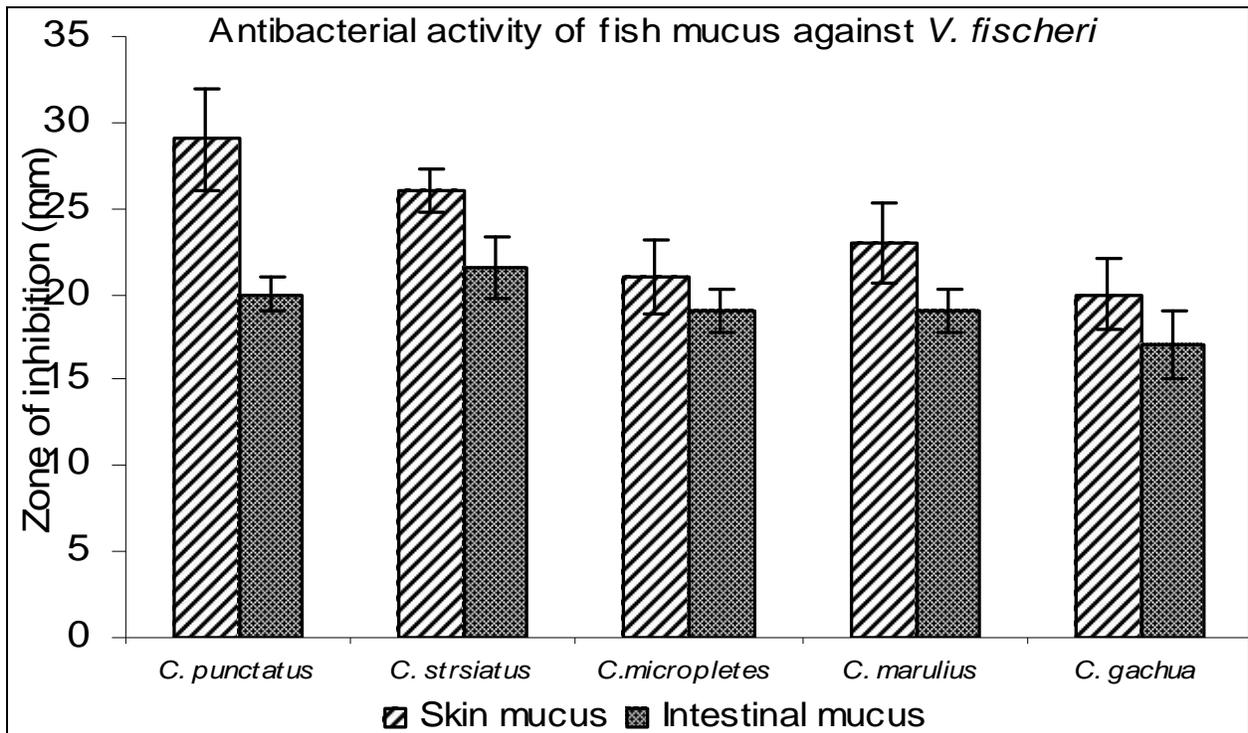


Figure 4. Antibacterial activity of skin mucus and intestinal mucus of five different *Channa* species against *V. fischeri*

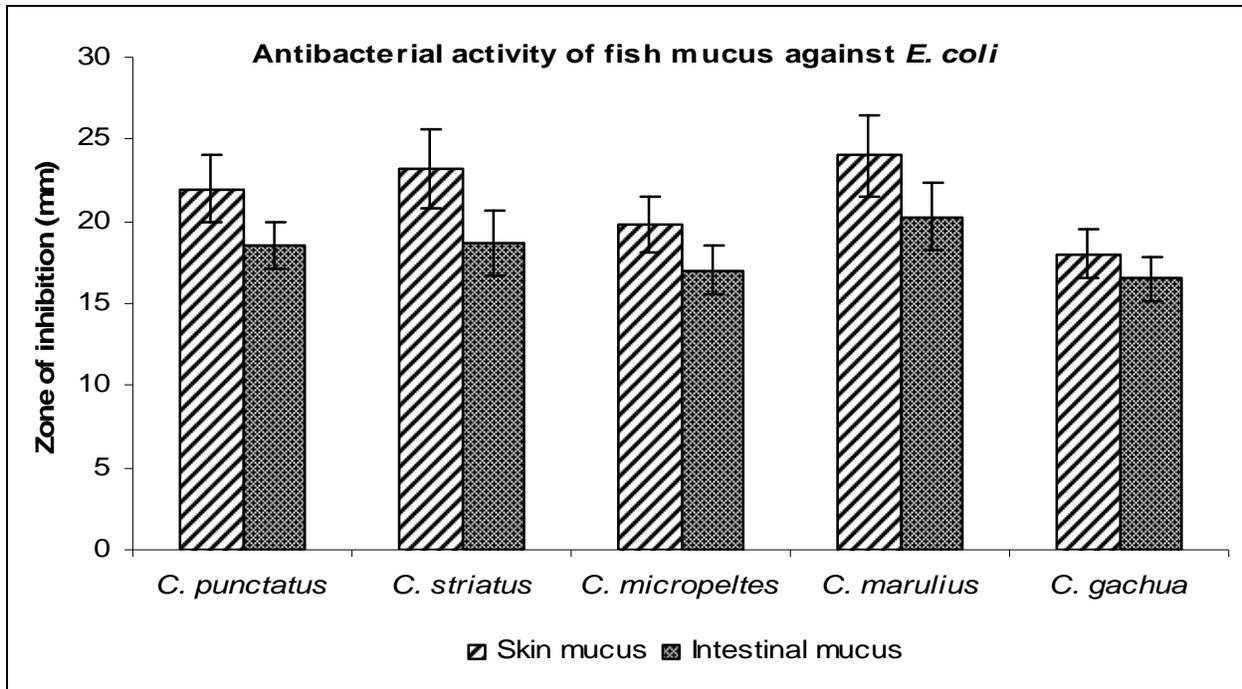


Figure 4. Antibacterial activity of skin mucus and intestinal mucus of five different *Channa* species against *E. Coli*.

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