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Antifungal activity of skin mucus of three cultivable fish species (*Catla-catla, cirrhinus mrigala* and *anguilla-anguilla*)

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Abstract
Fish are a diverse group of animals, highly specialized for their aquatic existence and comprising almost half the number of vertebrate species in existence today. Fish are in intimate contact with their environment, which can contain very high concentrations of bacteria and viruses. Mucus is a protective secretion of the epidermal membranes that cover the surface of fish as the first line of defense against invading pathogens using this approach present work explore for antifungal activity.
In present research, fish Mucus were collected by a modified Subramanian method. These collected samples were preserve in deep freezer at -80^{0}C to avoid bacterial growth and degradation and used for further study. Then mucus of three fishes *C. catla ,Anguilla-Anguilla* and *cirrhanus mrigla* were screened for the antifungal activity, allthe three fishes mucus sample exhibit antifungal activity against phytopathogenic fungi like *Aspergillus awamori and Colletotrichum falcatum.* In future fish mucus may be exploited as biocontrolling agent (BCA) for fungal diseases.

Keywords: fish mucus, antifungal activity, *aspergillus awamori, colletotrichum falcatum*

1. Introduction
Aquaculture is the process in which cultivation of aquatic animals like fishes, prawns, shell fishes, sea weeds etc. According to the intensive system of production of fishes to reduce the cost due to possibilities of exposure to high density of pathogens like bacteria, virus, fungus, parasites etc. (Laidler *et al*., 1999) [12]. In fact, these pathogens lead to high mortalities and reduce economical income for the fish farming industry (Munro 1933; Pilcher 1980) [14, 16]. In fact, bacterial and viral diseases of farmed fish have lead to high moralities and reduced economical income for the fish farming industry (Munro 1933; Pilcher 1980) [14, 16]. Avoiding disease epidermis in aquaculture is very important to get economic benefit and it is possible only when fish species are reared in a good environmental condition and given priority in fish welfare. Fish are in intimate contact with their environment through the large surface of their gills, skin, and of necessity they defecated into the medium in which they live, so water quality (in terms of dissolved oxygen, CO2, ammonia and pH) and the presence of contaminants (organic and inorganic pollutants) are probably the most critical aspects of the environment for fish welfare and also the best defined (Mellor and Stafford 2001) [13].
The epidermal mucus produced primarily by epidermal goblet or mucus cells are composed mainly of water and gel forming macromolecules including mucins and other glycoproteins (Shephard, 1993) [18]. The mucus substance secreted from the surface of fish performs a number of functions including disease resistance, respiration, ionic and osmotic regulation, locomotion, reproduction, communication, feeding and nest building (Ingram, 1980; Fletcher, 1978) [11, 8]. Fish mucus (slime layer) is the first physical barrier that inhibits entry of microbes from an environment into fish. It acts as a chemical barrier containing enzymes and antibodies which can kill invading disease causing organisms (Rottmann *et al*., 1992) [17]. Antimicrobial activity in mucus has been demonstrated in several fish species (Austin and Mcintosh, 1988) [2], yet this activity seems to vary from one fish species to the other and can be specific towards certain bacteria (Noga *et al*., 1995) [15]. An understanding on the antibacterial properties of skin mucus could be useful to gain insight in the involvement of mucus in innate host defense mechanism at organism-environment interfaces and could aid in the development of antibacterial agents for therapeutically application.

2. Materials and Methods
2.1 Mucus Collection
The healthy live fishes approximately 6 months old, weigh about 500 gms of each *Catla catla, Anguilla-anguilla* and *cirrhanus mrigala were* purchased from fish market, Latur (Maharashtra). Mucus of C. *catla, Anguilla-Anguilla* and *cirrhanus mrigala* was collected by a modified method of Subramanian *et al* (2008) [19]. The sample fish were killed with a sharp blow to the head. The fish were placed with ventral side facing downward in the "surgery-bed" and skin mucus from the dorsal side of the fish was collected by a cell-scraper. Mucus collected in the Eppendorf tubes and weighed (CONTECH, CB-Series). These samples were kept in at -80ºC for further use to avoid bacterial growth and degradation until used (Mohammad Mojibul Hoque Mozumder, 2005) [6].
Mucus was carefully scraped from the dorsal surface of the body using a sterile spatula. Mucus was not collected in the ventral side to avoid intestinal and sperm contamination. The collected fish mucus was stored at -80ºC for further study.

2.2 Antifungal Activity against Phytopathogens

The Fungal phytopathogenic strains were collected from the Department of Microbiology, Rajarshi Shahu Mahavidyalaya, and Latur (M.S). In *vitro* antifungal activity was carried out by disc diffusion method (Bauer *et al*. 1996) [4]. Whatman No.1 filter paper discs with 4 mm diameter were impregnated with known amount (5 µl) of test sample of fish mucus and a standard Mancozeb impregnated disc as control. The fungal plates were incubated at 30 ºC for 2 days and result was recorded by measuring zone of inhibition.

3. Results and Discussion

Skin mucus from *Catla catla*, Anguilla-anguilla *& Cirrhanus mrigala*were exhibited antifungal activity against phytopathogenic fungi like *Aspergillus awamori and Colletotrichum falcatum* (Table No.01 & Fig.1, 2, 3). In future fish mucus may be exploited as biocontrolling agent for fungal diseases.

The outermost surface of fish skin and gills are composed of epithelial cells. These outermost layers are covered with mucus layer composing of biochemically-diverse secretions from epidermal and epithelial cells. The mucus layer acts as a biological interface between fish and their aqueous environment. The skin mucous layer and epidermis are important in fish defense because they are the first sites of interaction between the host and potential pathogens.

Over the past years, it has also been shown that mucus plays a role in the prevention of colonization by parasites, bacteria and fungi and the antibacterial role of mucus has been known for many years (Austin and Mcintosh, 1988) [3]. Fish mucus was found as a source of antimicrobial products (Hellio *et al.*, 2002) [10].

Now days there are number of synthetic drugs and food preservatives are available, which are considerably affects on environment and ecosystem due to their higher persistency and constant accumulation in the biological system. To overcome this, considerable investigations are being carried out to develop safer source.

Table 1: The antifungal activity of fish skin mucus

S. No	Name of fungal Pathogen	Zone of Inhibition(in mm)			Control*Mancozeb* *(50µg/mL)*
		C.catla	*Anguilla-anguilla*	*C.mrigala*	
1	*Aspergillus awamori*	20	10	20	30
2	*Colletotrichum falcatum*	10	20	15	15
3	*Fusarium oxysporum*	13	10	10	12

Fig 1: Antifungal activity against *Colletotrichum falcatum*

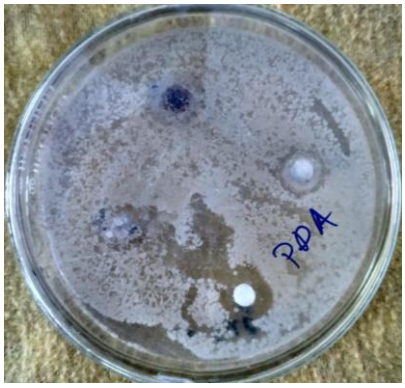

Fig 2: Antifungal activity against *Aspergillus awamori*

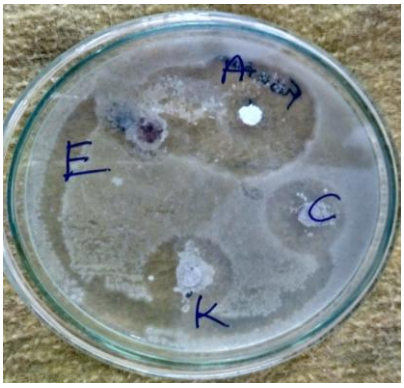

Fig 3: Antifungal activity against *Fusarium oxysporum*

4. Conclusion

The fish mucus contains antifungal agents which could be used to formulate new drugs for the therapy of infectious fungal diseases caused by pathogenic and opportunistic microorganisms.

These properties of mucus indicate that the fish mucus may be beneficial in aquaculture, human health related problem and agricultural field.

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