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Antibacterial activity of the mucus of mudskipper Boleophthalmus boddarti (Pallas, 1770) from Vellar Estuary

Velayudham Ravi, Kaila Kesavan, Sivakumar Sandhya, and Santhanam Rajagopal

CAS in Marine Biology, Annamalai University, Parangipettai-608502, Tamil Nadu, India; Corresponding author: V. Ravi, ravi.velayudham@gmail.com

Abstract. Mudskippers are known to secrete skin mucus that helps them survive in mudflats, acting as lubricant in locomotion and protecting against harmful microbes. Antibacterial activity in mucus has been demonstrated in several fish species and the activity can be specific towards certain bacteria. The present study was carried out to analyze antibacterial activity in mucus of the mudskipper, *Boleophthalmus boddarti* against several human pathogen bacterial strains. Out of the ten pathogen tested strains, eight proved to be sensitive to the antibacterial compounds contained by the mucus The study showed a positive progress in the study of the mudskippers mucus but further efforts are required for the purification and isolation of the active antimicrobial compounds in order to establish their possible applications.

Key Words: mudskipper, B. boddarti, antibacterial, Vellar, mucus.

Rezumat. Guvizii de nămol sunt cunoscuți pentru secrețiile de mucus de la nivelul pielii care îi ajută să supraviețuiască în zonele cu mâl. Mucusul acționează ca un lubrifiant pentru mișcările animalului și îi protejează pe aceștia împotriva infecțiilor bacteriene. Activitatea antibacteriană a acestui mucus a fost demonstrată la câteva specii de pești și această activitate poate fi specifică împotriva unei anumite bacterii. Aceast studiu a avut ca scop să analizeze activitatea antibacteriană a mucusului speciei *Boleophthalmus boddarti* asupra unor specii de bacterii patogene la om. Au fost testate zece tulpini ale agentului patogen studiat, din care opt s-au dovedit a fi sensibile la compușii antibacterieni conținuți de mucus. Cercetările au arătat un progres pozitiv în studiul mucusului secretat de guvizii de nămol, însă sunt necesare eforturi suplimentare pentru a purifica și izola compușii antimicrobieni activi în scopul stabilirii unor noi aplicații practice.

Cuvinte cheie: guvizi de nămol, B. boddarti, antibacterian, Vellar, mucus.

Introduction. Fish can maintain a healthy state by a complex system of innate defense mechanisms. Mucus is one among the dynamic coats, which passively flows over and covers the fish (Powell et al 1992). Mucus of the fishes acts as physical barrier through preventing mechanical absorption or deterrence of parasite, bacterial or viral access (Hughes & Munshi 1979), Also, the mucus has the ability to precipitate specific molecules like heavy metals, helps the secretion of immunoglobulin (Powell et al 1992) and electrolyte diffusion mechanisms in the presence of chloride cells (Shephard 1994). Mudskippers (Gobiidae: Oxudercinae) live in intertidal habitat of the mudflats and in mangrove ecosystem and these fishes are uniquely adapted to a completely amphibious lifestyle (Murdy 1989). They are quite active when out of water, feeding and interacting with one another and to defend their territories. Mudskippers are known to secrete skin mucus that helps them survive in mudflats, acting as lubricant in locomotion and protecting against harmful microbes. Antibacterial activity in mucus has been demonstrated in several fish species (Austin & Mcintosh 1988). Yet this activity seems to vary among fishes and can be specific towards certain bacteria (Noya et al 1995). The objective of the study is to analyze antibacterial activity in mucus of the mudskipper, B. boddarti against human pathogen bacterial strains.

Material and Method

Fish sample collection. Fish samples of the mudskipper *B. boddarti* (15 – 18 cm TL) were collected from the mudflats of Vellar Estuary (Lat. 11° 29' N; Long 79° 46' E) in Parangipettai, Tamil Nadu, South east coast of India. After collecting the fishes, the mucus was scraped gently from the body and the mucus was diluted with distilled water. Antibacterial assay. Anti bacterial assay were carried out by the disc diffusion technique in Petri dishes. Inocula of over night culture of each bacterial strain were streaked on the surface of Muller Hinton Agar plates. 20µl of the mucus extract was pipetted on a 6mm sterile paper disc. The solvent was allowed to evaporate and disc was placed on the surface of inoculated agar. The test plates were allowed for incubation for 24 hrs at 37 °C. Solvent controls was performed in each case. The normal distilled water used as the control. Areas of inhibited bacterial growth were observed as clear zones around the disc. The clear zone formation on the plates gives the effect of the sample on bacterial growth. Antibacterial activity was measured as diameter of zone of inhibition, excluding the paper disc diameter. (Muller-Hinton: Beef infusion - 3g; Casein acid hydrolyrate – 17.5g; Starch – 1.5g; Agar – 17g; pH – 7.3). The pathogen strains used in this study were Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi, Salmonella paratyphi, Klebsiella pneumonia, Proteous mirabilis, Klebsiella oxytoca, Lactobacillus bulgaricus, and Vibrio sp.

The zone of inhibition was measured by using scale, and its area of inhibition was calculated by using following formula:

Area = Π (R + r) (R-r)

Where, Π = 3.14 or 22/7; R = Zone of inhibition; r = Diameter of the disc

Results and Discussion. The crude aqueous extract from the skin mucus of *B. boddarti* were tested against 10 human pathogenic bacteria. *Viz., Pseudomonas aeruginosa, Vibrio* sp. *Escherichia coli, Staphylococcus aureus, Salmonella typhi, Salmonella paratyphi, Klebsiella pneumonia, Proteous microbilus, Klebsiella oxytoca,* and *Lactobacillus vulgaris.* zone of inhibition of different human bacterial pathogens were given. The zone formation was maximum in *Klebsiella pneumonia* (4.9 mm) followed by *Salmonella paratyphi* (4.3 mm) and *Salmonella typhi* (4 mm) but minimum zone in *Proteous microbilus* (3.2 mm). No zone formation was observed with *Escherichia coli* and *Klebsiella oxytoca.* The percentage contribution also reveals that *Klebsiella pneumonia* (15.8%) as maximum and the minimum was *Proteous microbilus* (10.3%). *Escherichia coli* and *Klebsiella oxytoca* has no contribution (Figure 1).

Bioactive substances from marine organisms have been studied for several decade and many chemicals have pharmacological properties (Kamath et al 1992). Fish mucus is multifunctional material, which plays a major role in communication, resistance to disease, respiration, ionic and osmotic regulation, feeding, nest building, reproduction and excretion (Ellis 1999). Mudskippers are known to secrete mucus but there is no much attention paid on antibacterial activity of the mucus in the Indian context. Mucus secretions from the fish epidermis are usually to be productive to the fish in various ways (Jakowska 1963). The mucus assists in locomotion by acting as a drag reducing polymer (Daniel 1981). The major components of the mucus layer are produced by goblet cells and these cells start to differentiate in the basal part of epidermis and then grow in size and move towards of the surface where they release their content (Pickering 1977).



Figure 1. Showing the percentage contribution of the species

More works have been documented that mudskipper are able to utilize aerial cutaneous respiration. Species with an ability for cutaneous respiration includes *B. pectinirostris* (Yokoya & Tamura 1992), *B. dussumieri* as *B. boddarti* and corrected by (Clayton & Snowden 2000; Al-Kadhomity & Hughes 1988) and some other species of (*Periophthalmus*, Schottle, 1931). In the present study it is noticed that the mudskipper, *B. boddarti* were also able to secrete mucus to protect themselves as they walk on the surface of the mudflat and frequently enter into their nesting burrows (Ravi 2005). They require lubricant material for these events not only for locomotion but also to protect themselves against pathogens and radiation. In the present study, the antibacterial activity was tested against human pathogens and it showed significant zone inhibition formation for some pathogens while tested aganist ten pathogens. Among the tested strains, *Klebsiella pnemoni* (4.9 mm) showed elevated zone formation followed by *Salmonella paratyphi* (4.3 mm) and *Salmonella typhi* (4 mm). But minimum zone formation was observed in *Proteous microbilus* (3.2 mm). There is no zone formation observed in *E. coli* and *Klebsiella oxytoca*.

Generally the fishes also posses a variety of relatively specific lytic molecules that causes cell lysis, and some of these materials are hydrolyses enzymes (lysozyme, chitinase, chitobiase) and the main actions of those are against bacteria and fungi. Many of the "defense" substances are present in skin mucus posses the capacity to react with potentially-infective microorganisms including parasites. Mucus thus acts as an immediate defense barrier to invasion and/or colonization of pathogens. The lysozyme isolated from fish was an enzyme with bacteriolytic properties and was ubiquitous in its distribution among living organisms (Hellio et al 2002). This enzyme had antibacterial and anti-inflamatory properties.

Conclusions. Many of the organisms have antimicrobial propreties, although most of the antibacterial agents that have been isolated from marine sources have not been active enough to compete with classical antimicrobials obtained from microorganisms. In the present study, mudskipper's mucus showed antibacterial activity which is potentially useful for mankind. A detailed study of the purification of the mucus is needed. Thus the present study is innovative in mucus studies of mudskipper against pathogen.

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Tamil Nadu, India, phone: +91 (0)4144 243223, ravi.velayudham@gmail.com;

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Velayudham Ravi, Centre of Advanced Study in Marine Biology, Annamalai University, Parangipettai-608 502,

Kaila Kesavan, Centre of Advanced Study in Marine Biology, Annamalai University, Parangipettai-608 502, Tamil Nadu, India, phone: +91 (0)4144 243223, k7til@yahoo.co.in;

Sivakumar Sandhya, Centre of Advanced Study in Marine Biology, Annamalai University, Parangipettai-608 502, Tamil Nadu, India, phone: +91 (0)4144 243223;

Santhanam Rajagopal, Centre of Advanced Study in Marine Biology, Annamalai University, Parangipettai-608 502, Tamil Nadu, India, phone: +91 (0)4144 243223, rajgopi52@yahoo.com.

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