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Screening of Antimicrobial activity of bodily fluid from three different local fish species around Madanapalle

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ABSTRACT

The fish skin acts as a protective shield against environments that are high in infectious agents. A thin layer of mucus which acts as a defence shield against colonization by aquatic parasites, bacteria and fungi which was mediated by peptides and polypeptides, was covered on the external body surface of the fish. In the present study, we had shown the activity of epidermal mucus of tap water, mineral water and saltwater fish exhibit strong antibacterial activity. Here, we have isolated supernatant of a fish Pamphlet (*Pomfret*), Tilapia (*Oreochromis niloticus*) and Catfish (*Clariasgariepinus*) mucus. This Bodily fluid (Fish mucus) activity was correlated with a strong antibacterial activity (minimal inhibitory concentration for the three fishes) against both Gram-ve and Gram +vebacteria. In this study, the mucus isolated shows an inhibiting effect on the selected microorganisms. The antibacterial activity of fish mucus may be due to the presence of antibacterial glycoproteins and able to kill bacteria by forming large pores in the target membrane. 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 defence barrier. Our results suggest that fish secrete antibacterial which are able to kill bacteria in the target membrane.

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INTRODUCTION

Fish are mostly cold-blooded aquatic vertebrates usually having scales and breathing through gills with the Composition of minerals, enzymes, pigments/flavors, proteins or are wealthy in items (Hellio *et al.*, 2002). Utilization of bodily fluid (Fish Mucus) for research on natural obstruction among fish and their watery circumstance contains of a bodily fluid layer made out of biochemically assorted emissions from epidermal and epithelial cells (Ellis, 1999). High potential mucus production is necessary for the Protection from abrasion injury (Blackstock and Pickering, 1982) and to limit frication against the water, the bodily fluid layer covers the surface of the exterior body (Bressler and Bressler, 1989). Its have an assortment of naturally dynamic components in the bodily fluid, in reality, go about as humoral barrier factors, considering the fact that the fish invulnerability is less modern than that of greater creatures (Alexander and Ingram, 1992). During the past years, a fish bodily fluid has additionally been assumes a function in the aversion of parasites, microscopic organisms, and fungi (Ebran *et al.*, 2000).

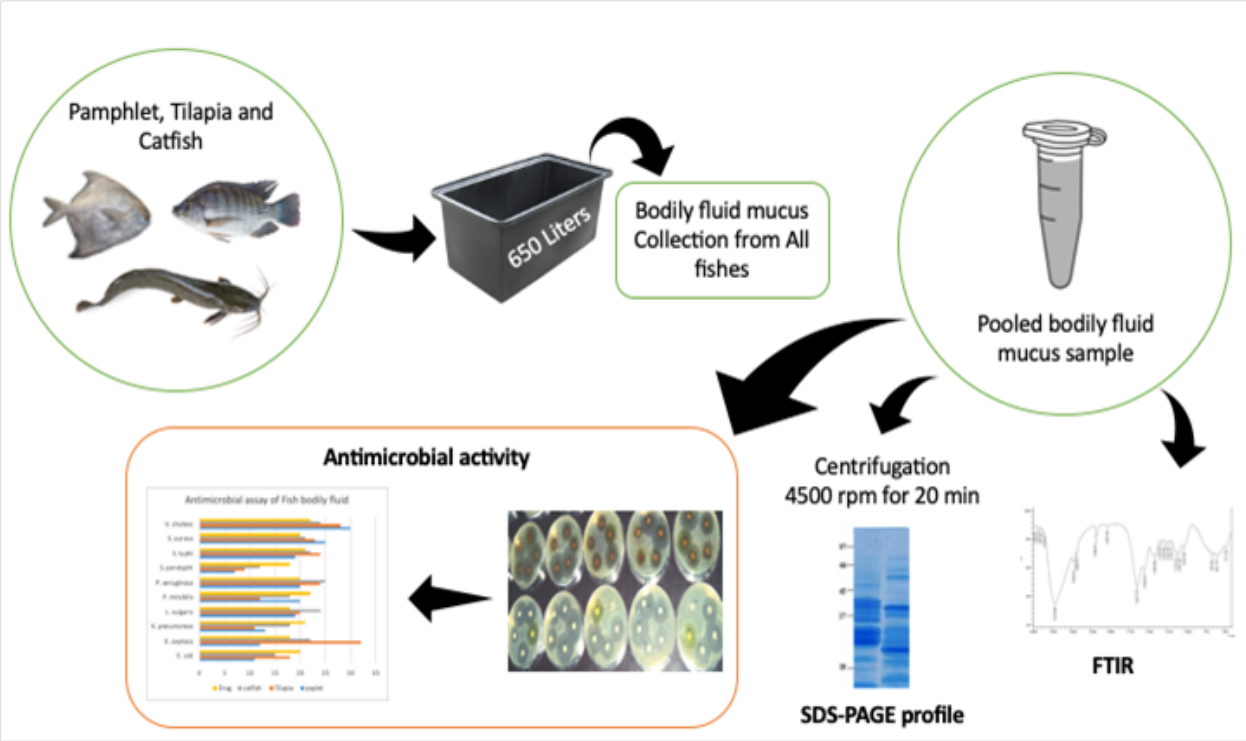

Figure 1: Screening process of Antimicrobial activity from three different species of fish.

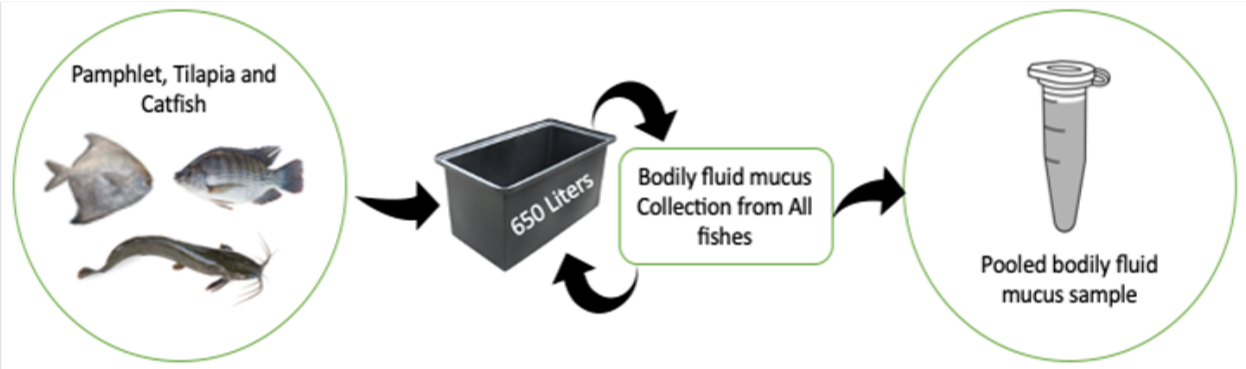

Figure 2: Bodily fluid Mucus collection

The antimicrobial agent play an important role in aquatic organisms which includes fishes, which are continually expressed to pathogenic microorganisms through the surrounding water. The antibacterial activity of fish bodily fluid was once acknowledged for a lengthy time then again, previous deals with antibacterial assessments has been coordinated toward marine microbial strains. As per the studies conducted by Dalmo *et al.* (1997) an inflammatory response such as elevated production of antimicrobial substances is often encountered (Dalmo *et al.*, 1997). It used to be accounted for that epithelial tissues produce antimicrobial which fills in as the mainline of hosts safeguard against microbial intrusion in an assortment of vertebrates along with humans (Ganz, 1999). In this existing study, a series of solvent extracts of mucus from three marine fishes have been display screen for their invitro recreation in opposition to terrestrial Gram negative positive, gram positive bacteria.

MATERIALS AND METHODS

Collection of Fish

Fish Collection and Maintenance of Mucus sample used to be accumulated from a Pamphlet (*Pomfret*), Tilapia (*Oreochromis niloticus*) and Catfish (*Clariasgariepinus*) (Bodyweight; 160g ± 2.324 g) are formed at kaikaluru, that have been received from a neighborhood fish market in Madanapalli, Chittoor, Andhra Pradesh. Then they are saved in three rectangular plastic tanks which has a capacity of above 500 Litres. The fish accustomed to lab conditions in three different types of water, i.e., tap water, min-

eral water and saltwater and maintained for seven days (Fast, 2002; Austin and Intosh, 1988) by feeding them once a day with industrial feed with feeding strategies of ad libitum (Lekang, 2015). The cleaning of all three tanks was done once a day by changing of 50% water. After Seven days of acclimatization, the fish were used for bodily fluid(mucus) collection. Dead fish or fish with skin lesions are not considered for the collection of bodily fluid. They are separated from the tanks. Only wholesome fishes had been chosen for mucus collection.

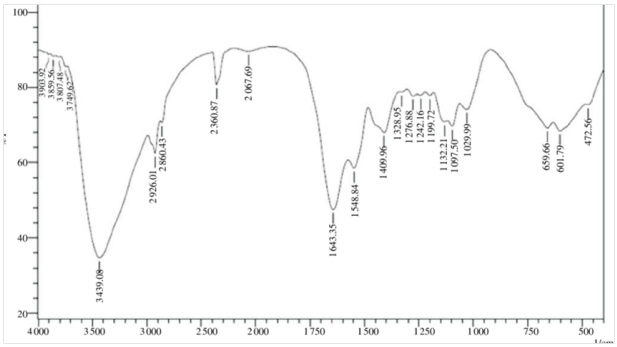

Figure 3: FT-IR samples of mucus from three marine fishes

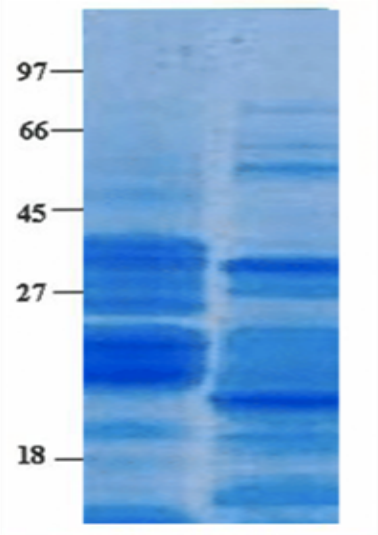

Figure 4: SDS-PAGE profile

Bodily fluid Collection

Bodily fluid collected by using way of a modified technique of (Subramanian *et al.*, 2008), as shown in Figure 2. Fish modified into starved for one day preceding to physical fluid series. At the day of bodily fluid collection, three fishes was once washed and transferred into a separate sterile polyethylene bag for 10-20 minutes and moved the front and lower

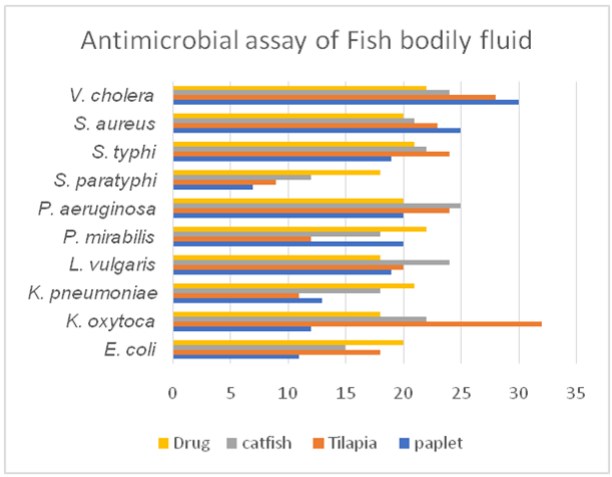

Figure 5: Antimicrobial assay of Fish bodily fluid

back to slough off the bodily fluid. Then, the individual fishes transferred again to restoration tanks. Physical fluids received from three fish used to be then pooled and saved in the fridge at 2°C till in similar use. The pooled bodily fluid mucus sample was further divided one by one with crude, acidic, and aqueous solvents as three elements (Diamond *et al.*, 1991).

Bacteria Culture Conditions

Antimicrobial activities of bodily fluid mucus extracts have been examined towards a wide variety of human and fish pathogens along with every gram +ve(*Lactobacillus vulgaris, Staphylococcus aureus*) and gram -ve bacterium (*Escherichia coli, Klebsiella oxytoca, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella paratyphi, Salmonella typhi, and Vibrio cholera*). All of the microbes have been grown at 38°C in Luria-Bertani (LB) broth and maintained at equal temperature, without fish pathogen aeromonas hydrophila. The fish pathogen turns out to be grown in nutrient broth at 38°C.

Bodily fluid mucus Extraction and Protein Quantification

For crude extract, 20 ml of bodily fluid was centrifuged at 4500 rpm for 20 minutes. The obtained supernatant are then saved at 4°C (Jais *et al.*, 1998). The aqueous extract of bodily fluid changed into organized use of a technique as mentioned through (Hellio *et al.*, 2002). 50 ml of bodily fluid blended with 50 ml of distilled water and homogenized with the use of a homogenizer. The aggregate used to be then centrifuged at 25,000 for forty minutes at 4°C (Remi, CPR-23 plus, RA-2313). The acquired Supernatant was collected and filtered with Whatman no.1 filter paper. The obtained fil-

trate was collected and stored in the refrigerator at 4^{o}C. The acidic extract of bodily fluid used to be geared up by using a modified approach of (Subramanian *et al.*, 2008). The acidic bodily fluid mucus mixture was prepared by 30ml of the bodily fluid was mixed with 30ml of 3% acetic acid and placed in a boiling electrical water bath for nearly 8mins. Later acidic mixture was cooled with the help of ice to reduce temperature and homogenized by the use of homogenizer. The combination used was then centrifuged at 25,000 rpm accurately 45 minutes by maintaining 4^{o}C temperature. The supernatant amassed and filtered by using a syringe with 0.22 μm clear out. Elutes which are obtained are again stored at in the refrigerator at 4°C. Protein quantification turns into determined based totally on Bradford protein assay (Bradford, 1976) via the utilization of bovine serum albumin as regularly occurring (Lowry *et al.*, 1951).

Microbial strains

Antimicrobial activity of bodily fluid changed into decided in opposition to 10 bacterial strains as per author (Subramanian *et al.*, 2008)*E. Coli, K. Oxytoca,L. Vulgaris, P. Mirabilis, P.Aeruginosa, S.Paratyphi, S.typhi S.Aureus, V.Cholera and Fungal strains A.Niger, C.Albicans, A.Flavus Mucor Sp., A.Alternata, Pencillium Sp., Rhizopus, T.Rubrum, T.Mentagarophytes, E.Floccosum*.are in experimental trial

Anti-microbial Assay

As mentioned by Rusell et al., antimicrobial activity was represented in diameter phrases by which area of inhibition is measured by using mm units The spectrum of antimicrobial activity grew to be described by means of way of (Russel and Fur, 1977) antimicrobial exercise become expressed in phrases of a diameter of area inhibition had been measured in mm via the usage of vernier callipers and recorded. The Inoculated micro-organism are incubated at 35^{o}C for 24Hr into a nutrient broth, fungal cultures have been incubated in potato dextrose broth at 25^{o}C for 48Hr. Those cultures had been spread-plate on Mueller Hinton agar antibiotic susceptibility checking out by means of the usage of sterile cotton and with the help of (1 cm), borer wells are made within the plates. The test bodily fluid (0.1ml) was delivered into the well and the plates should be incubated.

FTIR studies

FT-IR spectroscopy samples of mucus from three marine fishes relied on a pattern (5 mg) combined with 50 mg of dried potassium bromide (Kbr) and compressed in addition to preparing as a pellet for studying spectrum as sown in Figure 3.

RESULTS

Protein level

The bodily fluid become predicted for its protein stage and it effects 0.724mg/ml of protein content

Protein Quantification

The protein quantification was used to identify and monitor proteins during purification and to access the homogeneity by using Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis. SDS-PAGE of bodily fluid based on molecular size ranging >20KDa showed many protein bands shown in Figure 4.

Antimicrobial assay

To identify the antibacterial activity of bodily fluid extracted from three fishes *pamphlet, Tilapia and catfish* were used for inhibition assay on thin agar plates. The antibacterial activity of mucus of the *pamphlet, Tilapia and catfish* are represented in the Graph. The bodily fluid shows a strong inhibition in the growth of tested bacteria. Maximum zone of inhibition was observed against *Vibrio cholera* (26mm), *Staphylococcus aureus* (23mm)and *Salmonellaparatyphi*(12 mm), whereas the other bacteria show asignificant inhibition in their growth *Salmonella typhi* (17mm), *Lactobacillus Vulgaris*(17 mm), *Pseudomonas aeruginosa* (20 mm), *Proteus mirabilis* (20mm), *Klebsiella oxytoca*(12mm), *Escherichia coli* (11mm) and *Klebsiella pneumonia*(13 mm). The comparative antibacterial effect of the mucus of the fishes *pamphlet, Tilapia and catfish* with standard drug Chloramphenicol are shown in Figure 5 and Figure 6.

DISCUSSION

The antibacterial activity in mucus samples of three fishes *pamphlet, Tilapia and catfish* were tested among different bacteria strains of E.coli, Lactobacillus anguillarum, Clastridiumglutamicum and Staphylococcus aureus, later it was compared against different samples of bacterial cultures. Based on growth curves of bacteria and water was considered as negative controls. The antibacterial activity of fish mucus may be due to the presence of antibacterial glycoproteins and able to kill bacteria by forming large pores in the target membrane (Ebran *et al.*, 1999). In the serum and mucus, number of antibacterial factors were been founded in three fishes in which immunization acts against microbial diseases. (Rainger and Rowley, 1993) Based on the fish epidermis and epidermal mucus study author Hellio *et al.* (2002)

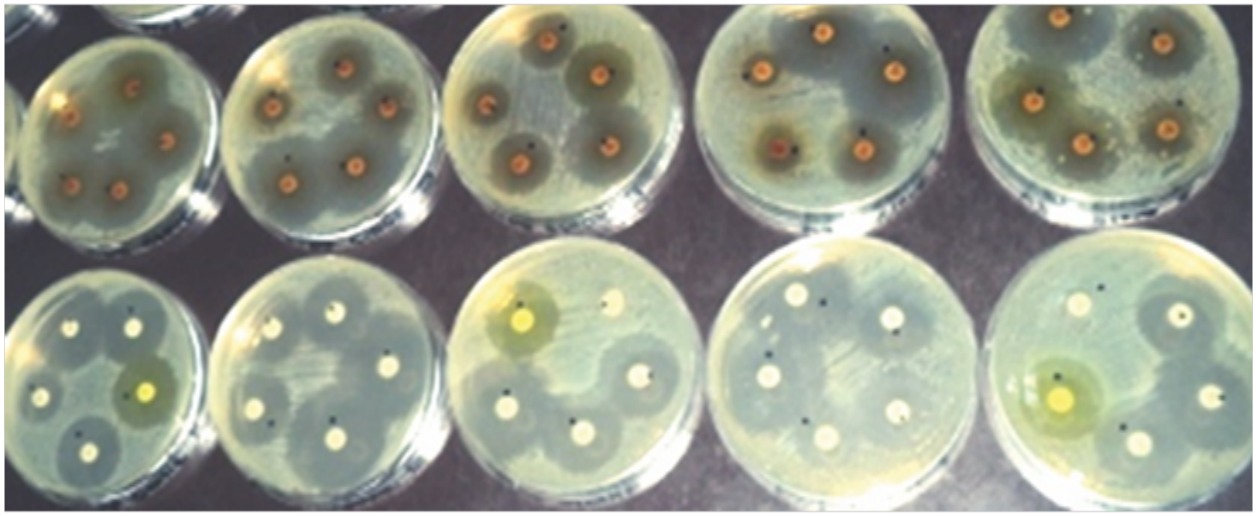

Figure 6: Antibacterial activity of bodily fluid extracted from three fishes

had found antifungal by conducting different activities of antibacterial, antifungal and cytotoxic of body fluid extracts from fish. Bodily fluid from solid phase extraction of three fishes was tested against E.coli, Lactobacillus anguillarum, Clastridiumglutamicum and Staphylococcus aureus for antibacterial activity. Its resulted L.anguillarum and C.glutamicum were noticed most sensitive micro-organisms during the activity.

CONCLUSIONS

In this research, results suggest that fish mucus have bactericidal properties and thus play important role in the protection of fish and showed that mucus had high potential source of an antimicrobial activity on against the invasion of pathogens. Further study will surely open new window to formulate new drugs for the therapy of infectious diseases caused by pathogenic and opportunistic microorganisms. These properties of mucus suggest that it may be beneficial in aquaculture and human health-related applications.

Conflict of Interest

None.

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REFERENCES

Alexander, J. B., Ingram, G. A. 1992. Noncellular nonspecific defence mechanisms of fish. *Annual Review of Fish Diseases*, 2:249–279.

Austin, B., Intosh, D. M. 1988. Natural antibacterial compounds on the surface of rainbow trout, Salmo gairdneri Richardson. *Journal of Fish Diseases*, 11(3):275–277.

Blackstock, N., Pickering, A. D. 1982. Changes in the Concentration and Histochemistry of Epidermal Mucous Cells During the Alevin and Fry Stages of the Brown Trout Salmo-Trutta. *Journal of Zoology*, 197:463–471.

Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72(1-2):248–254.

Bressler, R. S., Bressler, C. H. 1989. Functional anatomy of the skin. *Clin Podiatr Med Surg*, 6(2):229–275.

Dalmo, R. A., Ingebrigtsen, K., Bogwald, J. 1997. Nonspeciûc defence mechanisms in ûsh, with particular reference to the reticuloendothelial system (RES). *J. Fish Dis*, 20:241–273.

Diamond, G., Zasloff, M., Eck, H., Brasseur, M., Maloy, W. L., Bevins, C. L. 1991. Tracheal antimicrobial peptide, a cysteine-rich peptide from mammalian tracheal mucosa: peptide isolation and cloning of a cDNA. *Proceedings of the National Academy of Sciences*, 88(9):3952–3956.

Ebran, N., Julien, S., Orange, N., Auperin, B., Molle, G. 2000. Isolation and characterization of novel glycoproteins from fish epidermal mucus: correlation between their pore-forming properties and their antibacterial activities. *Biochimica et Biophysica Acta (BBA) - Biomembranes*, 1467(2):271–280.

Ebran, N., Julien, S., Orange, N., Saglio, P., Lemaître, C., Molle, G. 1999. Pore-forming properties and antibacterial activity of proteins extracted from epidermal mucus of fish. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 122(2):181–189.

Ellis, A. E. 1999. Immunity to bacteria in fish. *Fish & shellfish immunology*, 9(4):291–308.

Fast, M. 2002. Skin morphology and humoral nonspecific parameter of mucus and plasma in rainbow trout, coho and atlantic salmon. *Comp Biochem Physiol*, 132:645–657.

Ganz, T. 1999. Defensins and host defense. *Science*, 286(5439):420–421.

Hellio, C., Pons, A. M., Beaupoil, C., Bourgougnon, N., Gal, Y. L. 2002. Antibacterial, antifungal and cytotoxic activities of extracts from fish epidermis and epidermal mucus. *International Journal of Antimicrobial Agents*, 20(3):214–219.

Jais, A. M. M., Matori, M. F., Kittakoop, P., Sowanborirux, K. 1998. Fatty Acid Compositions in Mucus and Roe of Haruan, Channa Striatus, for Wound Healing. *General Pharmacology: The Vascular System*, 30(4):561–563.

Lekang, O. I. 2015. Feeding equipment. *Feed and Feeding Practices in Aquaculture*, pages 349–368.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J. 1951. Protein measurement with the Folin phenol reagent. *J Biol Chem*, 193(1):265–275.

Rainger, G. E., Rowley, A. F. 1993. Antibacterial activity in the serum and mucus of rainbow trout, Oncorhynchus mykiss, following immunisation with Aeromonas salmonicida. *Fish & Shellfish Immunology*, 3(6):475–482.

Russel, A. D., Fur, J. R. 1977. Antibacteria activity of a new chloroxylenol prepration containing ethylenediamine tetra acetic acid. *J. Appl. Bacteroil*, 43:253–260.

Subramanian, S., Ross, N. W., MacKinnon, S. L. 2008. Comparison of antimicrobial activity in the epidermal mucus extracts of fish. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 150(1):85–92.