ORIGINAL ARTICLE

The Epidermal Mucus from Clarias *batrachus* Inhibits Bacteria Growth

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ABSTRACT

Introduction: **The widespread bacterial resistance towards antibiotic had caused many researches to be done in searching of new alternative drug, such as antibacterial peptide. An element of the innate immune system with a broad range of activities is the antibacterial peptide. Recent research has revealed that fish epidermal mucus is a biological source that is particularly rich in antibacterial peptides.**

Method: **This study examines the possibility for *Clarias batrachus* epidermal mucus to serve as a source of an antibacterial peptide. An epidermal sample of *C. batrachus* is used to collect mucus, which is then extracted using aqueous extraction techniques. The spectrophotometric approach was used to assess the crude epidermal mucus extract of *Clarias batrachus* for its antibacterial activity. The findings of this investigation demonstrate the presence of antibacterial action from crude mucus epidermal extract at a dosage of 20 mg per ml toward the growth of the two bacterial strains being examined, Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853.**

Result: **The findings of this study support the idea that mucus functions as a component of the body's immune system, with antibacterial peptide serving as its primary building block. As a result, these findings could pave the way for the creation of a new class of medication that would be a market-wide solution to antibiotic resistance.**

KEY WORDS

***E. coli*, *Pseudomonas aeruginosa*, MIC, bioactive peptides, antibacterial agent**

INTRODUCTION

Many fish has a robust host defense system to enable them to survived infection in an aquatic environment rich in waterborne pathogens[1,2]. The first line of defense and one of the elements of the natural immune system that guards fish against the increased risk of pathogen infection brought on by repeated contact to microbes is the mucus from its epidermis[3]. Also, three cells called club, goblet and sacciform cells are the three cells that secretes skin mucus from fish. The club cells majorly release proteinaceous components. The goblet cells are located on the gills' surface and on their outer surfaces. These cells also produce mucus granules, which are made up of glycoproteins. Goblet cells' secretions are combined with those produced by sacciform cells[4,5]. Fish epidermal mucus contains antimicrobial peptides, proteolytic enzymes, lectins, lysozymes, flavoenzymes, immunoglobulins, and C-reactive proteins as natural immunological components[3,6]. Pelteobagrin, parasin I, myxinidin, pardaxin, and pleurocidin are a few of the antibacterial peptides that have been found in fish epidermal mucus[3,7].

The wood catfish or known as *Clarias batrachus* is a native catfish species that can be found in the Southeast Asian region[8]. This fish is regarded as a national priority as a result of its high demand, and declining nature with the aquatic environment[9]. A promising aquatic organism, C. batrachus is also hardy, grows well, has a high nutritional profile, and has a high commercial value. Due to its high degree of appeal with good flavor, medicinal value, and displays high popularity among consumers, it is regarded as one of the most economically vital freshwater species in Asia[10-12]. It is a fish species that does not have scales, thus it is completely dependent on epidermal mucus as the first mechanical resistance against aquatic pathogens. Its ability to breathe in air when there is no water makes it a species of fish that has high resistance. Wood catfish also have a high economic value due to the low cost of farming and simple farming methods. The study of the potential of wood catfish as a source of bioactive products is still under-explored in the scientific field. It is intriguing to investigate the ability of the mucus of wooden catfish to prevent and control bacterial infections given the importance of mucus as the primary line of defense for non-scaled fish. The current study was carried out to examine the antibacterial activity of epidermal mucus from *Clarias batrachus* against certain bacterial strains in the hunt for novel antimicrobial agents from aquatic environments.

METHOD AND MATERIALS

The Ulu Langat catfish farmers provided the 10 kg of Clarias batrachus, or wooden catfish. Both the Pseudomonas aeruginosa (ATCC 27853) and Escherichia coli (ATCC 29522) utilized in this investigation were obtained from the Faculty of Health Sciences' Biomedical Science Laboratory. Mueller-Hinton broth was used to prepare culture media at a temperature of 37℃. Before the trial, this was adjusted by 0.5 McFarland turbidity standards.

Fish sampling and mucus collection

Fresh catfishes were first cleaned with sterile distilled water. After that, a total of 500 mL of sterile distilled water was added into a con-

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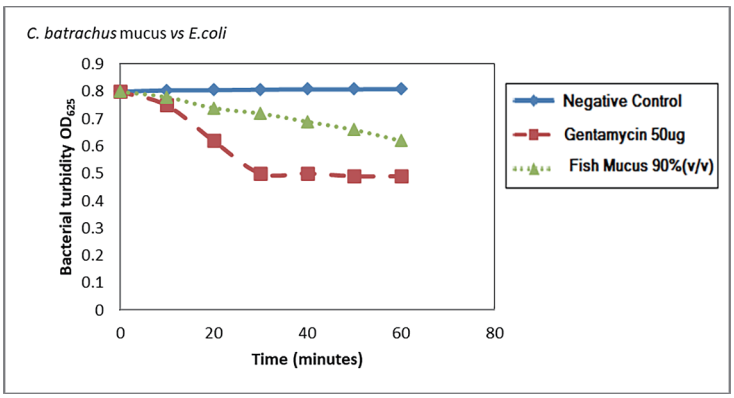

Figure 1: Effects of *C. batrachus* epidermal mucus extract against *E. coli* utilizing gentamycin as the active ingredient.

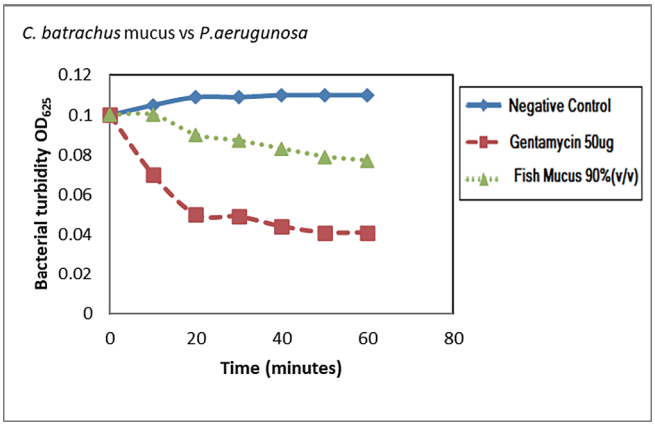

Figure 2: Effect of *C. batrachus* epidermal mucus extract against *P. aeruginosa* using Gentamycin as the positive control.

tainer filled with fish. The water in the container was swirled gently and the water was later removed and new clean distilled water was again added to the fish. This cleaning process was carried out three times to ensure the fishes were thoroughly cleaned. After the cleaning process, the clean fishes were left in water overnight in a cold room at 4°C.

The mucus was collected the following day by gently scraping a spatula across the surface of the fish after it had been cleaned with distilled water. Each fish had 5-10 mL of mucus collected during this operation. Centrifuging the gathered mucus at 4°C for 20 minutes at 10,000 rpm. After centrifugation, a sterilized membrane filter (0.45 m) was used to filter the mucus, which was then carefully collected with a sterile pipette. To prevent enzymatic degradation of the sample, the collected sterile mucus was promptly kept at -4°C[3].

Determination of antibacterial activity of Clarias batrachus epidermal mucus

A bacterial broth suspension was prepared and incubated for 3 to 4 h at 37°C to obtain bacterial growth in the early log growth phase. Then, the broth was centrifuged at a speed of 4000 rpm for 10 min using an eppendorf tube. By adding 10 mL of sterile 0.1 M phosphate buffered saline (PBS) solution, the bacterial pellet was supplied after the supernatant was withdrawn from the centrifuge tube. At a measurement of 0.5 OD625, the concentration of the resulting bacterial suspension was balanced.

The prepared bacterial solution is then divided into three parts, namely negative control, positive control and test. The positive control contains 40 µg of Gentamycin together with the test bacteria strains (*P. aeruginosa* ATCC 27853 and *E. coli* ATCC 29522), while for the negative control only the bacterial suspension was in the flask. The test tube containing *C. batrachus* mucus with the test bacteria (listed above) was placed in a water bath at 37°C. At the time intervals of 10, 20, 30, 40, 50 and 60 min, the sample tube was taken and tested it turbidity with a spectrophotometer at a wavelength of 625 nm. A graph of the bacteria turbidity versus time were plotted to observe changes in the growth of bacteria used above.

Table 1: the minimum inhibitory concentration (MIC) of the mucus extract from *C. batrachus* against *P. aeruginosa* and *E. coli*.

Bacteria	MIC (%)
E. coli	70
P. aeruginosa	60

Minimum Inhibitory Concentration (MIC) Test

The minimum inhibitory concentration (MIC) of the fish mucus extract in the presence or absence of PMBN were measured essentially by the broth micro-dilution method using Muller Hinton (MH) medium (Difco Lab. USA). A batch of wells containing MH broth with decrease percentage of concentrations from the *C. batracus* mucus extract was prepared (90% -50% v/v). The mixture was inoculated with test bacterium inoculums (103 cells/ml), and the microtiter plates were then incubated at 37°C overnight. Three copies of this process were completed. The MIC of fish mucus was defined as the lowest concentration at which the isolate was completely eliminated, as demonstrated by the absence of any discernible bacterial growth.

Scanning Electron Microscopy (SEM)

Preparation was done according to the method of[3]. A Glass slide was cleaned with alcohol solution (70%). Then, a tiny solution of test bacterial suspension (50 µL) was dropped on the glass slide and mixed with a dropped of the mucus solutions (90%). The mixture for the reaction was kept at room temperature. Subsequently, a portion of the mixture was pipetted (20 µL) and dropped on clean cover slips. A smear was made a left the mixture on the cover slips to dry. These glass cover slips are then immersed in a 10% saline formalin solution for two to four hours for bacterial fixation. Then it was transferred into an alcohol solution at several concentrations namely 90%, 70%, 50%, 30% and 10% each for several min. The final steps involved attaching the glass cover slips to a "stub" and coated with gold. It is then observed under an electron microscope.

RESULTS

The effect of C. batrachus epidermal mucus extract against *E. coli*

The antibacterial action of *C. batrachus* epidermal mucus extract on *E. coli* is depicted in Figure 1. From the results, the mucus from *E. coli* showed antibacterial action compared to the negative control from 20 to 80 min of the experiment. Also, at 20 min, *C. batrachus* mucus extracts shows similar activity with the gentamicin treated group.

Plate 1a. Control *E.coli*

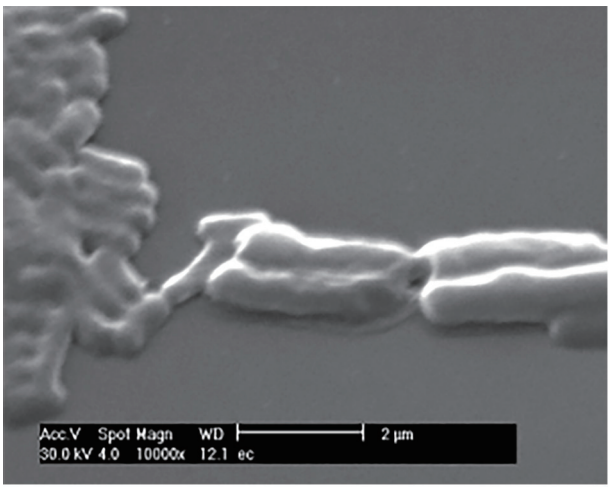

Plate 1b. Treated *E.coli* with mucus

Plate 2a. Control *Ps aeruginosa*

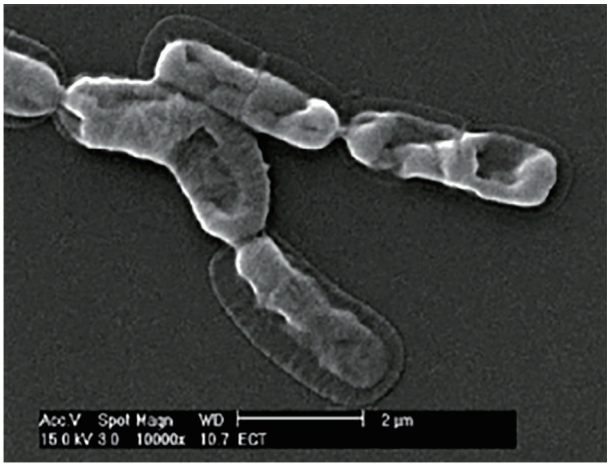

Plate 2b. Treated *Ps. aeruginosa* with mucus

Determination of the antibacterial activity of *C. batrachus* epidermal mucus extract against *P. aeruginosa*

The effect of mucus extracts of *C. batrachus* on *P. aerogenosa* is shown on Figure 2. The mucus extract of *C. batrachus* displayed antibacterial actions against *P. aeruginosa* when compared to the negative control. From the result, the mucus extract significantly decreased bacterial turbidity from 20 to 60 min of the experimental duration.

Scanning electron microscope (SEM)

Plate 1 shows the scanning electron microscope for the effect of *C. batrachus* epidermal mucus extract against *E. coli*. Plate 1a shows an active *E. coli*, which serve as a negative control whereas, *E. coli* treated with *C. batrachus* epidermal mucus extract are shown in Plate 1b. In this plate, the bacteria are less motile and not discrete.

The effect of *C. batrachus* epidermal mucus extract on *P. aeruginosa* is shown on Plate 2. The negative control shows active and mobile *P. aeruginosa*. The *P. aeruginosa* treatment with the mucus extract shows less active and immotile bacteria.

DISCUSSION

This study was undertaken to determine whether C. batrachus' epidermal mucus could be utilized as a source of antibacterial agent to treat bacterial illnesses. Mucus that has been extracted through the aqueous extraction method[14,15]. It has been documented that water-soluble components in *C. batrachus* mucus consist of bioactive substances such as peptides, proteins, glycoproteins, lysozymes and proteases[6]. In line with this, mucus from fishes were proposed to serve as promising avenue for new antimicrobial agents.

Similarly, the results from this study, the growth rate of *E. coli* and *P. aeruginosa* was decreased following treatment with mucus of C. batrachus compared to the negative control which is bacteria without mucus. The effect of the mucus reaction against this bacteria shows that there are bacterial cells that are destroyed or lysis occurs which results in the decreased growth of the bacteria observed in this study. The antibacterial action exhibited by the *C. batrachus* could be linked to the biological compounds that have documented to be present in the mucus extract. This includes compounds such as peptides, proteolytic enzymes, lectins, lysozymes, flavoenzymes[3,6,7] (Subramaniam *et al*. 2008; Dash *et al*., 2018; Tiralongo *et al*., 2020). The possible employed by the compounds found in the mucus extract from *C. batrachus* could be through cell membrane targeting, among other possible mechanisms. Additionally, the *E. coli* exposed to *C. batrachus* mucus showed higher MIC value of 70% compared to *P. aeroginosa* treated with the same extract. This is an indication the mucus displayed better antibacterial action against *P. aeroginosa* (60%) compared to *C. batrachus*. To further show the antibacterial action of *C. batrachus* mucus extract, SEM shows that both the *E. coli* and *P. aeroginosa* were less active and immotile when exposed to epidermal mucus extract of *C. batrachus*.

The findings of this study support the idea that mucus functions as a component of the body's immune system, with antibacterial peptide serving as its primary building block. As a result, these findings could pave the way for the creation of a new class of medication that would be a market-wide solution to antibiotic resistance.

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