Antimicrobial property of the epidermal mucus of Tilapia *Oreochromis* spp.

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ABSTRACT

This study was conducted to determine and compare the antimicrobial property of the epidermal mucus of Tilapia (*Oreochromis* spp.) from two environmental conditions, the fish tank and fishpond. The antimicrobial property was determined using Filter Paper Disc Diffusion Method with Amoxicillin and Nystatin as the positive controls and distilled water as the negative control. Results showed significant differences in the effects of the treatments when tested against *Escherichia coli, Staphylococcus aureus*, *Bacillus cereus, Bacillus subtilis, Bacillus megaterium, Pseudomonas aeruginosa, Candida albicans, Aspergillus niger* and *Aspergillus flavus* ($p<0.05$). The Duncan's Multiple Range Test further proved that the mucus of Tilapia from fishpond was significantly higher in terms of antibacterial property compared to mucus of Tilapia from fish tank. On the other hand, both of the epidermal mucus of Tilapia from fishpond and fish tank did not show any inhibitory effect against *P. aeruginosa* and *A. niger*. The epidermal mucus of Tilapia showed bacteriostatic, fungistatic and bactericidal effects against test microorganisms. Based on the results, the mucus of *Oreochromis* spp. from fishpond and fish tank are potential sources of antimicrobial compounds.

Keywords: Tilapia, *Oreochromis* spp., antimicrobial property, epidermal mucus, fish tank, fishpond

INTRODUCTION

Fish farming is the growing of fish in a controlled environment (concrete or earthen ponds), vats (wooden or fiber glass) and plastics (Nwokoye et al. 2007; Osawe 2007). Fish tanks allow the culturist to manage environmental parameters such as water temperature, dissolved oxygen concentration, pH and waste that can be adjusted to promote maximum production (DeLong et al. 2009). Since fish have limited access to natural foods in tanks, they must be fed a complete diet containing vitamins and minerals (Rakocy 2005). On the other hand, fish on ponds ranges from extensive systems using only organic or inorganic fertilizers, to intensive systems using high-protein feed, aeration (Rakocy and McGinty 1989) and water exchange rich in oxygen and some nutrients (Nandlal and Pickering

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2004). Studies show that a special set of water chemistry requirements is essential to a healthy, balanced, and functioning aquaculture system (DeLong et al. 2009). The growth and health of different fish species are also influenced by a different range of factors, among them water quality parameters (Makori et al. 2017). In the Philippines, Tilapia is the second most farmed fish species next to milkfish (Guerrero III 2019).

Tilapia *(Oreochromis* spp.) is a freshwater fish belonging to the Family Cichlidae. The group consists of three important genera, *Oreochromis, Sarotherodon* and *Tilapia* (Tower 2005). They are native to Africa, but were introduced into many tropical, subtropical and temperate regions of the world, including the Philippines, during the second half of the 20th century (Pillay 1990). Tilapia has a number of characteristics that make them attractive for tank. They prefer water temperatures between 29°C and 31°C and tolerate wide range of salinity. They can tolerate the crowding and handling that is required in a tank-based facility (DeLong et al. 2009) and also low dissolved oxygen and high ammonia concentrations better than most aquaculture species (Boyd 1990). They are more resistant to viral, bacterial and fungal diseases than other aquaculture species (World Seafood Market 2005) and can inhibit the spread of Vibrio and other pathogenic bacteria through the secretion of its mucus (Caipang et al. 2011; Wibowo et al. 2015).

The fish skin mucus is the slimy and slippery layer covering the epithelial surfaces which provides a stable physical or chemical barrier against the invading pathogens (Dash et al. 2018). Mucus is the material that makes fish slippery to touch. Its slipperiness is the result of its high water content and the presence of high-molecular-weight, gel-forming macromolecules (Shephard 1994). Several roles for this sticky layer have been suggested. This layer acts as a lubricant (Rosen and Cornford 1971) and has mechanical protective function (Cameron and Endean 1973) involved in osmoregulation and locomotion playing a possible immunological role (Fletcher 1978) and controls the intra-specific chemical communication (Saglio and Blance 1989). There is also a growing evidence that lectins from the skin mucus of fish have the ability to agglutinate, opsonize and/or suppress microbial growth (Suzuki et al. 2003; Dutta et al. 2005; Tsutsui et al. 2006, 2007; Argayosa et al. 2011). The antimicrobial activity of epidermal mucus extracts against a broad range of microbial pathogens was observed by Hellio et al. (2002). Many researchers have proven that the mucus substances are good defense which can inhibit the spread of *Vibrio* and other pathogenic bacteria (Caipang et al. 2011; Wibowo et al. 2015) but not in the difference on the effects of mucus from Tilapia grown in the fish tank and in the fishpond.

Thus, this present study aimed to evaluate the antimicrobial property of the epidermal mucus of Tilapia. Specifically, this study aimed (1) to determine the antimicrobial property of the epidermal mucus of Tilapia from

two environmental conditions namely concrete tanks and fishpond against *Escherichia coli* (Escherich, 1885), *Staphyloccocus aureus* (Ogston, 1880), *Bacillus subtilis* (Ehrenberg, 1835), *Bacillus cereus* (Ehrenberg, 1835), *Pseudomonas aeruginosa* (Schroeter, 1872), *Bacillus megaterium* (Ehrenberg, 1835), *Aspergillus flavus* (Link, 1809), *Aspergillus niger* (Tiegh, 1867), and *Candida albicans* (Berkhout, 1923); and (2) if antimicrobial property is present, determine which of the epidermal mucus from two environmental conditions would show the higher antimicrobial property.

METHODS

Sample Collection and Locale of the Study

This study was conducted in November 2016 at the Microbiology Laboratory of the Western Philippines University-Puerto Princesa Campus (WPU-PPC), Puerto Princesa City, Palawan, Philippines. The epidermal mucus was obtained from Tilapia grown in fish tanks of Aquatic Sciences Laboratory, WPU-PPC and in fish pond of Iwahig Penal Farm which are both locally located in Puerto Princesa City, Palawan. The mucus samples were freshly collected by scrapping at the dorsal part of the fish using a sterile spatula. For each treatment, four fish samples were collected with common sizes ranging from 15 to 20 cm in total length. Mucus from the four samples for each treatment was mixed together to make one replicate and were stored in a sterilized vial at 4°C. It was then placed in a cooler and immediately transported to the laboratory for analyses. Four biological replicates of epidermal mucus were prepared for each environmental condition. The freshly collected mucus were assayed directly for antimicrobial property.

Culture Media Preparation

Culture media (Nutrient agar, Nutrient broth and Potato Dextrose agar) preparation was done following the protocol provided by the manufacturer (HIMEDIA).

Preparation of Microorganisms

The test microbes were the opportunistic *E. coli, B. cereus, P. aeruginosa, A. flavus* and *C. albicans*; disease causing bacteria *S. aureus* and *A. niger;* and non-disease causing bacteria *B. subtilis* and *B. megaterium*. The test microorganisms were purchased from stock cultures of the Mindanao State University, Marawi City. These cultures were used as representatives for the four groups of microorganisms such as Gram-negative bacteria, Gram-positive bacteria, molds and yeast. A 10 ml of the previously prepared nutrient broth was poured into test tubes with cotton plugs for sterilization at 121°C,

15 psi for 15 minutes. After sterilization, the culture medium was allowed to cool down. A loopful (0.01 ml) of each test bacterium from the selected microbes was then inoculated aseptically on the pre-labeled broth medium and covered with cotton plugs. The microbial subcultures were placed in the incubator for 24 hours at 37°C.

Antimicrobial Assay

The filter paper disc diffusion method was used to determine the antimicrobial property of the epidermal mucus of Tilapia. For the positive controls, a 500 mg Amoxicillin, which was dissolved in 10 ml sterile distilled water for the bacteria, and Nystatin (100, 000 units/ml) for fungi were used. On the other hand, 10 ml sterile distilled water was used as the negative control. The treatment designations were the following: T1 (positive control), T2 (negative control), T3 (Tilapia mucus from fish tank) and T4 (Tilapia mucus from fishpond). A loopful (0.01 ml) of test organisms, *E. coli, S. aureus*, *B. cereus, B. subtilis, B. megaterium, P. aeruginosa, A. niger, A. flavus* and *C. albicans* from the subcultures were inoculated into the sterile nutrient agar (20 ml) by direct seeding before pouring it into Petri dishes and allowed to solidify. The previously sterilized filter paper discs (cut by paper puncher to 6 mm diameter) were soaked to the freshly collected mucus samples (from fishpond and fish tank) and in the positive and negative control using sterile forceps. These impregnated discs were placed on the designated areas (4 discs on 1 plate) (Figure 1). Four replicates for each treatment were prepared. The Petri plates were incubated for 24 hours to allow microbial growth. After 24 hours, the plates were examined and zones of inhibition were measured using standardized transparent ruler (in mm).

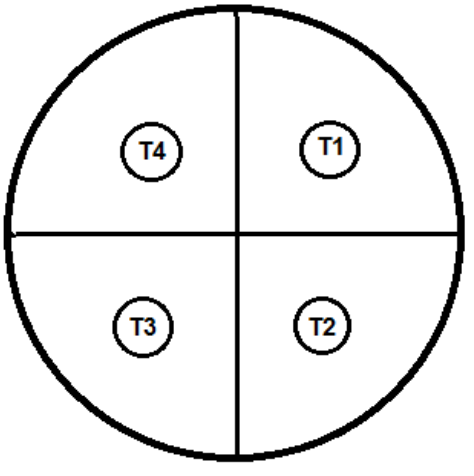

Figure 1. Filter Paper Disc Diffusion Test

Statistical Analyses

The data on the zones of inhibition of the treatments against *E. coli, S. aureus*, *B. cereus, B. subtilis, B. megaterium, P. aeruginosa, A. niger, A. flavus* and *C. albicans* were analyzed using one-way analysis of variance (ANOVA) to test the significant differences. The data were subjected to Duncan's Multiple Range Test (DMRT) to compare the means using IBM SPSS Statistics 20 software.

Diameter of Zones of Inhibition Interpretative Standard for Test Microorganisms

The zones of inhibition were interpreted using the Laboratory Manual of Standardized Methods for Antimicrobial Sensitivity Tests (Tendencia 2004; Table 1).

Table 1. Zones of Inhibition Interpretative Standard for Test Microorganisms.

Test Microorganisms	Interpretative Criteria		
	Sensitive	Intermediate	Resistant
Escherichia coli	≥18	14-17	≤13
Staphyloccocus aureus	20	-	19
Bacillus subtilis	≥18	14-17	≤13
Bacillus cereus	≥18	14-17	≤13
Bacillus megaterium	≥18	14-17	≤13
Pseudomonas aeruginosa	≥18	14-17	≤13
Candida albicans	≥18	14-17	≤13
Aspergillus niger	≥15	10-14	≤10
Aspergillus flavus	≥15	10-14	≤10

RESULTS

The epidermal mucus of Tilapia from fishpond showed higher inhibitory effect against *S. aureus*, *B. subtilis, B. cereus* and *C. albicans* compared to the effect of epidermal mucus of Tilapia from fish tank. It also showed inhibitory effect against *E. coli, B. megaterium* and *A. flavus* while the epidermal mucus of Tilapia from fish tank did not. Both of the epidermal mucus didn't show any zone of inhibition when tested against *P. aeruginosa* and *A. niger*. Table 2 shows the mean zones of inhibition of the treatments against the nine test microorganisms which were subjected to ANOVA and Post Hoc test (Duncan's test).

Table 2. Inhibitory effects of the treatments against Test Microorganisms. ** - highly significant at α= 0.01; * - significant at α= 0.05; Different letters signify significant difference at α=0.05.

Test Microorganisms/Treatment	Mean ± sd (mm) zones of inhibition	DMRT*	F-value	P-value
Escherichia coli				
Positive control	37.5±2.68	A	33.96**	0.000
Negative control	0	C		
Mucus of Tilapia from fish tank	0	C		
Mucus of Tilapia from fishpond	11.5±2.68	B		
Staphyloccocus aureus				
Positive control	15±1.19	A	52.82**	0.000
Negative control	0	C		
Mucus of Tilapia from fish tank	8.375±1.19	B		
Mucus of Tilapia from fishpond	8.75±1.19	B		
Bacillus subtilis				
Positive control	40±1.60	A	153.54**	0.000
Negative control	0	D		
Mucus of Tilapia from fish tank	9.25±1.60	C		
Mucus of Tilapia from fishpond	15.25±1.60	B		
Bacillus cereus				
Positive control	41.25±2.68	A	57.87**	0.000
Negative control	0	D		
Mucus of Tilapia from fish tank	12.75±2.68	C		
Mucus of Tilapia from fishpond	20±2.68	B		
Bacillus megaterium				
Positive control	20±0.88	A	226.68**	0.000
Negative control	0	C		
Mucus of Tilapia from fish tank	0	C		
Mucus of Tilapia from fishpond	8.5±0.88	B		
Pseudomonas aeruginosa				
Positive control	8.5±0.35	A	289.00**	0.000
Negative control	0	B		
Mucus of Tilapia from fish tank	0	B		
Mucus of Tilapia from fishpond	0	B		
Candida albicans				
Positive control	19.5±1.70	A	15.02**	0.000
Negative control	0	C		
Mucus of Tilapia from fish tank	13.6±1.70	B		
Mucus of Tilapia from fishpond	13.75±1.70	B		
Aspergillus niger				
Positive control	14.5±0.35	A	841.00**	0.000

Test Microorganisms/Treatment	Mean ± sd (mm) zones of inhibition	DMRT*	F-value	P-value
Negative control	0	B		
Mucus of Tilapia from fish tank	0	B		
Mucus of Tilapia from fishpond	0	B		
Aspergillus flavus				
Positive control	20±0.72	A		
Negative control	0	C	335.74**	0.000
Mucus of Tilapia from fish tank	0	C		
Mucus of Tilapia from fishpond	7.75±0.72	B		

Effects of Treatments against *E. coli*

In this study, epidermal mucus of Tilapia from fishpond showed zones of inhibition when tested against *E. coli* while the epidermal mucus from fish tank did not (Figure 2; ECR4). ANOVA proved that there were significant differences in the effects of the treatments when tested against *E. coli* while DMRT showed that the antimicrobial property of epidermal mucus from fishpond differ significantly compared to other treatments although not comparable to positive control when tested against *E. coli.*

Effects of Treatments against *S. aureus*

The epidermal mucus of Tilapia from fish tank and fishpond showed zones of inhibition when tested against *S. aureus* (Figure 2; SAR1). The ANOVA showed that there were significant differences in the effect of the treatments when tested against *S. aureus*. DMRT proved that the epidermal mucus from fishpond and fish tank are both comparable to each other but not to positive control when tested against *S. aureus* (Table 2).

Effects of Treatments against *B. subtilis*

The epidermal mucus of Tilapia from fishpond and fish tank both showed zones of inhibition against *B. subtilis* (Figure 2; BSR2). Between the two treatments that showed inhibitory effects, the epidermal mucus of Tilapia from fishpond showed the higher zones of inhibition. The ANOVA showed that there were significant differences in the effects of the treatments when tested against *B. subtilis*. DMRT further proved that epidermal mucus form fishpond was significantly higher in terms of antibacterial property but not comparable to positive control (Table 2).

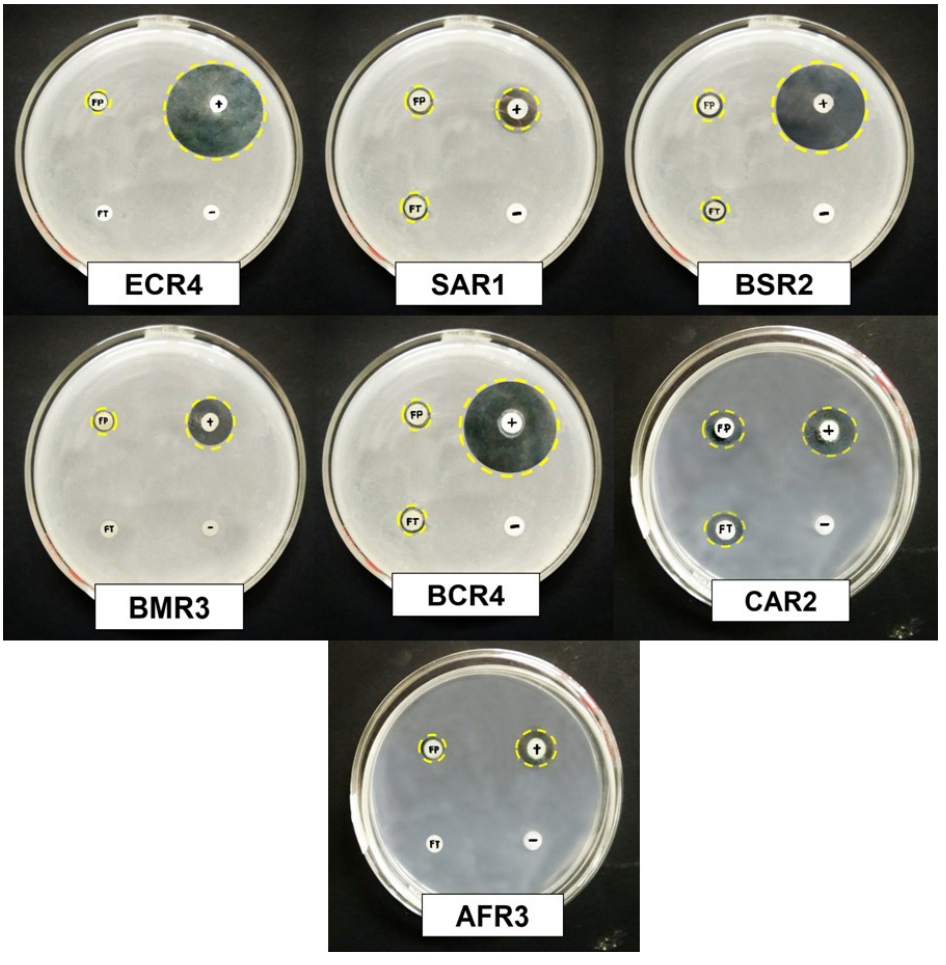

Figure 2. A representative replicate of epidermal mucus showing zones of inhibition against test microorganisms: *Escherichia coli* (ECR4), *Staphyloccocus aureus* (SAR1), *Bacillus subtilis* (BSR2), *Bacillus megaterium* (BMR3), *Bacillus cereus* (BCR4), *Candida albicans* (CAR2) and *Aspergillus flavus* (AFR3). FP stands for mucus of tilapia from fishpond and FT stands for mucus of tilapia from fish tank. Circles highlight the zones of inhibition.

Effects of Treatments against *B. cereus*

Both of the epidermal mucus from fish tank and fishpond showed zones of inhibition when tested against *B. cereus* (Figure 2; BCR4). The ANOVA showed that there were significant differences in the effects of the treatments against *B. cereus*. Duncan's test proved that the epidermal mucus of Tilapia from fishpond was significantly higher compared to epidermal mucus of Tilapia from fish tank but not comparable to positive control when tested against *B. cereus* (Table 2).

Effects of Treatments against *B. megaterium*

In this study, the epidermal mucus of Tilapia from fishpond showed zones of inhibition when tested against *B. megaterium* while the epidermal mucus of Tilapia from fish tank did not (Figure 2; BMR3). The ANOVA showed that there were significant differences in the treatments against *B. cereus*. DMRT proved that the epidermal mucus of Tilapia from fishpond is significantly higher from epidermal mucus from fish tank but not comparable to positive control when tested against *B. megaterium* (Table 2).

Effects of Treatments against *P. aeruginosa*

The epidermal mucus of Tilapia from fishpond and fish tank did not show any zone of inhibition against *P. aeruginosa* (Table 2).

Effects of Treatments against *C. albicans*

In this study, both of the epidermal mucus of Tilapia from fishpond and fish tank showed zones of inhibition against *C. albicans* (Figure 2; CAR2). The ANOVA showed that there were significant differences in the treatments against *C. albicans*. DMRT proved that the epidermal mucus of Tilapia from fishpond and the epidermal mucus of Tilapia from fish tank were not significantly different from each other and not comparable to positive control when tested against *C. albicans* (Table 2).

Effects of Treatments against *A. niger*

Both the epidermal mucus of Tilapia from fishpond and fish tank did not show any inhibitory effect towards *A. niger* (Table 2).

Effects of Treatments against *A. flavus*

The epidermal mucus of Tilapia from fishpond showed zones of inhibition when tested against *A. flavus* while epidermal mucus of Tilapia from fish tank did not (Figure 2; AFR3). ANOVA proved that there were significant differences in the effects of the treatments when tested against *A. flavus*. DMRT proved that the antifungal property of epidermal mucus from fishpond is not comparable to positive control when tested against *A. flavus* (Table 2).

Table 3 shows the average zones of inhibitions by the treatments where it shows that *P. aeruginosa* and *A. niger* were the most resistant test microorganisms while *B. subtilis* and *B. cereus* are the most susceptible.

Table 3. Average zones of inhibitions by the treatments towards the test microorganisms and their interpretations.

Test Microorganisms	Environmental Conditions	Mean ±sd (mm) of Zone of Inhibitions	Interpretation
Escherichia coli	Fishpond	11.5±2.68	Resistant
	Fish tank	0	Resistant
Staphyloccocus aureus	Fishpond	8.75±1.19	Resistant
	Fish tank	8.375±1.19	Resistant
Bacillus subtilis	Fishpond	15.25±1.60	Intermediate
	Fish tank	9.25±1.60	Resistant
Bacillus cereus	Fishpond	20±2.68	Sensitive
	Fish tank	12.75±2.68	Resistant
Bacillus megaterium	Fishpond	8.5±0.88	Resistant
	Fish tank	0	Resistant
Pseudomonas aeruginosa	Fishpond	0	Resistant
	Fish tank	0	Resistant
Candida albicans	Fishpond	13.75±1.70	Intermediate
	Fish tank	13.6±1.70	Intermediate
Aspergillus niger	Fishpond	0	Resistant
	Fish tank	0	Resistant
Aspergillus flavus	Fishpond	7.75±0.72	Resistant
	Fish tank	0	Resistant

DISCUSSION

The epidermal mucus of Tilapia collected from two environmental conditions, the fishpond and fish tank showed antimicrobial property. Fishes, like Tilapia, excrete mucus on their epidermal and epithelial cells (Pickering 1974; Ellis 1999) that acts as a lubricant (Rosen and Cornford 1971) and has its mechanical protective function (Cameron and Endean 1973) which involved in osmoregulation and locomotion, playing a possible immunological role (Fletcher 1978) that controls their intra-specific chemical communication (Saglio and Blance 1989). Mucus layer is a biological interface between fish and their aqueous environment that consists of biochemical diverse secretions. Over the past years, it has also been shown that mucus plays a pivotal role in the prevention of colonization by parasites, bacteria and fungi (Bragadeeswaran 2011) which this study has also proven.

Rao et al. (2015) found that Tilapia mucus has high protein content and acidic extract compared to other freshwater fish species. The acidic mucus extracts of Tilapia also showed potent bactericidal activity against a wide range of fish and human pathogens (Subramanian et al. 2008). One inherent property of tilapia such as the antimicrobial components in the acidic mucus

extracts of tilapia mucus are believed to have a key role in host defense against pathogenic infection (Rao et al. 2015) such as *Vibrio harveyii* (Forlenza et al. 2008) in the aqueous environment. Aside from the mucus, the skin also expresses genes that may enhance immune system including antimicrobial peptides, cytokines, complements, major histocompatibility complex (MHC) and immunoglobulins. These genes that are located in the skin produce substances which are then released to the surface and integrate with the mucus, thereby enhancing the first line of defense in fish against pathogens (Gonzalez et al. 2007).

The functional property of the mucus depends on its capacity to form gel on the epithelial surface (Bragadeeswaran 2011). This mucus is secreted by the epidermal goblet cells, composed mainly of water and gel forming macromolecules such as mucins and other glycoproteins (Martinez et al. 2006). In addition, fish mucus also contains a variety of biologically active substances such as lysozyme, lectins, flavoenzymes and immunoglobulin. It was reported that the epithelial tissues produce antimicrobial molecules which serve as the first line of a host's defense against microbial invasion in a variety of vertebrates (Villarroel et al. 2007).

In this study, the epidermal mucus of Tilapia obtained from fishpond has higher antimicrobial effect compared to epidermal mucus of Tilapia from fish tank. The two environmental conditions have differences in their environmental parameters (e.g., water temperature, dissolved oxygen concentration, pH) suggesting that there is a variation in type and level of innate immune factors in mucus between species inhabiting different ecological niches (Jensen 2015). Ponds have a wide variety of microbial life. Nutrients are brought to the pond by streams that feed into, run off during rain, or by the human anthropogenic activities (Ehiagbonare and Ogunrinde 2010). The water in soil, animal waste and decaying plant matter in the pond are broken down and used to fuel the pond ecosystem. Many animals that live in the surrounding area, migrating birds and nearby plants depends on these ponds for a rich source of nutrient and water (Ehiagbonare and Ogunrinde 2010). The presence of the following bacterial genera *Aeromonas, Klebsiella, Micrococcus, Alcaligenes, Vibrio, Flavobacterium*, *Bacillus, Pseudomonas* and *Coryneforms* and fungal species *Mucor, Aspergillus, Microsporum, Trichophyton* and *Chrysosporum* were more prevalent in pond water (Okaeme and Olufemi 1997). Some of these microbes have been implicated as the major causative organisms of known diseases of fish (Nahiduzzaman et al. 2000; Sarkar and Rashid 2012) and could also trigger the fish pond fishes to elicit protective mechanisms such as antimicrobial compounds in mucus which is more potent. On the other hand, using fish tanks allows the fish culturist to manage stocks and have a good deal of control over environmental parameters that can be adjusted to promote maximum production (DeLong et al. 2009). Since Tilapia has limited access to natural foods in tanks, they must

be fed a complete diet containing vitamins and minerals (Rakocy and McGinty 1989), though in this study, tilapia were only fed with floating pellets. In small tanks, it is practical and economical to treat diseases with therapeutants applied to the culture water (DeLong et al. 2009) suggesting that Tilapia may have depended their defense mechanism on this. Unlike on fishpond, fish are fed with rice bran, flour, peanut cake or pellet (Sarker et al. 2011).

Diets with functional ingredients are becoming a part of the preventive health strategy in fish farms (Bricknell and Dalmo 2005; Covello 2012). The strengthening of the skin and mucus layer through dietary modulation could play a role in preventing damage, parasite attachment, promote faster recovery of damaged skin (Jensen 2015) and could be source of nutrients and substrate for growth by certain bacteria (Shoemaker and LaFrentz 2015). In addition, the composition and rate of mucus secretion has been observed to change in response to microbial exposure or to environmental perturbation such as hyperosmolarity and acidity (Ellis 1999). The structure of fish skin is highly adapted to the aqueous environment (Jensen 2015). There is also evidence that the mucus composition varies with season, smoltification, salinity, stress, disease, parasite attack (Schrock et al. 2001; Mustafa et al. 2005; Roberts and Powell 2005; Easy and Ross 2009, 2010; Lü et al. 2012; Guardiola et al. 2014) and environmental conditions (Blackstock and Pickering 1982). Living in a pathogen-rich environment makes fish vulnerable to infections, and therefore reliant on a potent first defense line (Jensen 2015).

Differences in activities of antimicrobial enzymes, such as lysozyme and proteases, and how they relate to the structure and composition of mucus and epidermal layers, may also relate to the differences observed in disease resistance (Mozumder 2005) which may explain the observation why the epidermal mucus of Tilapia from fishpond smelled more fishy compared to the epidermal mucus of Tilapia from fish tank. The mucus holds some proteases (serine protease, cysteineprotease, metalloprotease and trypsin like protease) having strong antibacterial activity (Fast et al. 2002) such as in Tilapia (Sriket 2014).

Rao et. al (2015) compared the bactericidal activity of Tilapia to other fishes and it was found out that Tilapia and bagrid catfish have showed a broad spectrum of bactericidal activity against *E. coli*. The acidic mucus extract of tilapia and bagrid catfish were found to inhibit most of the human pathogens such as *E. coli*, *Streptococcus entericaserovar typhimirium*, *S. entericaserovar enteritidis*, *Klebsiella pneumoniae, P. aeruginosa, Methicillin-resistant S. aureus* (MRSA), *Micrococcus luteus, B. subtilis* and *Aeromonas hydrophila*. Similarly, the acidic mucus extracts of brook trout, haddock and hagfish showed bactericidal activity against a wide range of fish and human pathogens (Subramanian et al. 2008). This suggests that antimicrobial components in the acidic mucus extracts may have a key role in

host defense against pathogenic infection in the aqueous environment. Previous studies have shown a variety of antimicrobial proteins such as (paradaxinand pleurocidin) from fish mucus that is potentially involved in the protective function against invading pathogens (Cole et al. 1997).

On the other hand, this study found that the epidermal mucus of Tilapia from fishpond showed antimicrobial effects against most of test microorganisms except when tested against *P. aeruginosa* and *A. niger* for both exhibit cytotoxicity (Rivera et al. 2014) while epidermal mucus of Tilapia from fish tank showed only antimicrobial property when tested against *S. aureus*, *B. cereus, B. subtilis,* and *C. albicans*. The epidermal mucus of Tilapia from fishpond showed the higher antimicrobial property against *S. aureus*, *B. cereus, B. subtilis*, *C. albicans, E. coli, B. megaterium* and *A. flavus*. Gram-negative bacteria have an effective permeability barrier, comprised of a thin lipopolysaccharide exterior membrane, which could restrict the penetration of the epidermal mucus while Gram positive bacteria have a mesh-like peptidoglycan layer which is more accessible to permeation of epidermal mucus (Zgurska et al. 2015).

The present work supports the view that fish mucus could be a source of antimicrobial agent for animal pathogens. Further purification of the bioactive compounds is necessary in order to identify their chemical nature and to evaluate their potential as novel drug. Similar study can be done using the same or different species including other environmental conditions such as natural lakes.

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