ROLE OF MUCUS IN PROTECTION OF SOME BACTERIAL FISH DISEASE

Eissa, I.A.M.; Mona Abd El-Wahab; Derwa, H. and Bakry, M.*
Dept. of Fish Diseases & Management, Fac. of Vet. Med., Suez Canal University
**Animal Health Research Institute, Ismailia branch.*

Abstract

A total number of 50 fish were represented as 25 wild African catfish (Clarias gariepinus) each about 300g and 25 of cultured Nile tilapia (Oreochromis niloticus) each about 250g, alive and apparently healthy were collected randomly. The cutaneous mucus of these fishes were collected each in test tube with cover. A buffer solvent was added to the mucus and then centrifuged in cooling centrifuge then take the supernatant to determine the different protein concentrations and detect the antimicrobial activity. The total protein, albumin and globulin of C.gariepinus were 1.25, 0.4 and 0.86 g/dl respectively, while in O.niloticus were 2.3, 1.08 and 1.26 g/dl respectively and A/G ratio was 0.8 in C.gariepinus while in tilapia was 0.75. The antimicrobial activity was tested against (Aeromonas hydrophila, Pseudomonas fluorescence and Staphylococcus aureus). The C.gariepinus mucus was very effective at the first 6hrs and its action prolonged for 12hr and the activity was nearly equal against the three strains. The O.niloticus mucus was not effective in the first 6 hours. After the first 6hrs its action become so potent in inhibition of bacterial growth for the all strains.

Introduction

Aquaculture has an important role in the development and meeting the increase demand of animal protein.

A great deal of research supports the notion that layers of mucus accumulate on the skin and gills of fish that are stressed by diseases, adverse environmental conditions and handling (Lebedeva et al 2002,Palaksha et al 2008).

The antibacterial activity in mucus has been demonstrated in several fish species and the activity can be specific towards certain bacteria *(Velayudham et al. 2010)*.

The present study was planned to compare the protein concentrations in fish mucus of wild African catfish (Clarias gariepinus) and cultured Tilapia (Oreochromis niloticus). besides, testing the antibacterial activity in external mucus of both species against some bacterial fish pathogens.

Materials And Methods

Fish:

A total number of 50 fishes were represented as 25 cultured Nile tilapia (O.niloticus) and 25 wild African catfish (C. gariepinus) were randomly collected alive and apparently healthy in summer season. Both fish species were kept in large tanks filled with their natural water supplied with aerators. They were transported immediately to the wet laboratory, Animal Health Research Institute, Ismailia.

Collection and preparation of mucus samples:

The catfish (C.gariepinus) were placed with the ventral side of the body facing downward and cutaneous mucus from the dorsal side of the fish was collected and transferred to a plastic tube with cover. The tilapia were de-scalled after killing, then cutaneous mucus was collected from the dorsal side of the fish by a strile blunt end of scalpel and transferred to a plastic tube with cover. *According to Mozumder (2005)* with modification, 50 mM NaAc, pH 6.0 (v\v) was added to each mucus sample. Samples were centrifuged in cooling centrifuge at 4°C for 15 minutes at 14,000 rpm and the supernatants were transferred to new tubes. The pellet was resuspended again with 50 mM NaAc buffer, pH 6.0 (v\v) and centrifuged again at 14,000 rpm for 15 minutes. The supernatants were transferred to the first supernatant and then the prepared samples were stored at –73°C until analyzed.

Measurement of protein concentrations in fish mucus

Total protein measurement

10 fish of each species were examined for total mucus proteins according to Stoscheck (1990) using available kits from Pierce (Rockford, Ill.).

Determination of mucus albumin

Mucus albumin was determined according to Reinohold (1953) using commercially available kits of chemoroy

Calculation of globulin

Globulins were calculated by subtract the total mucus albumin from total mucus proteins.

Bacterial refreshment

Well identified bacterial isolates (Aeromonas hydrophila, Pseudomonas fluorescence and Staphylococcus aureus) were kindly supplied from Dept. of Fish Diseases and Management, Fac. of Vet. Medicine, Suez Canal University. One bacterial colony from each bacterial isolates on the Nutrient plate was transferred to 5 ml nutrient broth under complete aseptic condition (Tryptocase Soya broth added for Aeromonas hydrophila) and then shake over night at room temperature. The next morning 20 µl of that solution was transferred again into 5 ml nutrient broth and shake for 2 hours. then using McFarland technique make solution contain 40.000 cell/ml. These suspensions were used in the antibacterial testing.

Antibacterial activity testing

15 fish of each species were examined. The antibacterial test was performed in test tubes designed in 3 raw each contained 11 test tube. 500µl of nutrient broth containing the bacteria added to each tube i.e. each bacterial strain in single raw. The examined 15 fish were divided into 3 groups each contained 5 fish and each group examined against single bacterial strain. 500µl of the prepared sample was added to the different strains except the negative control, 500µl distilled water to each bacterial strain. According to Mozumder (2005) with modification, bacterial growth was assayed by Easy Bioscreen Experiment Programme (for 48 hours). Antibacterial activity was determined by reading the optical density of spectrophotometer at wave length 578 nm. The optical density was measured for each tube each 6hrs and curves for

bacterial growth were drawn for each sample.

Results

1-Mucus protein concentrations in Oreochromis niloticus:-

The maximum total mucus proteins of the 10 fish mucus was observed as 3g/dl while the lowest was 1.8g/dl and the mean was 2.3g/dl. The maximum albumin concentration was 1.2g/dl while the lowest was 0.9g/dl and the mean was 1.08g/dl. The maximum globulin concentration was 1.8g/dl in the lowest was 0.6g/dl and the mean was 1.26g/dl. The maximum A/G ratio was 2 and in the lowest was 0.6 and the mean was 0.75.

2-Mucus protein concentrations in Clarias gariepinus:-

The maximum total protein observed was 1.8g/dl while the lowest was 0.6g/dl and the mean was 1.25g/dl. The whole 10 examined fish was containing equal albumin concentration 0.4g/dl. The maximum globulin concentration was 1.4g/dl while the lowest was 0.2g/dl and the mean was 0.86g/dl. The maximum A/G ratio observed was 2 while the lowest was 0.3 and the mean was 0.8.

3-Antibacterial activity

The growth of bacteria in the nutrient broth cause increasing the turbidity in the solution which could be measured in term of optical density (OD) using the spectrophotometer. As the growth of bacteria increased the (OD) would increase. Lowering of the (OD) reading of the bacteria with the mucus sample than the bacteria alone indicating antibacterial activity of mucus. In tilapia mucus, due to high initial (OD) reading which give false result. We draw a third curve represented as (t*) by subtracting the initial reading from each following reading.

In Nile tilapia mucus with A. hydrophila and P. fluorescence the initial reading was (0) in the 0hr then the reading of bacteria with mucus become higher than bacteria alone after the first 6hr. then the difference decline at 12hr. then the reading of bacteria with mucus become lower than bacteria alone. While in S. aureus with mucus of tilapia there were a decrease in reading after the first 6hr. In African catfish mucus the reading of bacteria with mucus become higher than bacteria alone in 0hr. then the difference decline at 6hr. then the reading of bacteria with mucus become lower than bacteria alone at 12hr. then the reading of bacteria with mucus become higher than bacteria alone after 18hr.

Discussion

The present work showed that the total mucus protein concentrations of O.niloticus were ranged from 1.8 to 3g/dl. These findings are nearly similar with the results obtained by *Mai et al. (2008)*. while the total mucus protein concentrations of catfish (C.gariepinus) were ranged from 0.6 to1.8 g/dl. These results are nearly similar with that given by *Manivasagan et al.(2009)*. From the above mentioned results, it was established that O.niloticus mucus samples contained more total protein concentrations than C.gariepinus mucus samples. The total mucus protein in Bluegill (Lepomis macrochirus), Carp (Cyprinus carpio) and discus fish (Symphysodon spp.) were estimated 1.8mg/ml, 0.5g/dl and 0.63mg/ml respectively reviewed by *(Hashimzue et al.2005, Lebedeva et al.2002 and Chong et al.2005)*. The albumin contents in O.niloticus mucus were ranged from 0.9 to 1.2g/dl while the globulin was 0.6-1.5g/dl. while the albumin contents in the mucus of catfish was 0.4g/dl and the globulin contents was 0.2-1.4g/dl.

To the best of my knowledge, there no records involving the concentrations of albumin or globulin in mucus genus of fish.

The present study revealed that the mucus samples of both fishes inhibited the growth of bacteria either Gram +ve bacteria or Gram –ve bacteria. The catfish(C.gariepinus) mucus was very effective during the first 6hrs and its action has prolonged for 12 and up to 24hrs the activity was nearly equal against the three strains.

Staphylococcus aureus growth decreased by the mucus samples of catfish No.1,2,3,4. While mucus of catfish No.5 have shown very week antimicrobial action. The action of mucus of catfish No.2 was clear in the first 6hrs then become weak till 24hrs. The growth of Aeromonas hydrophila and Pseudomonas fluorescence decreased by the action of mucus of catfish No. 1 and 4, but the action of other mucus samples was not clear. Moreover, the action of catfish No. 1 appeared during the first 6hrs only.

The O.niloticus mucus was not effective in the first 6 hrs Furthermore, it may be considered to be helpful for bacterial growth. After the first 6hours its action become so potent in inhibition of bacterial growth for the all strains. The action of tilapia mucus on Staphylococcus aureus seems to be bactericidal effect due to the sharp fall in its growth curve, while on the other strains it only inhibited its growth (bacteriostatic).

The antibacterial activity of fish mucus and other aquatic organisms were detected by several authors.

Bergsson et al. (2005) found an acidic extract from the epidermal mucus of the Atlantic cod (Gadus morhua) exhibit antimicrobial activity against Bacillus megaterium, Escherichia coli and Candida albicans No lysozyme activity was detected in the extract . Moreover, in a study for *Subramanian et al.(2008)* the epidermal mucus samples from Arctic char (Salvelinus alpinus), brook trout (S. fontinalis), koi carp (Cyprinus carpio sub sp. koi), striped bass (Morone saxatilis), haddock (Melanogrammus aeglefinus) and hagfish (Myxine glutinosa) were extracted. The acidic mucus extracts of brook trout, haddock and hagfish exhibited bactericidal activity. The organic mucus extracts of brook trout, striped bass and koi carp showed bacteriostatic activity. There was no activity in the aqueous mucus extracts.

A bioactive protein found in the mucus layer of the flounder (Platichthys stellatus), which showed antibacterial activity against Staphylococcus epidermidis, Staphylococcus aureus and methicillin-resistant S. aureus *(Kasai 2010)*. Furthermore, *Tvete and Haugan (2008)* found that plaice (Pleuronectes platessa) epidermal mucus contained at least three antibacterial proteins in addition to lysozyme.

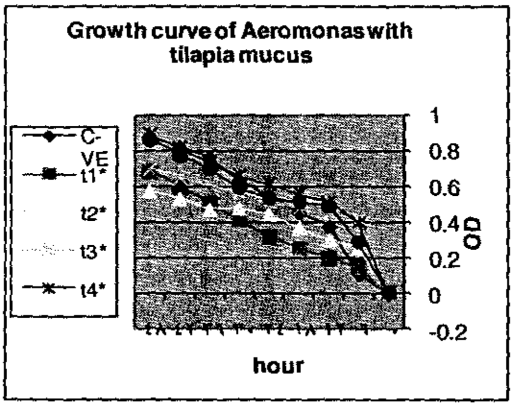
Table (1) showing protein concentrations in O. niloticus mucus samples

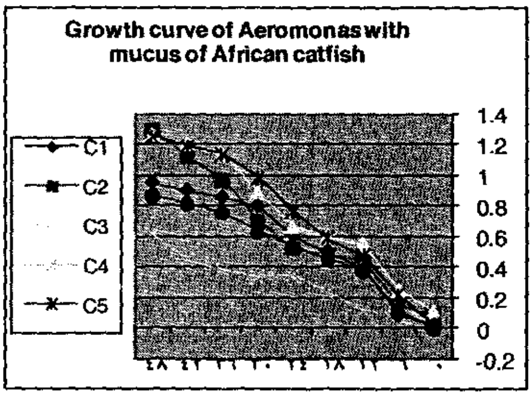
Group NO	Total protein g/dl	Albumin g/dl	Globulins g/dl	A\G Ratio
Minimum	1.8	0.9	0.6	0.6
Maximum	3.0	1.2	1.8	2.0
Mean ±S.E	2.3±0.05	1.08±0.03	1.26±0.1	0.75±0.05

Table (2) showing protein concentrations in mucus samples of

SAMPLE NO	Total protein g/dl	Albumin g/dl	Globulin g/dl	A\G Ratio
Minimum	0.6	0.4	0.2	0.3
Maximum	1.8	0.4	1.4	2.0
Mean ±S.E	1.25±0.08	0.4	0.86±0.05	0.8±0.04

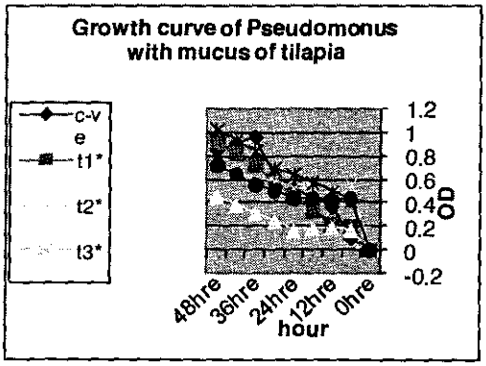
C. gariepinus:-

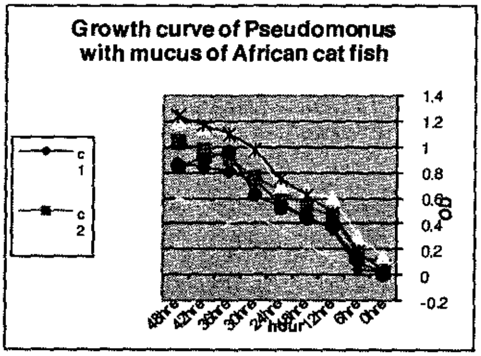




T1=growth curve of bacteria with tilapia mucus no. 1
T2=growth curve of bacteria with tilapia mucus no. 2
T3=growth curve of bacteria with tilapia mucus no. 3
T4=growth curve of bacteria with of tilapia mucus no. 4

T5=growth curve of bacteria with tilapia mucus no. 5
C1=growth curve of bacteria with catfish mucus no. 1
C2=growth curve bacteria with catfish mucus no. 2
C3=growth curve bacteria with catfish mucus no. 3
C4=growth curve of bacteria with of catfish mucus no. 4
C5=growth curve of bacteria with mucus catfish no. 5
C-ve= growth curve of bacteria alone





T1=growth curve of bacteria with tilapia mucus no1
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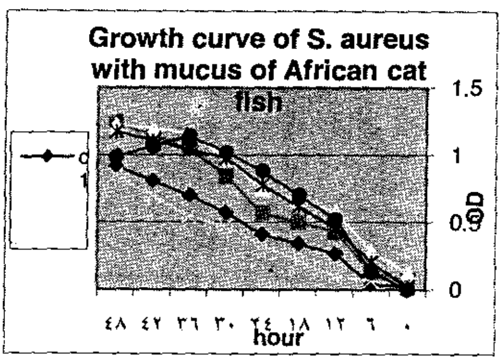
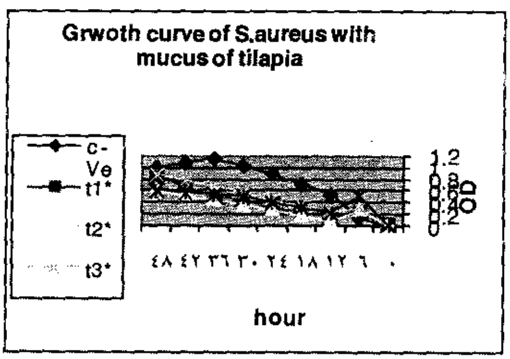
T3=growth curve of bacteria with tilapia mucus no. 3
T4=growth curve of bacteria with of tilapia mucus no. 4
T5=growth curve of bacteria with tilapia mucus no. 5
C1=growth curve of bacteria with catfish mucus no. 1
C2=growth curve bacteria with catfish mucus no. 2
C3=growth curve bacteria with catfish mucus no. 3
C4=growth curve of bacteria with of catfish mucus no. 4
C5=growth curve of bacteria with mucus catfish no. 5
C-ve= growth curve of bacteria alone





T1=growth curve of bacteria with tilapia mucus no. 1
T2=growth curve of bacteria with tilapia mucus no. 2
T3=growth curve of bacteria with tilapia mucus no. 3

T4=growth curve of bacteria with of tilapia mucus no. 4
T5=growth curve of bacteria with tilapia mucus no. 5
C1=growth curve of bacteria with catfish mucus no. 1
C2=growth curve bacteria with catfish mucus no. 2
C3=growth curve bacteria with catfish mucus no. 3
C4=growth curve of bacteria with of catfish mucus no. 4
C5=growth curve of bacteria with mucus catfish no. 5
C-ve= growth curve of bacteria alone

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الملخص العربي

دور المخاط في حماية الأسماك ضد العدوى البكتيرية

إسماعيل عبد المنعم محمد عيسى *، منى محمد عبد الوهاب، حسن إبراهيم محمد،
محمد احمد بكرى*

قسم أمراض الأسماك و رعايتها, كلية الطب البيطري, جامعة قناة السويس

**معمل بحوث صحة الحيوان بالإسماعيلية*

تم تجميع ٥٠ سمكة حية وسليمة ظاهريا بواقع ٢٥ من البلطي النيلى المستزرع تزن كل واحدة ٢٥٠جم و ٢٥ من القرموط الافريقي البرى تزن كل واحدة ٣٠٠ جم بصورة عشوائية من مياة عذبة تم تجميع المخاط الخارجي لكل سمكة على حدة وترقيمها ثم إضافة محلول مذيب للمخاط وتعريضة لجهاز الطرد المركزي البارد ثم الاحتفاظ بالجزء السائل لقياس نسب البروتينات المختلفة وتقييم الأثر المثبط للمخاط علي النمو الميكروبي. كانت نسب كلا من البروتين الكلي والالبيومين والجلوبيولين في القرموط الافريقى ١,٢٥ و ٠,٤ و ٠,٨٦ جم/ديسلتر على التوالي بينما في البلطي المستزرع كانت ٢,٣ و ١,٠٨ و ١,٢٦جم/ديسلتر على التوالي. اما نسبة الالبيومين /الجلوبيولين فكانت ٠,٨ فى القرموط الافريقى و ٠,٧٥ فى البلطي النيلى. ثبط مخاط السمك المعزول من اسماك القرموط النمو البكتيري لميكروبات(ايروموناس هيدروفيلا و سيدوموناس فلوريسنس و ستافيلوكوكس اوريس) في الستة ساعات الأولى بصورة كبيرة ويستمر لمدة ١٢ ساعة بينما لم يظهر مخاط السمك المعزول من البلطي اى اثر مثبط في هذة الفترة. بعد مرور الستة ساعات الأولى يبدأ الأثر المثبط لمخاط اسماك البلطي في الظهور ويستمر لمدة ٢٤ ساعة ثم يزول ذلك الأثر المثبط.