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STUDIES ON THE MORPHOLOGICAL, BIOLOGICAL AND BIOCHEMICAL CHARACTERS OF FLAVOBACTERIUM SP., ISOLATED FROM DISEASED FISHES (LABES NILOTICUS) IN UPPER EGYPT

(With 3 Tables)

By

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دراسات مورفولوجية وبيولوجية وبيوكيميائية عن ميكروب الفلافوبكتـــــر
المعزول من الأسماك المريضة (اللبيس النيلى) في صعيد مصـــــــــر

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تم أخذ عشرين عينة من أسماك اللبيس النيلى بطريقة عشوائية · تمثلت الأعـــــراض الإكلينيكية في تغير لون الخياشيم بالإضافة إلى أعراض نقص الأكسجين في خمسة أسمـــاك من هذه المجموعة · كما تم عزل سبع عترات من ميكروب الفلافوبكتر من الخياشيم · وقـــد أجريت دراسة الصفات المورفولوجية والبيولوجية والبيوكيميائية للعترات المعزولة من هـــذه الإسماك · وقد تم إجراء إختبارات الحساسية بالنسبة للمضادات الحيوية المختلفة وأوضحـــت الدراسة : أن الميكروب حساس لكل من :ـ الاريثرومايسين ، النتروفيورنتيون ، الأوكـــى تتراسيكلين ، النتراسيكلين والفيومايسين · وغير حساس للمضاد الحيوى الترايسيوبريـــم ـ سلفا ميثاكســـول ·

SUMMARY

Twenty fish samples from labes niloticus were collected randamly from River Nile. Five samples showed pale colouration of the gills with signs of oxygen deficiency. Seven isolates of Flavobacterium Sp. were isolated from the gills. The morphological, biological and biochemical feature were discussed. The bacterial isolates were highly sensitive to erythromycin, nitrofurantion, oxytetracycline, tetracycline, neomycin and resistent to trimethoprim-sulfamethoxale.

INTRODUCTION

Gram-negative pigmented rods is one of the most important bacteria affecting fish. This group include Cytophaga, Flavobacterium, Flexibacter, Myxobacterium and Sporocytophaga (AUSTIN and AUSTIN, 1987). The most important characteristics of these bacteria are gliding movement or swarming on surface of agar media. According to BUCHANAN and GIBBSON (1974), the non motile members of the genus Flavobacterium are closely releated to group of gliding bacteria. There are many investigations

about Flavobacterium which have been previously isolated from freshwater and marine fishes by (BRISOU, et al. 1964; BULLOCK, et al. 1971; ROBERTS, 1978; ACUIGRUP, 1980 and WAKABAYASHI, et al. 1980). The earliest reports described Flavobacterium Balustinum as pathogen of marine fish namely halibut (HARRISON and SADLER, 1929).

MEYER, et al. (1959) considered Flavobacterium piscicida is the causative agent of redtide in marine fish species. ACUIGRUP (1980) discussed Flavobacteriosis in coho salmon which cause 20-25% mortality in salmon during summer. The best decumented case of flavobacteriosis concerned an outbreak which firstly appears in Japan during (KIMURA, et al. 1978 and WAKABAYASHI, et al. 1980).

FARKAS (1985) metioned that Flavobacterium Sp. occurs in Europe both in salmonids and warm-water also during winter. The outher isolated the Flavobacterium Sp. from gills of apparently silber carp during winter but have never isolated during summer.

The aim of this investigation is to isolate the Flavobacterium Sp. from Labes niloticus in Upper Egypt.

MATERIAL and METHODS

Twenty fish from Labes niloticus were collected randomly in December 1987 from River Nile at Assiut governorate. The weight of fish was about 100-150 grams and were brought as quickly as possible to laboratory of fish diseases, department of Vet. Med. & Int. Dis., Fac. of Vet. Med., Assiut University.

The fish were directly examined for clinical and postmortem finding according to AUSTIN and AUSTIN, 1987.

Gills, liver, kidney and intestine of examined fish were taken under aseptic conditions for bacteriological examination these samples were cultivated on cytophaga agar (ANACKER and ORDAL, 1959), blood agar and nutrient agar (CRUICKSHANK, et al. 1975) and incubated at 22 C for 48 hours.

The bacterial isolates were identified by morphological feature of colony and microscopic examination. The biochemical tests were performed according to WAKABAYASHI, et al (1980) and FARKAS (1985) table No. (1).

The growth of the isolated strain were studied in different percentage of sodium chloride (Table 2).

Mycological and parasitological studies were done by using FUHRMANN (1983) and KABATA (1985).

The antibiotic sensitivity test, of bacterial isolates were performed by method of FUHRMANN (1983) and was carried out on sheep blood agar. The antibiotic tests are showed in table (3).

RESULTS

Five fish from collecting samples were seen swiming near the surface of the water and easily to be catch by the hand. Signs of hypoxia associated with pale colouration of gills were evident. The other samples were assumed to be apparently healthy.

Parasitiological examination:

Microscopical examination of gills smear of samples revealed free from any type of parasites .

Mycological examination:

No fungal growth peared on the cytophaga agar.

Bacteriological examination:

Seven isolates of Flavobacterium species were isolated only from gills of all samples while kidney, liver and intestine were free. Five out seven isolates were isolated from pale coloured gills fish (5).

The growth of bacterial isolates were appeared on cytophaga-blood-nutrient-agar at 22°C for 48 hours.

The character of colonies were rounded, mucoid and yellowish orange. The bacterial colonies did not glide or swarm on this media.

It was noticed that, cytophaga and nutrient-agar were best and good media for the growth and facilitate the identification with its special colour for the Flavobacterium Sp.

It was observed that the optimum for Flavobacterium Sp. were 22°C and 30°C than 37°C.

The morphological, physiological and biochemical characters of Flavobacterium Sp. were shown in the table (1).

The study revealed that all the isolated strains were grown on different percentage of sodium chloride except isolates No. 1 and 7 were not grown on percentage 2% and 3% of sodium chloride (Table 2).

Antibiotic sensitivity test for seven isolates from Flavobacterium Sp. are recorded in table (3).

DISCUSSION

It is important to indicate that, the cause of gills disease could be one of the following agents, myxobacteria, fungi, parasites, deficiency of panthotenic acid, chemical pollution and pesticides (BULLOCK, et al. 1971).

From the obtained results neither fungal infection nor parasitic infestation were demonstrated. However, Flavobacterium Sp. was isolated. We can say that Flavobacterium Sp. considered one of the causes of gills diseases in fishes. On the other side the major clinical signs of Flavobacterium infection which were reported previously by BRISOU, et al. 1964; MEYER, et al. (1959); BULLOCK, et al. (1971); KIMURA, et al. (1978) and ACUIGRUP (1980) in different species of fish were not noticed in the present study.

Bacteriological examination revealed the presence of seven Flavobacterium isolates from gills. These isolates have the following characters, Gram-negative bacilli, colonies were rounded, mucoid, yellowish-organe in colour, and did not glide or swarm on the cytophaga-blood-nutrient-agar. The optimum temperature for growth was 22°C and 30°C. The obtained results agreed with those described by WAKABAYASHI, et al. (1980) and FARKAS (1985).

The results of the biochemical reactions of the isolated Flavobacterium Sp. are demonstrated in table (1). These results were agreed in general with ACUIGROUP (1980), WAKABAYASHI, et al. (1980); ALLEN, et al. (1983) and FARKAS (1985).

The sensitivity test of Flavobacterium Sp. to antibiotics is shown in table (2). From that table it appeares that the isolated bacteria were highly sensitive to erythromycin, nitrofurantion, oxytetracline, tetracycline and neomycin. The results are in aggreement with those mentioned by FARKAS (1985). The bacteria were resistent to trimethoprimsulfamethoxale as shown FUHRMANN (1983).

As a conclusion, the present investigations represents a preliminary study concerned with the isolation and identification of Flavobacterium Sp. from freshwater fish. Under our local environment, the available litrature revealed no evidence on the isolation of Flavobacterium Sp. from fishes in Upper Egypt.

Further investigations will clarify the pathogenicity of Flavobacterium Sp. and factors which enhanced the spreading of the infection under our local environment.

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Table (1)

Morphological, physiologiol and biochemical characteristics of Flavobacterium Sp.

Biochemical tests	1	2	3	4	5	6	7
Grow on							
Sheep blood agar	+	+	+	+	+	+	+
Cytophaga agar	+	+	+	+	+	+	+
Nutrient agar	+	+	+	+	+	+	+
Mocconckey agar	-	-	-	-	-	-	-
Gliding movement	-	-	-	-	-	-	-
Swarming	-	-	-	-	-	-	-
Motility by hanged drops	-	-	-	-	-	-	-
Motility in semisolid media	-	-	-	-	-	-	-
Grow at 22°C	+	+	+	+	+	+	+
30°C	+	+	+	+	+	+	+
37°C	+	+	+	+	+	+	+
Gram stain	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Oxidase	+	+	+	+	+	+	+
O/F - test	+/+	(+)/+	-/(+)	-/(+)	+/+	+/(+)	+/-
Gelatin hydrolysis	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+
Flexirubin-test	+	+	+	+	+	+	+
Esculin	+	+	+	+	+	+	+
Indole production	-	+	+	+	-	-	+
H2S production	-	-	-	-	-	-	-
Methyl red	-	-	-	-	-	-	-
Acid from							
Glucose	(+)	(+)	-	+	+	(+)	+
Sucrose	+	+	+	+	(+)	+	+
Salicin	-	(+)	-	-	(+)	-	(+)
Fructose	+	+	+	+	+	+	(+)
Arabinose	(+)	+	(+)	-	+	(+)	+
Lactose	-	-	-	-	+	+	-
Trehalose	+	+	+	+	+	+	+
Galactose	(+)	+	+	(+)	(+)	+	+
Insitol	-	-	-	-	-	-	-
Mannitol	-	-	-	-	-	-	-

\+ Positive. (+) Weakly positive.

\- Negative.

Table (2)
Effect of sodium chloride on growing of the isolated strain

Test	1	2	3	4	5	6	7
Nacl 1%	+	+	+	+	+	+	+
Nacl 2%	-	+	+	+	+	+	-
Nacl 3%	-	+	+	+	+	+	-

\+ growth - no growth

Table (3)
Sensitivity of seven isolates of Flavobacterium Sp. to antibiotics (ug/ml)

		1	2	3	4	5	6	7
Erythromycin	(15)	++	++	++	++	++	++	++
Nitrofurantoin	(300)	++	++	++	++	++	++	++
Colistine	(10)	+	+	R	+	R	R	R
Oxytetracycline	(30)	++	++	++	++	++	++	++
Kanamycine	(30)	+	+	R	R	R	R	R
Tetracycline	(30)	++	++	++	++	++	++	++
Doxcycline	(30)	++	R	++	++	++	++	++
Chloramphenicol	(30)	+	R	+	+	+	+	+
Neomycine	(30)	++	++	++	++	++	++	++
Trimethoprim-sulfamethoxazole		R	R	R	R	R	R	R

++ Highly sensitive.
\+ Moderate sensitive.
R Resistent.