ISOLATION OF *ENTEROCOCCUS FAECALIS* FROM TILAPIA IN LAKE TEMSAH IN ISMAILIA GOVERNORATE


Departement of Microbiology., Faculty of Vet. Med. Suez Canal University, Egypt*.
Departement of Fish Disease, Central Laboratory For Aquaculture Research, Abbassa, Sharkia, Egypt**.
National Institute of Oceanography and Fisheries, "NIOF"***.

ABSTRACT

This study was applied on 100 naturally diseased *Oreochromis niloticus* and *Tilapia zilli* (50 fish each) collected from Lake Temsah in Ismailia governorate to investigate the most prevalent bacterial pathogen. Clinical signs and postmortum findings of the diseased fishes were done, samples were taken from fins, tails and skin ulcer, liver, kidney, spleen, brain and mouth lips for bacteriological examination which indicated the presence of different bacterial species including *Enterococcus faecalis*. The percentage of infection in Nile tilapia with *Ent. faecalis* was 23.76%. The percentage of infection in *Tilapia zilli* with *Ent. faecalis* was 31.90%. Antibiogram test of bacterial isolates recovered from naturally infected Nile tilapia and *Tilapia zilli* revealed that Amoxicillin (Ax10) and Penicillin (P10) were the drug of choice against *Ent. faecalis*. The results of Polymerase Chain Reaction (PCR) showed appearance of specific bands of *Enterococcus spp.* in the kidneys of both tilapias and the brain of Nile tilapia which were identified by multiple sequence alignments of published 16S rRNA sequences and gave a specific band at 733 basepair (bP).
INTRODUCTION

Bacterial diseases are the most common diseases in intensive fish rearing facilities (Kusuda and Salati., 1999).

Streptococcosis, is a septi-
cemic disease affecting fresh and marine water fishes. The disease was firstly reported in rainbow trout (Hoshina et al., 1958). In Egypt, Streptococcosis was recorded among Nile tilapia (Badran, 1994; Ebt-
sam, 2002; and Dena, 2004 ), African catfish (El- Rafaee, 2005), Silver carp (Safinaz, 2006), Grey mullet (Ebrahim et al., 2007). In recent years, authors have described molecular methods for the detection and identification of Enterococcus species by the use of labelled oligo-
nucleotide probes based on 16S and 23S rRNA genes (Deasy et al., 2000). This study was done to reveal the isolation of *Enterococcus faecalis* from naturally infected diseased Tilapia found in Lake Temsah where it is facing many pollution sources; identification of the isolated bacteria using biochemical tests, antibiogram test and PCR (Poly-
merase Chain Reaction).

MATERIALS & METHODS

Lake Temsah is located on the north of the Suez Canal. The Lake is the end point where some municipal, agricultural and industrial wastewaters are discharged. The Lake is also an important source of fish in Ismailia governorate (El-
Sherif, 2009).

A total number of 100 clinically diseased Nile tilapia (*Oreochromis niloticus*) and redbelly tilapia (*Tilapia zilli*) each of 50 fish were collected alive from Lake Temsah in Ismailia governorate in a period from April to December 2008. Under aseptic conditions, samples were taken from fins, skin ulcers, liver, kidneys, spleen, brain and mouth lips for bacteriological exam-
ination. A total of 550 samples were collected from both clinically infected Nile tilapia (*O. niloticus*) and Redbelly tilapia (*T. zilli*) under aseptic conditions and submitted for bacteriological studies. A sterile bacteriological loop was inserted through the sterilized organ and then inoculated into Tryptic soya broth, incubated at 29-30°C for 18-24 hours, then streaked onto Strept-
ococcus selective agar, and incubated at 29-30°C for 24-48 hrs. The suspected pure colonies were picked up and streaked onto the same specific media for further purification. Isolated pure colonies were transferred into nutrient agar slant for further identification. Gram staining and biochemical tests were done according to Facklam and Carly (1985), Ravelo et al., (2001). The isolated *Ent. faecalis* were tested for their susceptibility against different antibiotics using disc diffusion method according to Baur et al.,(1966). PCR was carried out according to Deasy et al.,(2000) which were unique to Enterococci species and were identified by multiple sequence alignments of published 16S rRNA sequ-ences.
Table (1): Primer details.

<table>
<thead>
<tr>
<th>Name of oligonucleotide</th>
<th>Sequence</th>
<th>Primer length</th>
<th>Primer location</th>
<th>Product length (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enter - F</td>
<td>5′- TCA ACC GGG GAG GG T -3′</td>
<td>15</td>
<td>632-646</td>
<td>733 bp</td>
<td>Deasy et al. (2000)</td>
</tr>
<tr>
<td>Enter - R</td>
<td>5′- ATT ACT AGC GAT TCC GG -3′</td>
<td>17</td>
<td>1353-1369</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

RESULTS

Photo 1: Showing *O. niloticus*, fish 1 in the bottom with normal signs of healthy fish; (2), (3) with hemorrhages on the dorsal, anal, above the anal fins.

Photo 2: Diseased Nile tilapia showing deep haemorrhagic ulcer on the caudal peduncle.
Photo 3: Diseased *Tilapia zilli* showing ulceration on the head region and excessive mucous on the head.

Photo 4: P.M lesions of diseased *Tilapia zilli* showing enlarged haemorrhagic liver reached nearly the size of gonads and haemorrhages at the kidney.
A total of 176 isolates showed characters of *Enterococcus faecalis* with a percentage of 26.66%, 101 isolates were isolated from *Oreochromis niloticus* with a percentage of 23.76% and 75 isolates were isolated from *Tilapia zilli* with a percentage of 31.90%. The colonies on Streptococcus Selective agar at 29-30 °C for 48hrs showed dew drops like, white colonies, very small, rounded with entire edges or creamy rounded, large colonies (2-3mm).

The isolated *Enterococcus faecalis* stained with gram's stain were gram-positive arranged in pairs and some-times short chains, oxidase negative, catalase negative, glucose fermentative, vogus proskauer positive, Bile-Esculine hydrolysis positive. Only two strains were positive for starch hydrolysis, lactose fermentation, Sucrose positive, Arabinos negative, Indole test negative, Growth at 0-6.5 %NaCl positive, grew at temperatures 10-45 °C.

### Table 2: Distribution of *Enterococcus faecalis* isolates among various tissues and organs of naturally infected *Oreochromis niloticus*:

<table>
<thead>
<tr>
<th>Examined organs</th>
<th>No. of isolates</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fins and Skin ulcers</td>
<td>50</td>
<td>49.5</td>
</tr>
<tr>
<td>Liver</td>
<td>17</td>
<td>16.83</td>
</tr>
<tr>
<td>Spleen</td>
<td>12</td>
<td>11.88</td>
</tr>
<tr>
<td>Kidneys</td>
<td>16</td>
<td>15.84</td>
</tr>
<tr>
<td>brain</td>
<td>6</td>
<td>5.94</td>
</tr>
<tr>
<td>Total</td>
<td>101</td>
<td>100</td>
</tr>
</tbody>
</table>
Table 3: Distribution of *Enterococcus faecalis* isolates among various tissues and organs of naturally infected *Tilapia zilli*:

<table>
<thead>
<tr>
<th>Examined organs</th>
<th>No. of isolates</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fins and Skin ulcers</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td>Liver</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td>Spleen</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td>Kidneys</td>
<td>25</td>
<td>33.3</td>
</tr>
<tr>
<td>Brain</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mouth</td>
<td>5</td>
<td>6.67</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>75</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

The antibacterial biogram revealed that *Enterococcus (Streptococcus) faecalis* was highly sensitive to Amoxicillin (Ax$_{10}$), Penicillin (p$_{10}$), and showed medium sensitivity to Ciprofloxacin (Cip$_{5}$), Tetracycline(Te$_{30}$), Streptomycin(S$_{10}$) and showed resistance to Colistin (Cl$_{10}$), Nalidixic acid (NA$_{30}$), Rifa-

mpicin(RA$_{5}$), Lincomycin(L$_{2}$). By identifying three bacterial isolates from the diseased fishes (Nile tilapia, *Tilapia zilli*), the results showed presence of specific bands of *Enterococcus spp.* obtained from the kidneys of diseased Nile tilapia, *Tilapia zilli* and from the brain of Nile tilapia.

**Photo 6: Showed the sources of the isolates :**

Lane (M): (M) = Marker. DNA molecular weight ladder of 100 bp + 2 Kb + 3 Kb
Lane (1): Positive control (*Enterococcus faecalis* collected from dairy products).
Lane (2): Negative control (*Staphylococcus Sp.*)
Lane (3): Bacterial isolate from the kidney of *Tilapia zilli*.
Lane (4): Bacterial isolate from the kidney of *O. niloticus*.
Lane (5): Bacterial isolate from the brain of *O. niloticus*. 
DISCUSSION

In this study, the species *Enterococcus faecalis* was isolated from Tilapia collected from a brackish water. These results agree with those of *El-Rafae (2005), Safinas (2006), Eman (2007)*.

The results of biochemical tests disagree with those of *Fac-klam and Carly (1985), Ravelo et al (2001)* who reported that the isolates were lactose fermentation positive, starch hydrolysis negative.

However, the present study shows that lactose fermentation were negative in some isolates and starch hydrolysis was positive in two cases. These results agree with those of *Eman (2007)*. Table 2 reveals that the tissue distribution of *Enterococcus faecalis* in infected *O. niloticus* which was high in fins and skin ulcers (49.5%) as the predominant organ followed by the liver (16.83%), kidney (15.84%), spleen (11.88%) and brain (5.94%). The target tissues used for isolation of the bacterium agree with those of *Ebtsam (2002), El-Rafae (2005), Eman (2007)* but disagree with them in point where the kidney was the best organ for isolation of *Streptococcus faecalis*. Our results also disagree with those of *Eman (2007)* who revealed that there were no isolates in brain. The difference between authors in tissue distribution, regardless the prevalence percentage of tissue distribution of each bacterium, may be due to the different number of diseased fishes, different number of isolated samples and different culturing methods of the bacteria. The results of Antibio-gram sensitivity agree with those of *Al-Harbi (1994)* but partially disagree with those of *Dena (2004)* who revealed that the bacteria were sensitive to Oxytetracyclin and resistant to Streptomycin, Nalidixic acid, *El-Rafae (2005)* who revealed that *Streptococcus faecalis* was sensitive to Nalidixic acid, Amoxicillin and Penicillin and Streptomycin and resistant to Ciprofloxacin and Tetracycline. The steps of PCR protocol were done: initial denaturation was done at 95 °C for 5 min, Denaturation at 94°C for 30 seconds, annealing at 45 °C for 30 seconds, Extension at 72 °C for 30 seconds and Final extension at 72 °C for 5 min, then these steps were repeated for 30 cycles for amplification of DNA in a DNA thermal cycler. These steps were partially similar to the steps done by *Deasy et al., (2000)* who performed the steps as follows: denaturation at 94 °C for 1 min, annealing at 60 °C for 1min and amplification was repeated for 25 cycles in a DNA thermal cycler. The difference between the present study methods and the author were according to *Rychlik and Rhoads, 1989* who reported that the melting temperature (Tm) is calculated as the following simple rule is: $Tm = 4(G+C)+ 2(A+T)$ and
according to (Innis and Gelfand, 1990) who reported that the annealing temperature (Ta) chosen for a PCR about $5^\circ C$ below the lowest Tm of their pair of primers to be used as follows : $Ta = 4(G+C)+2(A+T)-5$.

By applying this formula on the primer taken by Deasy et al., (2000):

The results of melting temperature (Tm) after calculation of the forward primer sequence were: $Tm = 4(7+3)+2(3+2) - 5 = (40+10)-5 = 45^\circ C$.

REFERENCES


and and Management). Faculty of Veterinary medicine Moshtohor, Benha University.


**الملخص**

عزل ميكروب *الإنتيروكوكس فيكالز* من أسماك البلطي من بحيرة التمساح في محافظة الإسماعيلية.

أجريت هذه الدراسة على 50 سمكة مريضة طبيعياً من البلطي النيلي و 50 من البلطي الأخضر وذلك للتعرف على أهم العوامل البكتيرية الموجودة في بحيرة التمساح في محافظة الإسماعيلية، وقد تم الفحص الإكلينيكي والصفة التشريحية للأسماك المريضة، وأخذت العينات من تقرحات الجلد والزعانف- الكبد- الطحال- الكلي- المخ- الفم وذلك للفحص البكتريولوجي. وأوضحت النتائج وجود بكتريا الإنتيروكوكس فيكالز بنسبة (67.32%) من...
البلطي النيلي ونسبة (1,9%) من البلطي الأخضر. وبإجراء اختبار الحساسية لكل من البكتريا المعزولة من أسماك البلطي النيلي والبلطي الأخضر وجد أن ميكروب الإنتيروكوكس فيكالز اشد حساسية للأموكسيسيلين والبينيسيلين. وأبرزت نتائج انزيم البلمرة المتسحل عزل بكتريا (الإنتيروكوكس فيكالز) من الكلي في كل النوعين (أسماك البلطي النيلي وأسماك البلطي الأخضر)، ومن المخ في أسماك البلطي النيلي، وأكدت النتائج بوجود الرابط المخصص لهذه البكتريا عند BP 733. وتم ذلك بناء على وجود الجين المخصص لها 16S rRNA.