Isolation and Identification of Pathogenic Bacteria from Red Tilapia in Cage-Cultured System and its Environment

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Abstract

Bacteria were isolated from the brain, eye and kidney of red tilapia, as well as water and debris samples. The weight and length of red tilapia were measured and the water quality as well. API test were done to identify the type of bacteria from the isolates. In Kenyir Lake, bacterial isolates that predominated in the fish were *Micrococcus* spp. and *Aeromonas hydrophila* at 13.64 %, in water samples it was *Staphylococcus xylosus* at 40% and in the debris samples, *Pseudomonas aeruginosa* and *Enterobacter cloacae* at 50%. In the Semantan River, the predominant bacteria in fish and debris samples were *Aeromonas hydrophila* at 23.53 % and 90 % respectively. In the water samples, *Staphylococcus lentus* and *Staphylococcus xylosus* were the predominant bacteria with 30 and 20%, respectively. The ammonia, sulphide, iron and nitrite-nitrogen levels in the Semantan River were over the acceptable limits and this may lead to high fish mortality. This study concluded that *Aeromonas hydrophila* and *Staphylococcus spp.* were the most predominant bacteria in red tilapia and poor water quality played a major role in red tilapia succumbing to infections by pathogenic bacteria.

Keywords: Bacteria, red tilapia, API test, water quality.

Introduction

Red tilapia (*Oreochromis niloticus* hybrid) was first introduced into Malaysia in the mid 1980's. It was initially considered to be hardy and resistant to diseases (Siti-Zahrah, 2004). The incidence of microbial pathogens in fish, especially those of bacterial origin is one of the most significant factors affecting fish culture (Post, 1989; Zorrilla et al., 2003).

Sugita et al. (1982) mentioned that *Aeromonas* spp. and *Pseudomonas* spp. were the predominant bacterial genera in the tilapia fish. In contrast, Chowdhury et al. (1989) found that the predominant bacteria recovered from tilapia are *Micrococcus*.

Atwood et al. (2001) found that small-sized weighted Nile tilapia were more tolerant to nitrite than larger fish. LaDon (1992) cited that more ammonia was excreted by heavy fish as waste.

One of the factors affecting fish culture is the quality of water, which in turn, determines the incidence of microbial pathogens particularly those of bacterial origin (Austin and Austin, 1999; Owens, 2003).

The objectives of the present study are to isolate and identify pathogenic bacteria from red tilapia, water and feces or debris and to determine the water parameters from cage-cultured tilapia in Kenyir Lake, Terengganu and Semantan River, Pahang.

Materials and Methods

Fish, water and debris sampling and water quality measurement

At each site, 30 cage-cultured red tilapia fish were randomly caught, 10 water and 10 debris samples were randomly taken from fish cages. The temperature, pH and dissolved oxygen and conductivity were measured using YSI 556. The ammonia, iron, sulphate and nitrites were determined using a Hach Spectrophotometer (DR 2800 Portable Spectrophotometer). All fish were measured and weighed. External observations on fish were also recorded.

Bacterial isolation from fish and debris at sampling site

Incision was made on the eye surface using sterile scalpel blade and then, a sterile wire loop was used to obtain a sample from the eye. The samples were cultured onto the blood agar (BA). The same procedures were also applied to other organs such as brain and kidney. Any organ abnormalities were recorded. Debris or fecal sample were picked using sterile wire loop and cultured onto blood agar. The inoculated BA media were incubated at room temperature, ~22°C.

Subculture of fish and debris bacterial colonies from BA medium

Inoculated BA medium were observed for the presence of bacterial growth after 24 hours incubation. The bacterial colonies present were sub-cultured onto BA medium and incubated at 30°C in an incubator for 18-24 hours.

Isolation of bacteria from water samples into BHI broth

One milliliter of water sample taken using a sterile pipette was inoculated into 9 mL of brain heart infusion broth (BHIB) in a tube media and mix well using a rotator. The inoculated broth was later placed in an incubator shaker at 30°C for 18 hours. Growth in the tubes was indicated by the presence of turbidity of the broth.

Subculture of bacteria from water samples in BHI broth onto BA medium

The broth samples were mixed using vortex mixer. One hundred microliters of the broth was then dropped onto BA medium and streaked with a sterile wire loop. The inoculated medium was then incubated at 30°C for 18-24 hours.

Second sub-culture of pure bacterial colonies onto BA and TSA medium

The bacterial colonies were sub-cultured onto Trypticase Soya Agar (TSA) medium for Gram-stain, catalase and oxidase tests. The bacterial colonies were sub-cultured onto BA medium for second time to get pure colonies of bacteria for API (Analytical Profile Index) test.

Gram staining, catalase and API test

Gram staining was done using the pure culture of bacterial colonies grown on TSA medium. For Gram-negative bacteria, API 20E was used. For Gram-positive bacteria, catalase test was done. Catalase positive bacteria were subjected to API 20 Staph test. Catalase negative bacteria were tested with API 20 Strep test. Oxidase tests were done on Gram-negative bacteria colonies grown on TSA medium. All the API test kits used were incubated in normal incubator at 30°C for 24 hours. Identification of bacteria was made by using API test software.

Results and Discussion

Kenyir Lake

The bacterial isolates from tilapia of Kenyir Lake are mostly predominated by *Micrococcus* spp. and *Aeromonas hydrophila* at 13.64 % of both bacteria and followed by non-fermenter sp. at 9.09%. In water samples, the most predominant bacteria isolates are *Staphylococcus xylosus* with percentage of 40%, followed by *Staphylococcus lentus*, *Klebsiella terrigena* and *Kocuria varians* at 20%. In debris samples, there were 2 species of bacterial isolates have been isolated there, which are *Pseudomonas aeruginosa* and *Enterobacter cloacae* at 50%.

Semantan River

The most predominant bacterial isolates in the tilapia there are *Aeromonas hydrophila* with 23.53%, followed by *Staphylococcus xylosus* and *Staphylococcus caprae* both with 11.76%. In water samples, the most predominant bacteria isolates are *Staphylococcus xylosus* with percentage of 40%, followed by *Staphylococcus lentus*, *Klebsiella terrigena* and *Kocuria varians* with percentage of 20% of all bacteria, respectively. While, the 90% of bacterial isolates in debris samples of Semantan River are *Aeromonas hydrophila* and the remaining isolates which are 10% are *Staphylococcus lentus.*

Water quality

Only pH and conductivity between Kenyir Lake and Semantan River have no significant difference. All of the parameters of water quality in Semantan River were higher than in Kenyir Lake except for temperature, DO and pH References

The findings of *A. hydrophila* being the predominant bacterial genera in tilapia fish in the current study are in agreement with the report of Sugita et al. (1982).

The internal organ of *A. hydrophila* infected fish were found congestion or swollen of the kidneys, softening of brain tissues and enlargement of liver. These findings are in agreement with Huizinga et al. (1979).

*Micrococcu*s spp. is the most predominant of *Staphylococcus* spp. that being isolated from other studies conducted by Siti-Zahrah et al. (2008) and Chowdhury et al. (1989). This is in agreement with the result at Kenyir Lake.

No abnormal findings on external organs were noted. This is in agreement with the findings from previous study by Siti-Zahrah et al. (2008), who noted that grossly sampled tilapia affected by *Micrococcus* spp. did not show little or no clinical signs.

Ammonia level in Kenyir Lake and Semantan River were exceeded the acceptable limit (0.02 ppm) for fish. The high level of ammonia in the present study is due to the high level of ammonia in the feed as found in Semantan River where goat faeces and visceral organs of chickens used as a source of feed.

The tolerance of tilapia to nitrite may also influenced by the fish size. Atwood et al. (2001) found that small-sized weighted Nile tilapia were more tolerant to nitrite than larger fish. From this study, the weight of the fish in Semantan River is more than Kenyir Lake fish, indicative of low tolerance to nitrite. This may also explained why high mortality occurred at Semantan River fish.

Thus, the environment also contributes to the existence of some pathogenic bacteria in red tilapia especially contaminants from feed, water and debris. The poor water quality may also cause fish more succumb to diseases or infection eventually death due to environmental stress and attacked by opportunistic pathogens such as *A. hydrophila*.

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