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RESEARCH ARTICLE

ISOLATION AND IDENTIFICATION OF BACTERIA FROM INFECTED FRESHWATER FISH *CATLA CATLA*

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ABSTRACT

The present investigation infected fresh water fish Catla catla were collected from Chidambaram area, Tamil Nadu and bacterial species were isolated. Totally five different bacteria were isolated from the infected carp gill, intestine and muscles samples, namely species of Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Yersinia species and Enterobacter aerogen. These bacterial strains were caused by diseases in the fishes.

INTRODUCTION

Bacterial diseases are responsible for heavy mortality in both wild and cultured fish. The actual role of these micro-organisms may vary from that of a primary pathogen to that of an opportunist invader of a host rendered moribund by some other disease process" (Richards and Roberts, 1978). Generally bacteria play two major roles as beneficial bacteria and pathogenic forms, beneficial bacteria are helpful in nutrient recycling and organic matter degradation and thus clear the environment (Moriarty, 1997). The Food and Agricultural Organization of the United Nations (FAO, 2009), state that illness due to contaminated food is perhaps the most widespread health problem in the contemporary world and an important cause of reduced economic productivity (Edema *et al*., 2005). Biological contaminants such as bacteria, viruses, protozoa, fungi and helminthes constitute the major cause of food borne diseases such as cholera, *E. coli* gastroenteritis, salmonellosis, typhoid fever and poliomyelitis with varying degrees of severity, ranging from mild indisposition to chronic or life threatening illness (Phyllis, 2007). Various types of diseases such as ulcer type disease including epizootic ulcerative syndrome, bacterial hemorrhagic septicaemia, tail and fin rot, bacterial gill rot, dropsy, columnaris disease, fungal disease and parasitic disease are important limiting factors for sustainable fish production (Chowdhury, 1997). Carps affected by these diseases were than taken from the culture ponds and microbial pathogen was controlled in the laboratory conditions.

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Many pathogens species encountered in this study are no doubt potentially pathogen to human. *Bacllus sp. E. coli, Salmonella sp. Streptococcus sp* and *S. aureus* were also implicated in fish-borne (Babu, 2000). The pathogenic bacteria cause diseases in carp fishes. In their natural condition carps become diseased but most of these bacterial diseases pass unnoticed because of lesser occurrence or disease might not have caused mass mortality. The bacterial diseases of carps in culture systems are more obvious. The reason being it may either affect carp crop production or lead to mass mortality. Therefore through knowledge of bacterial diseases, symptoms, provisional diagnosis may help the aquaculturist in preventing the economical loss. Most of the organisms are healthy in their natural habitat, but when these organisms are cultured, we may not be able to maintain all the environmental factors, so the organisms become susceptible for stress, which leads to disease (Reddy, 2004).

Fish pathogenic bacteria have developed many strategies for infection to host fish species. Adhesion to the epithelial tissue of host, resistance against body surface mucus and serums of host are the important mechanisms during initial stages of infection (Thune *et al*., 1993). In recent years, the carp culture industry has been facing serious problems due to microbial diseases in the coastal belts of Tamil Nadu, India. In view of this, the present investigation has been carried out to study the outbreak diseases in the fish *Catla catla* cultured in semi-intensive ponds in the Chidambaram area of Cuddalore District.

MATERIALS AND METHODS

Collection of Fish samples: Infected carp *Catla catla* were collected from carp culture pond at Chidambaram area Cuddalore district, Tamil Nadu.

Isolation of bacteria: From serial dilution, bacterial species were isolated and 0.1 ml of the sample taken from 10^{-6} and 10^{-7} tube and spread aseptically over on the nutrient agar medium. Over plate maintain as a control without sample. All the nutrient agar plates were incubated 37°C for 48 h. After incubation the plates were observed for the bacterial growth (Aneja, 2001).

Identification of bacteria: The isolated bacterial species were identified by the following the (Aneja, 2001) morphological and biochemical characteristics of the individual colony was recorded. The individual colony was transferred to nutrient agar. The isolates were subjected to following test pure culture, gram staining, motility Test (Hanging Drop method) and biochemical test (Indole test, Methyl red test, Voges proskauer test, Citrate Utilization test, Catalase test, Oxidase test, Triple Sugar Iron test, Carbohydrate fermentation test and Urease test). The bacteria were identified by using standard manuals of bacteria. Photographs were taken by using Nikon microscope (Nikon, Japan).

RESULTS

Isolation of bacteria from infected fish *Catla catla*

The survey on the infected fish in the culture ponds showed that Chidambaram area. The survey on the infected *Catla catla* in culture ponds system indicated that Chidambaram area. Among the isolates bacteria of *Escherichia coli* species was found to be dominant microbes. The bacterial strains were isolated from the infected shrimp Gills-4; Intestine-2 and Muscles-2. Totally 5 different bacterial strains were observed on the cultural characteristics (Table 1 and Plate 1). Bacterial species were isolated by serial dilution method were plated in nutrient agar medium. The culture plates were incubated at 37°C for 24 to 48 hours.

Physical Analysis (Motility Test)

Hanging drop method was carried out for motility test. The bacterial culture plate B5 resulted was showed non-motile and other cultures were motile (Table 2).

Biochemical Analysis

The different species of bacteria were identified by using the biochemical tests.

Gram staining: All the bacterial cells have been stained by counter stain safranin and appeared red in color. The culture B1, B2, B3, B4 and B5 were resulted in gram negative (Table 2). Among these five bacterial species were rod shaped.

Indole Test : Ring formations were observed only B2 and B3 other broth culture were indicate negative results in B1, B4 and B5. Broth cultures showed no ring formation so it indicates the negative result.

Methyl red test: Addition of methyl red indicated to the MR broth cultures of such as B3 and B4 showed the presence of pink color, which indicated positive results and there was no colour change and other broth culture were showed negative result.

Voges proskauer test: Addition of methyl red indicated to the VP broth cultures of such B3, B4 and B5 showed the presence of pink red color. Which indicated positive results and there was no color change in other culture strains which showed negative result.

Citrate Utilization Test: The culture tube B1was observed the no color change, the other broth culture was observed the green to blue, and hence it indicates positive result.

Urease Test: The broth culture such as B1 showed yellow to pink color change positive result other culture strains no color changes showed the negative results.

Catalase Test: The appearance of gas bubbles was observed the broth culture such as B1, B4 and B5 and there was no gas bubbles observed B2 and B3 strains.

Oxidase Test: The formation of dark purple colour within 30 seconds B1 and B2 were showed positive results. No colour change was observed in oxidase sterile disc B3, B4 and B5 starins. Hence it indicates negative result.

TSI (Triple Sugar Iron Test) : The production of acid slant and acid butt were showed in the broth culture B1, B4 and B5. The production of alkaline slant and acid butt was showed in B2 and B3.

H_2S Production : The production of gas showed the broth culture in B2 and B3. No gas production was observed in B1, B4 and B5 strains.

Carbohydrate fermentation : All the broth cultures were showed positive (Table 2).

Table 1. Isolation of bacteria from infected carp of *Catla catla*

S.No.	Name of the bacteria	Gills	Muscles	Intestine
1.	*Escherichia coli*	+	-	+
2.	*Proteus mirabilis*	+	-	+
3.	*Pseudomonas aeruginosa*	-	+	-
4.	*Yersinia species*	+	-	-
5.	*Enterobacter aerogen*	+	+	-

Identification of bacteria

The isolated different bacterial strains were identified based on the cultural morphology and biochemical characteristics. The table results were compared with Bergey's manual of systemic bacteriology.

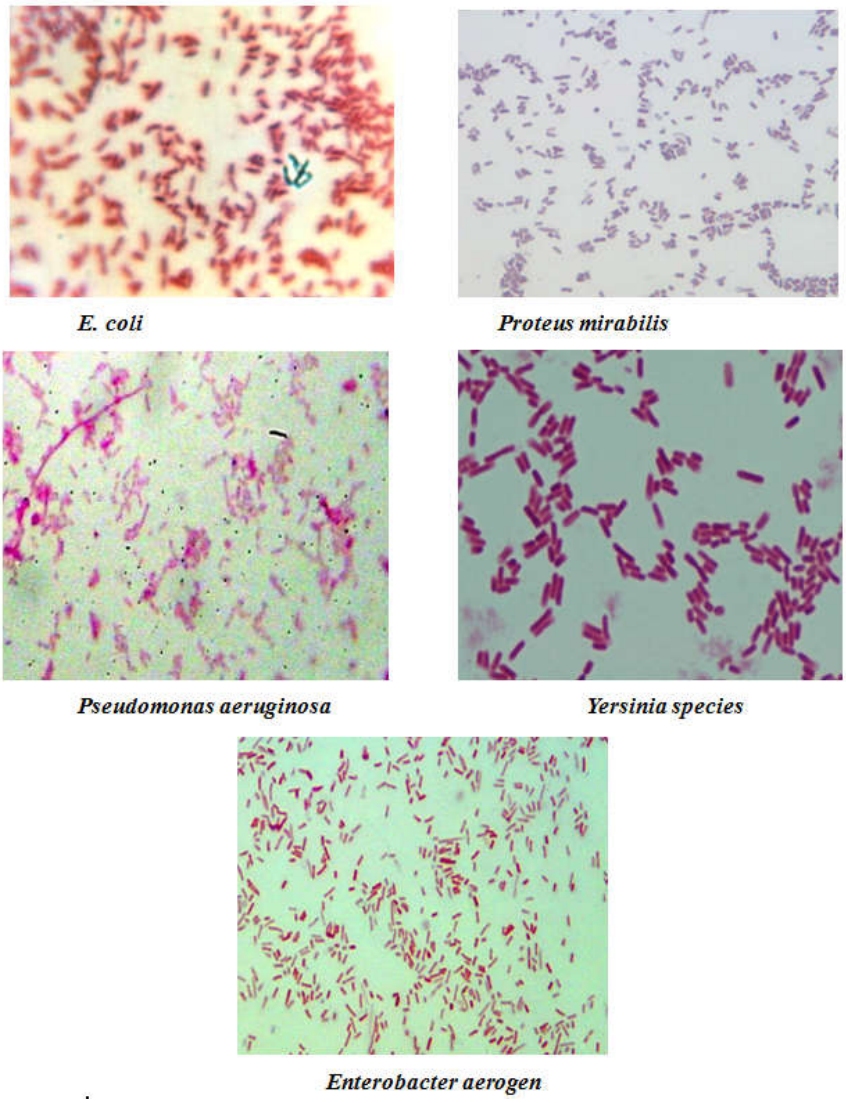

Fig. 1. Isolated bacteria from infected carp *Catla catla*

Based on the results were confirmed as *Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Yersinia species* and *Enterobacter aerogen.* The results were presented in Table 1, 2 and Fig. 1.

DISCUSSION

In the present study bacterial species were isolated from the infected carp gills, intestine and muscles. Totally 5 bacterial species were isolated by diluting plating technique. The infected carp *Catla catla* were collected from Chidambaram area of Cuddalore District. The investigated results were discussed with previous theoretical and biostatistical reports. The bacterial species were isolated such as *Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Yersinia species* and *Enterobacter aerogen*. The isolated all the bacterial species is very harmful pathogen. Isolated bacterial pathogen could be further exploited for biotechnological and molecular studies. The similar observations were reported by various workers Alauddin, *et al*., (1999) noted in isolation and identification of bacterial strains. The pathogenic bacteria were identified based on the cultural morphology and biochemical characteristics.

The results were compared with Bergey's manual of systematic bacteriology. Tests were carried out on each isolate following the procedures described by Prescott *et al.,* (2005) and Cheesbrough, (1994) to enable identification to the generic and species levels with the aid of the Bergey's manual of determinative bacteriology. The similer workers Holt *et al.,* (1994), and Aneja, (1994) were also noted in the bacterial isolation from the fresh water fishes. According to Pal and Dasgupta, (1991, and 1992) were reported the Pathogenic bacteria, including *E.coli* are rapidly destroyed by temperatures higher than 70^0C and contaminated fish do not pose a threat to human health if well cooked. However, one of the associated health hazards is the presence of antibiotic residues in food and the potential for resistant strains to develop that pass on or confer resistance factors to human bacteria such as *Salmonella typhi*, which causes typhoid. Fish can hold waterborne pathogens in the intestine for a long time. If farm animals excrete such resistant pathogens and the excrement is used in fish ponds, the pathogens can be retained in the fish and passed on to humans later. Olayemi *et al.* (1991) have reported that the presence of faecal coliform in fish intended for human consumption may constitute a potential danger not only in causing

disease, but also because of the possible transfer of antibiotic resistance from aquatic bacteria to human infecting bacteria from non-aquatic sources. According to Shiranee *et al.* (1993), was observed a higher bacterial load in the gut of fish has been observed than in the surrounding waters. According to Ogbondeminu, (1993) has been found in the *Escherichia coli* bacteria were isolated from in the gills, in the muscles and on the skin of fish. The micro-environment of bacteria associated with the gastrointestinal tract of an animal influences the host in many ways, including the metabolism of nutrients. Given the importance in digestion and health, the composition, diversity and morphological characteristics of the gut microflora in many species of marine and fresh water fishes and invertebrates have been extensively researched by Cahill (1990), Kuzmina and Skvortsova, (2002). According to Spanggaard *et al.* (2000) the isolated bacteria from fish gut were identified using the guidelines described in the Bergey's Manual of Systematic Bacteriology. Bacterial septicemia is responsible for millions of USD in annual losses to the cultured freshwater fish industry in the US, China and other countries (Shoemaker *et al*., 2002).

Conclusion

The bacteria are perhaps the most important pathogens in fish culture ponds causing severe mortalities and financial losses. In the present investigation, the bacterial species showed harmful effect on the culture organisms which needs to be addressed and create awareness among fish eating people. Therefore, present study suggests that carps should be cultured in good quality water with periodical examinations and excess feeding must be avoided to prevent the disease in carp culture ponds.

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