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IJPBS |Volume 2| Issue 4 |OCT-DEC |2012|53-58

Research Article
Biological Sciences

COMPARATIVE MICROBIAL INVESTIGATION ON SELECTED ORGANS IN FRESH WATER FISH *Channa punctatus* (Bloch, 1794) of PADITHURAI POND (MARIAMMAN KOVIL) AND COMMON POND (THIRUNAGESWARAM), THANJAVUR DISTRICT

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ABSTRACT

In this study, we examined the microbial population in different parts, gill, muscles and intestine region of Channa punctatus from two different fish culture farm. Based on their growth characteristics on specific culture media, the following human bacterial pathogen such as Achromobacter xylosoxidans (Gram +ve), Acinetobacter anitratus (Gram -ve), Bacillus subtilis (Gram +ve), Proteus vulgaris (Gram –ve), Pseudomonas aeruginosa (Gram –ve) and Klebsiella pneumonia (Gram – ve), and fungal pathogens like Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus, Aspergillus glaceus, Cunninghamella bertholetiae, Mucor sp. and Penicillium sp. were isolated. The fishes of different habitat were furthermore collected and examined for pathogens. However, There were six species of bacteria such as Achromobacter xylosoxidans (Gram +ve), Acinetobacter anitratus (Gram -ve), Bacillus subtilis (Gram +ve), Proteus vulgaris (Gram –ve), Pseudomonas aeruginosa (Gram –ve) and Klebsiella pneumonia (Gram – ve), exist in the gill region also in the muscles and intestine samples of sample 1 and sample 2. the fish organs contains seven species of fungal flora such as Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus, Aspergillus glaceus, Cunninghamella bertholatiae, Penicillium sp and Mucor sp. were isolated from gill, muscles and intestinal sample of C. punctatus. This research highlights the quality of Channa punctatus in fish culture farm and to create awareness amid fish eating population.

KEYWORDS

Channa punctatus, Bacterial and fungal investigation

1. INTRODUCTION

Aquaculture is an emerging industrial sector which requires continued research with scientific and technical developments, and innovation. In particular, fish is a rich source of animal protein and its culture is an efficient protein food production system from aquatic environment. An aquatic ecosystem (habitats and organisms) includes rivers and streams, ponds and lakes, oceans and bays, and swamps and marshes, and their associated animals. Aquatic habitats provide the food, water, shelter, and space essential for the survival of aquatic animals and plants. Aquatic biodiversity is the rich and harbors variety of plants and animals-from primary producer's algae to tertiary consumers large fishes, intermittently occupied by zooplankton, small fishes, aquatic insects and amphibians. The study of freshwater habitats is known as limnology. Unfortunately, human infections caused by pathogens transmitted from fish or the aquatic environment are quite

common depending on the season, patients' contact with fish and related environment, dietary habits and the immune system status of the exposed individual. Microbial investigation for characteristics of potential pathogenic microorganisms for fish will allow the application of adequate measures to prevent and control the main diseases limiting the production of fishes. Hence, the present study was examined the microbial population in different parts, gill, muscles and intestine region of *Channa punctatus* from two different fish culture farms.

DESCRIPTION OF THE EXPERIMENTAL PLANT

SCIENTIFIC CLASSIFICATION: *(Channa punctatus)*

Kingdom : Animalia
Phylum : Chordata
Class : Actinopterygii
Order : Perciformes
Family : Channidae
Genus : *Channa*
Species : *punctatus.*
Binomial Name : *Channa punctatus*

Channa is a genus of the Channidae family of snakehead fish. This genus contains about 29 species, but the most well known are probably the northern snakehead (*Channa argus*) and the giant snakehead (*Channa micropeltes*). The diets of various species of *Channa* include fish, frogs, snakes, rodents, birds, and insects. Some can move on land like snakes and breathe air. Species and species complexes of the genus *Channa* are native from southeastern Iran and eastern Afghanistan eastward through Pakistan, India, southern Nepal, Bangladesh, Myanmar, Thailand, Laos, Malaysia, Sumatra, Indonesia, Vietnam, Korea, and China northward into Siberia Of the currently recognized 26 species of *Channa*, 8 species and representatives of 4 species complexes occur in peninsular Malaysia, Sumatra, and/or Indonesia. This species occurs in lakes, ponds, pools and backwaters of large rivers, preferring large, slow-flowing or standing water bodies with vegetation In the wild, and their eggs, which are slightly heavier than water, develop while drifting downstream, kept in suspension by turbulence. *Channa punctatus* effects on their invaded environments are quite variable, with both desirable and undesirable effects.

2. SCOPE OF THE PRESENT STUDY

In the present investigation, it is proposed to study certain bacterial and fungal species were analyzed in different parts namely, gill, muscle and intestine of freshwater fish ***Channa punctatus*** collected from two different pond namely Common pond and Padithurai pond at Thirunageswaram and Mariamman Kovil in Thanjavur district, Tamil Nadu. The study period was during the months of March 2012 – August 2012.

3. MATERIALS AND METHODS

Collection of Water Sample

The data for the present study were collected during March 2012 to August 2012 in the freshwater fish pond sourced from Thirunageswaram and Mariamman Kovil at Thanjavur district, Tamil Nadu, India. The microbial investigation was carried out in various parts of collected fish *Channa punctatus,* namely, gill, muscles and intestine. The microorganisms such as bacteria and fungus in various parts of *Channa punctatus* were identified in the aforesaid study period.

Biochemical tests

Indole test

The test requires sterilized (121^{o}C for 15 min) tryptophan broth. The culture was inoculated in cooled sterilize broth. After 24 hrs of incubation, 0.3 ml of Kovac's reagent was added into the tubes for observe the result.

Methyl Red and Voges Proskauver's Test

The culture was inoculated into the tubes containing sterilized (121°C, 15 min at 15 lbs) MR–VP broth; Tubes were incubated at 37°C for 24 hrs; Add 0.5ml of MR reagent, 0.2ml of VP reagent A and B; After adding reagent to observe the result.

Citrate Utilization Test

Lightly inoculate a pure culture into a tube of sterilize (121°C, 15 min at 15 lbs) Simmon's citrate medium, using needle to stab, then streak the medium; Be careful not to carry over any nutrient material; incubated at 37°C for 24 hrs; After incubation were observe the results.

Urease Test

In this test 20% urea solution added and added on sterilized (121°C, 15 min at 15 lbs/Inch2) media and it was transferred into the slant tubes for slanting position solidification. The culture was inoculated into the tubes and incubated at 37°C for 24 hrs. After incubation observe the result.

Triple Sugar Ion Test

Inoculate pure culture by stabbing and streaking the triple sugar iron (TSI) slant tube; Incubate at 37°C for 24 hrs in an incubator. Read and record reactions;

Catalase Test

A drop of culture broth was placed on clean microscope slide; two or three drops of hydrogen peroxide solution were added to culture broth on the slide for observe air bubble formation.

Oxidase Test

Oxidase disc coated with 1% N–N tetra methylparaphenylene diamine dihydrochloride was placed at the centre of the clean microscope slide. A drop of culture broth was placed over the surface of the oxidase disc for observe the colour change.

Microbial test

The bacterial and fungal species were isolated from the gill, muscles and intestine region of *C. punctatus.*

Serial dilutions of the sample

The nutrient agar medium were prepared, sterilized and poured in sterile Petri plates and allowed to solidify. The 10gm of the sample was added to 90ml of the distilled water in a flask. It was shaking vigorously and 1ml was transferred in test tube containing 9ml of distilled water. The content was mixed well and 1ml was transferred from 10-1 dilution to the next dilutions up to 10-9 dilution. After solidifying, the nutrient agar plates with dilution 10-6 and 10-7 were taken. 0.1ml sample was poured in Petri plates using spread plate technique. The Nutrient agar plates with dilution 10-4, 10-5and 10-6 were taken 0.1ml sample was poured in Petri plates using spread using spread plate technique. The plates were incubated for bacteria at 37°C for 24 hrs.

Streak plate method

In this method a sterilized loop of transfer needle was dipped into a streak plate method offers in the most practical method of obtaining discrete colonies and pure cultures. Suitable diluted suspension of organisms, which is then streaked on the surface of an already solidified agar plate to make a serious of parallel. The aim of this method is to check whether the organisms are growing i.e., growth derived from a single or spore.

Isolation of bacteria by gram staining

Gram staining was done by the method of Hans Christian's gram.

Sabouraud dextrose agar method

The Rose Bengal agar and Sabouraud dextrose agar medium were prepared and sterilized. The medium was poured in sterile Petri plates and allowed to solidify. For primary isolation of the fungus, 10gm of the sample was added to 90ml of the distilled water; shaked vigorously and 1ml was transferred 9ml of distilled water. The

content was mixed well and 1ml was transferred from 10-1 dilution to the next dilutions up to 10-9 dilution. After solidifying, the nutrient agar plates with dilution 10-2, 10-3 and 10-4 were taken. 0.1ml sample was poured in Petri plates using spread plate technique. The Sabouraud dextrose agar plates with dilution 10-2, 10-3 and 10-4 were taken 0.1ml sample was poured in Petri plates using spread using spread plate technique. The plates were incubated at 37°C for 72 hrs.

Lactophenol cotton blue method

One or two drops of lacto phenol cotton blue stain were placed on the clean glass slide. Tuft of fungus suspension was mixed with the stain. A clean cover slip was placed over the preparation without the formation of any air bubbles and observed fungus under the microscope.

4. RESULT AND DISCUSSION

The results and observations of identification and isolation of certain bacteria and fungi in gill, muscle and intestine of the fresh water fish *Channa punctatus* were carried out. The fortnightly data of present study were observed, recorded and tabulated during the month of March 2012 to August 2012. The data for the present study were tabulated.

ISOLATION AND IDENTIFICATION OF TEST ORGANISMS

The bacterial populations were isolated from the gills, muscles and intestinal region of *C. punctatus* which were collected from two different areas**.** The bacterial species namely, ***Achromobacter xylosoxidans*** (Gram +ve), ***Acinetobacter anitratus*** **(Gram -ve),** ***Bacillus subtilis*** (Gram +ve), ***Proteus vulgaris*** (Gram –ve), ***Pseudomonas aeruginosa*** (Gram –ve) and ***Klebsiella pneumonia*** (Gram – ve), were observed.

FUNGAL POPULATIONS

The present study investigates the isolates of fungal flora of fresh water fish *Channa punctatus*. The table clearly depicts the seven species of fungal flora in gill, muscle and intestinal region of fresh water fish *Channa punctatus*. The identified fungus such as ***Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus, Aspergillus glaceus, Cunninghamella bertholetiae, Mucor sp. and Penicillium sp.*** were recorded.

The present study revealed the identified and confirmed bacterial and fungal species, isolated from gill, muscle and intestine region of *C. punctatus*. The fish were collected from two fresh water ponds in Thanjavur district. They were named as **sample 1 and sample 2** and hence used in the results to follow.

Table: 1 showing the Bacterial Population in gill, muscle and intestinal Region of fresh water fish *Channa punctatus* (Sample 1)

Name of the Organisms	Gill	Muscle	Intestine
Achromobacter xylosoxidans	+	+	-
Acinetobacter anitratus	-	-	-
Bacillus subtilis	-	+	-
Proteus vulgaris	-	+	-
Pseudomonas aeruginosa	+	+	+
Klebsiella pneumonia	-	-	-

(+) denote Present (-) denote absent

Table: 2 showing the Fungal Population in gill, muscle and intestinal Region of fresh water fish *Channa punctatus* (Sample 1)

Name of the Organisms	Gill	Muscle	Intestine
Aspergillus Niger	-	+	-
Aspergillus fumigatus	+	+	-
Aspergillus glaceus	-	-	-
Aspergillus flavus	-	+	-
Cunninghamella bertholatiae	+	+	-
Penicillium sp.	+	-	+
Mucor sp	+	+	-

(+) denote Present (-) denote absent

Table: 3 showing the Bacterial Population in gill, muscle and intestinal Region of fresh water fish *Channa punctatus* (Sample 2)

Name of the Organisms	Gill	Muscle	Intestine
Achromobacter xylosoxidans	+	+	-
Acinetobacter anitratus	-	-	+
Bacillus subtilis	-	-	-
Proteus vulgaris	-	+	-
Pseudomonas aeruginosa	+	+	-
Klebsiella pneumonia	-	-	+

(+) denote Present (-) denote absent

Table: 4 showing the Fungal Population in gill, muscle and intestinal Region of fresh water fish *Channa punctatus*(Sample 2)

Name of the Organisms	Gill	Muscle	Intestine
Aspergillus niger	-	-	+
Aspergillus fumigatus	+	-	-
Aspergillus glaceus	-	+	-
Aspergillus flavus	-	+	-
Cunninghamella bertholatiae	+	-	-
Penicillium sp.	+	-	-
Mucor sp	-	+	+

(+) denote Present (-) denote absent

5. CONCLUSION

Based on the present study, it may be concluded that the microbial characteristics were found to be optimum level when examined. The microbial characteristics were found to be differing in each organ of two fish samples according to the ecological influence. So, we conclude that the proper management and monitoring the levels of microbes plays the vital roles in the maintenance of the bacterial and fungal outbreaks in the culture ponds.

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