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Research Article

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Microflora in different parts of *Cobitis elongata* (Balkan loach) collected from Anathalai river in Rajapalayam, Western Ghats of India.

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Abstract

The present investigation was on testing for the microflora in different organs of *Cobitis elongate* collected from anathalai river and western ghats of india. Ten fish samples of size weighing about 3±0.5g were used for the microbial study.*E.coli, Salmonella sp, Bacillus spp, Proteus spp, Vibrio spp, Aeromonas spp, Pseudomonas spp, Achromobacter spp*, un identified bacteria were distributed in different parts of the fish with varying percentages occurrence.The total viable count (TVC) of edible portion was1.0×10^{l} to11×10^{l} CFU/g where as it was 2.0×10^{3} to 8.0×10^{4} CFU/g in surface, 2.8×10^{3} to 8.0×10^{4} CFU/g in gills and 3.0×10^{6} to 4.0×10^{7} CFU/g in gut sample respectively.Nutrient agar was used for the isolation of bacteria and 110 strains were obtained which were obtained which were identified up to genus level using biochemical identification methods as per Bergey's manual of determinative bacteriology.

Keywords: *Cobitis elongata*, Microflora, Total viable count, *E. coli, Salmonella spp, Bacillus spp, Proteus spp, Vibrio spp, Aeromonas spp, Pseudomonas spp, Achromobacter spp*

Introduction

Many species are predominantly consumed as protein foods worldwide. Fish contains vitamins and minerals, which are essential proteins for the body. Fish offers very significant minerals and micronutrients for physical growth and healthy diets. There are 96 recognized species are available in the genus *Cobitis*. It is a freshwater fish found in paddy fields, ponds, rivers. *C. longata* fishes have been giving growth to children. *C. longata* helps to control blood flow, prevent blood clots and maintain a healthy heart. Vitamin E is present in this fish, helps to improves skin health. It is in the least concern listed by IUCN (International Union for Conservation of Nature). Red list threatened species. It needs ingredients such as blood worms, microalgae and organic materials in the sand. From January to July the breeding season and from April to June the spawning season. Air is evacuated via the anus during intestine breathing. It uses a sophisticated branching device for filtering feed and Sediments are highly oxygenated. This calcium-based fish with many minerals is useful for cardiac issues and helps to treat cold, it is a fish with a rich source of fatty acids omega-3 and omega-6. These fish produce eggs from 300 to 1500, and larvae from four to six days. That fish is around the mouth with 6

barbells however, several studies have also shown that the intestinal content of freshwater fish in particular is far more diverse than had previously been assumed (Ringo et al. 2006), (Cantas et al., 2012) Allochthonous microbiota intestinal tract architecture is autonomous and affected by environmental variables such as temperature, salinity, pH, etc (Liu et al.,2008). Food comfort, shape, and changes in a fish gut may alter bacterial diversity (Ringo & Strom). Bacterial colonization in the early stages (Ringo et al., 1995) and the reflection of surrounding water were demonstrated in their variety of the fish bowel's microbiota. Fishes, like human beings and other animals, play a crucial role in nutritional metabolic homeostatic and immunological defense (Sullam et al., 2012). Fish origin more than 600 million years ago comprises over half of all vertebrates (Nelson, 2006). The microbial intestinal flora of fish was investigated by several scientists for not similar goals. Included are the microbial spoilage description (Josep et al. 1988), the relationship between environment and micro-field fish (Horsely 1973), change in fish shape (Allen et al. 1973), and micro-flower as a foodstuff to the fish (Kamjunke et al. 2002). (Span et al., 1989). For these reasons, it is vital to investigate intestinal bacterial flora. This study is aimed at isolating and estimating the bacteria flora from the Gills and Gut.

Materials and Methods

Site

The fish were taken in Rajapalayam, Western Ghats of India, from the Anathalai river (9,4965 N, 77,5276 E). Wild squirrel Sanctuary is located near Rajapalayam, Tamilnadu, India.

Sample collection

The drift net was used to capture the fish sample. The fish were caught at 3 feet deep, placed in sterile polythene bags and transferred securely to the Fish Microbiology Laboratory for further study at CAS Marine Biology, Annamalai University, Parangipettai.

Sample process

For further analysis, Adapting aseptic procedures, samples were taken from surface, edible portion, gills and gut.

Isolation of microbes

The samples of fish from *Cobitis elongata* are collected in rajapalayam, southwest of India from anathalai river. The stomach and gill section of fish separated from the fish in order to perform serial dilution amounts to up to $10^{-}1$, further dilution is up to $10^{-}8$. The petriplate was aerobically incubated to count bacterial colonies for 24 hours at 37 °C. The well grown and isolated colonies with distinctive morphology were noticed after the plate had been taken from the incubator and then selected for further analysis.

Screening

The isolated bacteria are screened in the necessary number with nutrient agar. The incubator was then used for 24-48 hrs for screening. The test tube with the appropriate quantity is collected for further analysis for the nutrition agar.

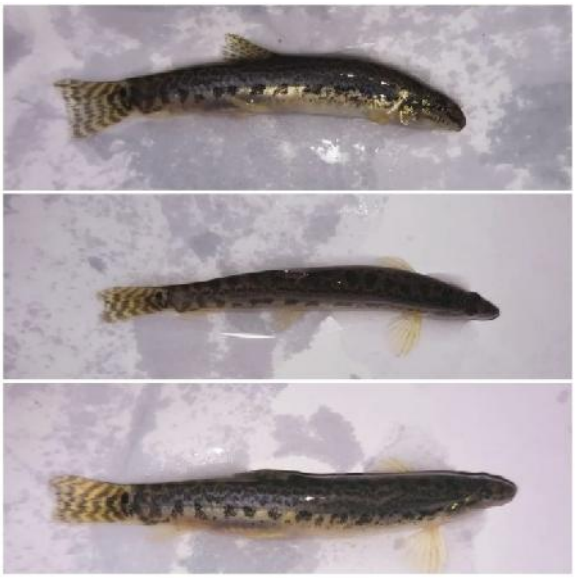
Identification and characterization of microbes

Size, structure, adaptability carried out by the viewing of petri-plate bacteria are indicated on the morphological microbial characters. Displays biochemical properties of Indole output (gram positive and gram-negative organism identification and differentiation) (Indole test), Methyl red test (gain of stable glucose fermentation acids), Voges - Proskauer reaction – to utilise glucose citrate (It breaks down citrate to oxaloacetate and acetate). Gram dye (different from their diverging cell wall-constituent test oxidase are many major groupings of bacteria (identity bacteria that can yield cytochrome c oxidase), catalase test (it breaks down of hydrogen peroxide into oxygen and water), coagulase test (yield of coagulase enzyme) used as standard for the identification of species by Bergy’s manual of systematic Bacteriology (Hotet et al., 1994)

Results and Discussion

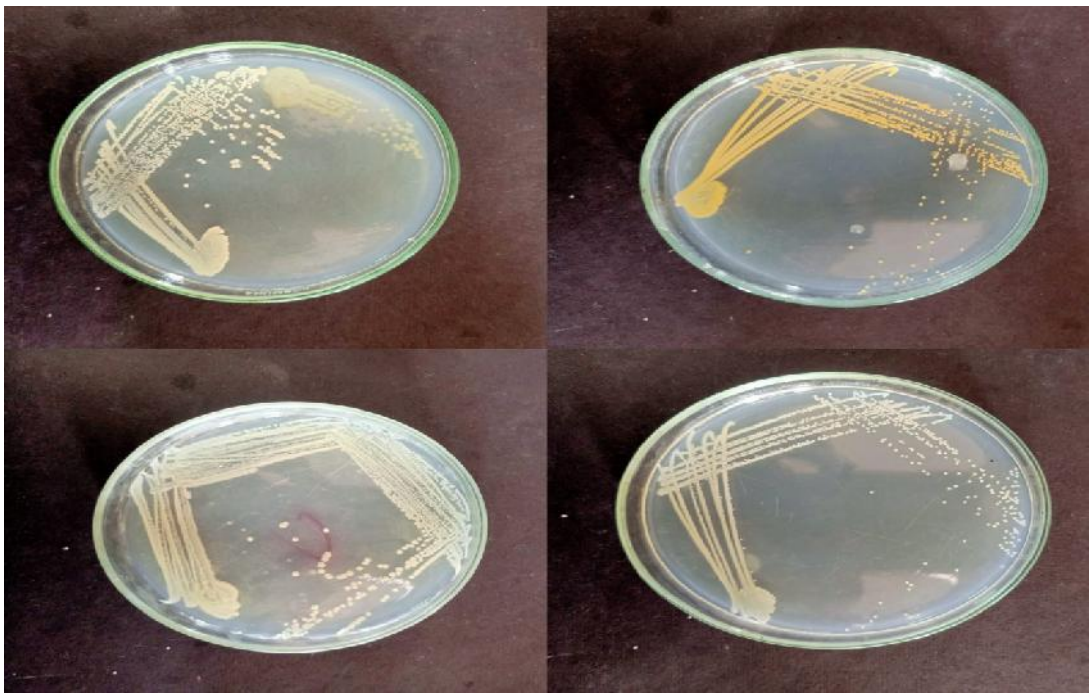
Sample collection

The Fish sample *Cobitis elongata* are collected from anathalai river and Rajapalayam, South Western Ghats of India.Female fishes are live up to five years and male fishes up to three live. This fishes are threat to humans Harmless. (Froese et al., 2016). Fish take feeds like Cladocera, Chironomidae (larvae and pupae), Copepods, Ostracods other macro invertebrates and detritus.



Kingdom: Animalia
Sub kingdom: Bilateria
Infrakingdom: Deuterostomia
Phylum: Chordata
Subphylum: Vertebrata
Infraphylum: Gnathostomata
Superclass: Osteichthyes
Class: Actinopterygii
Subclass: Neopterygii
Infraclass: Teleostei
Superorder: Ostariophysi
Order: Cypriniformes
Superfamily: Cobitoidea
Family: Cobitidae
Subfamily: Cobitinae
Genus: *Cobitis*
Species: *Cobitis elongata*

Isolation and identification of microbes



Fish are known to contain infectious pathogens in a natural environment (Pillay, 1990). A limited numbers of fish species have been identified for bacterial flora isolated from eggs, skin, gills, and intestines. The range of isolated bacterial stages is generally related to the fish's aquatic habitat and changes with conditions such as habitat, salinity and bacterial loads in the water (Cahill et al., 1990). Fish digestive system micro flora is an important factor in the development of disease resistance by generating antibacterial compounds to prevent pathogens from entering an organism (Sugita et al., 1988). Micro flora of the skin, gills, digestive systems, etc. have been identified as fish with their microbial variety, frequently reflecting allochthonous or autochthonous bacterial populations in the surrounding water. (Froese et al., 2021)In this research isolation of bacteria on nutrient agar after incubation the density was calculated on Total viable count Table-2. The present study result showed that fish Gill and Gut. (Table-1or Fig-1) identify the genus after the biochemical analysis (Table-3)

Table -1. Persentage occurrence of bacterial flora in different body parts of *Cobitis elongata*

S.No	Bacteria Name	Edible	Surface	Gills	Gut
1	*E.coli*	0%	3.2%	4.8%	8.0%
2	*Salmonella spp*	0%	8.0%	4.8%	9.6%
3	*Bacillus spp*	0%	8.0%	8.0%	8.0%
4	*Proteus spp*	0%	9.6%	3.2%	9.6%
5	*Vibrio spp*	0%	9.6%	9.6%	10.2%
6	*Aeromonas spp*	0%	1.6%	9.6%	10.2%
7	*Pseudomonas spp*	1.6%	6.4%	0%	4.8%
8	*Achromobacter spp*	3.2%	4.8%	0%	4.8%
9	Un identified	3.2%	6.4%	8.0%	8.0%

Table 2. Total viable count (TVC) of bacterial count in *Cobitis elongata* (in CFU/g)

S.No	Sample Name	Minimum	Maximum
1.	Edible portion	1.0×10^{l}	11×10^{l}
2.	Surface	2.0×10^{3}	8.0×10^{4}
3.	Gills	2.8×10^{3}	8.0×10^{4}
4.	Gut	3.0×10^{6}	4.0×10^{7}

Table: 3. Biochemical analysis

Test /pathogens	*Pesudomonas spp.*	*E.coli*	*S.aureus spp.*	*Salmonella spp.*	*Bacillus spp.*	*Vibrio spp.*	*Klebsilla spp.*
Indole	-ve	+ve	-ve	-ve	-ve	-ve	-ve
MR-Test	-ve	+ve	+ve	+ve	+ve	+ve	-ve
VP-Test	-ve	-ve	+ve	-ve	+ve	-ve	+ve
Citrate	+ve	-ve	-ve	-ve	+ve	+ve	+ve
TSI	-ve	-ve	-ve	+ve	-ve	-ve	-ve
Gram	-ve	-ve	+ve	+ve	+ve	-ve	-ve
Oxidase	+ve	-ve	-ve	-ve	+ve	+ve	+ve
Motility	+ve	+ve	-ve	+ve	+ve	+ve	-ve

Conclusion

Fish is in high demand as food, food additives, and supplements as they are a rich source of carbon, proteins, vitamins, and minerals. In this work, the isolation of and identification of the micro flora from the different organ of culturable fresh water fish *Cobitis elongata* have been shown to be a source of microorganisms of public health significance for the gills and gut of fresh water fish. If not properly cooked, fresh fish might induce food borne illnesses by eating polluted water. As the relationship between pollution and the diversity of microbiomas separated from fish exists, it is crucial that the fish habitats are properly cared for and publicly healed.

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Compliance with ethical standards

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest

The authors declare no conflict of interest.

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