



**Special Issue (NSAZ-2022)**

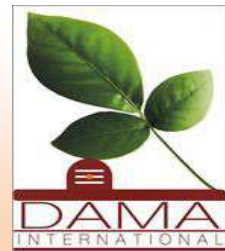


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# **Applied Zoology, Profitable Animal Production, and Health: Current Status and Future Progress (NSAZ-2022)**

**Organized by**

**Department of Zoology and Fishery Science,  
Rajarshi Shahu Mahavidyalaya (Autonomous),  
Latur- 413531, Maharashtra**

**On**

**23<sup>rd</sup> & 24<sup>th</sup> September- 2022**

**Volume 11, Issue 1(2022), ISSN: 2319-474X (p); 2319-4758 (e)**

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(Conference Special Issue)**

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**Published By:**

Publisher: DAMA International

E-ISSN: 2319-4758

Print ISSN: 2319-474X

URL: [www.sciencejournal.in](http://www.sciencejournal.in)

Chief Editor: Dr Laxmikant B. Dama

Contact email: [trendsres@gmail.com](mailto:trendsres@gmail.com)

Address: 15 B. Vijaynager, Z.P. Colony, Bijapur Road, Solapur (M.S.), India.

Country: India

**Volume 11, Issue 1(2022)**

**ISSN:** 2319-474X (p); 2319-4758 (e)

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## Isolation, Identification and Biochemical Characterization of Probiotic Bacteria Isolated from Fresh Water Fishes of Gharani River

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### ABSTRACT

The microbiota plays a fundamental role on the induction, training, and function of the host immune system. It is recognized that the gastrointestinal microbes of homeothermic animals serve several functions, such as digestion and development of the mucosal system, angiogenesis, and as a protection barrier against disease. In present investigation some Probiotics bacteria were isolated from common carp gut further The morphological characteristics of bacterial isolates were recorded. The bacterial isolates were also analyzed for various biochemical tests like oxidase tests, catalase test, motility test, indole producing test, and carbohydrates fermentation (arabinose, fructose, galactose, lactose, mannitol, salicin, sucrose and trehalose). These characteristics are considered for the species level identification of bacterial isolates. Among the 90 isolates, cultural characteristically distinct 8 isolates were from *Cyprinus carpio*, isolates morphologically characterized, were gram-positive, non-motile, the shape of the isolates were varied rod, stout rod, bacilli, short bacilli. total isolated colony are grouped as 10 sample from *Cyprinus carpio* which named as CP-A to CP -J by considering first letter of genus *Cyprinus* and P for probiotic .

**Key word:** Gharni River, Probiotics, Isolation, Major Carp.

### 1. Introduction

Fish disease is a common problem encountered even in these modern days, which is said to be the period of scientific development and awareness of hygiene. Vaccines are being developed and marketed and they generally cannot be used as a universal disease control measure in aquaculture. Juvenile fish are fully immune-competent and do not always respond to vaccination. Vaccination with the help of injection, sometimes are the only effective path of vaccine delivery, its impractical when supplied to small fish or a large number of fish. This situation is avoided by an alternative in the production system through the use of beneficial bacteria to fight against pathogenic bacteria i.e., through the use of Probiotics which is an acceptable practice in aquaculture.

The intestinal micro organisms investigations of fish have been motivated by the possible use of the biologically significant bacteria as supposed Probiotics [1] Attempts carried out on the intestinal micro organisms in fishes have shows that bacterial inhabitants influence the establishment of pathogenic microorganisms in the intestinal tract [2,3]. Further the focus had been given on the stability of the intestinal flora in intestine .as the pathogens enters in intestine the

natural resistance of fish to infections produced by bacterial pathogens in the digestive tract (Ringo *et al.*, 2003). Global aquaculture production was approximately 121365 tons while capture production was 23905 tons for grey mullet of the world in the year of 2002 [4].

The use of commercial probiotics made in market used to feed fish is relatively fruitless since most of the commercial preparations are obtained or prepared by using probiotic source from the strains from non-fish sources.

These micro organisms unable to survive or viable at high cell density in the gut environment of fish during the active growth phase of the fish [5]. For this reason, there is elegant logic in isolating assumed probiotics from the host in which the probiotic is intended for use. Such strains should perform better because they have already adhered to the gut wall of the fish. consequently, they are well-adapted to compete with pathogens for nutrients. Presumably, strains that develop dominant colonies in the fish intestine are good candidates for preventing the adhesion of pathogens to the gut wall. Biological control has been described as the utilization of natural enemies to reduce the damage caused by noxious organisms to tolerance limits [6] or more precisely, the control or regulation of pest populations by natural enemies [7] Strictly speaking, probiotics ought not to be classified as a biological agent, since a Probiotics microorganism doesn't necessarily attack the noxious agent (pathogen).

Keeping these views in the mind the present research work is designed to assess prospective probiotics bacteria from the gut region of the fresh water fishes of Gharni River

## **2: Materials and Methods**

### **2.1. Sample collection and fish acclimatization**

Fresh water fishes were collected from Gharni River (Latur). The healthy Fishes were isolated from infected ones and used for the identification of probiotic bacteria Fishes Were Selected randomly using a cast net. All healthy fishes were then examined at the laboratory (RSML, Latur 18.4088° N, 76.5604° E) for detail study of probiotic bacteria.

### **2.2. Isolation of culturable intestinal micro organisms.**

The culturable micro organisms was isolated from the intestinal content of freshwater fishes (viz. *Catla catla*, *Labeo rohita*, *Cyprinus carpio*) at Gharni; the river has its origin near wadval and flows through Chakur taluka in latur district . The acclimatized live fish were brought to the laboratory alive further these all fishes were sacrificed. The belly region was sterilized by using 70% ethanol and the gut region was aseptically removed from the fish abdominal cavity. The healthy fingerlings of both the species were selected and sacrificed in the laboratory; the bacterial samples were isolated by using sterile swabs on MRS agar, MRS broth, TCBS agar, and SAA. The agar plates and broth were incubated at 37°C for 24-48 hours after that the bacterial colonies had growth on plates was examined for further characterization and identification.



## **II: Endospore staining**

The endospores were isolated by Schaeffer–Fulton stain technique. Bacterial strains placed on a slide heat well to fix it. Slide allowed to steam in water bath covering porous paper over it. Malachite green applied to the slide, which help to penetrate the walls of the endospores. After five minute, slide was removed from the steam, and the paper towel was removed. it allows for cooling then slide was rinsed thoroughly by using water up to thirty second. Slide was stained by diluted safranin for two minute; it stains other micro organic bodies red or pink. Slide then rinsed again, blotted dry with the help of bibulous paper. Allowed it for drying. Prepared slides were observed under light microscope. Endospores observed were green in color. Bacterial bodies look red in color.

## **III. Motility test**

Motility test was done by preparing a dilute suspension of fresh bacterial culture on a clean glass slides further it had covered by using cover slip. Prepared slide were observed under microscope having camera attached to the computer monitor (MIPS-OLYMPUS)

## **IV: Morphological /Biochemical identification of the potential probiotic strain**

The morphological characteristics of bacterial isolates were recorded. analyzed for various biochemical tests *viz.* catalase test, oxidase tests, motility test, indole producing test, and carbohydrates fermentation (Arabinose, Fructose, Galactose, Lactose, Mannitol, Salicin, Sucrose And Trehalose). These characteristics are considered for the species level identification of bacterial isolates. entire biochemical analysis further were confirmed by using Enterobacteriaceae as well as Manuallt Kit.

## **3. Results**

From the 8 gut samples collected, 90 isolates were isolated. The maximum *Lactobacillus* isolates were observed in *Cyprinus carpio* isolates as compare with the *catla catla* and *labeo rohita* which indicates that the probiotic bacterial distribution may be varies according to the generic variation of fishes.

Among the 90 isolates, cultural characteristically distinct 8 isolates were from *Cyprinus carpio*, isolates morphologically characterized, were gram-positive, non-motile, the shape of the isolates were varied rod, stout rod, bacilli, short bacilli. total isolated colony are grouped as 10 sample from *Cyprinus carpio* which named as CP-A to CP -J by considering first letter of genus *Cyprinus* and P for probiotic .

## **Primary and biochemical identification of CP-C**

The strain CP-C was Gram Positive, Aerobic, Endospores forming and motile, while spores are Ellipsoidal and central in position CP-C was positive for the Vogues-Proskaeur reaction and negative for the methyl red test. The able strain produced Catalase, Indole and capable of utilizing citrate and reduce nitrate to nitrite. It is negative for Oxidase. It cannot utilize Hydrogen Sulphide. The sugar fermentation test confirms the capability of CP-C to utilize Glucose, Maltose, Sucrose, Mannitol, and Fructose as the Carbohydrate source for their growth (Table 1)

**Table 1: Biochemical identification of CP-C isolate**

S.No.	Characteristic features	Observation
1	Colony shape	Round
2	Colony margin	Entire
3	Colony texture	Moist
4	Colony color	White, shiny
5	Gram staining	+
6	Shape of cell	Rod shape
7	Endospores formation	+
8	Cell size	1.5~3.0 $\mu\text{m}$
9	Catalase	+
10	Oxidase	-
11	Methyl red test	-
12	Voges-proskauer test	+
13	Indole	+
14	Triple sugar ion agar	No $\text{H}_2\text{S}$ production
15	Citrate utilization	+
16	Urea	-
17	Sugar utilization test	
	Glucose	+ve
	Sucrose	+ve
	Fructose	+ve
	Lactose	-ve
	Mannitol	+ve
	Mannitol	+ve



beneficial effects of probiotics use in fish aquaculture are growth performance improvement the fish intestine is a favorable ecological niche for microorganisms, the colony is more than in the surrounding water. In the present investigation, isolation of effective probiotic bacteria from the intestinal tract of *Cyprinus carpio* indicate diverse occurrence of bacterial strains.

Earlier studies suggest that, microorganisms form an important dietary component for deposit feeding animals. Near about 15–30% of organic basis in the stomach of *Cyprinus carpio* has been contribute by microorganisms. Such occurrence of the diversified population of bacteria also indicates their possible role in the breakdown of plant matter as the gut content of mullets also harbour sizable quantity of plant matter. Similar studies on the intestinal content of mullets were carried out by Moriarty . He reported that the muramic acid in the gut of fish was high; indicating ingestion of bacterial strains associated with sediments thus forming an important component of detritus based food chain. He also reported that the microbial community in the intestinal tract comprises of both gram positive and gram negative bacteria. Occurrence of diseases in fish culture systems poses a great threat to fish farmers, in terms of heavy loss. Biochemical identification of CP-C were confirmed s *Lactobacillus acidophilus*. coastal fishes bivalves [9]

## Discussion :

The present study concluded that the isolates were normal in microflora in fresh water fishes *Catla catla*, *Labeo rohita*, From the above said results were compare to the Bergey's Manual of Systematic Bacteriology, Sec. Ed., Vol.3 [9] the Isolate – 1 is *Bacillus spp*, These isolates may have high potential to adhere to fish mucus. herefore, it seems that isolated organisms have high potential probiotic, so these organisms are further studied by molecular characterization.

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