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# Microbial isolation from the gut of *Channa punctata* (Goroi fish) and study of their glycosidic activity

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**Abstract**
Microbial isolation from the gastro-intestinal tracts (only the midgut and hindgut) of *Channa punctata* have been performed to detect their importance in the digestion process of the experimental fish. Three types of microbes (bacteria and fungi) were separated from the GI tract of *Channa punctata*. Pure bacterial cultures were grown on Nutrient Agar (NA) media and were identified as Gram –ve cocci shaped bacteria through morphological test. While pure cultures of fungi were grown on Potato Dextrose Agar (PDA) media and were identified as *Penicillium sp*. and white sterile mycelia with no spore formation through morphological test. Intestinal isolates were checked for extra-cellular enzyme producing abilities by plate screening method. Amylolytic and cellulolytic microbes were found in the experimental fish's gut. Cellulase activity was shown by all microbial isolates, while amylase activity was exhibited by the bacterial isolates and *Penicillium sp*. isolated from the GI tract of *Channa punctata*. Thus, from the experimented study, it can be concluded that, there would be a possibility of the digestive enzymes to be supplemented by the microbes of the experimental fish's gut, which would be helpful in the preparation of diets, constituting or including the feeds that are non-conventional ones. Moreover, the microbes of the fish gut may help in the digestion process of the fish, and this study have isolated and identified the enzyme-producing microbes.

**Keywords:** Gastrointestinal, nutrition, microbes, pure cultures, plate screening, aquaculture

## 1. Introduction
*Channa punctata* is a species of snakehead fish, which are distributed in the Indian Subcontinent and nearby areas of Afghanistan, Pakistan, India, Sri Lanka, Nepal, Bangladesh, Myanmar and Tibet. They live in swamps, ponds and brackish water systems and normally grow up to 15 cm in length. They are listed as Least Concern in IUCN. It is an important commercial fish for aquaculture, having high food value and used as an ornamental fish.

Fishes, as they are aquatic, therefore they are repeatedly susceptible to micro-organisms present in the aquatic surroundings. As the fishes are rich in nutrients, the gastro-intestinal tract of fishes grants an advantage for the growth of the micro-organisms. According to Cahill 1990 [2]; Horsley 1977 [5], the intestinal tract of fish is generally colonized by a good number of heterotrophic bacteria and ecological studies on the gut micro biota of fish have been also presented by them. The gut bacteria having roles in nutrition, growth and disease susceptibility in fish has been established for homoeothermic species by Floch *et al*. 1970 [3]. For the intensive rearing of fish, an understanding of the persistent micro-organisms is very much important as they might help to improve the feeding and other conditions of the fish. Microorganisms are rich in source of many new metabolites, which have a wide range of biological functions and important practical applications. The protective effect of the gut bacteria are termed as bacterial antagonism is a significant component of host defence against pathogens. Gut bacteria shows antagonistic activity against pathogenic bacteria and fungi.

According to Bairagi *et al*. 2002 [1]; Ghosh *et al*. 2002 [4]; Saha *et al*. 2006 [10], in all higher vertebrates, digestion of food material occurs through the dual action of the enzyme system of the host itself and also contributed by the intestinal micro biota. There is paucity of information in the field of enzyme producing bacteria in the fish gastrointestinal (GI) tract. There needs a proper investigation of the gastro-intestinal tract microbes in order to have an understanding of the digestive enzyme, which has the ability of producing, that will help to determine their possible functions in fish nutrition and digestion. Therefore, an effort has been done in the present experimental study to isolate the enzyme-producing microbes from the

gastro-intestinal tracts of *Channa punctata*, identification of the isolated microbes and determining the glycosidic activity by assessing for amylolytic and cellulolytic producing microbes.

## 2. Materials and Methods

Taxonomic position of experimental fish
Kingdom: Animalia
Phylum: Chordata
Class: Actinopterygii
Order: Perciformes
Family: Channidae
Genus: *Channa*
Species: *C. punctata*

**2.1 Collection of sample:** Fishes (*C. punctata*) of moderate sizes were collected from Lalganesh fish market, one day before prior to its dissection. After collection of fishes, they were kept in well maintained aquarium containing water and left for 24 hours of starvation. The starvation was done so as to make their gastro-intestinal tract clear and also to eradicate or remove the microbes that were transient in nature.

**2.2 Dissection of midgut and hindgut of the experimental fish**

After starvation period, the fishes were dissected aseptically and the GI tracts (midgut and hindgut) were removed carefully, separated and cut into pieces.

**2.3 Preparation of experimental fish's gut stock**

The cut pieces of midgut and hindgut were first sterilised with 70% alcohol for 3 minutes. Then the pieces were crushed with 10 ml of sterile distilled water (sterile distilled water was obtained by autoclaving the distilled water) in a sterile mortar-pestle. Now, this stock was kept in a glass vial. The stock was used for streaking purpose in NA and PDA plates.

**2.4 Isolation of gut microbes from experimental fish's gut stock**

From the fish's gut stock, with the help of sterile inoculating loop, streaking was made on NA and PDA plates and kept for 1-2 days and 3-4 days respectively at 37 °C in the incubator.

**2.5 Preparation of culture media**

**2.5.1 Preparation of Nutrient agar (NA) for bacterial growth**

150 ml of Nutrient agar was prepared. 4.65 g of nutrient agar was dissolved in 150 ml of distilled water and was autoclaved for sterilization. After autoclave, the media was poured on sterile petri plates under the laminar air flow. The plates were then kept to solidify. After solidification, one plate was kept in the incubator for contamination check.

**2.5.2 Preparation of Potato Dextrose Agar (PDA) for fungal growth**

150 ml of PDA media was prepared. 5.85g of PDA was dissolved in 150 ml of distilled water and were micro waved for proper mixing of the PDA. After that, the PDA was autoclaved for sterilization. After autoclave, an antibiotic named Ampicillin was added to the PDA media and mixed well. Addition of antibiotic to PDA resists the growth of bacteria. The media was then poured on sterile petri plates under the laminar air flow. The plates were then kept to solidify.

**2.6 Enzymatic activity test of isolated microbes by plate screening method**

**2.6.1 Amylolytic Activity Test**

Amylolytic activity test for bacterial and fungal isolates were performed by plate screening method. In the experiment, isolates were transferred to starch agar plates containing 2% starch, 5% peptone, 5% beef extract, 5% NaCl, 20% agar powder at pH 7. Culture plates were maintained for 72 hours at 30 °C. After that, the plates were taken out of the incubator and flooded with 1% Lugol's iodine solution to check if there is any formation of clear zone around the fungal or bacterial colonies against a blue background, which was taken as a positive test for amylase activity [7]. The clear zones which were formed was observed by naked eyes and documented by taking photographs.

**2.6.2 Cellulolytic Activity Test**

For detecting the extra-cellular cellulase activity of bacterial and fungal isolates, plate screening method was done. In the experiment, isolates were transferred into a media containing each of 2% CMC, 5% beef extract, 5% NaCl, 5% peptone and 20% agar powder at pH 7 and kept for 6-8 days at 28 °C. After that, the plates were taken out of the incubator and flooded with 1% Congo red dye. Formation of clear transparent area in the fungal or bacterial colonies against the background, was taken as a positive test for cellulase activity [9]. The clear zones which were formed was observed by naked eyes and documented by taking photographs.

**2.7 Identification of bacteria by Gram staining**

The grouping of two principle groups of bacteria, i.e., Gram (+) bacteria and Gram (-) bacteria, is based on the reaction of bacteria to Gram's stain (Christian Gram, 1884). The pure cultures of bacterial colonies were first taken on a clean glass slide and smears were prepared by using another slide, in such a way that, the second slide was inclined to the first one at an angle 45° to each other at the base, and then smearing was done by sweeping it with the help of the second slide. Secondly, the smears prepared were air dried and then heat fixed by using a flame. After heat fixing, the slides were allowed to cool down. Then, the smears were flooded with crystal-violet dye and allowed to stand for one minute. After one minute, the slides were washed with distilled water, and treated with Gram's Iodine Mordant for 1 minute. After one minute, again the slides were washed with distilled water. Now, the smears were decolorized by using Gram's decolourizer. Then, counter staining were performed using Safranin for one minute. Again the slides were washed with distilled water and air dried. Finally, they were observed under compound microscope, and also documented by taking photographs.

**2.8 Fungal identification**

A small portion of the pure cultures of fungal colonies were taken on a clean slide and stained with Aniline blue. After that, one drop of lactophenol was added and covered it with a coverslip. Then, the slide was observed under microscope.

## 3. Results

**3.1 Bacterial Colonies:** After streaking the experimental fish's gut stock on the NA plates and keeping it in the incubator for 1-2 days, it was observed that bacterial colonies appeared on the plates (Fig 1). As a result, the bacterial colonies were separated and streaked separately by using an inoculating loop on NA plates to obtain pure cultures (Fig 2).

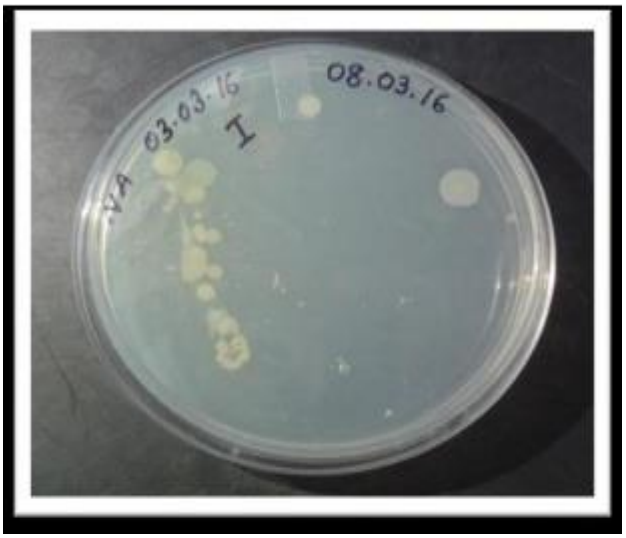


**Fig 1:** Bacterial colonies (1) obtained on NA plates from experimental fish's gut

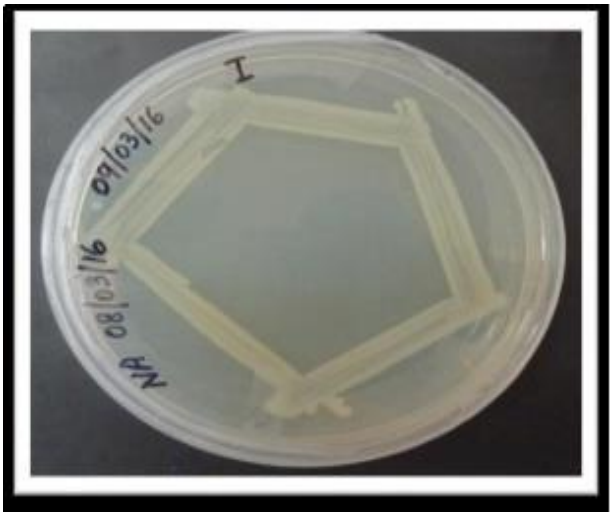


**Fig 2:** Bacterial pure culture obtained from the above bacterial stock (1)

### 3.2 Bacterial identification

By Gram's staining technique, the bacteria were identified as Gram –ve and the shape of the bacteria was coccus (Fig 3).

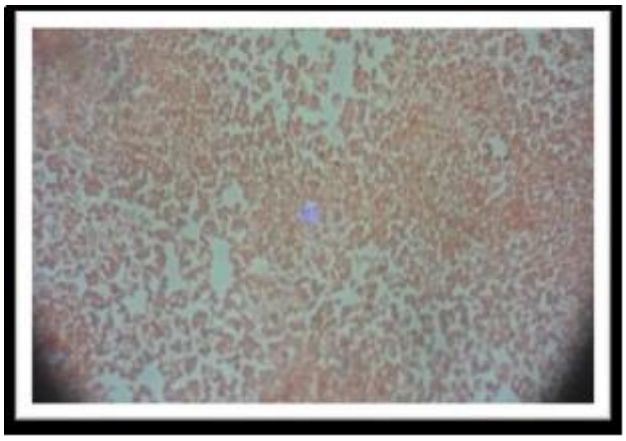

**Fig 3:** Gram –ve cocci shaped bacteria observed under light microscope (40X).

**3.3 Fungal Colonies:** After streaking the experimental fish's gut stock on the PDA plates and keeping it in the incubator for 3-4 days, it was observed that fungal colonies appeared on the plates (Fig 4 & 5). The fungal colonies were isolated and streaked separately on PDA plates to obtain pure cultures (Fig 6 & 7).

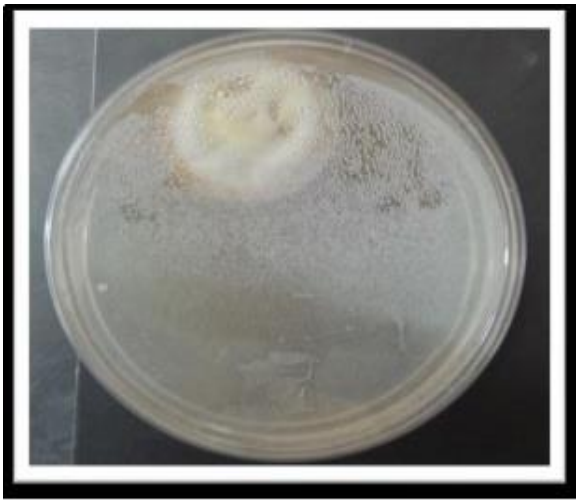

**Fig 4:** Fungal colonies (2) obtained on PDA plates from experimental fish's gut

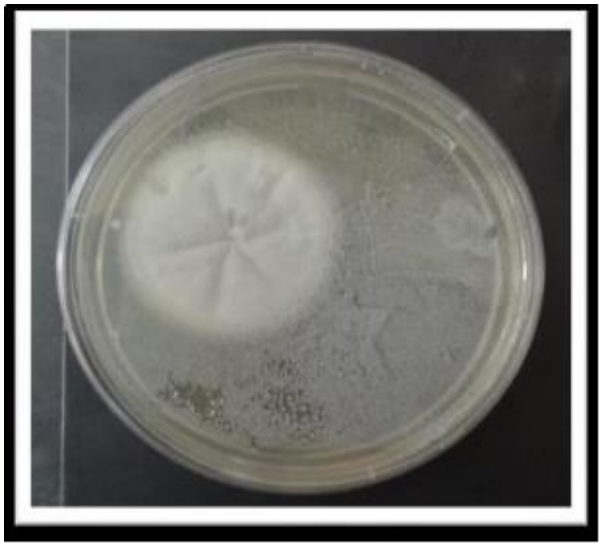

**Fig 5:** Fungal colonies (3) obtained on PDA plates from experimental fish's gut


**Fig 6:** Fungal pure culture obtained from the above fungal stock (2).

**Fig 7:** Fungal pure culture obtained from the above fungal stock (3).

### 3.4 Fungal identification

The fungi observed were *Penicillium sp*. (Fig 8) and White sterile mycelia & With no spore formation (not identified) (Fig 9).

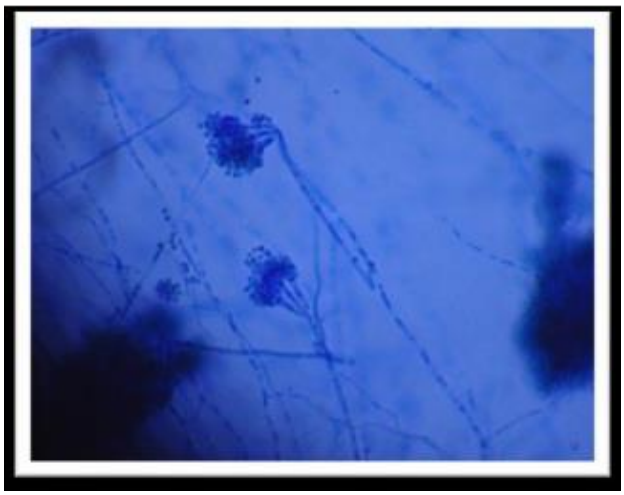

**Fig 8:** *Penicillium sp*. observed under light microscope [40X] (obtained from No.2 fungal colony)

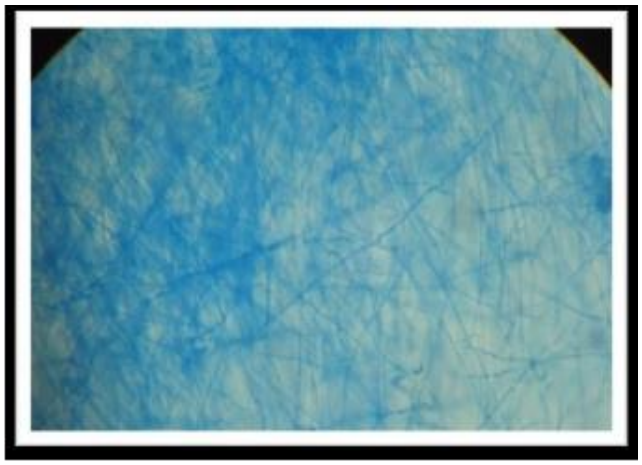

**Fig 9:** Sterile mycelia with no spore formation was observed under light microscope [40X] (obtained from No.3 fungal colony)

### 3.5 Enzymatic activity test by plate screening method

**3.5.1 Amylolytic Activity Test:** After keeping the starch agar plates in the incubator for 72 hours, i.e., for 3 days at 30 °C, the plates were taken out of the incubator, and then flooded with 1% Lugol's iodine solution. Clear zones were formed around the bacterial and fungal colonies, which was taken as a positive test for amylase activity (Fig 10, 11 & 12).

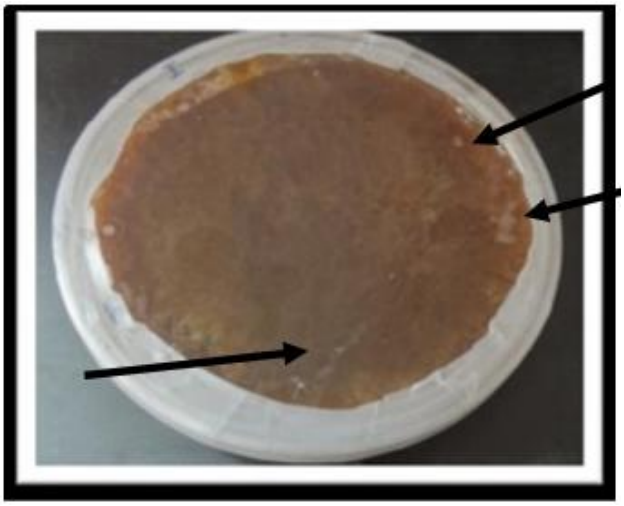

**Fig 10:** Starch agar plates for Gram –ve cocci shaped bacteria showing clear zones formed around the bacterial colonies, is a positive test for amylase activity.

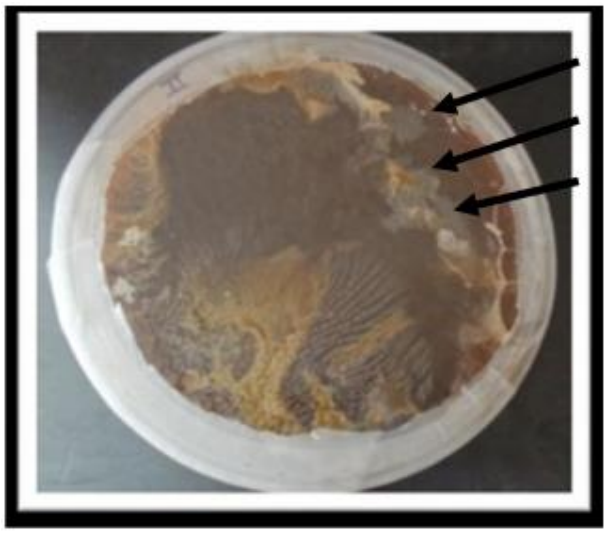

**Fig 11:** Starch agar plates for *Penicillium sp*. showing clear zones formed around the bacterial colonies, is a positive test for amylase activity.

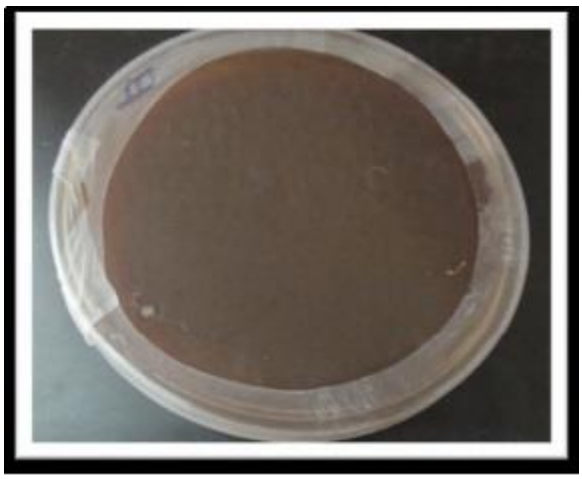

**Fig 12:** Starch agar plates for sterile mycelia with no spore formation. Here, clear zones were not formed around the fungal colonies and was taken as a negative test for amylase activity.

**3.5.2 Cellulolytic Activity Test:** After keeping the cellulase agar plates in the incubator for 6-8 days at 28 °C, the plates were flooded with 1% Congo red dye. Clear transparent zones were formed in the bacterial and fungal colonies by reacting with the enzyme secreted by the isolates and the chromogenic substances, which was taken as a positive test for cellulase activity (Fig 13, 14 & 15).

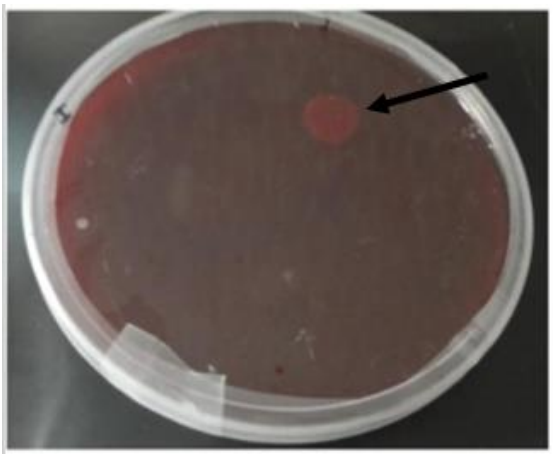

**Fig 13:** Cellulase agar plates for Gram –ve cocci shaped bacteria Here, clear zones were formed around the bacterial colonies and was taken as a positive test for cellulose activity

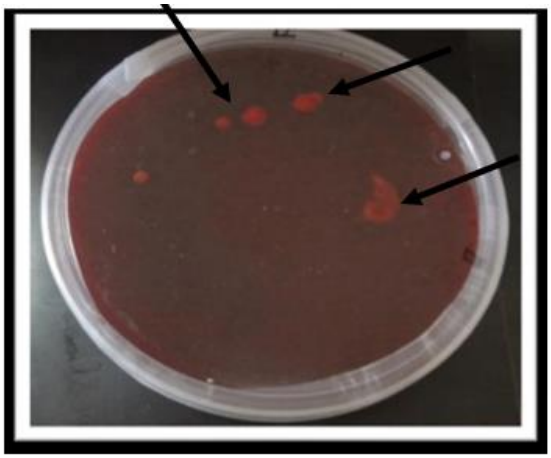

**Fig 14:** Cellulase agar plates for *Penicillium sp*. Here, clear zones were formed around the fungal colonies and was taken as a positive test for cellulase activity.

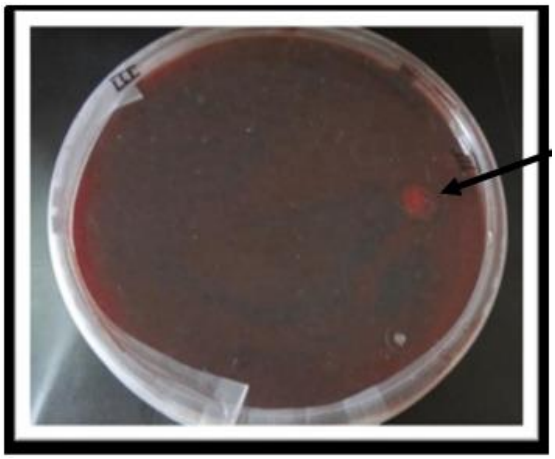

**Fig 15:** Cellulase agar plates for sterile mycelia with no spore formation Here, clear zones were formed around the fungal colonies and was taken as a positive test for cellulase activity.

## 4. Discussion

In this present experimented study, it has been found that there is a prominent body of indication about the digestive enzymes in the experimented fish. The intestinal microbes in the fish has been classified as indigenous or persistent, in which they are capable of colonizing the gut ecosystem, by which they only passes through the gastro-intestinal tract without colonizing. Moreover, in this experimented study, the experimental fishes were first starved for 24 hours, before the preparation of pure cultures from their gastro-intestinal tracts (only midgut and hindgut) for the separation of the indigenous microbes. Therefore, it reveals that microbial population found in this experimented study formed an innate and sustained population in the GI tracts.

Physiological and biochemical characterization of the isolated microbes from the gastro-intestinal tract are significant as because they help in explaining their function in the GI tract. In this experimental study, the isolated microbes from the gut of the fish were identified as gram negative cocci shaped bacteria, *Penicillium sp*. and white sterile mycelia with no spore formation. Kar *et al*. 2008 [6], however, reported presence of gram positive bacteria in both the fishes, i.e., *Labeo rohita* and *Channa punctatus.*

Moreover, the microbial isolates were able to exploit a wide range of carbohydrates, which included amylose and cellulose. The gut of the experimental fish producing the enzymes may play a role in the digestion process of the fish, like cellulose, which are also present in phytoplankton (one of the primary food of fish). Our results corroborates with Lesel *et al.* 1986 [8], who detected both amylolytic and proteolytic bacteria in the gut of gold fish (Phytophagus fish).

From this experimental study, it is revealed that bacterial isolates have their capability for extra-cellular amylase and cellulase production. Amylase and cellulase activities were shown by the bacterial and fungal isolates. Cellulolytic and amylolytic bacteria in the gut of *Channa punctata* also revealed about their omnivorous mode of feeding. This finding can be inferred from Ghosh *et al.* 2002 [4], who studied the occurrence of proteolytic, cellulolytic and amylolytic bacteria in the gut of rohu and suggested an omnivorous feeding aptitude of the fish.

There could be a mutual association between the bacteria consumed by the experimental fish in its diet, which may become suitable for the gastro-intestinal tract. The present experimental study of isolation of microbes (bacteria and fungi) showed that they were capable of growing in the temperature of upto 37 °C and pH 7. And due to this ability, they could probably adapt themselves with the GI environment of the experimented fish. The findings of Kar *et al*. 2008 [6] regarding the temperature and pH of the isolated strain from the gut of *C. punctata* are comparable to our results.

## 5. Conclusion

From the present experiment, it is revealed that the microbes isolated from the GI tract of *C. punctata* are capable of producing extra-cellular enzymes and also have a beneficial effect in the digestive processes of the fish. Characterization of the microbial populations in the intestinal microenvironment of the fish and understanding the physiological interactions between the indigenous micro biota and the host may have important implications. The present experiment revealed that the enzyme producing gut microbes are able to utilize carbohydrates, such as cellobiose, amylose,

cellulose. These substances are mainly found in plant feedstuffs. Therefore, cellulase and amylase activities by the gut microbes indicates their ability in the digestion process of the plant feedstuffs. These beneficial bacteria could be introduced in commercial aquaculture by incorporating them into formulated fish diets, or in the form of bacteria biofilm to achieve colonization in the fish GI tract at a higher degree. The enzyme-producing gut bacteria isolated in the present experiment might be used beneficially for fish especially in the larval stages. Therefore, further research of such types of beneficial microbes could be carried out in future for having a baseline idea for evaluation of their efficacy and to explore the potential in the field of aquaculture.

## 6. Acknowledgement

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