

**CELLULOLYTIC AND PROTEOLYTIC BACTERIA IN GI TRACT OF *GAMBUSIA AFFINIS*
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ABSTRACT

Bacterial ecology of gastrointestinal tract of *Gambusia affinis* (Mosquito Fish) has been studied to investigate the enzymatic activity of intestinal bacteria in physiology of digestion and also the possibility of enzyme supplementation in aquaculture. Cellulolytic and Proteolytic bacteria from Gastro-intestinal tract of *Gambusia affinis* were isolated. Their antibiotic activities were also tested. Extracellular enzyme producing capacities of selected intestinal isolates were analyzed. The outcome of the study holds significant importance because of the fact that the reports of cellulase activity in fish intestine are rare. Recently *Gambusia* has been reported to be harmful to other fish inhabiting the same habitat due to increased competition. *Gambusia* feeds on Mosquito larvae. The study of GI tract bacterial ecology of *Gambusia* can have two fold advantages. It can be used to develop supplements to support *Gambusia*'s growth. It can also be used to develop antibiotics so as to curb the growth in cases where *Gambusia* competes with other inhabitants.

KEY WORDS: cellulose, gastrointestinal tract, Mosquito Fish.

INTRODUCTION

Microflora of fish intestines appear to vary with the complexity of the fish digestive system. The genera present in the gut generally seem to be those from the environment or diet which can survive and multiply in the intestinal tract, although there is evidence for a distinct intestinal microflora in some species. Fish receive bacteria in digestive tract from aquatic environment through water and food that are populated with bacteria. Being rich in nutrient, the environment of the digestive tract of fish confers favorable culture environment for the microorganism. The importance of the intestinal bacteria in the nutrition and well-being of their host has been established for homeothermic species such as birds and mammals (Floch *et al.*, 1970). Endogenous digestive enzymes in fish have been studied by several workers (Dhage, 1968; Kawai and Ikeda, 1972; Das and Tripathi, 1991). However, information regarding the enzyme producing intestinal bacteria, their source and significance in fish is scarce. The enzymatic activities of the intestinal bacteria in herbivorous and carnivorous fish have been reported to be remarkably different. The scanning electron microscopic studies on interaction of bacteria with the intestine of *Labeo rohita* have been carried out by Ghosh *et al.* (2010). Bacteria were observed to adhere to the gut enterocyte surfaces. Population level of enzyme-producing bacteria was higher in the midgut and hindgut regions than that observed in the foregut region. Furthermore, dense amyolytic and cellulolytic bacterial population in comparison to the proteolytic population was noticed in the GI tract of *L. rohita*.

The studies on Intestinal microorganisms and their enzymatic activities hold importance in view of possible use as probiotics. These probiotics, apart from their use as nutritional supplements, may also act as biological control agents in aquaculture (Verschuere *et al.*, 2000). In view of the available knowledge so far, it appears interesting to investigate the enzymatic activity of intestinal bacteria from *Gambusia affinis* (Feeds on mosquito larva). Enzymatic activity in intestinal bacteria from *Gambusia affinis*, commonly known as Mosquito fish, has not been investigated so far.

MATERIALS AND METHODS**Fish Examination**

Mosquito fish as the name suggests, feeds on mosquito larvae. The fish were collected with the help of fishing net from the fish tank in the botanical garden of the premises of S. N. Arts, D. J. M. Comm. & B. N. S. Sci. College, Sangamner. During the sampling periods, the water temperature varied between 25°C and 28°C. The feeding habits (Jhingran, 1997), average weight, total length (L) and gut length (L) of the fish studied are presented in Table 1. Relative gut length has been reported as the ratio of total length to the length of the intestine.

Post Mortem Examination

To isolate stable aerobic heterotrophic bacterial population from the GI tracts, three fish of each species from each pond were starved for 24 hours in order to make their intestinal tract clear and also to eliminate the bacteria that were transit in nature. After starvation period, the fish were sacrificed and GI tracts were removed. A homogenate solution was made by adding GI tracts with 0.89% sodium chloride (NaCl) solution (10:l; volume:weight) (Das and Tripathi, 1991). Serial dilutions were made by mixing this homogenate solution with sterilized distilled water using vortex mixer to use as inoculums.

Microbial culture

Diluted samples (0.3 ml) were poured aseptically within a laminar airflow on sterilized Luria Agar to determine, the total heterotrophic bacterial population. The cellulolytic bacteria were isolated on CMC Agar and proteolytic bacteria were isolated on PGA. Spread plate technique was employed for the purpose. Culture plates were incubated at 37°C overnight and examined for development of bacterial colonies after the incubation period. Two well separated colonies (IG1 & IG2) with apparently different morphologies were selected and were streaked on separately on Luria Agar to get pure culture and stored at 4°C for further studies.

Table 1: Average weight, Total length, Gut length and relative gut length of the fish examined. The results are mean±S. E of the three observation

Body weight	Total Length (L _t) (cm)	Gut Length (L _g) (cm)	Relative Length (L _t / L _g)	Gut Feeding Habit
0.64±0.06	3.16±0.17	5.46±0.12	1.72±0.06	Feeds on zooplankton, small insects and detritus

Qualitative enzyme producing capacity

The intensity of extracellular enzyme production by the isolated bacterial strains was analyzed on agar plates with selective media. For Cellulase production the isolates were spot inoculated on CMC Agar and incubated at 37°C for 48 hrs. The culture plates were flooded with Congo red for 15 minutes. The plates were destained by 2% NaCl solution. The appearance of clear zone indicated the presence of cellulose activity. Similarly, for extracellular protease, the isolates were spot inoculated on the PGA and incubated at 37°C for 24 hours. The appearance of clear zones after flooding with 15% HgCl₂ indicated the presence of proteolytic activity.

Morphology of the isolates:

The morphology of the two isolates was determined by using Gram's staining Kit (HIMEDIA LABORATORIES PVT. LTD.). The stained isolates were observed under oil immersion (100x magnification).

Media Composition:

LA medium (gL⁻¹): Tryptone (Pancreatic digest of Casein) 10.00; Sodium Chloride 10.00; Yeast Extract 5.00; Bacteriological Agar.15.00; pH 7.

CMC Agar(gL⁻¹): Carboxymethylcellulose 5.00 ; NaNO₃ 10.00; K₂HPO₄ 10.00; KCl 10.00; MgSO₄ 5.00; yeast extract 5.00; glucose 10.00; Agar 15.00; pH 7

PGA: Peptone 10.00; NaCl 5.00; Gelatin 10.00; Agar 20.00; pH 7

RESULTS

Morphology of the isolates:

The isolate IG1 was found to be Gram positive bacillus. (Fig 1). The second isolate was found to be a Gram positive coccus. (Fig 2)

Relative gut Length:

The relative gut length (L_g/ L_t) was significantly shorter (1.72 ± 0.06). This suggests predominantly carnivorous feeding habit in *Gambusia affinis*.

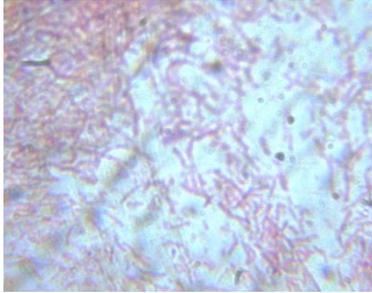


Fig 1: Gram Positive Bacillus

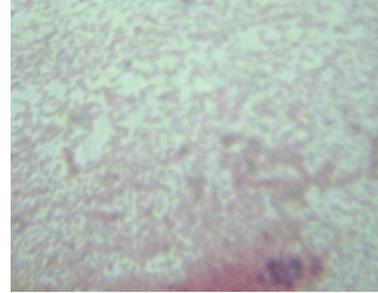


Fig 2: Gram Positive Cocci.

Enzyme producing capacities of the isolates:

Strain IG1 showed endocellulase activity as well as protease activity while the isolate IG2 showed only proteolytic activity (Fig 3a and 3b).

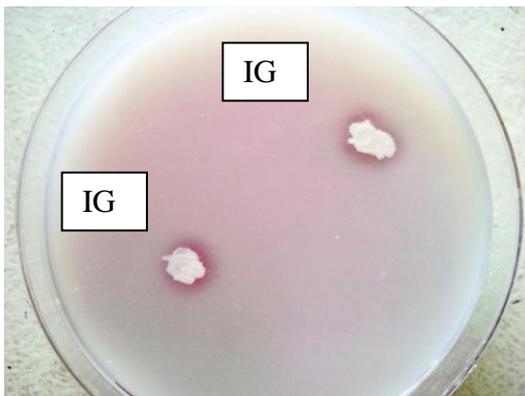


Fig 3a: Protease activity in IG1 and IG2

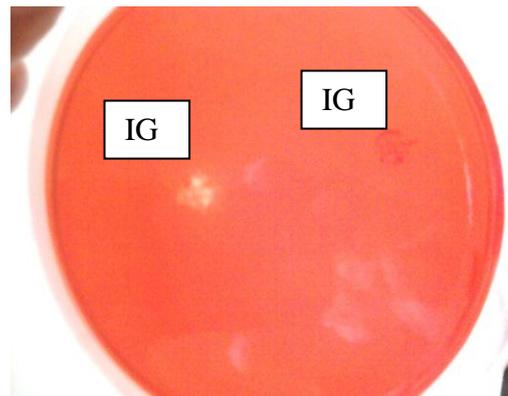


Fig 3b Cellulase activity of IG1

DISCUSSION

Presence of the proteolytic and cellulolytic bacteria in the intestine of *Gambusia affinis* suggests predominantly carnivorous feeding habit of the fish. This is also supported by the relative gut length. The digestive tract of *Gambusia* appears to be small. The bacteria present in the intestine have shown extra cellular protease activity and feeble endocellulase activity. *Gambusia* has been famously used in the control of mosquito. The findings help in the possible role of the isolates in the physiology of digestion. The information generated from the present study can contribute to the incorporation of these bacteria in the aquaculture feed production and probiotics.

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