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Bacterial Flora of the Intestines of Farm Raised Freshwater Fishes *Catla catla, Labeo rohita* and *Ctenopharyngodon idella*

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Bacterial flora associated with the intestines of farm raised freshwater fishes such as *Labeo rohita, Catla catla* and *Ctenopharyngodon idella* have been analysed. The mean population of total heterotrophic bacteria (THB) of the intestine of *L. rohita* was $3.2x10^6$ colony forming units (CFU) per gram and that of *C. catla* and *C. idella* were $4.79x10^6$ and $1.13x10^7$ CFU respectively. Morphological and physiological grouping of the isolates revealed dominance of gram negative rod shaped bacteria capable of elaborating various hydrolytic enzymes such as amylase, lipase and gelatinase. Ureolytic forms were relatively few. Characterisation of the genera revealed the dominance of Aeromonas in the intestine of these fishes. Other genera encountered were Corynebacterium, Micrococcus, Alcaligenes, Vibrio, Moraxella, Bacillus, Lactobacillus, Acinetobacter, Flavobacterium and members of the family Enterobacteriaceae.

Key words: Bacterial flora, freshwater fish, intestine

The microbial population in the digestive tract of fish are rather dense, with the number of micro-organisms much higher than the surrounding water, indicating that the digestive tract provides favourable ecological niche for these organisms (Austin & Al-Zahrani, 1988; Sakata, 1990). The gastrointestinal micro flora of fish appear to be simpler when compared to endotherms and are dominated by aerobes and facultative anaerobes (Trust & Sparrow, 1974; Horseley, 1977; Sakata, 1990).

The bacterial population in the gastrointestinal tract of fishes has been studied qualitatively and quantitatively by several workers. Sugita *et al.* (1982) and Sakata *et al.* (1980) reported that facultative anaerobic bacteria, Vibrio-Aeromonas group and obligatory anaerobic bacteria, Bacteroides type A and type B were major components of the micro flora present in the intestine of cultured freshwater fishes. However, most studies are restricted to species belonging to temperate regions or cold water fishes such as salmon, rainbow trout and Arctic charr. There is dearth of information on the intestinal micro flora of farm raised freshwater fishes of the tropical region such as *Labeo rohita* (Hamilton), *Catla catla* (Hamilton) and *Ctenopharyngodon idella* (Valenciennes). In the present study, an attempt has been made to characterize the micro flora associated with the gastrointestinal tract of these fishes in order to find out the generic composition and the role of intestinal micro flora in the digestive process of these fishes.

Materials and Methods

Samples were collected from Maharaja Aqua Farms, located at Bhavani, Tamil Nadu. The culture pond selected was a semi intensive fresh water aquaculture pond practising mixed culture of *L. rohita, C. catla* and *C. idella.*

Fishes were caught by cast net. Fishes of medium size were taken for analysis assuming that they might have a well established pattern of intestinal micro flora. Fishes were transferred to water collected from the pond and brought to the laboratory in live condition.

Upon reaching the laboratory, the intestines of the fishes were dissected out aseptically within an hour, so as to get the full stomach contents. The intestine was then homogenized in a sterile homogeniser, serially diluted and plated in nutrient agar to find out the total heterotrophic bacteria (THB) population. The plates were incubated at room temperature for 48 hours and counts estimated.

Morphologically, different colonies were picked at random from the plates and restreaked to ensure purity and maintained on nutrient agar slants. Around 40 colonies were selected for each fish as it was sufficient to obtain a representative diversity of bacterial communities (Bianchi & Bianchi, 1982). The isolates were characterized up to genus level on the basis of Gram stain, spore stain, motility, oxidase, catalase and O/F tests and grouped into various genera as per Buchanan & Gibbons (1979). The ability of the isolates to elaborate various hydrolytic enzymes such as amylase, gelatinase and lipase were determined by plate assay. Urea splitting ability was determined by inoculating into Christiansen's urea agar medium.

Results and Discussion

Total heterotrophic bacterial population in the intestines of farm raised *L. rohita*, *C. catla* and *C. idella* is presented in Table 1. The mean population of THB was greater than 1 x 10^6 CFU/g of the intestine in *C. catla* and *L. rohita*. The THB load in the intestine of *C. idella* was 1 log higher than THB population of the intestine of *C. catla* and *L. rohita*. THB population of such magnitude were reported earlier (Sugita *et al.*, 1983) in the gastrointestinal tract of fishes collected from rivers. However the THB load was less than those reported in the intestines of salmonids (Trust & Sparrow, 1974; Kamei *et al.*, 1985) as well as that of Arctic charr in the natural environment (Ringo & Strom, 1994). This may be due to the controlled environmental conditions in the aquaculture farm as the fishes acquire their intestinal micro flora from the ambient environment. Till recently, it was believed that the larval gut was sterile until first feeding (Muroga *et al.*, 1987; Tansomvang & Muroga, 1989). However during the hatching process, the water often contains heavy bacterial load (Hansen & Olafsen, 1992) and during drinking, larvae ingest bacteria.

Table 1. Mean and range of population of total heterotrophic bacteria (THB) in the intestine of *Catla catla*, *Labeo rohita* and *Ctenopharyngodon idella*

Sample source	Mean population (CFU / g)	Range of THB
Intestine of *C. catla*	4.79 x 10^6	3.2 x 10^5 - 1.1 x 10^7
Intestine of *L. rohita*	3.2 x 10^6	1 x 10^5 - 4.1 x 10^7
Intestine of *C. idella*	1.13 x 10^7	1 x 10^6 - 4.1 x 10^7

Table 2. Percentage incidence of various morphological and physiological groups in the intestine of *Catla catla*, *Labeo rohita* and *Ctenopharyngodon idella*.

Morphological and physiological characteristics	Percentage incidence of various morphological and physiological groups in the intestines of		
	L.rohita (*n*=38)	*C.catla* (*n*=38)	*C.idella* (*n*=47)
Gram negative	66	68	70
Gram positive	34	32	30
Rods	71	87	98
Cocci	29	13	2
Motile	76	48	64
Oxidase positives	76	92	89
Catalase producers	63	98	87
Glucose fermenters	97	71	91
Amylolytic forms	66	65	85
Gelatinolytic forms	74	79	91
Lipolytic forms	66	75	87
Ureolytic forms	42	23	28

Morphological and physiological grouping of bacteria found in the intestine of *L. rohita*, *C. catla* and *C. idella* is presented in Table 2. There was predominance of Gram negative rods in the intestines of these fishes. The occurrence of Gram negative rods was around 70% in the intestines of *C. idella*, *L. rohita* and *C. catla*. Predominance of Gram negative rods in the intestines of various fishes such as Arctic charr (Ringo, 1993; Ringo & Strom, 1994), chum salmon (Trust,

1975), rainbow trout (Nieto *et al.*, 1984) and salmonids (Yoshimizu *et al.* 1976) has been reported earlier.

Table 3. Percentage occurrence of various genera in the intestine of *Catla catla*, *Labeo rohita* and *Ctenopharyngodon idella*

Sample source	Genera	Percentage occurrence
Intestine of *C. catla*	*Aeromonas*	52
	Corynebacterium	17
	Micrococcus	13
	Alcaligene	6
	Vibrio	6
	Moraxella	2
	Bacillus	2
	Unidentified	2
Intestine of *L. rohita*	*Aeromonas*	50
	Staphylococcus	29
	Lactobacillus	5
	Moraxella	5
	Vibrio	3
	Enterobacteriaceae	3
	Unidentified	5
Intestine of *C. idella*	*Aeromonas*	64
	Corynebacterium	28
	Acinetobacter	2
	Moraxella	2
	Micrococcus	2
	Flavobacterium	2

Ability of the bacterial isolates to elaborate various hydrolytic enzymes indicates that majority of them are capable of utilizing various substrates such as starch, gelatin and lipid (Tween 80). Urea splitting forms were relatively less (Table 2). The beneficial effects of micro-organism in the digestive process of terrestrial animals are well documented (McBee, 1977; Goldin, 1986; Moriarty, 1990). Some investigators have also suggested that micro-organisms exert a beneficial effect on the digestive process of fish (Danulat & Kausch, 1984; Kono *et al.*, 1987). Flora of the digestive tract can act on lipolysis by way of contributing to triglyceride breakdown. Lipolytic activity has been previously reported in bacterial isolates from gastrointestinal tract of grass carp (Trust *et al.*, 1979). Flora of the digestive tract can act on lipolysis in different ways by contributing to triglyceride break down through bacterial action and by changing pancreatic lipase secretion or inactivating it with bacterial proteases. Bacterial lipase synthesis is stimulated by lipids such as lard, butter, olive oil and fatty acids (Finnerty, 1989).

Occurrence of various bacterial genera in the gastrointestinal tract of *L. rohita*, *C. catla* and *C. idella* is presented in Table 3. *Aeromonas* was the dominant genera in the intestines of all these fishes. While *Corynebacterium* was not encountered in the intestine of *L. rohita* it was the second dominant genera in the intestines of *C. catla* and *C. idella*. *Moraxella* was present to the extent of about 5% in the intestine of all the three fishes under study. Other genera present were *Micrococcus*, *Staphylococcus*, *Alcaligenes*, *Vibrio*, *Acinetobacter*, *Lactobacillus* and members of the family Enterobacteriaceae. Predominance of *Aeromonas* spp in the intestines of freshwater fish (Tilapia) is reported earlier (Sakata & Koreeda, 1986). Findings of this study also agree with the observations of Sugita *et al.* (1983) who has reported *Vibrio-Aeromonas* group as the dominant genera encountered in the micro flora of the intestine of fresh water fishes such as carp, crucian carp, common minnow and brackish gobi. They had also reported that in the gastrointestinal contents of fresh water fishes, facultative anaerobes were always predominant and obligate anaerobes failed to be isolated from all fish samples. However, isolation of obligate anaerobes was not attempted during this investigation.

Staphylococcus was the second dominant genera in the intestine of *L. rohita*. It has been reported that the bacterial flora of the fish intestine is a reflection of ambient micro flora and the presence of staphylococci in farm raised fishes is reported to be due to human activity around the pond (Ayub Ali *et al.*, 1992).

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