International Journal of Current Science Research
www.drbgrpublications.in

Volume: 2; Issue: 1; January-2016; pp 180-197. ISSN: 2454-5422

Enzyme producing bacteria in the gastro intestinal tracts of C*hanna striatus*

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Abstract

Research on the metabolic process related is digestion in rearing fish is still in progress and in those species currently formed, the status of research on digestive physiology is far from a complete picture on the process of nutrient hydrolysis. Therefore, further investigations on digestive enzymes are required to improve knowledge existing on their interaction with different factors intrinsic to fish nutrition (Such as dietary composition (or) growth stage); all these features can offer interesting perspectives for further studies, with exciting and promising applicative purposes for aquaculture development. The clarification of aspects intrinsic to the digestive physiology, such as the definition of the enzymatic pattern typical of a selected fish species, the chronobiology of the digestion and the evolution of the digestive organs during fish growth can provide useful contributions to the fields of fish nutrition.

Keywords: *Channa striatus*, Digestive enzymes, Proteases, Lipases

Introduction

Fish receives Bacteria in the digestive tract from the aquatic environment through water and food that are populated with bacteria. Being rich in Nutrient, the environment of the digestive tract of fish confers a favourable culture environment for the micro organisms. 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 in scarce. Studies aimed at investigating the functioning

of the digestive tract in different species can provide relevant tools for the optimization of the relative percentage of their dietary macro nutrients; therefore, knowledge of digestive enzymes of fish has important practical implications for their Nutrition. The ability of fish to metabolize a diet depends on the availability of appropriate digestive enzymes, which mediate specific degradation pathways, as well as on both physical and chemical nature of food. The measurement of specific activities (proteases, carbohydrases and lipases) may provide information about the whole digestive capacity and the efficiency of species reared to use feeding components.

Several studies have shown that the distribution and activity of digestive enzymes within the gut are affected by feeding habits. Carnivorous fish have a short intestine with higher levels of proteases compared with herbivores fish, while also amylase and lipase are represented in minor percentage in their digestive tract. The predominant bacterial species isolated from most of the fish digestive tracts have been reported to be aerobes (Trust and sparrow 1974; Bairagi *et al* 2002; Saha *et al* 2006). Studies aimed at investigating the functioning of the digestive tract in different species can provide relevant tools for the optimization of the relative percentage of their dietary macronutrients, therefore, knowledge of digestive enzymes of fish has important practical implications for their nutrition. In the present study, an attempt has been made to investigate the relative amount of protease, amylase and cellulose producing bacteria in the gastrointestinal (GI) tracts of fresh water Murrel, namely the *Channa striatus*.

Materials and Methods

Carnivore, bottom feeder murrel, *Channa striatus* was sampled by gill–net from the local ponds and analysed separately for the present study. Fish were collected from two ponds designated as pond A and pond B. During the sampling periods, the water temperature varied between 25^{o}C and 28^{o}C. The feeding habits (Jhingran 1997), average weight, total length (LT) and gut length (LG) of the fish studied are presented (Table 1). Relative gut length is reported as the ratio of the gut length to the total length. [LG/LT].

To isolate stable aerobic heterotrophic bacterial population from Gastro intestinal tracts, three fishes from each pond were starved for 21 hours in order to make intestinal tract clear and also to 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 solution (NaCl) [10:1; I volume: weight] (Das by Tripathi 1991). Serial dilutions were made by mixing this homogenate solution with sterilized distilled water using vortex mixture to use as inoculums.

Microbial culture of the homogenized GI tracts of fish from each pond was carried out separately for isolation of Bacteria. Diluted samples (0.3ml) were poured aseptically within a laminar airflow on sterilized Tryptone soya agar [(TSA), Himedia, India]. To determine, the total heterotrophic bacterial population. To isolate and enumerate protease, cellulose and amylase producing population, diluted samples (0.3ml) were poured on Peptone Gelatin Agar (PG), Carboxy Methyl Cellulose agar (CMC), and Starch Agar (SA) plates respectively, in triplicate. Spread plate technique was employed for the purpose. Culture plates were incubated at 37˚C overnight and examined for the development of bacterial colonies after the incubation period. It was assumed that the microflora, which had formed colonies on the SA plate, had amylolytic activity. Similarly, it was assumed that the microflora grown on CMC and PG plates had cellulolytic and proteolytic activities respectively (Ghosh *et al* 2002) water and bottom sediments of the collection ponds were also analyzed subsequently for total and specific enzyme producing bacterial population. Single isolated colonies from the streaked plates were transfused to TSA plates as pure culture and maintained at 4˚C in the refrigerator to further study.

The intensity of extra cellular enzyme production by the isolated bacterial strains was analysed on agar plates with selective media. For Extracellular amylase production, isolates were inoculated on SA plates and incubated at 37˚C for 48 hours. The culture plates were then flooded with 1% Lugol's iodine solution to identity amylase activity by formation of transparent tone surrounding the colony (Jacob and Gerstein 1960). Similarly for Extra – cellular protease, the isolates were inoculated on PG plates and incubated at 37˚C for 15 hours. The appearance of a clear zone around the colony after flooding the plate with 15% $Hgcl_2$ indicated the presence of proteolytic activity (Jacob and Gerstein 1960). For determination of cellulose production, isolates were ground on CMC plates at 37˚c for 24 hours and flooded with Congo red dye prepared with 0.7% agarose (Teather and wood, 1982) (Plate.4a). Congo red selectively binds with unhydrolysed CMC (Plate 4b). Appearance of clear halo due to the presence of hydrolyzed CMC surrounding bacterial colony in the medium. Statistical analysis of the experimental data

was made by analysis of variance (ANOVA) followed by scheffe's F-test for multiple comparison (Gas and Das 1993).

Result

Analysis of bacterial flora in the gut of the fish examined showed higher aerobic bacterial population on TSA plate in *Channa striatus* irrespective of the pond proteolytic bacterial flora was detected abundantly in the fish species examined while enumerating specific enzyme producing bacteria it was observed that the relative abundance of enzyme producing bacteria followed the same pattern in the murrel, *channa striatus* collected from both ponds. Proteolytic (4.0±0.12) and cellulolytic (4.5±0.05) strains are higher densities than the Amylolytic (3.2±0.12) Strains in the *channa striatus*, collected from pond A. However, in pond B, cellulolytic (3.0±0.01) strains & Amylolytic (3.5±0.05) strains higher than the proteolytic (2.0±0.09) strains In *channa striatus*. In Pond A, cellulolytic activities are higher than the Amylolytic and Proteolytic strains. In pond B, Amylolytic strains are higher than the Proteolytic and cellulolytic strains. Protease and Amylase producing capacity is higher in the strains CSA1 and CSA2 isolated from *Channa striatus*, collected from pond A. However in pond B, cellulase and amylase producing capacity is higher in CSB1 and CSB2. Amylase producing capacity was found to be very low in CSA1 and CSA2. Protease producing capacity was found to be very low in CSB1 and CSB2 (Table 3).

The strains CSA1, CSA2 and CSB1, CSB2 showed their capacity to produce all the three studied enzymes, viz., protease, amylase and cellulase. However, the bacterial strains CSA1 and CSB1 isolated from *Channa striatus* respectively exhibited better enzyme producing capacities in comparison to the other isolates.

Discussion

The intestinal microbiota of fish and bacterial content of the water has been demonstrated by several authors (Horslay 1997; Blanch *et al* 1997). Dabrowski and Glogowski (1977) reported increase in proteolytic enzyme activity, considerably, when common carp fry were provided with bovine trypsin in their diet. The endogenous amylase activities in the intestine of herbivorous carp were much more intense than in carnivorous species (Sarbahi 1951; Dhage 1968). However,

reports on microbial amylase activity in fish gut are scanty (Sugita *et al* 1997; Bairagi *et al* 2002; Ghosh *et al* 2002).

Reports on the existence of cellulase activity in the digestive system of fish are rare and conflicting with contradictory result. Fish (1957) and Yokoi and Yasumasu (1964), believed that fish do not posses endogenous cellulose. Shcherbina and Kazlaalene (1971), indicated the presence of microbial cellulase in the posterior portion of digestive tract of carp. Further, Lindsay and Harris (1980), showed cellulase activity in the digestive tract of fish and suggested the source of cellulase activity from the microbial population, although they discarded the idea of maintenance of stable cellulolytic microflora in fish. Later, Lesel *et al* (1986) reported cellulolytic flora in grass carp. Das and Tripathi (1991) assumed the cellulose–producing bacteria as a part of persistence intestinal flora in fish. In addition to the endogenous sources, enzymes from the intestinal microflora potentially could have a significant role in digestion, especially for substrates such as cellucose, which few animals can digest and also for other substrates (Smith 1989). All the information might contribute to the incorporation of these bacteria in commercial aquaculture as supplement in formulated fish fed or in form of bacteria biofilm to achieve colonization in the fish gut at a higher degree.

Bacteria present in the aquatic environment may influence the composition of gut microbiota in fish (Cahill 1990). The result of the present study showed variation in bacteria load from murrel collected from different ponds. This may be due to varied bacterial load of the collection ponds. Possible correlation between the intestinal microbiota of fish and bacterial load has been reported by several authors (Horslay 1997).

The maximum density of proteolytic bacteria was detected in *Channa striatus*. Maximum protease producing capacity was observed within a strain from the same species (CSB2). Several authors have reported adaptive changes in the relation to the type of the diet. The occurrence of proteolytic bacteria in the gut of murrel *Channa striatus* in high intensity also seems to support the presence of diet dependent towards animal matter. On the other hand, the intestine of fish is short and bears a distinct stomach indicating production of endogenous protease and also their carnivores feeding aptitude. Colonization of amylolytic and cellulolytic bacteria in high intensity suggest that supplementation of amylase and cellulase serves as the basis for the symbiotic relationship between the bacterial flora and the fish, endogenous carp are much more intense

than in carnivorous species (Sarbahi 1951; Dhage 1968;) However reports on microbial amylase activity in fish gut are scanty (Sugita *et al* 1997; Bairagi *et al* 2002., Ghosh *et al* 2002). In the present study, a considerable population of amylolytic bacteria was detected in the fish species with studied.

Bairagi *et al* (2002) could not detect cellulolytic bacteria in the gastro intestinal tract of carnivorous cat fish and murrels. However, the result of the present investigation showed the presence of cellulolytic bacteria in Murrels. Stickney (1975) looked at cellulase activity in a number of fresh water species and concluded that herbivores are unlikely to have the enzyme, but omnivores and carnivores may pick it up from invertebrates, that harbour the bacteria producing the enzyme. This may explain the occurrence of both cellulolytic and amylolytic bacteria in the digestive tract of a supposed carnivore fish species, the murrel.

From this present study, the bacteria present within the gut of *Channa striatus* were capable of producing various extracellular enzymes. Bacteria in the surrounding environment and feeding habit may have influence on the composition of the gastrointestinal microbiota in fish. In addition to the endogenous sources, enzymes from intestinal micro flora potentially could have a significant role in digestion, especially for substrates such as cellulose, which few animals can digest, and also for other substrates (Smith 1989). The use of such beneficial bacteria has a long tradition in the animal husbandry (Starvrie and Kornegay 1995). The information generated from the present investigation might contribute to the incorporation of these bacteria in commercial aquaculture as supplement in formulated fish feed (or) in form bacteria biofilm to achieve colonization in the fish gut at a higher degree. However, further research involving potent bacterial strains should be conducted for evaluating their efficacy under actual farm conditions.

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Table.1 Average weight, total length, relative gut length and feeding habit of fish examined. Results of mean ± S.E of the three observations.

Fish Species	Collected Pond	Body Weight (g)	Total length (LT) (CM)	Weight of the gut (g)	Gut length (LG) (CM)	Relative Gut length (LG/LT)	Feeding habit
Channa striatus	A	77±2.35	17. ±1.32	3.27±0.89	8.5±0.35	0.49±0.04	Insects, zooplanktons, Insect larvae,Smaller fish, Waterbugs
	B	88.6±3.75	18.1±1.04	3.61±0.72	9.8±1.65	0.54±0.06	

Values with the same superscript in the same column are not significantly different (P.0.05).

Table: 2. Aerobic heterotrophic bacterial count in fish digestive tracts. Results are mean ±S.E. of the three determinations.

Fish Species	Collection Pond	Bacterial Populations ($CFUg^{-1}$ digestive tract)			
		In TSA Plate ($x\ 10^6$)	Proteolytic ($x\ 10^4$)	Amylolytic ($x10^4$)	Cellulolytic ($x\ 10^4$)
Channa striata	Pond A	0.45±0.02	4.0 ± 0.12	3.2 ± 0.12	4.5 ± 0.05
Channa striata	Pond B	0.23 ± 0.02	2.0 ± 0.09	3.5 ± 0.05	3.0 ± 0.01

Values with the same superscript in the same column are not significantly different (P.0.05).

Table – 3

Qualitative extra cellular enzyme producing capacities of the bacterial strains isolated from fish gut. Result represents impression of three determinations.

Fish Species	Collection pond	Strain No	ENZYME PRODUCING CAPACITY		
			Protease	Amylase	Cellulose
Channa striata	Pond A	CSA_1	+ + + + + +	+ +	+ + + + + +
		CSA_2	+ + + +	ND	+ + +
	Pond B	CSB_1	+ + +	+ + +	+ + + + +
		CSB_2	ND	+ + +	+ +

With pure cultures of the intestinal isolates.

ND – Not detected, number of '+' Sign indicates the intensity of enzyme production.