Turkish Journal of Fisheries and Aquatic Sciences 8: 115-120 (2008)

Enzyme Producing Bacteria in the Gastrointestinal Tracts of *Labeo rohita* (Hamilton) and *Channa punctatus* (Bloch)

Nibedita Kar[1], Koushik Ghosh[1,*]
[1] The University of Burdwan, Department of Zoology, Aquaculture Laboratory, Golapbag, Burdwan 713104, West Bengal, India.

* Corresponding Author: Tel.: +91.342 2657595
E-mail: kghosh_bu@yahoo.co.in

Received 17 March 2007
Accepted 05 March 2008

Abstract

Isolation and enumeration of heterotrophic bacteria from the gastrointestinal tracts of rohu, *Labeo rohita* and murrel, *Channa punctatus* have been carried out to find out their importance in the nutrition of the host fish. Amylolytic, proteolytic and cellulolytic bacteria were detected in the fish gut. Among specific enzyme producing bacteria, proteolytic and cellulolytic bacteria were present at higher number within the gut of murrel and rohu, respectively. Selected intestinal isolates were analyzed for extracellular enzyme producing capacities. Protease and cellulase activities were exhibited by all bacterial isolates, while amylase production remained poorly detected by the strains isolated from murrel. Results of the study indicated the probability of digestive enzyme supplementation by the intestinal bacteria in the fish gut. The information generated from the present investigation might contribute to the utilization of these extracellular enzyme-producing bacteria in commercial aquaculture.

Key words: Rohu, murrel, gastrointestinal bacteria.

Introduction

Fish receive 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 microorganisms. The importance of intestinal bacteria in the nutrition and well–being of their hosts has been established for homeothermic species, such as birds and mammals (Floch *et al.*, 1970). However, there is limited information for fish, the poikilothermic vertebrates. Though the digestive tract of endotherm that is mainly colonized by obligate anaerobes (Finegold *et al.*, 1983), the predominant bacterial species isolated from most of the fish digestive tracts have been reported to be aerobes or facultative anaerobes (Trust and Sparrow, 1974; Bairagi *et al.*, 2002; Saha *et al.*, 2006).

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. In the present study, an attempt has been made to investigate the relative amount of protease, amylase and cellulase producing bacteria in the gastrointestinal (GI) tracts of two fresh water teleosts, namely the Indian major carp rohu, *Labeo rohita* (Hamilton) and the murrel, *Channa punctatus* (Bloch). Further, intestinal isolates were evaluated for extracellular enzyme producing capacities. Data suggest that the composition of the specific enzyme producing bacterial flora in the fish digestive tracts may have correlation with their feeding habits.

Materials and methods

Fish Examined

Herbivore, column feeder rohu and carnivore, bottom feeder murrel were sampled by gill–net from the local ponds and analyzed 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°C and 28°C. The feeding habits (Jhingran, 1997), average weight, total length (L_T) and gut length (L_G) of the fish studied are presented in Table 1. Relative gut length is reported as the ratio of the gut length to the total length (L_G/L_T).

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.

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

Fish species	Collected pond	Body weight (g)	Total length (L_T) (Cm.)	Weight of the gut (g)	Gut length (L_G) (Cm)	Relative gut length (L_G/L_T)	Feeding habit*
Labeo rohita	A	$69.3^{a}\pm3.06$	$14.5^{a}\pm0.83$	$3.78^{a}\pm0.54$	$152.25^{b}\pm3.62$	$10.46^{b}\pm0.35$	Microscopic plants, decaying higher plants, Vegetable debris, detritus, mud
	B	$68^{a}\pm2.16$	$17.9^{b}\pm1.42$	$3.51^{a}\pm0.56$	$181.62^{c}\pm3.87$	$10.18^{b}\pm0.57$	
Channa punctatus	A	$78^{b}\pm2.35$	$17.3^{b}\pm1.32$	$3.27^{a}\pm0.89$	$8.5^{a}\pm0.35$	$0.49^{a}\pm0.04$	Insects, Zooplanktons, Insect larvae, Smaller fish
	B	$87.6^{c}\pm3.65$	$18.1^{b}\pm1.04$	$3.61^{a}\pm0.72$	$9.8^{a}\pm1.65$	$0.54^{a}\pm0.06$	

* Adapted from: "Fish and Fisheries of India" by V.G. Jhingran (1997).
Values with the same superscript in the same column are not significantly different ($P<0.05$).

Microbial Culture

Microbial culture of the homogenized GI tracts of fish from each pond was carried out separately for isolation of bacteria. Diluted samples (0.3 ml) 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, cellulase and amylase producing bacterial population, diluted samples (0.3 ml) were poured on peptone gelatine agar (PG), Carboxymethylcellulose 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 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.

Nine well-separated colonies of intestinal bacteria having apparently different morphological appearance were selected and were streaked separately on TSA plates for obtaining pure cultures. Single isolated colonies from the streaked plates were transferred to TSA plates as pure culture and maintained at 4°C in the refrigerator to further study.

Qualitative Enzyme Producing Capacity by the Selected Isolates

The intensity of extracellular enzyme production by the isolated bacterial strains was analyzed on agar plates with selective media. For extracellular amylase production, isolates were inoculated on SA plates and incubated at 37°C for 48 h. The culture plates were then flooded with 1% Lugol's iodine solution to identify amylase activity by formation of transparent zone 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 h. 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 cellulase production, isolates were grown on CMC plates at 37°C for 24 h and flooded with Congo red dye prepared with 0.7% agarose (Teather and Wood, 1982). Congo red selectively binds with unhydrolysed CMC. Appearance of clear halo due to the presence of hydrolysed CMC surrounding bacterial colony indicated cellulase production in the medium.

Media Composition

TSA medium (g L^{-1}): Pancreatic digest of casein, 15; Papaic digest of soyabean meal, 5; NaCl, 5; Agar, 15; pH 7. SA medium (g L^{-1}): Beef extract, 5; Peptone, 5; NaCl, 5; Starch (soluble), 2; Agar, 20; pH 7. CMC medium (g L^{-1}): Beef extract, 5; Peptone, 5; NaCl, 5; Carboxymethyl cellulose, 2; Agar, 20; pH 7. PG medium (g L^{-1}): Beef extract, 3; Peptone, 5; Gelatin, 4; Agar, 20; pH 7.

Statistical Analysis

Statistical analysis of the experimental data was made by analysis of variance (ANOVA) followed by Scheffe's F-test for multiple comparison (Das and Das, 1993).

Results

Relative Gut Length

The digestive tract is relatively longer in rohu (152.25±3.62 cm and 181.62±3.87 cm in pond A and B, respectively) than the murrel (8.5±0.35 cm and 9.8±1.65 cm in pond A and B, respectively). Mean±S.E of relative gut length (L_G / L_T) was significantly shorter in murrel than rohu (Table 1).

Bacteria in Fish Gastrointestinal Tract

Analysis of bacterial flora in the gut of the fish examined showed higher aerobic bacterial population on TSA plate in *L. rohita* irrespective of the pond.

Proteolytic, amylolytic and cellulolytic bacterial flora was detected abundantly in both fish species examined. While enumerating specific enzyme producing bacteria, it was observed that the relative abundance of the enzyme-producing bacteria followed the same pattern in rohu and murrel collected from both ponds. Proteolytic strains were present at higher densities in the *C. punctatus*. However, the cellulolytic population exhibited maximum densities in rohu, *L. rohita*. Amylolytic population was detected in both fish, but it was dominated in murrels. In general, fish samples collected from Pond A exhibited higher bacterial population than the samples collected from Pond B, except cellulase producing bacteria. Cellulolytic population was higher in rohu collected from Pond B, while among murrels it was dominant in fish collected from the Pond A as for other enzyme producing bacteria (Table 2).

Pond Bacteria

Aerobic or facultative aerobic bacterial population on TSA plate exhibited maximum density in the water and bottom sediment of Pond A compare to the Pond B. While analyzing specific enzyme producing bacterial flora, it was also evident that proteolytic and amylolytic bacterial populations were present in higher densities in water and bottom sediment of pond A than pond B. However, cellulolytic bacterial population in the pond water was comparatively higher in Pond B than Pond A. Though cellulolytic population in the bottom sediment exhibited higher density in Pond A (Table 3).

Extracellular Enzyme Producing Capacity of the Intestinal Isolates

Protease producing capacity of CpB2 isolated from *C. punctatus* found to be the greatest and was followed by the strains isolated from *L. rohita*, LrBl and LrA2 respectively. However, the strain isolated from *L. rohita*, LrAl showed the best amylase producing capacity and was followed by another isolate from *L. rohita*, LrBl. Amylase production by the strains isolated from *C. punctatus* remained poorly detected. Cellulase producing capacity was found to be the greatest in CpB2 and was followed by LrB1.

The strains LrA1, LrB1, LrB2, CpA2 and CpB2 showed their capacity to produce all the three studied enzymes, viz., protease, amylase and cellulase. However, bacterial strains, LrB1 and CpB2, isolated from rohu and murrel respectively exhibited better enzyme producing capacities in comparison to the other isolated strains (Table 4).

Discussion

In the present investigation, both fish species examined exhibited considerable amylolytic, proteolytic and cellutolytic bacterial population (Table 2). This can be correlated with their feeding habit. Being an herbivore fish species, occurrence of protease, amylase and cellulase producing bacterial population is noteworthy in the digestive tract of rohu. Lesel *et al.* (1986) detected both amylolytic and proteolytic bacteria in the gut of gold fish (Phytophagus fish). Therefore, the occurrence of

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

Collection pond	Fish species	Bacterial populations (CFU g^{-1} digestive tract)			
		In TSA plate ($\times10^7$)	Proteolytic ($\times10^5$)	Amylolytic ($\times10^5$)	Cellulolytic ($\times10^5$)
Pond A	*Labeo rohita*	2.5^{d}±0.09	2.6^{c}± 0.11	1.3^{c}±0.04	3.2^{c}±0.13
	Channa punctatus	0.49^{b}±0.02	2.9^{cd}± 0.12	3.4^{d}±0.15	2.8^{b}±0.08
Pond B	*Labeo rohita*	1.6^{c}±0.06	0.34^{a}± 0.01	0.17^{a}±0.01	5.9^{d}±0.21
	Channa punctatus	0.23^{a}±0.01	2.0^{b}± 0.09	0.85^{b}±0.04	1.8^{a}±0.05

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

Table 3. Aerobic heterotrophic bacterial count in the pond water and bottom sediment. Results are mean ± S.E. of the three determinations

Collection pond	Bacterial population in pond water (CFU ml^{-1})			
	In TSA plate ($\times10^6$)	Proteolytic ($\times10^3$)	Amylolytic ($\times10^4$)	Cellulolytic ($\times10^4$)
Pond A	8^{b}±0.25	6.6^{b}±0.31	6.7^{b}±0.28	1.3^{a}±0.05
Pond B	2.5^{a}±0.10	3.3^{a}±0.15	0.2^{a}±0.01	2.6^{b}±0.11
	Bacterial population in bottom sediment (CFU g^{-1})			
	In TSA plate ($\times10^7$)	Proteolytic ($\times10^5$)	Amylolytic ($\times10^6$)	Cellulolytic ($\times10^6$)
Pond A	10^{b}±0.38	8.3^{b}±0.31	6.6^{b}±0.23	9.3^{b}±0.46
Pond B	1.4^{a}±0.06	2.3^{a}±0.11	0.26^{a}±0.01	1.8^{a}±0.07

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

Table 4. Qualitative extracellular enzyme producing capacities of the bacterial strains isolated from fish gut. Result represents impression of three determinations

Collection pond	Fish species	Strain No.	Enzyme producing capacity*		
			Protease	Amylase	Cellulase
Pond A	*Labeo rohita*	Lr A1	+ +	+ + + +	+ +
		Lr A2	+ + + + +	ND	+ + +
	Channa punctatus	Cp A1	+ +	ND	+ +
		Cp A2	+ +	+ +	+ +
Pond B	*Labeo rohita*	Lr B1	+ + + + + +	+ + +	+ + + + +
		Lr B2	+ + +	+	+ +
	Channa punctatus	Cp B1	+ + +	ND	+ + + +
		Cp B2	+ + + + + + +	+ +	+ + + + + + +
		Cp B3	+ + + +	ND	+ + + +

* With pure cultures of the intestinal isolates
ND= Not detected, number of '+' sign indicates the intensity of enzyme production.

proteolytic, cellulolytic and amylolytic bacteria in the gut of rohu suggests an omnivorous feeding aptitude of the fish as has been studied by Creach (1963) and Ghosh *et al.* (2002). Higher number of cellulolytic bacteria in the digestive tracts of rohu collected from both ponds and the best amylase producing capacity by a strain from rohu (LrA1) may also indicate their preference towards plant matter. This has been further supported by the value of relative gut length. The digestive tract of rohu appears to represent a relatively long gut (Table 1), that is widely recognized in herbivorous (as compared with carnivorous) fish (Okeyo, 1989; Hugueny and Pouilly, 1999; Drewe *et al.*, 2004).

Bacteria present in the aquatic environment may influence the composition of the gut microbiota in fish (Cahill, 1990). The result of the present study showed variation in the relative abundance of the enzyme-producing bacteria in rohu and murrel collected from different ponds. This may be due to varied bacterial load of the collection ponds (Table 3). Possible correlation between the intestinal microbiota of fish and bacterial content of the water has been demonstrated by several authors (Horslay, 1997; Blanch *et al.*, 1997). Between the two fish species studied, rohu exhibited higher bacterial population than murrels irrespective of collection ponds. This is may be due to the relatively longer intestine in the digestive tract of rohu and neutral or an alkaline environment therein probably because of the absence of stomach.

The maximum density of proteolytic bacteria was detected in *C. punctatus*. Maximum protease producing capacity was observed within a strain from the same species (CpB2). Several workers have reported endogenous protease activity in fish. Kawai and Ikeda (1972) reported adaptive changes in the proteolytic enzyme in common carp (*Cyprinus carpio*) in relation to the type of the diet. Dabrowski and Glogowski (1977) reported to increase proteolytic enzyme activity considerably when common carp fry are provided with bovine trypsin in their diet. Thus, the occurrence of proteolytic bacteria in the gut of murrel in high intensity also seems to support the presence of diet dependent microbial population indicating their feeding towards animal matter. On the other hand, the intestine of this fish is short and bears a distinct stomach indicating production of endogenous protease and also their carnivorous feeding aptitude. Colonization of amylolytic and cellulolytic bacteria in such a high intensity may suggest that supplementation of amylase and cellulase serves as the basis for the symbiotic (mutual) relationship between the bacterial flora and the fish species. Among the fish, endogenous amylase activities in the intestine of herbivorous 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. Though, being carnivore species higher densities of amylolytic bacteria in the digestive tracts of murrels than rohu in both ponds is surprising.

Reports on the existence of cellulase activity in the digestive system of fish are rare and moreover are conflicting with contradictory result. Fish (1951), Barrington (1957) and Yokoi and Yasumasu (1964) believed that fish do not posses endogenous cellulase. Shcherbina and Kazlawlene (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 cellulose 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 cellulase-producing bacteria as a part of persistent intestinal flora in fish. The presence of considerable cellulolytic bacterial population has been observed in fish digestive tracts in the present investigation. Such abundance of cellulolytic bacteria has gained further support from the reports made by Das and Tripathi (1991) in grass

carp, Saha and Ray (1998) and Ghosh *et al.* (2002) in rohu fingerlings. Other vertebrates including ruminants have also been shown to possess a cellulolytic microflora (Crobsy and Reid, 1971). Bairagi *et al.* (2002) could not detect cellulolytic bacteria in the gastrointestinal tract of carnivorous catfish 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.

It has been revealed from the present study that the bacteria present within the gut of *L. rohita* and *C. puntatus* 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 the intestinal microflora 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 (Stavric 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 of 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.

Acknowledgements

The authors are grateful to the Head, Department of Zoology, The University of Burdwan, West Bengal, India and The Department of Science and Technology (FIST programme), New Delhi, India for providing research facilities, and UGC Minor Research Project F. No. 32-523 / 2006 (SR) for partial financial support.

References

Bairagi, A., Ghosh, K., Sen, S.K. and Ray, A.K. 2002. Enzyme producing bacterial flora isolated from fish digestive tracts. Aquaculture International, 10: 109-121.

Barrington, E.J.W. 1957. The alimentary canal and digestion. In: M.E. Brown (Ed.), The Physiology of Fishes. Vol. 1, Academic Press, New York: 109-161.

Blanch, A.R., Alsina, M., Simon, M. and Jofre, J. 1997. Determination of bacteria associated with reared turbot (*Scophthalmus maximus*) larvae. Journal of Applied Microbiology, 82: 729-734.

Cahill, M.M. 1990. Bacterial flora of fishes: a review. Microbial Ecology, 19: 21-41.

Creach, P.V. 1963. Les enzymes proteolytiques des poisons. In La Nutrition Chez les Poecilothermes. CNRS (Ed.), Paris, France: 375-471.

Crobsy, N.D. and Reid, R.G.B. 1971. Relationship between food, phylogeny and cellulase digestion in the Bivalvia. Canadian Journal of Zoology, 49: 617-622.

Dabrowski, K. and Glogowski, J. 1977. Studies on the role of exogenous proteolytic enzymes in digestion processes in fish. Hydrobiologia, 54: 129-134.

Das, D. and Das, A. 1993. Statistics in Biology and Psychology, Academic Publishers, Calcutta, India.

Das, K.M. and Tripathi, S.D. 1991. Studies on the digestive enzymes of grass carp, *Ctenopharyngodon idella* (V). Aquaculture, 92: 21-32.

Dhage, K.P. 1968. Studies of the digestive enzymes in the three species of the major carps of India. Journal of Biological Science, 11: 63-74.

Drewe, K.E., Horn, M.H., Dickson, K.A. and Gawlicka, A. 2004. Insectivore to frugivore: changes in gut morphology and digestive enzyme activity in the Characid fish *Brycon guatermalensis* from CostaRican rain forest streams. Journal of Fish Biology, 64: 890-902.

Finegold, S.M., Sutter, V.L. and Mathisen, G.E. 1983. Normal indigenous intestinal flora, In: D.J. Hentgens (Ed.), Human Intestinal Microflora in Health and Disease. Academic Press, New York: 3-31.

Fish, G.K. 1951. Digestion in Tilapia esculenta. Nature, 167: 900-901.

Floch, M.N., Gorbach, S.L. and Lucky, T.D. 1970. Symposium: The intestinal microflora. American Journal of Clinical Nutrition, 23: 1425-1540.

Ghosh, K., Sen, S.K. and Ray, A.K. 2002. Characterization of bacilli isolated from the gut of rohu, *Labeo rohita* fingerlings and its significance in digestion. Journal of Applied Aquaculture, 12(3): 33-42.

Horslay, R.W. 1997. A review of the bacterial flora of teleosts and elasmobranches, including methods for its analysis. Journal of Fish Biology, 10: 529-553.

Hugueny, A.M. and Pouilly, M. 1999. Morphological correlation of diets in an assemblage of West African freshwater fishes. Journal of Fish Biology, 54: 1310-1325.

Jacob, M.B. and Gerstein, M.J. 1960. Hand book of Microbiology. D. Van Nostrand Co. Inc. Princeton. New Jersey, USA, 61 pp.

Jhingran, V.G., 1997. Fish and Fisheries of India. 3rd Edition. Hindustan Publishing Corporation, Delhi, India, 335-337.

Kawai, S. and Ikeda, S. 1972. Studies on digestive enzymes of fishes. Effect of dietary change on the activities of digestive enzymes in carp intestine. Bull. Jpn. Soc. Sci. Fish., 38: 265-270.

Lesel, R., Fromageot, C. and Lesel, M. 1986. Cellulase digestibility in grass carp, *Ctenopharyngodon idella* and in gold fish, *Carassius auratus*. Aquaculture, 54: 11-17.

Lindsay, G.J.H. and Harris, J.E. 1980. Carboxymethyl cellulase activity in the digestive tracts of fish. Journal of Fish Biology, 6: 219-233.

Okeyo, D.O. 1989. Herbivory in freshwater fishes: a review. Israeli J. of Aquaculture Bamidgeh, 41: 77-97.

Ringø, E., Olsen, R.E., Mayhew, T.M. and Myklebust, R. 2003. Electron microscopy of the intestinal microflora of fish. Aquaculture, 227: 395-415.

Saha, A.K. and Ray, A.K. 1998. Cellulase activity in rohu fingerlings. Aquaculture International, 6: 281-291.

Saha, S., Roy, R.N., Sen, S.K. and Ray, A.K. 2006. Characterization of cellulase-producing bacteria from the digestive tract of tilapia, *Oreochromis mossambica* (P) and grass carp, *Ctenopharyngodon idella* (V). Aquaculture Research, 37: 380-388.

Sarbahi, D.S. 1951. Studies on the digestive enzymes of goldfish *Carassius auratus* (L) and large mouth black bass *Micropterus salmoides* (L). Biological Bulletin, 100: 244-257.

Shcherbina, M.A. and Kazlawlene, O.P. 1971. The reaction, of the medium and the rate of absorption of nutrients in the intestine of carp. Journal of Icthyology, 11: 81-85.

Smith, L.S. 1989. Digestive functions in teleost fishes. In: J.E. Halver (Ed.), Fish Nutrition, 2nd Ed. Academic Press, San Diego: 331-421.

Stavric, S. and Kornegay, T. 1995. Microbial probiotics for pigs and poultry. In: R.J. Wallace and A. Chuson, (Eds.), Biotechnology in Animal Feeds and Animal Feeding. Weinheim, New York: 205-231.

Stickney, R.R. 1975. Cellulase activity in the stomachs of freshwater fishes from Taxas. Proc. of southeast Association of Game fish Commission, 29: 282-287.

Sugita, H., Shibuya, K., Harada, H. and Deguchi, Y. 1997. Antibacterial abilities of intestinal microflora of the river fish. Fisheries Science, 63: 378-383.

Teather, R.M. and Wood, P.J. 1982. Use of Congo-red polysaccharide interactions in enumeration and characterization of cellulolytic bacteria from the bovine rumen. Applied Environmental Microbiology, 43: 777-780.

Trust, T.J. and Sparrow, R.A.H. 1974. The bacterial flora in the alimentary tract of fresh water salmonid fishes. Canadian Journal of Microbiology, 20: 1219-1228.

Yokoi, Y. and Yasumasu, I. 1964. The distribution of cellulase in invertebrates. Comparative Biochemistry and Physiology, 13**:** 223-228.