



## **Analysis of Total Microbial Count in the Gut of Fresh Water Fish *Cyprinus carpio* fed with isolated probiotics.**

**K.Parvathi<sup>1\*</sup> and J. Karthegaa<sup>2</sup>**

<sup>1</sup>Assistant Professor in Zoology, Erode Arts and Science College (Autonomous), Erode, India.

<sup>2</sup>Ph. D Scholar, Erode Arts and Science College (Autonomous) Erode, India.

\*Corresponding author: [karthegaas@gmail.com](mailto:karthegaas@gmail.com)

### **Abstract**

The aim of the present study was to count the total heterotrophic microbial count in the gut of fresh water fish *Cyprinus carpio*. Probiotics are bio-friendly agents that can be introduced into the cultured environment to promote the growth of cultured organism. The experiment was conducted in two ponds such as control pond (without probiotics) and an experimental pond (*Bacillus spp*). The total heterotrophic microbial count in the fish gut was analyzed for three months with the interval of 30 days. From the present results, it is concluded that the fish fed with probiotic bacteria (*Bacillus spp.*) shows higher microbial count in the gut of *Cyprinus carpio* than the fish fed in control feed.

**Keywords:** *Cyprinus carpio*, Isolated probiotics, Total microbial count.

### **Introduction**

Fish are the major food components from ancient time (Noor *et al.*, 2013) which are highly perishable food, due to its quick perishability leads the main difficulty in its preservation (Okoro *et al.*, 2010; Musa *et al.*, 2010; Dewi *et al.*, 2011) easy digestible and high nutritional value is an advantage of fish (Leisner *et al.*, 2001). Probiotics is one of the technique to increase the yield with the demand of eco- friendly aquaculture. The probiotics is "Organisms and substances which contribute to intestinal microbial balance" (Parker, 1974). Freshly caught fish microflora is largely a reflection of microbial quality of the water from where they are cultured. Gastrointestinal bacteria take part in the decomposition of nutrients with the help of active materials such as enzymes, aminoacids and vitamins (Bairagi *et al.*, 2004; Wache *et al.*, 2006; Wang, 2007; Wang and Xu, 2006). The probiotics in aquaculture has more beneficial effects. It improves water quality,

increases growth, enhance immune response of host and enhancement of nutrition of host through the supplemental digestive enzyme (Thomas *et al.*, 1999, Verschuere *et al.*, 2000, Carnevali *et al.*, 2006).

Majority of the probiotics are non pathogenic, non-toxic and can survive in the intestinal gut and remain potent for long period of days under storage condition (Ramakrishnan *et al.*, 2008). Karthegaa *et al.*, (2016) observed higher growth rate in *Cyprinus carpio* when the fish fed with isolated gut microbes along with the formulated feed. The aim of the present study was to analysis the total heterotrophic count in the gut of fresh water fish *Cyprinus carpio*.

## Materials and Methods

The experiment was conducted in two ponds situated in Berruhalli village which is located at Pochampalli Taluk (12.33°N 78.36°E) Krishnagiri district in Tamil Nadu, India. Among the two ponds, pond A is control (without probiotics) and pond B is an experimental (*Bacillus spp*).

### Feed preparation and feeding

To prepare the diet, the following ingredients such as (rice bran, ground nut oil cake, dry fish, soya, gelatin, vitamin & mineral mix and fish oil) were purchased from local Erode market, Tamil Nadu, India. All the ingredients were mixed and powdered by a machine and were made into dough with the help of distilled water (Sivakumar *et al.*, 2014).

### Analysis of gut microflora

Fish were randomly selected from each pond and collected in sterile plastic bags and carried to laboratory for further biological analysis. The fish were starved for 24hrs before sampling. The surface of the *Cyprinus carpio* was disinfected with alcohol (70%), dissected under aseptic conditions and intestines were taken out and cut into small pieces and homogenized in 10ml distilled water by Bergey's manual method (Holt *et al.*, 1996). The dilution was spread on nutrient agar medium followed by 24 hr

incubation at 37°C to count total colony of bacteria (Paludan – Muller *et al.*, 1999).

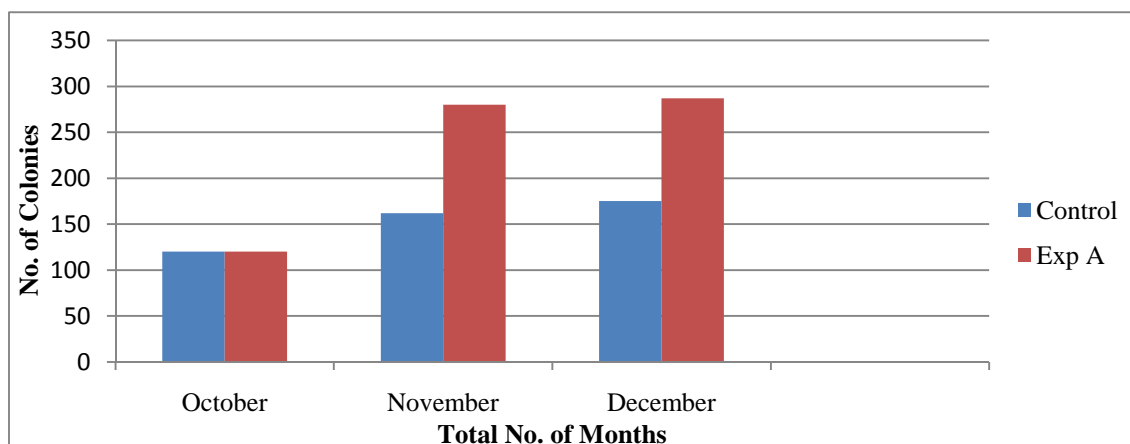
## Results and Discussion

The total bacterial load of gut sample in *Cyprinus carpio* was analysed for three months with the interval of 30 days. The bacterial population in the gut of the fish generally varies due to the hydrobiological fluctuations occurring in the natural systems (Rheinheimer, 1985). Probiotics are live microbial cells that are administrated to intestinal tract as a feed supplement and improving its intestinal microbial balance and health (Fuller, 1989). Probiotic might be used in two ways in aquaculture; through the diet or by introducing the beneficial bacteria into water. Wadher *et al.*, (2010) explains that probiotic bacteria used to preserve intestinal integrity and reduce the effects of colitis, alcoholic liver disease, irritable bowel syndrome.

The total heterotrophic count (THP) in gut of control fish for 3 months recorded as  $1.20 \times 10^6$ ,  $1.62 \times 10^6$ ,  $2.80 \times 10^6$  where as the total heterotrophic microbial count in the experimental fish was recorded as  $2.20 \times 10^6$ ,  $1.75 \times 10^6$ ,  $2.87 \times 10^6$  respectively ( Table 1 and Figure 1). Supplementary components with probiotics in fish feed have strong adhesive and growth abilities (Mukhopadyya and Paul 1996). Bairagi *et al.* (2002) stated that *Bacillus* species isolated from the gut of *Cyprinus carpio* have high amount of extracellular amylolytic, lipolytic and proteolytic activity.

**Table 1: Total count of gut microbes in fresh water fish *Cyprinus carpio***

S. No	Pond	No. of Colonies			Dilution No
		October	November	December	
1	Control	$1.20 \times 10^6$	$1.62 \times 10^6$	$1.75 \times 10^6$	$10^6$
2	A	$2.20 \times 10^6$	$2.80 \times 10^6$	$2.87 \times 10^6$	



**Fig 1: Effect of probiotics on gastro intestinal tract of *Cyprinus carpio*.**

From the present result fish fed with probiotics in the diet improves food conversion and nutrition retention hence leading to higher growth of fish. Higher growth rate was recorded in our study also. It is also concluded that the fish fed with probiotic bacteria (*Bacillus spp.*) shows higher microbial count in the gut of *Cyprinus carpio* than the fish fed in control feed.

## References

1. Wadher KJ, Mahore JG, Umekar MJ. Probiotics: living medicines in health maintenance and disease prevention. Int J Pharm Bio Sci 2010; 1(3): 1-9.
2. Carnivali, O.D., Sulpizio, L., Gicchini, R., Olivotto, I.G. and Silvi, S. (2006) Growth improvement by probiotic in European sea bass juveniles (*Dicentrarchus labrax*, L.), with particular attention to IGF- 1, myostatin and cortisol gene expression. Aquaculture, 258, 430-438.
3. Ramakrishnan, C.M., Haniffa, M.A., Mahonar, M., Dhanraj, M., Jesu Arockiaraj, A., Seetharaman, S. & Arunsingh, S.V. (2008) Effects of probiotics and spirulina on survival and growth of juvenile common carp (*Cyprinus carpio*). Isr. J. Aquacult., 60, 128- 133.
4. Bairagi, A., Sarkar Ghosh, K., Sen, S.K. & Ray, A.K. (2002) Duckweed (*Lemna polyrhiza*) leaf meal as a source of feedstuff in formulated diets for rohu (*Labeo rohita*) Ham. fingerlings after fermentation with a fish intestinal bacterium. Bioresour. Technol., 85, 17-24.
5. Parker, R. B., 1974. Probiotics, the other half of the story. Animal Nutrition and Health 29, 4-8.
6. Paludan - Muller C, Huss, HH and Gram L. Characterization of lactic acid bacteria isolated from a Thai low-salt fermented fish product and the role of garlic as substrate for fermentation. Int. J. Food Microbiol. 46, 1999, 219- 229.
7. Mukhopadhyay P.K. and Paul B.N. (1996) value addition components in aquaculture feeds. Fishing Chimes, 16: 15-16.
8. Verschuere, L., G. Rombaut, P. Sorgeloos and W. Verstraete, 2000. Probiotic bacteria as biological control agents in aquaculture. Microbiol. Mol. Biol. Rev., 64; 655- 671.
9. Fuller R., 1989. Probiotics in man and animals. J. Appl. Bacteriol., 66; n 365- 378.
10. Leisner, J.J., Vancanneyt, M., Rusul, G., Pot, B., Lefebvre, K., Fresi, A. and Tee, L.T., 2001. Identification of lactic acid bacteria constituting the predom, mating microflora in an acid fermented condiment (tempoyak) popular in Malaysia. Inter. J. food Microbiol. 63:147-157.
11. Musa, U.S., Hati, S., Adam, Y.I. and Mustapha, A., 2010. Pesticide residues in smoked fish samoles from North- Eastern Nigeria. J. Applied Sci, 10:975-980.
12. Noor, R., Acharjee, M., Ahmed, T., Das, K.K., Paul, L., Munshi, S.K., Urmi, N.J., Rahman, F. and Md. Zahangir Alam., 2013. Microbiological study of major sea fish available in local markets of Dhaka city, Bangladesh. J. Microbiol. Biotechnol. Food Sci. 2(4): 2420-2430.
13. Okoro, C.C., Aboaba, O.O. and Babajide, O.J., 2010. Quality Assessment of a Nigerian Marine Fish, Mullet (*Liza falcipinnis*) under different Storage Conditions. New York Science J., 3(8): 2128.
14. Rheinemer, G., 1985. Aquatic Microbiology, 3rd ed. University of Kiel, West Germany. Wiley, Chichester, New York, Brisbane, Toronto, p. 257.
15. Dewi, R.S., Huda, N. and Ahmed, R., 2011. Changes in the physicochemical properties, microstructure and sensory characteristics of shark dendeng using different drying methods. Am. J. Food Technol, 6: 149-157.
16. Karthegaa. J., Parvathi. K., Jaya Prakash. S., 2016. The effect of isolated probiotic bacteria on the growth performance of fresh water fish *Cyprinus carpio*. Int. J. of Res. In Pharmacology & Pharmacotherapeutic Vol-5(4) 2016.
17. Bairagi, A., Sarkar Ghosh, K., Sen, S.K., Ray, A.K., 2004. Evaluation of the nutritive value of *Leucaena leucocephala* leaf meal, inoculated with fish intestinal bacteria *Bacillus subtilis* and *Bacillus circulans* in formulated diets for rohu, *Labeo rohita* (Hamilton) fingerlings. Aquac. Res. 35, 436-446.
18. Wacheø, Y., Auffray, F., Gatesoupe, F.J., Zambonino, J., Gayet, V., Labbeø, L., Quentel, C., 2006. Cross effects of the strain of dietary *Saccharomyces cerevisiae* and rearing conditions on the onset of intestinal microbiota and digestive enzymes in rainbow trout, *Onchorhynchus mykiss*, fry. Aquaculture 258, 470-478.
19. Wang, Y.B., 2007. Effect of probiotics on growth performance and digestive enzyme activity of the shrimp *Penaeus vannamei*. Aquaculture 269, 259-264.
20. Wang, Y.B., Xu, Z.R., 2006. Effect of probiotics for common carp (*Cyprinus carpio*) based on growth performance and digestive enzyme activities. Anim. Feed Sci. Technol. 127, 283-292.

21. Sivakumar P, Rajan MR, Ramachandran P. Effects of probiotics on growth performance of common carp *Cyprinus carpio* var communis. Int J Pharm Bio Sci. 5(1), 2014, 835- 839.
22. Holt, J.G., Krieg, N.R., Sneath, P.H.A., Staley, J.T. and Williams, S.T. 1994. Bergey's Manual of Determinative Bacteriology. 9th edition.

Access this Article in Online	
	Website: <a href="http://www.ijarbs.com">www.ijarbs.com</a>
	Subject: Zoology
Quick Response Code	
DOI: <a href="https://doi.org/10.22192/ijarbs.2017.04.07.021">10.22192/ijarbs.2017.04.07.021</a>	

How to cite this article:

K.Parvathi and J. Karthegaa. (2017). Analysis of Total Microbial Count in the Gut of Fresh Water Fish *Cyprinus carpio* fed with isolated probiotics. Int. J. Adv. Res. Biol. Sci. 4(7): 169-172.

DOI: <http://dx.doi.org/10.22192/ijarbs.2017.04.07.021>