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Effect of Commercial Aquaculture Probiotic and Fish Gut Antagonistic Bacterial Flora on the Growth and Disease Resistance of Ornamental Fishes *Carassius auratus* and *Xiphophorus helleri*

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Özet: ***Akuakültür probiyotiği ve mide antogonistik bakteriyel florasının* Carassius auratus *ve* Xiphophorus helleri*'de gelişim ve hastalık direncine etkisi.*** Bu çalışmada ticari bir akuakültür biyotiğinin (CA probiyotik) ile *Lactobacillus sp.* ve *Bacillus sp.* (FG probiyotik) gibi balık midesinde bulunan antogonistik bakteriyel floranın bir karışımının süs balıklarından *Carassius auratus* ve *Xiphophorus helleri* türlerinin gelişimi ve hastalık direnci üzerine etkisi araştırılmıştır. 5g/kg seviyesinde CA probiyotik ile beslenen *C. auratus*'ta gelişiminde olumlu bir etkisi olmadığı ve aksine kontrol grubu ile karşılaştırıldığında büyümede yavaşlama gözlenmiştir. Benzer şekilde *X. helleri*'de de toplam yaş ağırlık kazanımı, besin tüketim oranı (FCR) ve spesifik büyüme oranı (SGR) üzerinde önemli bir etkisi bulunamamıştır. Bir başka açıdan, FG probiyotik içerikli besinler ile beslenen *C. auratus* bireylerinde FCR dışında ($p<0.04$) gelişim parametrelerindeki değişimler istatistiksel açıdan önemli değildir. Buna karşın aynı probiyotikli besinin *X. Helleri*'de toplam yaş ağırlık ve FCR açısından kontrol grubu ile önemli farklılıkları tespit edilmiştir. CA ve FG probiyotikli besinler, balıklarda *Pseudomonas fluorescens* 58C enfeksiyonuna karşın bir koruma sağlamamıştır ($p>0.05$). Ancak FG probiyotik ilaveli besleme, kontrol grubu ile karşılaştırıldığında yüzgeç ve kuyrukta oluşan çürümelerde önemli farklılıklar göstermiştir.

Anahtar Kelimeler: *Carassius auratus, Xiphophorus helleri,* probiotik, *Lactobacillus* sp., *Bacillus* sp., hastalık direnci.

Abstract: Effect of commercial aquaculture probiotic (CA probiotic) and a mixture of fish gut antagonistic bacterial flora such as *Lactobacillus* sp. and *Bacillus* sp. (FG probiotic) on the growth and disease resistance of ornamental fishes *Carassius auratus* and *Xiphophorus helleri* was investigated. The CA probiotic at a level of 5g / kg feed had no effect on the growth rate of *C. auratus,* rather it reduced the growth compared to control group. Also it had no significant effect on the total wet weight gain, food conversion ratio (FCR) and specific growth rate (SGR) of *X. helleri*. On the other hand, in FG probiotic feed fed *C. auratus,* except FCR ($P<0.04$), the variations in growth parameters were statistically insignificant. While in *X. helleri*, there existed significant differences in the total wet weight gain and FCR of FG probiotic feed fed and control groups. The CA and FG probiotic feed fed fishes did not show any significant protection ($P>0.05$) against *Pseudomonas fluorescens* 58C infection. However, marked differences in the fishes exhibiting fin and tail rot were noticed between the FG probiotic fed and control groups

Key Words: *Carassius auratus, Xiphophorus helleri,* probiotic, *Lactobacillus* sp., *Bacillus* sp., disease resistance.

Introduction

Intensive aquafarming accompanies several disease problems often due to opportunistic pathogens as evident from general aquaculture. High stocking densities, high food inputs and other organic loads stimulate the selection and proliferation of opportunistic bacteria (Austin *et al*. 1995). Due to this negative balance of the microbial community in rearing water as well as in fish gut, the aquaculturists often face mass mortality of their stocks. However, with changing scenario farmers are emphasizing on diagnosis and prevention of infection to promote health and production efficiency. The fish farm health management has now become an integral part of ornamental fish Quality Assurance programme. Though the use of antibiotics and chemotherapy remains the method of choice as disease control strategy, the abuse of chemotherapeutics, especially antibiotics has resulted in development of multiple antibiotic resistant bacteria. Increased concern about antibiotic resistant microorganisms has led to several alternatives including use of non-pathogenic microorganisms as probiotic. India with a vast resource in the form of natural water bodies and species diversity has a great potential to uplift the production of ornamental fish. India shares only 0.007% of global trade in ornamental fish that can be raised to 0.1% in the next 5 years. The use of probiotics in aquaculture (Kozasa 1986; Gatesoupe 1991; 1994; Uma *et al.* 1999; Irianto and Austin 2002), and freshwater ornamental fish culture (Abraham *et al*. 2007a, b; Abraham 2008) is well documented. This communication reports the effect of commercial aquaculture probiotic and a mixture of fish gut antagonistic bacterial flora (*Lactobacillus* sp. and *Bacillus* sp.) on the growth and disease resistance of ornamental fishes *Carassius auratus* and *Xiphophorus helleri.*

Materials and Methods

The experiemental fishes goldfish, *Carassius auratus* (Linnaeus, 1758) and swordtail, *Xiphophorus helleri* (Heckel,

1848) were procured respectively from commercial goldfish breeders of Santragachi, Howrah district and swordtail breeders of Amtala, South 24 Parganas district, West Bengal, India. A commercial probiotic for aquaculture application, which contained 2.82×10^8 cfu of live probiotic cells/g product, comprising *Lactobacillus sporogens, L. acidophilus, Bacillus subtilis, B. licheniformis, Streptococcus faecium, Saccharomyces cerevisiae* together with vitamins and minerals was procured locally for experiment 1. Two antagonistic bacterial strains, viz., *Lactobacillus* sp. P21 (LP21) and *Bacillus* sp. P3 (BP3) isolated respectively from *Cirrhinus mrigala* gut and *Carassius auratus* gut as described in Abraham *et al.* (2007a) were used as probiotic strains in experiment 2. A commercial fish feed containing crude protein (Min 41%), crude fat (Min 6%), crude fiber (Min 3%) and moisture (Max 11%) was used for feeding the experimental fishes. The basic ingredients as per the manufacturer of the feed include: fishmeal, fish lipid oil, fish solubles, squid liver powder, wheat flour and soya meal, lecithin, vitamin-C and vitamin and mineral premixes. The binder used was of the brand Trubind (Wockhardt, Mumbai, India). Each 10 g binder contained 100 mg protein, 25 mg cholesterol, 10 mg calcium, 20µg vitamin D3 and 50µg carotenoid as per the manufacturer of the binder.

The commercial aquaculture probiotic was admixed with the basal dry feed at a level of 5g / kg feed using binder (CA probiotic feed). The probiotic stains LP21 (10^6 cells / g feed) and BP3 (10^5 cells / g feed) were added into the basal feed and admixed with binder (FG probiotic feed) as described earlier elsewhere (Abraham *et al.* 2007b). The binder was used at the rate 10-ml / 100 g feed. In control feed, binder alone was added as in test feeds. After admixing the ingredients using binder, the feeds were air dried for 1–2 days and placed in airtight plastic containers separately at room temperature (26-32^{0}C).

Fifteen gold fish, *C. auratus* of size ranging from 1.36 - 1.58 g weight and 46.75 – 48.20 mm length were introduced into each of six glass aquaria of 50-liter capacity, which contained 35-liter bore well water. Likewise, twenty-swordtail, *X. helleri* of size ranging from 1.37 – 1.52 g weight and 46.65 – 48.80 mm length were introduced into each of six glass aquaria containing 35-liter bore well water. During the study period of 30 days with continuous aeration, *C. auratus* and *X. helleri* were fed with CA probiotic feed. The fishes of control tanks were fed with control feed in triplicate.

Fifteen gold fish, *C. auratus* of size ranging from 0.4 – 0.5g weight and 28 – 29 mm length were introduced into each of six glass aquaria of 50-liter capacity, which contained 35-liter bore well water. Likewise, twenty-swordtail, *X. helleri* 0.18 – 0.22g weight and 23 – 26 mm length were introduced into each of six glass aquaria containing 35-liter bore well water. During the study period of 30 days (60 days for *X. helleri*) with continuous aeration, *C. auratus* and *X. helleri* were fed with FG probiotic feed containing a mixture of *Lactobacillus* sp. P21 and *Bacillus* sp. P3. The fishes of control tanks were fed with control feed in triplicate.

In all the cases, feeding was done daily at the rate 5% of the body weight for *C. auratus* and 3% of the body weight for *X. helleri,* in 2 split doses. The wastes and faecal matter were siphoned out and 50% of the water was exchanged on every 3rd day. The fishes were observed for mortality daily and the dead ones removed immediately and weighed. The length and weight of the fish of all categories were noted at regular intervals. From these data, the survival percentage, wet weight gain, feed conversion ratio (FCR) and specific growth rate (SGR) were estimated.

A pathogenic bacterium *Pseudomonas fluorescens* 58C was used in the challenge experiment by immersion assay (Austin *et al.* 1995). Ten fishes each from CA probiotic feed fed and control groups of *C. auratus* and *X. helleri* from experiment 1 were introduced respectively into the tanks (G1 – G4 and X1 – X4) containing 20L bore well water. Likewise, ten fishes each from FG probiotic feed fed and control groups of *C. auratus* and *X. helleri* from experiment 2 were introduced respectively into the tanks (G5 – G8 and X5 – X8) containing 20L bore well water. To facilitate infection, two or three scales were removed from five fishes from each tank and reintroduced into the respective tanks. The cell suspension of *P. fluorescens* 58C was inoculated into odd numbered tanks in such a way to get a level of 10^7 cells/ml rearing medium. The even numbered tanks served as control for both probiotic feed fed and control groups of experiment 1 and 2. The experiment was carried out for a period of 30 days in duplicate and the fishes were fed daily with basal diet on demand. The dead fishes were removed immediately. The accumulated wastes and faecal matter were siphoned out on every 5th day. Mortality, external signs of infection and behavioural abnormalities were recorded daily. Chi-square (χ^2) test was followed to determine the significance of difference in the survival and disease resistance of the treatment and control groups (Snedecor and Cochran 1974).

Results and Discussion

Use of probiotics in aquaculture began with the commercial preparation meant for terrestrial animals. With increasing intensification in commercial aquaculture, many products are being made available for aquaculture purpose with varying success rate. The results of the present study (Table 1) revealed that the commercial aquaculture probiotic at a level of 5g / kg feed had no effect on the growth rate of *C. auratus* and *X. helleri.* Rather, it reduced the growth rate of *C. auratus* compared to control group. The CA probiotic feed also had no significant effect on the total wet weight gain, FCR, SGR of *X. helleri* ($P>0.05$). In FG probiotic feed fed *C. auratus,* except FCR ($P<0.04$), the variations in growth parameters were statistically insignificant. There existed significant differences in the total wet weight gain and FCR of FG probiotic feed fed and control groups of *X. helleri*.

Many workers have used commercially available products to improve the growth performance of fish successfully. The spores of *Bacillus toyoi* and other *Bacillus*

sp. when used as feed additive increased the growth rate of yellow tail, *Seriola quinquiradiata* (Kozasa 1986), turbot *Scophthalmus maximus* (Gatesoupe 1991; 1994), common snook, *Centropomus undecimalis* (Irianto and Austin 2002) and *Penaeus monodon* (Vaseeharan and Ramasamy 2003). The commercial preparations of *Streptococcus faecium* and a mixture of bacteria and yeast improved the growth and food conversion efficiency of *Cyprinus carpio* (Bogut *et al.* 1998) and *Catla catla* (Mohanty *et al.* 1996), respectively. The results of Lara-Flores *et al*. (2003) also indicated that the *Oreochromis niloticus* fry subjected to diets with a probiotic supplement exhibited greater growth than those fed with the control diet. The recent reports on the use of *Lactobacillus* spp. and *Bacillus* spp. (Salinas *et al*. 2005; Balca´zar and Rojas-Luna, 2007; Aly *et al*. 2008) also demonstrated the beneficial effects of stimulating the gut immune system and the growth improvements in the fish larvae. The results of the study with FG probiotic corroborate the observations of Carnevali et al. (2004), who recorded a significantly decreased larvae and fry mortality when *Lactobacillus fructivorans* (AS17B), isolated from sea bream (*Sparus aurata*) gut, was used a probiotic.

The results of the CA probiotic of the present study, however, are in accordance with few of the earlier studies (Epifario 1979; Uma *et al*. 1999; Murthy and Naik 2002) conducted on a variety of aquatic animals. For example, reduced growth due to poor digestion of oyster *Crassostrea virginica* fed with higher proportion of yeast (Epifario 1979), reduced growth due to catabolic effect at higher dose of Biovet-YC (Murthy and Naik 2002) in *C. mrigala* have been amply documented. Uma *et al.* (1999) investigated the efficiency of commercial probiotic (Lactosacc) containing organisms similar to CA probiotic feed and observed a systematic reduction in the growth of *Penaeus indicus* when fed with higher dose of lactosacc due to poor digestion and assimilation of yeast and excessive faecal loss.

Table 1. Growth performance of *Carassius auratus* and *Xiphophorus helleri* fed with commercial aquaculture probiotic, and fish gut probiotic feed containing *Lactobacillus* sp. and *Bacillus* sp.

Growth parameters	*Carassius auratus*		*Xiphophorus helleri*	
Experiment 1	**CA probiotic feed**	**Control**	**CA probiotic feed**	**Control**
Total wet weight gain (g)	15.26 ± 0.77[a]	16.57 ± 0.08[a]	6.30 ± 3.94	5.67 ± 1.37
Mean survival (%)	100.00 ± 0.00	100.00 ± 0.00	93.33 ± 2.36	96.67 ± 2.36
Food conversion ratio	2.65 ± 0.05	2.44 ± 0.21	3.21 ± 1.16	3.59 ± 0.86
Specific growth rate	1.83 ± 0.53	1.96 ± 0.14	1.03 ± 0.36	0.91 ± 0.25
Experiment 2	**FG probiotic feed**	**Control**	**FG probiotic feed**	**Control**
Total wet weight gain (g)	4.29 ± 0.27	3.75 ± 1.03	6.91 ± 1.26[c]	4.93 ± 1.15[c]
Mean survival (%)	55.53 ± 6.32	51.07 ± 3.16	71.67 ± 4.71	68.33 ± 4.71
Food conversion ratio	1.47 ± 0.0[b]	2.16 ± 0.40[b]	1.64 ± 0.21[d]	2.40 ± 0.40[d]
Specific growth rate	2.53 ± 0.02	2.25 ± 0.48	1.03 ± 0.36	0.91 ± 0.25

Values sharing common superscripts within rows are significantly different. a: P<0.04, t= -2.95, df=4; b: P<0.04, t= -2.95, df=4; c: P<0.0066, t= 12.22, df=4; d: P<0.043, t= -2.91, df=4.

Table 2. Disease resistance in *Carassius auratus* and *Xiphophorus helleri* fed with commercial aquaculture probiotic and fish gut probiotic feed containing *Lactobacillus* sp. and *Bacillus* sp.

Treatment	Survival (%)		Infectivity* (%)	
	Infected stock	**Uninfected stock**	**Infected stock**	**Uninfected stock**
Carassius auratus				
CA probiotic feed	95.00. ± 5.00	100.00 ± 0.00	15.00 ± 5.00	10.00 ± 0.00
Control	95.00 ± 5.00	100.00 ± 0.00	25.00 ± 5.00	10.00 ± 0.00
Xiphophorus helleri				
CA probiotic feed	85.00 ± 5.00	90.00 ± 0.00	30.00 ± 10.00	10.00 ± 0.00
Control	85.00 ± 5.00	90.00 ± 0.00	40.00 ± 10.00	10.00 ± 0.00
Carassius auratus				
FG probiotic feed	100.00 ± 0.00	100.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Control	100.00 ± 0.00	100.00 ± 0.00	10.00 ± 0.00	5.00 ± 5.00
Xiphophorus helleri				
FG probiotic feed	90.00 ± 10.00	100.00 ± 0.00	15.00 ± 5.00	0.00 ± 0.00
Control	85.00 ± 5.00	95.00 ± 5.00	35.00 ± 5.00	5.00 ± 5.00

**: Percentage of fish exhibited tail / fin rot in 30 days of experimental infection. Infected with Pseudomonas fluorescens 58C at a level of 2.10x10^7 cells / ml*

Both probiotic feed fed fishes did not show any significant protection (P>0.05) against *P. fluorescens* 58C, although there was marked difference in the fishes exhibiting fin and tail rot (Table 2). Likewise, Robertson *et al.* (2000) and Abraham et al. (2007b) observed less evidence of minor health problems such as fin and tail rot in probiotic fed group. The results of Uma *et al.* (1999) indicated low mortality rate in lactosacc fed *P. indicus* than control group. The fact is that the aquatic animals are quite different from the land animals for which the probiotic concept was developed. In finfish and shellfish, gram-negative facultative anaerobes prevail in the digestive tract and symbiotic anaerobes may be dominant in

the posterior intestine of some herbivorous tropical fish. *Aeromonas, Plesiomonas* and Enterobacteriaceae are dominant in freshwater fish (Sakata 1990). Most microbes are transients in aquatic animals and may change rapidly with the intrusion of microbes coming from water and food. A consequence of specificity of aquatic microflora is that the most efficient probiotics for aquaculture may be different from those of terrestrial species. Many of the earlier studies used commercial probiotic for land animals and also demonstrated the interest on the use of bacterial addition in aquaculture feeds. But, the survival of probiotic microbes is uncertain in the gastrointestinal tract of aquatic animals and so also the desired beneficial effect as has been observed in CA probiotic feed fed groups. After the pioneer studies by Maeda and Liao (1992), attempts have been aimed at seeking autochthonous bacterial strains with probiotic properties. Although the results of the present study with antagonistic strains *Lactobacillus* sp. P21 and *Bacillus* sp. P3 isolated from fish gut are encouraging, further studies are required to elucidate their usefulness for commercial application in ornamental fish production. The results of the present study would form the basis for future research and development.

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