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Full Length Research Paper

Effect of probiotics on growth and microbiological changes in snakehead *Channa striatus* challenged by *Aeromonas hydrophila*

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The snakehead *Channa striatus* is widely preferred by consumers and is often affected by the dreadful disease Epizootic Ulcerative Syndrome (EUS) and encounters losses in capture as well as culture fisheries. Antibiotics, chemotherapeutants and vaccines are now commonly used in aquaculture to prevent/treat fish diseases. Meanwhile an alternative safer way to tackle the disease problem eco-friendly is to improve the resistance of target species by application of probiotics as growth promoters and immunomodulators. The present investigation was attempted to find out the effects of common probiotic strains *Bacillus subtilis, Bacillus coagulans* and *Saccharomyces cerevisiae* and commercial probiotic Efinol® FG on growth performance and microbiological changes in normal striped murrel *C. striatus* and also those challenged by *Aeromonas hydrophila*. Probiotics were added to the autoclaved semi moist feed and fed to the fingerlings. Feeding experiment was conducted for 45 days. Growth measurements and total microbial count (TMC) were made once in 15 days for a total period of 45 days. Best SGR and FCR were estimated as a function of Efinol® FG diet and the difference in weight gain (16.37 g), SGR (4.22) and FCR (2.28) was found to be significantly different (P<0.05) between Efinol® FG diet and other probiotic diets. TMC is higher in Efinol® FG fed diet (7.7 × 10^8 CFU g^{-1}) than other fed diets. After that the fingerlings from each group were divided into non-challenged (NC) and challenged by *A. hydrophila* by oral injection (OI) and intramuscular injection (IM). Growth measurements and TMC were made again once in 7 days for a total period of 21 days. The challenged group of control diet (OI and IM) showed a drastic increase from day 1 (3.5 × 10^8 CFU g^{-1}) to day 21 (7.1 × 10^9 - 8.8 × 10^9 CFU g^{-1}) whereas the OI and IM groups of all probiotic diets showed a drastic decrease. In the case of OI group Efinol® FG diet, the decrease was from 7.7 x10^8 CFU g^{-1} on day 1 to 2.0 × 10^8 CFU g^{-1}on day 21. The corresponding decrease in IM group was from 7.7 × 1 0^8 CFU g^{-1} to 4.5 × 10^8 CFU g^{-1}. Regarding the equilibrium between competing beneficial Efinol® FG and pathogenic *A. hydrophila,* extension of experimental duration / increase in Efinol® FG concentration may help to arrive at a conclusion.

Key words: *Channa striatus,* probiotics, Efinol® FG, growth parameters, total microbial count.

INTRODUCTION

In aquaculture the concept of biological disease control or bioremediation using microbiological modulators for disease prevention has received wide spread attention all over the world. Probiotics are live microbial cells. When administered to the gastrointestinal tract of hosts as feed supplements, they improve intestinal microbial balance and health ultimately (Gatesupe, 1999). Murrels commonly called snakeheads constitute the most common and dominant group of air–breathing freshwater fish and are highly regarded as food fish in south-east Asian countries (Wee and Tawn, 1982, Haniffa et al., 2006). They are often affected by the dreadful disease

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Table 1. The composition of control diet.

Ingredient	(%)
Fishmeal, Anchovy	26.9
Soybean flour	25
Jawala, Acetes sp.	20
Tapioca meal	10.9
Wheat flour	10
Sunflower Oil	5.8
Mineral Premix	0.5
Monosodium Phosphate	0.5
Aqua Saver	0.3
Vitamin and Mineral premix [a,b]	0.1
Ascorbic acid	0.01

[a] Vitamin mixture providing the following concentration per kilogram diet; vitamin A 5000 IU; vit D 400 IU; vit E 20 mg; thiamin mononitrate (B1) 4 mg; riboflavin (B2) 6 mg; nicotinamide 50 mg; pyridoxine hydrochloride 3 mg; calcium pentohenate 10 mg; cyanocobalamine (B12) 2mg; ascorbic acid (vit C) 100 mg; biotin 0.1 mg. [b] Trace mineral mix use providing the following concentration (ppm) copper 10; iron 100; manganese 50; zinc 50; cobalt 0.05; and iodine 0.1.Probiotic diets – wheat flour was replaced by D1 – Efinol FG, D2 – *B.subtilis,* D3 – *B.coagulans,* D4 – *S.cerevisiae* and D5 – control.

Epizootic Ulcerative Syndrome (EUS) and encounter losses in capture as well as culture fisheries. The primary causative agent is the fungus *Aphanomyces invadans* (Karunasagar et al*.,* 1995) and the opportunistic bacterial pathogen *Aeromonas hydrophila* characterized as virulent (Karunasagar et al*.,* 1995) or cytotoxic (Yadav and Indira Ansari, 1992) invades the blood stream and causes lethal septicemia and this paves the way for drastic mortality in EUS affected fish (Llobrera and Gacutan, 1987; Pal and Pradhan, 1990).

Antibiotics, chemotherapeutants and vaccines are now commonly used in aquaculture to prevent/treat fish diseases. Meanwhile an alternative safer way to tackle the disease problem eco-friendly is to improve the resistance of target species by application of probiotics (Ringo and Birkbeck, 1999; Irianto and Austin 2002). The present investigation was attempted to find out the effects of common probiotic strains *Bacillus subtilis, Bacillus coagulans* and *Saccharomyces cerevisiae* and commercial probiotic Efinol®FG on growth performance and microbiological changes in normal striped murrel *C. striatus* and also those challenged by *A. hydrophila*.

MATERIALS AND METHODS

The probiotic bacteria *B.subtilis* (strain No.359217), *B. coagulans* (strain No.3592174) and yeast *S.cerevisiae* (strain No.592-AC) and commercial probiotic Efinol®FG (strain No.52451) were obtained from Bentoli Agri Nutrition Inc. USA. Pathogenic bacteria *A.hydrophila* were isolated from diseased *C.striatus* collected from the wild. Subculture was maintained on Tryptone Soy Agar slopes (Himedia) at 4°C and was routinely tested for pathogen icity at CARE laboratory. A stock culture in Tryptone Soy Broth was stored in freezer (-20^{O}C) to provide stable inoculam throughout the study.

C.striatus fingerlings (2.5 ± 0.75 g) produced at CARE Aquafarm were used for the present study. The feed ingredients were sterilized and allowed to cool at 50^{O}C. After that the probiotics were added and semi-moist pellets were prepared manually and stored at -20°C until use. The concentration of probiotics u sed was 10^{6} colony forming units per gram (CFU g^{-1}) and in the case of Efinol®FG, the same was 3 g/kg feed (Arthi Manju et al*.,* 2011). Feeding experiment was conducted for 45 days using cement tanks (300 L) each with 45 fingerlings. After an acclimatization period of 7 days on basal control diet (Table 1), the first group was fed on control diet and the remaining groups were fed on Efinol®FG, *B. subtilis*, *B. coagulans* and *S. cerevisiae* incorporated diets. Growth measurements and microbiological analyses were made once in 15 days for a total period of 45 days of phase 1 growth studies. After that the fingerlings from each group were classified into 3 categories, namely non-challenged (NC) and challenged by *A. hydrophila* by oral injection (OI) and intramuscular injection (IM) for phase 2 studies on microbiological analyses for 21 days. Student's t-test was applied to evaluate the difference in performance if any as a function of quality of probiotic diet. Growth data were subjected to one way ANOVA and Duncan (1995) multiple range test. Difference at 5% ($P<0.05$) level was calculated following statistical software version 7 software package.

RESULTS AND DISCUSSION

Incorporation of probiotics in diets produced elevated values in growth parameters of *C. striatus* fingerlings. For instance *C. striatus* fingerlings fed on control diet showed

Table 2. Growth parameters of C.*striatus* fed on four different probiotic diets (diet 1 to 4) and control basal diet (diet 5). All values are given as mean±SD. Means in the same row with different alphabets are significantly different (P<0.05).

Diet	Initial weight (g)	Final weight (g)	Weight gain (g)	Specific growth rate	Food conversion ratio
Control	2.56±0.08	15.91±0.03	13.19±0.09	3.91±0.01	3.91±0.01
Efinol® FG	2.88±0.09	19.25±0.02	16.37±0.08	4.22±0.09[a]	2.28±0.09
B. subtilis	2.79±0.04	17.52±0.16	14.73±0.12	1.07±0.02	2.56±0.02
B. coagulans	2.75±0.06	16.98±0.05	14.23±0.02[b]	4.04±0.02[b]	2.64±0.02[b]
S. cerevisiae	2.56±0.08	15.91±0.03	13.19±0.009	4.17±0.01	2.78±0.01

13.19 g weight gain, whereas those supplied with Efinol® FG gained 16.37 g body weight followed by test individuals fed on *B. subtilis* (14.73g) and *B. coagulans* diets (14.23 g) (Table 2). Concerning the growth performance of *Oreochromis niloticus* treated with *S. cerevisiae* and *B. subtilis*, the results revealed higher growth rate due to probiotic supplemented diet (0.0096 g/day) than basal diet (0.0046 g/day) (Marzouk et al*.,* 2008). The feed conversion ratio (FCR) of *C.striatus* on basal diet (control) was higher (3.9) than diet supplemented with probiotics, Efinol® FG diet (2.28). In the present study, the best SGR of 4.22 and FCR of 2.28 were estimated as a function of Efinol® FG diet and the difference in weight gain, SGR and FCR was found to be significantly different (P<0.05) between Efinol® FG diet and other probiotic diets (Table 2).

According to Marzouk et al*.* (2008), FCR of *O. niloticus* on basal diet (control) was higher (5.8) than diet supplemented with probiotics, *S. cerevisiae* and *B. subtilis* (4.7) due to probiotic supplementation by improved feed utilization. Similarly, *S. cerevisiae* is a protein source by conventional definition (Cheng et al*.*, 2004) and in non-salmonids dietary supplementation of *S. cerevisiae* and other yeast species has improved fish growth (Lara-Flores et al*.,* 2003; Li and Gatlin 2003, 2004). Similar observations have also been made in Indian carp, *Labeo rohita* (Ghosh et al*.,* 2003), *Carnegiella strigata* (Gomes et al*.,* 2008) and common carp *Cyprinus carpio* (Yanbo and Zirong, 2006). El-Haroun et al. (2006) reported that commercial probiotic Biogen improved SGR (1.48 %/day) and FCR (1.67) in *C.carpio.* Noh et al*.* (1994) and Bogut et al*.* (1998) too showed that commercial probiotic preparation of *Streptococcus faecium* has improved the growth and FCR of Israeli carp, *C. carpio* (Ahilan et al*.,* 2004).

After challenged by *A. hydrophila,* decrease in weight gain of fingerlings was observed in both OI and IM groups. For instance the challenged groups (OI and IM) of Efinol® FG diet showed poor weight gain (3.75 and 2.75 g) when compared to the non-challenged group (8.35 g). The challenged groups (OI and IM) of other probiotic diets also showed similar trend of decrease in weight gain when compared to non-challenged groups (P<0.0.5) (Table 3). When weight gain was analyzed as a function of probiotic diets, maximum weight gain of 8.35 g was noticed for Efinol® FG diet followed by *B. subtilis* (7.48 g) and *B. coagulans* (5.0 g) diets. Not only weight gain but also SGR and FCR showed a similar trend. Maximum SGR of 1.7 was noticed in non challenged group of Efinol® FG diet and the same was the lowest in control diet (1.04) as well as *S. cerevisiae* diet (1.04). SGR showed a decrease not only as a function of probiotic quality, but also due to challenge (OI and IM) by *A. hybrophila* (P<0.05). Non-challenged group of control diet showed FCR of 2.97, whereas the same was 2.78 in the case of Efinol® FG fed individuals. As far as FCR was considered, there was not much difference between Efinol® FG group (2.78) and *B. subtilis* group (2.81); similarly between control and the other two probiotic groups also (Table 3).

In the present investigation it is clear that, in *C.striatus* challenged *by A. hydrophila,* the growth performance was poor and this could be due to toxins produced by *A. hydrophila* altering normal physiological activities and ultimately affecting the survival and growth of the host. Meanwhile among the challenged individuals, growth performance was better in the case of those fed on probiotic diet suggesting that production of metabolites by probiotics suppresses the pathogenicity of *A. hydrophila* and ultimately results in better survival and growth of the host. The non specific immune system can be stimulated by probiotics. Rengpipat et al*.* (2000) reported that the use of *Bacillus* species (strain S 11) provided disease protection by activating both cellular and humoral immune defences in Tiger shrimp.

Total microbial count (TMC) on tryptic soy agar showed an increase from day 1 to 45th day as a function of Efinol® FG ($5.4 \times 10^4 - 7.7 \times 10^8$ CFU g^{-1}), *B.subtilis* (5.4×10^4 - 6.8×10^8 CFU g^{-1}), *B.coagulans* ($5.4 \times 10^4 - 4.4 \times 10^8$ CFU g^{-1}) and *S.cerevisiae* diet ($5.4 \times 10^4 - 3.1 \times 10^7$ CFU g^{-1}) (Table 4). Among the 4 diets, high TMC was evidenced in Efinol® FG fed diet (7.7×10^8 CFU g^{-1}). Our results are supported by Ramakrishnan et al. (2008) and Wache et al. (2006) who reported increased bacterial

Table 3. Growth performance of *C.striatus* fed on control and probiotic diets after challenged by *A.hydrophila.*

	NC	OI	IM
Control			
WG	3.84±0.06	1.34±0.02	1.09±0.05
SGR	1.04±0.004	0.38±0.004	0.33±0.004
FCR	2.97±0.004	4.28±0.009	4.31±0.004
Efinol® FG			
WG	8.35 ±0.04[a]	3.75±0.04[a]	2.75±0.09[a]
SGR	1.7±0.02	0.85±0.04[a]	0.66±.02[a]
FCR	2.78±0.01	3.88±0.009	3.98±0.009
B. subtilis			
WG	7.48±0.24	3.33±0.06[a]	2.48±0.09[a]
SGR	1.66±0.01	0.81±0.004[a]	0.62±0.004[a]
FCR	2.81±0.08	3.90±0.04	3.99±0.04
B. coagulans			
WG	5.00±0.23	1.19±0.38[ab]	0.69±0.004[ab]
SGR	1.23±0.01	0.28±0.004[b]	0.19±0.004[ab]
FCR	2.97±0.03	4.1±0.04	4.0±0.04
S. cerevisiae			
WG	4.14±0.39	2.22±0.04[a]	2.02±0.08[b]
SGR	1.04±0.004	0.57±0.004[a]	0.52±0.004
FCR	2.98±0.004	4.21±0.04	4.15±0.008

WG-weight gain (g), SGR-Specific growth rate, FCR-Food conversion ratio, NC-non challenge, OI-oral ingestion, IM-intra muscular injection, Values are presented as mean±SD of triplicate observations (P<0.05).

Table 4. Total microbial count (CFU/g) on Tryptic soy agar.

Diet	Day 1	Day 15	Day 30	Day 45
Control	5.4×10^{4} ± 0.75	6.20×10^{7} ± 0.94	2.20×10^{7} ± 0.11	3.4×10^{8} ± 0.29
Efinol ® FG	5.4×10^{4} ± 0.75	6.12×10^{7} ±1.11	3.60×10^{8} ± 0.23	7.7×10^{8} ± 1.55
B.subtilis	5.4×10^{4} ± 0.75	4.17×10^{8} ±0.68	6.13×10^{8} ± 1.00	6.8×10^{8} ± 1.05
B.coagulans	5.4×10^{4} ± 0.75	6.81×10^{6} ±1.34	4.63×10^{7} ± 0.57	4.4×10^{8} ± 0.55
S.cerevisiae	5.4×10^{4} ± 0.75	5.63×10^{6} ± 0.82	3.90×10^{6} ± 0.21	3.1×10^{7} ± 0.23

count in common carp (*Cyprinus carpio*) and rainbow trout fed with probiotic diets. Probiotics promoted colonization of bacteria in the fish gut for a prolonged period and had capacity to adhere and grow well *in vitro* in the intestinal mucus from turbot (Makiridis et al., 2000). Robertson et al. (2000) observed a constant increase in Probiont (*Carnobacterium* sp.) population in the gut of rainbow trout and Atlantic salmon fingerlings fed with probiotic diet. Rainbow trout (*Oncorhynchus mykiss*) was fed with the diet supplemented with probiotic, *Bacillus* species. The count of bacteria was higher ($3.39\pm2.06 \times 10^{7}$ CFU g^{-1}) than the control ($12.5 \pm 1.08 \times 10^{7}$ CFU g^{-1}) (Bagheri et al*.,* 2008). He et al*.* (2009) indicated that with increasing the supplementation levels of DVAqua (*S. cerevisiae* as culture supplement), the count of beneficial bacteria increased.

After challenges, on day 21, NC groups of Efinol® FG (7.3×10^{8} CFU g^{-1}), *B. subtilis* (7.4×10^{8} CFU g^{-1}), *B.*

Table 5. Total microbial count (CFU/g) on Tryptic soy agar after challenged by *A.hydrophila.*

Diet	Day 1	Day 7	Day 14	Day 21
		Control		
NC	$3.4 \times 10^{8} \pm 0.04$	$8.0 \times 10^{7} \pm 1.08$	$2.3 \times 10^{8} \pm 0.58$	$1.8 \times 10^{8} \pm 0.81$
OI	$3.4 \times 10^{8} \pm 0.04$	$5.0 \times 10^{9} \pm 0.85$	$8.0 \times 10^{8} \pm 0.47$	$8.8 \times 10^{9} \pm 0.49$
IM	$3.4 \times 10^{8} \pm 0.04$	$7.8 \times 10^{10} \pm 0.48$	$5.2 \times 10^{8} \pm 0.78$	$7.1 \times 10^{9} \pm 0.77$
		Efinol® FG		
NC	$7.7 \times 10^{8} \pm 0.12$	$5.3 \times 10^{8} \pm 1.05$	$1.9 \times 10^{7} \pm 0.49$	$7.3 \times 10^{8} \pm 0.72$
OI	$7.7 \times 10^{8} \pm 0.12$	$6.7 \times 10^{9} \pm 0.78$	$1.0 \times 10^{7} \pm 0.54$	$2.0 \times 10^{8} \pm 0.58$
IM	$7.7 \times 10^{8} \pm 0.12$	$8.3 \times 10^{9} \pm 0.33$	$7.2 \times 10^{6} \pm 1.12$	$4.5 \times 10^{8} \pm 0.69$
		B. subtilis		
NC	$6.8 \times 10^{8} \pm 0.04$	$4.7 \times 10^{7} \pm 0.77$	$6.2 \times 10^{8} \pm 1.03$	$7.4 \times 10^{8} \pm 0.74$
OI	$6.8 \times 10^{8} \pm 0.04$	$3.0 \times 10^{9} \pm 0.94$	$5.7 \times 10^{5} \pm 0.73$	$1.2 \times 10^{7} \pm 0.44$
IM	$6.8 \times 10^{8} \pm 0.04$	$5.2 \times 10^{9} \pm 0.32$	$6.1 \times 10^{5} \pm 0.24$	$4.3 \times 10^{7} \pm 0.67$
		B. coagulans		
NC	$4.4 \times 10^{8} \pm 0.04$	$1.1 \times 10^{9} \pm 0.36$	$7.2 \times 10^{8} \pm 0.92$	$5.3 \times 10^{8} \pm 1.29$
OI	$4.4 \times 10^{8} \pm 0.04$	$2.7 \times 10^{9} \pm 0.64$	$8.1 \times 10^{6} \pm 0.85$	$6.0 \times 10^{7} \pm 0.96$
IM	$4.4 \times 10^{8} \pm 0.04$	$4.8 \times 10^{9} \pm 0.93$	$5.0 \times 10^{6} \pm 0.99$	$6.5 \times 10^{6} \pm 0.37$
		S. cerevisiae		
NC	$3.1 \times 10^{7} \pm 0.04$	$3.9 \times 10^{8} \pm 0.99$	$1.5 \times 10^{8} \pm 0.83$	$3.0 \times 10^{6} \pm 0.86$
OI	$3.1 \times 10^{7} \pm 0.04$	$1.2 \times 10^{8} \pm 0.16$	$2.8 \times 10^{6} \pm 0.63$	$2.6 \times 10^{7} \pm 0.28$
IM	$3.1 \times 10^{7} \pm 0.04$	$6.1 \times 10^{9} \pm 1.07$	$5.1 \times 10^{5} \pm 1.79$	$6.5 \times 10^{7} \pm 0.69$

NC-non challenge, OI-oral ingestion, IM-intra muscular injection of *A.hydrophila*, Values is presented as mean±SD of triplicate observations.

coagulans (5.3×10^{8} CFU g^{-1}) and *S. cerevisiae* (3.0×10^{6} CFU g^{-1}) fed diets showed an increase in TMC whereas in challenged groups, the TMC decreased irrespective of the probiotic diet (Table 5); in the case of control diet, the NC group showed a drastic decrease from 3.4×10^{8} CFU g^{-1} on day 1 to 1.8×10^{8} CFU g^{-1} on day 21 (Table 5).

Increase in TMC of NC groups of probiotic diets could be due to the antagonistic effect of probiotics on pathogenicity of *A.hydrophila.* Bacterial antagonism is a common phenomenon in nature. Microbial interactions play a major role in the equilibrium between competing beneficial and potentially pathogenic microorganisms. According to Balcazar (2004), microbial manipulation constitutes a viable tool to reduce or eliminate incidence of opportunist pathogens. The challenged group of control diet (OI and IM) showed a drastic increase from day 1 (3.4×10^{8} CFU g^{-1}) to day 21 (7.1×10^{9} - 8.8×10^{9} CFU g^{-1}) whereas in the OI and IM groups of all probiotic diets showed a drastic decrease. For instance in the case of OI group Efinol® FG diet, the decrease was from 7.7×10^{8} CFU g^{-1} on day 1 to 2.0×10^{8} CFU g^{-1} on day 21. The corresponding decrease in IM group was from 7.7×10^{8} CFU g^{-1} to 4.5×10^{8} CFU g^{-1}. It is clear from the results that the antagonistic effect of Efinol® FG against *A.hydrophila* is better than that of the other probiotics, since the decrease on day 21 was not drastic as in the case of other probiotics. Regarding the equilibrium between competing beneficial Efinol® FG and pathogenic *A.hydrophila,* extension of experimental duration **/** increase in Efinol® FG concentration may help to arrive at a conclusion.

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REFERENCES

Ahilan B, Shine G, Santhanam R (2004). Influence of probiotics on the growth and gut microflora load of juvenile gold fish (*Carassius auratus*). Asian Fish. Sci., 17: 271–278.

Arthi Manju R, Haniffa MA, Arun Singh SV, Muthu Ramakrishnan C, Dhanaraj M, Xavier Innocent B, Seetharaman S (2011). Effect of dietary administration of Efinol® fg on growth and enzymatic activities of *Channa striatus* (Bloch, 1793). J. Anim. Vet. Adv., 10(6): 796-801.

Bagheri T, Aliakbar S, Yavari V, Alizade, M, Farzanfar, Ali (2008). Growth, survival and gut microbial load of rainbow trout (*Onchorhynchus mykiss*) fry given diet supplemented with probiotic during the two months of first feeding. Turk. J. Fish. Aquat. Sci., 8: 43-48.

Balcazar JL, Vendrell D, Ruiz-Zarzuela I, Muzquiz JL (2004). Probiotics: a tool for the future of fish and shellfish health management. J. Aquacult. Trop., 90:389-392.

Bogut I, Milakovic Z, Bukvic Z, Brkic S, Zimmer R (1998). Influence of probiotic (*Streptococcus faecium* M74) on growth and content of intestinal microflora in Carp (*Cyprinus carpio*). J. Anim. Sci., 43:231-235.

Cheng ZJ, Hardy RW, Huige JJ (2004). Apparent digestibility coefficients of nutrients in brewer's and rendered animal by-products for rainbow trout *Oncorhynchus mykiss* (Walbaum). J. Fish Dis., 25: 333-342.

Duncan DB (1995). Multiple range and multiple F-tests. Biometrics., 11: 1-42.

El-Haroun ER, Goda A, Kabir Chowdhury MA (2006). Effect of dietary probiotic Biogens supplementation as a growth promoter on growth performance and feed utilization of Nile tilapia, *Oreochromis niloticus* (L). Aquacult. Res., 37: 1473-1480.

Gatesupe FJ (1999). The use of probiotics in aquaculture. Aquacult., 180: 147-165.

Ghosh K, Kumar SK, Kumar RA (2003). Supplementation of an isolated fish gut bacterium *Bacillus circulans*, in: Formulated diets for Rohu, *Labeo rohita*, fingerlings. Isr. J. Aquaculture/Bamidgeh., 55(1): 13-21.

Gomes LC, Brinn RP, Marcon JL, Dantas LA, Brandão FR, Sampaio de Abreu J, McComb DM, Baldisserotto B (2008). Using Efinol®L during transportation of marbled hatchetfish, *Carnegiella strigata* (Günther). Aquacult. Res., 39: 1292–1298.

Haniffa MA, Dhanaraj M, Muthu Ramakrishnan C, Arthi Manju R (2006). Simple herbal treatment for epizootic ulcerative syndrome in murrels (snakehead). Aquaculture Asia., 3: 18-21.

He S, Zhou Z, Liu Y, Shi P, Yao B, Ringo E, Yoon I (2009). Effect of dietary *Saccharomyces cereviscia* fermentation product (DVAqua) on growth performance, intestinal bacterial community and non-specific immunity of hybrid tilapia (*Oreochromis niloticus*×*O. aureus*) cultured in cages. Aquaculture, 294: 99-107.

Irianto A, Austin B (2002). Use of probiotics to control furunculosis in rainbow trout, *Oncorhynchus mykiss* (Walbaum). J. Fish Dis., 25: 333-342.

Karunasagar I, Sugumar G, Karunasagar I (1995). Virulence characteristics of *Aeromonas sp*. isolated from EUS-affected fish. In *Diseases in* Asian Aquaculture Vol. II, ed. Shariff M, Arthur, J R and Subasinghe, Manila, Philippines: Fish Health Section, Asian Fisheries Society, pp. 307S–314.

Lara-Flores M, Olvera-Novoa MA,Guzman-Mendez BE, Lopez MadridW (2003). Use of bacteria *Streptococcus faecium* and *Lactobacillus acidophilus*, and the yeast *Saccharomyces cerevisiae* as growth promoters in the Nile tilapia (*Oreochromis niloticus*). Aquaculture, 216: 193-201.

Li P, Gatlin DM (2003) Evaluation of brewer's yeast (*Saccharamyces cerevisiae*) as a feed supplement for hybrid striped bass (*Morone chrysops* x *M. saxatilis*). Aquaculture, 219: 681–692.

Li P, Gatlin DM (2004). Dietary brewer's yeast and the probiotic influence, growth performance, immune responses and resistance of hybrid striped bass (*Morone chrysops* x *M. saxatilis*) to *Streptococcus iniae* infection. Aquaculture, 231: 445–456.

Llobrera A, Gacutan RQ (1987). *Aeromonas hydrophila* associated with ulcerative disease epizootic in Laguna de Bay, Philippines. Aquaculture, 67: 273-278.

Makridis P, Fjellheim AJ, Skjermo J, Vadstein O (2000). Colonization of the gut in first feeding turbot by bacterial strains added to the water or bio encapsulated in rotifers. Aquacult., Int., 8: 367–380.

Marzouk MS, Mosustafa MM, Mohamed M (2008). The influence of some probiotics on the growth performance and intestinal microbial flora of *Oreochromis niloticus*. ISTA8. pp: 1059-1071.

Noh SH, Han IK, Won TH, Choi YJ (1994). Effect of antibiotics, enzyme, yeast culture and probiotics on the growth performance of Israeli carp. Korean Anim. Sci., 36: 480–486.

Pal J, Pradhan K (1990). Bacterial involvement in ulcerative condition of air-breathing fish from India. J. Fish Biol., 36: 833–839.

Ramakrishnan CM, Haniffa MA, Manohar M, Dhanaraj M, Arockiaraj AJ, Seetharaman S Arunsingh SV (2008). Effects of probiotics and spirulina on survival and growth of juvenile common carp (*Cyprinus carpio*). Isr. J. Aquacult./Bamidgeh., 60: 128-133.

Rengpipat S, Rukpratanporn S, Piyatiratitativorakul S, Menasveta P(2000). Immunity enhancement in black tiger shrimp *Penaeus monodon ,* by a probiotic bacterium (Bacillus S11). Aquaculture, 191: 271-288.

Ringo E, Birkbeck TH (1999). Lactic acid bacteria in fish: A review. Aquaculture, 160: 177-203.

Robertson PAW, Dowd O, Burrells C, Williams C, Austin B (2000). Use of *Carnobacterium* sp. as probiotic for Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*, Walbaum). Aquaculture, 185: 235-243.

Wache Y, Auffray F, Gatesoupe FJ, Zambonino J, Gayet V, Labbe 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 tout, *Onchorhynchus mykiss* fry. Aquaculture, 258: 470–478.

Wee KL, Tawn AGJ (1982). A preliminary study on the dietary protein requirement of juvenile snakehead. J. Jpn. Soc. Sci. Fish, 48: 1463-1468.

Yadav MG, Indira Ansary (1992). Cytotoxin elaboration by *Aeromonas hydrophila* isolated from fish with epizootic ulcerative syndrome. J. Fish Dis., 15: 183-189.

Yanbo W, Zirong Z (2006). Effect of probiotics for common carp (*Cyprinus carpio*) based on growth performance and digestive enzyme activities. Anim. Feed Sci. Technol., 127: 283-292.