Journal of Animal and Veterinary Advances 10 (17): 2305-2311, 2011
ISSN: 1680-5593

The Effects on Growth, Survival and Tolerance against Environmental Stressor (High Temperature) of Different Concentrations Probiotic *Bacillus* sp., Fed to Angelfish (*Pterophyllum scalare* Schultze, 1823) Larvae

Amin Farahi, Milad Kasiri, Mohammad Sudagar and Fatemeh Alamshahi
Department of Fishery, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran

Abstract: This study was conducted for 4 weeks to determine the effects of different concentrations probiotic *Bacillus* sp., on angelfish growth, larvae survival rate and tolerance against environmental stressor (high temperature). This experiment was carried out in a completely random design. *Artemia urmiana* naupli was used as a vector to carry probiotic bacillus to digestive tract of *Pterophyllum scalare* larvae. Nauplii with three concentrations of bacteria, 1×10^7, 2×10^7 and 3×10^7 bacteria mL^{-1} in suspension of broth for 10 h were bioencapsulated and angelfish larvae were fed by them. Angelfish larvae were fed 4 times a day. The control treatment was fed by unbioencapsulated *Artemia nauplii*. At the end of experiment, half of larvae in each treatment were challenged by high temperature (36°C) and their survival was calculated. The results indicated that the probiotic bacillus could influence on growth, survival rate and viability against high temperature stress in angelfish larvae. The final body length in experimental treatments had significant difference in comparison to control ($p<0.05$). In experimental treatments the survival rate did not show the significant difference ($p>0.05$) in comparison with control. Nevertheless, viability against high temperature stress in T1 (bioencapsulated Artemia with 1×10^7 cfu mL^{-1}) was increased significantly ($p<0.05$) in comparison with control. The result indicate that different concentrations of bacteria could influence on growth, larvae survival rate and tolerance against environmental stressor (high temperature) in angelfish larvae and the findings can be useful in the performance of larviculture of this species.

Key words: Probiotic, larviculture, viability, angelfish, temperature, Iran

INTRODUCTION

The word probiotic is constructed from the Latin word pro (for) and the Greek word bios (life). The definition of a probiotic differs greatly depending on the source but the 1st generally, accepted definition was proposed by Fuller (1989) as a live microbial feed supplement which beneficially affects the host animal by improving its microbial balance. Animal gut microflora consists of hundreds of different bacterial strains (Walker and Duffy, 1998) able to promote digestion and absorption of nutrients to increase body resistance to infectious diseases (Tannock, 1988) to yield positive affects on growth and to improve general animal welfare (FAO/WHO, 2001). FAO has now designated the use of probiotics as a major means for the improvement of aquatic environmental quality (Subasinghe *et al*., 2003). In the last decade, the scientific community carefully examined roles and effects of probiotics in aquaculture as an alternative to antimicrobial drugs, demonstrating positive effects on fish survival (Villamil *et al*., 2002), growth (Burr *et al*., 2005), stress resistance (Smith and Davey, 1993; Rollo *et al*., 2006), immunosystem enhancement (Erickson and Hubbard, 2000; Picchietti *et al*., 2007) and finally general welfare (Silvi *et al*., 2008). Most studies on the effects of probiotics on cultured aquatic animals have emphasized a reduction in mortality or the improved resistance against putative pathogens (Irianto and Austin, 2002). However, the beneficial effects are sometimes temporal, depending on the time of exposure (Verschuere *et al*., 2000). As most fish contain a specific intestinal microbiota established at the juvenile stage (Olafsen, 2001), the colonization of probiotics to fish intestines requires adequate probiotics presented in ambient Microbial Community (MC) and their interaction with MC should not be neglected. In aquaculture, captive rearing conditions generally can be sources of stress, triggering high mortality, mainly during larval rearing. The use of natural prophylactic supplements in place of chemotherapeutics in aquaculture

Corresponding Author: Amin Farahi, Department of Fishery, Gorgan University of Agricultural Sciences and Natural Resources, Shahid Beheshti Avenue, Golestan, Postal Code 49138-15739, Gorgan, Iran

has received a great deal of attention in the past decade such preventive products include probiotics. These biotics can be applied through external bathing or dietary supplementation and have been demonstrated to improve growth performance, feed utilization, digestibility of dietary ingredients, disease resistance and stimulate the immune response of aquatic animals (Gatesoupe, 2008; Kesarcodi-Watson *et al*., 2008; Wang *et al*., 2008; Merrifield *et al*., 2010). Aquatic probiotics have been defined as live microbial supplements that can modulate microbial communities and improve microbial balance, thus providing benefits to the host (Gram *et al*., 1999). Furthermore, probiotic Bacillus species have been shown to improve digestive enzyme activities, growth and survival of crustaceans (Liu *et al*., 2009; Rengpipat *et al*., 1998, 2003; Wang, 2007; Zhou *et al*., 2009). The beneficial effects of these probiotics include higher growth and feed efficiency, prevention of intestinal disorders and pre-digestion of anti-nutritional factors present in the ingredients. Also, existing literature on probiotics usually focused on resistance to some aquatic pathogens such as *Vibrio* sp. (Villamil *et al*., 2003; Planas *et al*., 2006), *Amyloodinium ocellatum* (Li *et al*., 2005) and *Carnobacterium* sp. These studies mainly focused on effects of probiotics on enhancement of survival and nutritional parameters such as feed efficiency and feed conversion ratio. Bacillus can act positively on cultured organisms by enhancing survival and growth (Gomez-Gil *et al*., 2000) by stimulating the digestive (Ziaei-Nejad *et al*., 2006) and immune systems (Gatesoupe, 1999) and by improving water quality in terms of bioremediation (Kennedy *et al*., 1998; Moriarty, 1998). Several studies demonstrated these positive effects using a single or two probiotic strains and just few studies described the effects of a mixture of probiotics in fish and shrimp aquaculture (Balcazar, 2003; Lara-Flores *et al*., 2003; Ziaei-Nejad *et al*., 2006). Concurrently, Bacillus species can be found in marine environment and are part of the microflora of several marine species (Kennedy *et al*., 1998; Hovda *et al*., 2007). little studies had been carried out to incorporate probiotics into a freahwater species common carp, *C. carpio* (Yanbo and Zirong, 2006) and crustaceans, Indian white shrimp *Fenneropenaeus indicus* (Ziaei-Nejad *et al*., 2006) and shrimp *Penaeus vannamei* (Wang, 2007) based on growth performances and digestive enzyme activities. As stated above, the application of probiotics in aquaculture as the environment friendly treatments has been also increasing rapidly (Gatesoupe, 1999, 2002, 2007) and some papers were associated with the effect of probiotics in fish and other marine organisms (Mohanty *et al*., 1993; Sharma and Bhukhar, 2000). Probiotics is usually defined as live microbial feed supplements that are administered in such a way as to enter the gastrointestinal tract and to be kept alive; this beneficially affects the host animal by improving its intestinal microbial balance and in turn its health (Gatesoupe, 1999, 2007). As described by several researchers, probiotics in aquaculture have been demonstrated to have several modes of action: competitive exclusion of pathogenic bacteria through the production of inhibitory compounds; improvement of water quality; enhancement of immune response of host species and enhancement of nutrition of host species through the production of supplemental digestive enzymes (Thompson *et al*., 1999; Verschuere *et al*., 2000). The brine shrimp *Artemia* sp. are common live food organisms used for the rearing of marine fish larvae. These have been considered as possible vectors for the delivery of different substances such as nutrients and probiotics (Gatesoupe, 1991). This positive effect of probiotics may be attributed to their ability to outcompete other bacteria (Austin *et al*., 1995) or to produce micronutrients important for the development of fish larvae (Ringo *et al*., 1992). Several bacteria have been used as probiotics in the larval culture of aquatic organisms and they can be either delivered directly into the water or via live carrier such as *Artemia nauplii* and rotifers or else added to pelleted dry food (Gomez-Gil *et al*., 2000). The aim of this study was to evaluate the effects of probiotic bacillus on the growth, larvae survival rate and tolerance against environmental stressor (high temperature) in angelfish larvae.

MATERIALS AND METHODS

Preparing of probiotic bacillus: The probiotic bacillus was prepared from Protexin Co. (Iran-Nikotak). The five species of probiotic bacillus as bacterial blend under the commercial title of Protexin aquatic were used for bioencapsulation of *A. urmiana*. The blends of probiotic bacillii (*Bacillus licheniformis*, *Bacillus subtilis*, *Bacillus polymixa*, *Bacillus laterosporus* and *Bacillus circulans*) from suspension of spores with special media were provided. Three concentrations of bacterial suspension, 1×10^7, 2×10^7 and 3×10^7 bacteries mL^{-1} (cfu mL^{-1}) were provided by Protexin Co. and the Colony Forming Unit (CFU) of probiotic bacillii were tested by microbial culture in Tryptic Soy Agar (TSA) (Rengpipat *et al*., 1998).

Artemia cyst hatching and bioencapsulation: The cysts of *A. urmiana* from the center of Artemia and Aquatic Animals in Urmia (Iran) were used for this study. The corions of the cysts were removed chemically by using the methodology that proposed by Sorgeloos *et al*.

(1977). This process is known as decapsulation. Hatching of the decapsulated cysts was performed in glass cone with 1 L of seawater (3.0% salinity) at a density of 5.0 g L^{-1} and incubated at 30°C with constant illumination and aeration through setting air pump (Gomez-Gil *et al*., 1998). The bioencapsulation of *Artemia nauplii* were accomplished with density of 2 g live nauplii L^{-1} (Makridis *et al*., 2001) for 10 h and with three concentration of 1×10^7 (T1), 2×10^7 (T2) and 3×10^7 (T3) bacteries mL^{-1} in suspension of broth.

Experimental design: The experiments were conducted from June to August 2010 in Institute of Ornamental Fish Hatchery in Babol, Iran. After yolk sac absorption, larvae were divided to twelve glass aquaria (80×30×40 cm) with three replicates for experimental and control treatments. This experiment was conducted in a completely randomized design with four treatments (treatment 1-3 and control).

The density of fish larvae in per tank were 10 fish L^{-1}. Angelfish larvae in control and experimental treatments were fed 4 times a day (8.00, 12.00, 16.00 and 20.00). The control treatment was fed unbioencapsulated *Artemia nauplii*. Gentle aeration was provided by air stones. During the experiment, the water quality parameters were monitored during the trial and average value for temperature, dissolved oxygen, hydrogen ion concentration (pH) and salinity were 26±2°C, 5.7-7.7 mg L^{-1}, 6.9-7.8 units and 0.1 mg L^{-1}, respectively. Dark cycle of 12:12 h was maintained during the experiment. Angelfish larvae were reared for 30 days. Fish from each aquarium were counted and their length was measured to monitor growth and mortalities were recorded.

High temperature challenge: At the end of experiment for evaluation of the larval quality, half of the larvae (in each replicate) were challenged by high temperature. In this propose, larvae were transferred to other aquariums and temperature was increased to 36°C (10°C higher than cultivation temperature) and survival duration was calculated.

Calculations and statistical analysis: The following variable was calculated (Ai *et al*., 2006):

$$\text{Survival} = N_t \times 100\ N_0^{-1}$$

N_t and N_0 were final and initial numbers of larvae in each replicate, respectively and t is the experimental period in days. Results are presented as means±SD. Significant differences among treatments were determined by Analysis of Variance (ANOVA) and the differences between means were tested with Duncan's multiple-range test using SPSS 16.0 programme. Differences were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Effect of probiotic bacillus on growth and viability: Among the three different concentration of probiotic bacillus in bioencapsulation of *Artemia nauplii* which was fed by angelfish larvae, the highest results obtained in T1 (bioencapsulated Artemia with 1×10^7 cfu mL^{-1}). As shown in the Fig. 1, growth of angelfish larvae was significantly ($p<0.05$) affected by probiotic bacillus. In the experimental treatments, growth was significantly ($p<0.05$) higher than control treatment. The highest average of length was obtained in the experimental treatment of T1 however, there was no significant difference among experimental treatments ($p>0.05$). The survival rate of angelfish larvae at the end of experiment is showed in Fig. 2. The significant difference was not observed in experimental treatments in comparison with control ($p>0.05$).

Effect of probiotic bacillus on larval tolerance: As shown in the Fig. 3, differences in larval tolerance against high

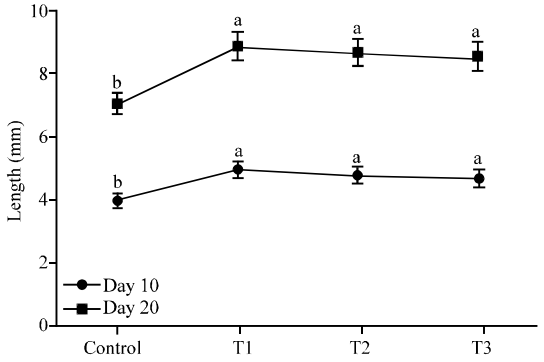

Fig. 1: Growth rate in experimental groups after 10 and 20 days

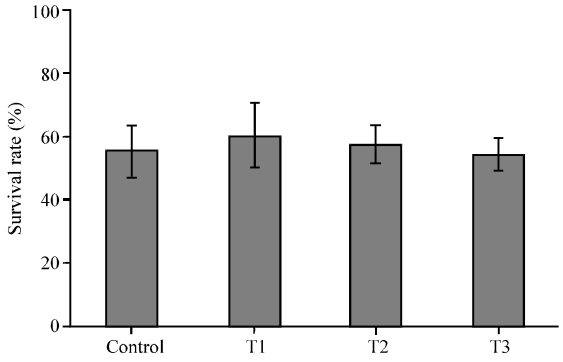

Fig. 2: Survival rate (%) of larvae in day 20th

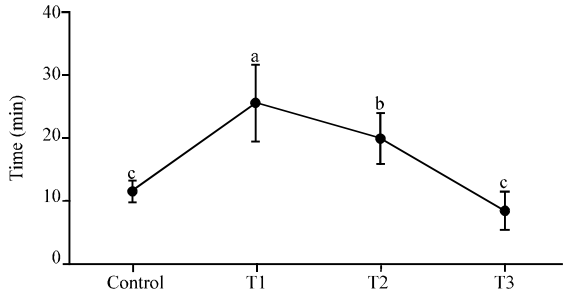

Fig. 3: Larval viability against high temperature stress

temperature stress (36°C) were observed among experimental groups and they were significant among them. The highest and lowest times of survival in 36°C were observed in T1 and T3, respectively.

The current study demonstrates different concentrations of bacteria for enrichment Artemia with commercial *Bacillus* sp. was significantly difference in growth between experimental treatments in comparison with control. Similar findings have previously been documented in preliminary trials on larval *H. gammarus* (Daniels *et al*., 2006). Also, this result indicates that different concentrations of bacteria not differed significantly in survival. Since, the 1st use of probiotics in aquaculture, a growing number of studies have demonstrated their ability to control potential pathogens and to increase the growth rates and welfare of farmed aquatic animals (Gatesoupe, 1991; Lara-Flores *et al*., 2003; Carnevali *et al*., 2004; Macey and Coyne, 2005; Wang *et al*., 2005; Yanbo and Zirong, 2006). Here, we report for the 1st time, an enhancement of the growth rate of the *Pterophyllum scalare*, one of the most important ornamental species in the Iran. The positive effect of Bacillus were observed by Gatesoupe (1991) in using *Bacillus toyoi* on turbot (*Scophthalmus maximus* Linnaeus, 1758), Swain *et al.* (1996) in Indian carps that improved the growth factors and feeding performance and Ghosh *et al.* (2003) on the Rohu. Noh *et al.* (1994) and Bogut *et al.* (1998) showed that a commercial probiotic preparation of *Streptococcus faecium* improved the growth and feed efficiency of carp (*Cyprinus carpio*).

Bagheri *et al.* (2008) found that supplementation of trout starter diet with the proper density of commercial Bacillus probiotic could be beneficial for growth and survival of rainbow trout fry. Ghosh *et al.* (2002) indicated that the *B. circulans*, *B. subtilis* and *Bacillus pamilus*, isolated from the gut of Rohu have extracellular protease, amylase and cellulose and play an important role in the nutrition of Rohu fingerlings. The photosynthetic bacteria and *Bacillus* sp. (isolated from the pond of common carp) was used in diet of common carp (*Cyprinus carpio* Linnaeus, 1758) by Yanbo and Zirong (2006). It is important to consider the possibility of using different species as suggested by Noh *et al.* (1994) and Bogut *et al.* (1998). Here, we studied the effects of a combination of *Bacillus* sp. (*Bacillus licheniformis*, *Bacillus subtilis*, *Bacillus polymixa*, *Bacillus laterosporus* and *Bacillus circulans*) at different concentrations of enrichment on growth and survival in angelfish larvae. The results indicated that always growth and survival rate do not increase with increasing concentrations of probiotics, similar results were reported by Jafaryan *et al.* (2010).

Cavalli *et al.* (2003) evaluated the effect of dietary supplementation of vitamins C and E on maternal performance and larval quality of the prawn *Macrobrachium rosenbergii*. They tested the tolerance of newly hatched and 8 days old larvae of *M. rosenbergii* to ammonia exposure. Their results shown newly hatched and 8 days old larvae tolerance tended to increase with increasing levels of AA and higher dietary levels of α-tocopherol acetate did not affect the tolerance to ammonia of newly hatched larvae but it positively augmented the ammonia tolerance of 8 days old larvae.

The study documented that larval survival duration against high temperature stress was promoted in T1 and T2 but resistance against stress in T3 was similar to control group. As a result larvae tolerance tended to decrease with increasing levels of probiotic.

CONCLUSION

In this study, this experiment indicated that the probiotic bacillus have the highest ability to promote the growth in *Pterophyllum scalare* larvae. Different concentrations of probiotic bacilluse had the same effects on the growth in angelfish larvae but the best survival duration against environmental steressor (high temperature) was obtained in T1 (bioencapsulated Artemia with 1×10^{7} cfu mL^{-1}). In general, the findings can be useful in the performance of larviculture of this species.

REFERENCES

Ai, Q., K. Mai, B. Tan, W. Xu, Q. Duan, H. Ma and L. Zhang, 2006. Replacement of fish meal by meat and bone meal in diets for large yellow croaker, *Pseudosciaena crocea*. Aquaculture, 260: 255-263.

Austin, B., L.F. Stuckey, P.A.W. Robertson, I. Effendi and D.R.W. Griffith, 1995. A probiotic strain of *Vibrio alginolyticus* effective in reducing diseases caused by *Aeromonas salmonicida*, *Vibrio anguillarum* and *Vibrio ordalii*. J. Fish Dis., 18: 93-96.

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

Balcazar, J.L., 2003. Evaluation of probiotic bacterial strains in *Litopenaeus vannamei*. Final Report, National Center for Marine and Aquaculture Research, Guayaquil, Ecuador.

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

Burr, G., D. Gatlin and S. Ricke, 2005. Microbial ecology of the gastrointestinal tract of fish and the potential application of prebiotics and probiotics in finfish aquaculture. J. World Aquac. Soc., 36: 425-436.

Carnevali, O., M.C. Zamponi, R. Sulpizio, A. Rollo and M. Nardi *et al*., 2004. Administration of probiotic strain to improve sea bream wellness during development. Aquacult. Int., 12: 377-386.

Cavalli, R.O., F.M.M. Batista, P. Lavens, P. Sorgeloos, H.J. Nelis and A.P. de Leenheer, 2003. Effect of dietary supplementation of vitamins C and E on maternal performance and larval quality of the prawn *Macrobrachium rosenbergii*. Aquaculture, 227: 131-146.

Daniels, C.L., D. Boothroyd, S. Davies, R. Pryor, D. Taylor and C. Wells, 2006. Bio-Mos® improves the growth and survival of cultured European lobster. Fish Farmer, 29: 24-27.

Erickson, K.L. and N.E. Hubbard, 2000. Probiotic immunomodulation in health and disease. J. Nutr., 130: 403-409.

FAO/WHO, 2001. Health and nutritional properties of probiotics in food including powder milk with live lactic. Report of a Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria, http://www.who.int/foodsafety/publications/fs_management/en/probiotics.pdf.

Fuller, R., 1989. Probiotics in man and animals. J. Applied Bacterol., 66: 365-378.

Gatesoupe, F.J., 1991. The effect of three strains of lactic bacteria on the production rate of rotifers, *Brachionus plicatilis* and their dietary value for larval turbot, *Scophthalmus maximus*. Aquacult., 96: 335-342.

Gatesoupe, F.J., 1999. The use of probiotics in aquaculture. Aquaculture, 180: 147-165.

Gatesoupe, F.J., 2002. Probiotic and formaldehyde treatments of Artemia nauplii as food for larval pollack, *Pollachius pollachius*. Aquacult., 212: 347-360.

Gatesoupe, F.J., 2007. Live yeasts in the gut: Natural occurrence, dietary introduction and their effects on fish health and development. Aquaculture, 267: 20-30.

Gatesoupe, F.J., 2008. Updating the importance of lactic acid bacteria in fish farming: Natural occurrence and probiotic treatments. J. Mol. Microbiol. Biotechnol., 14: 107-114.

Ghosh, K., S.K. Sen and A.K. Ray, 2002. Characterization of Bacillus isolated from the gut of Rohu, *Labeo rohita*, fingerlings and its significance in digestion. Applied Aquacult., 12: 33-42.

Ghosh, K., S.K. Sen and A.K. Ray, 2003. Supplementation of an isolated fish gut bacterium, *Bacillus circulans*, in formulated diets for rohu, *Labeo rohita*, fingerlings. Israeli J. Aquacult., 55: 13-21.

Gomez-Gil, B., A. Roque and J.E. Turnbull, 2000. The use and selection of probiotic bacteria for use in the culture of larval aquatic organisms. Aquaculture, 191: 259-270.

Gomez-Gil, B., M. Herrera-Vega, A. Abreu-Grobios and A. Roque, 1998. Bioencapsulation of two Different *Vibrio* species in nauplii of brine shrimp (*Artemia franciscana*). Applied Environ. Microbia, 64: 2318-2322.

Gram, L., J. Melchiorsen, B. Spanggaard, I. Huber and T.F. Nielsen, 1999. Inhibition of *Vibrio anguillarum* by *Pseudomonas fluorescens* AH 2, a possible probiotic treatment of fish. Applied Environ. Microbiol., 65: 969-973.

Hovda, M.B., M. Sivertsvik, B.T. Lunestad, G. Lorentzen and J.T. Rosnes, 2007. Characterisation of the dominant bacterial population in modified atmosphere packaged farmed halibut (*Hippoglossus hippoglossus*) based on 16S rDNA-DGGE. Food Microbiol., 24: 362-371.

Irianto, A. and B. Austin, 2002. Review: Probiotics in aquaculture. J. Fish Dis., 25: 633-642.

Jafaryan, H., M. Taati and N.M. Makhtumi, 2010. The effects of probiotic bacillus for promotion of growth and feeding parameters in beluga (*Huso huso*) larvae via feeding by bioencapsulated Artemia. Int. J. Bioflux Soc., 3: 273-280.

Kennedy, S.B., J.W. Tucker, C.L. Neidic, G.K. Vermeer, V.R. Cooper, J.L. Jarrell and D.G. Sennett, 1998. Bacterial management strategies for stock enhancement of warm water marine fish: A case study with common snook (*Centropomus undecimalis*). Bull. Mar. Sci., 62: 573-588.

Kesarcodi-Watson, A., H. Kaspar, M.J. Lategan and L. Gibson, 2008. Probiotics in aquaculture: The need, principles and mechanisms of action and screening processes. Aquaculture, 274: 1-14.

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

Li, P., G.S. Burr, J. Goff, K.W. Whiteman and K.B. Davis *et al*., 2005. A preliminary study on the effects of dietary supplementation of brewers yeast and nucleotides, singularly or in combination, on juvenile red drum (*Sciaenops ocellatus*). Aquac. Res., 36: 1120-1127.

Liu, C.H., C.S. Chiu, P.L. Ho and S.W. Wang, 2009. Improvement in the growth performance of white shrimp, *Litopenaeus vannamei*, by a protease-producing probiotic, *Bacillus subtilis* E20, from natto. J. Applied Microbiol., 107: 1031-1041.

Macey, B.M. and V.E. Coyne, 2005. Improved growth rate and disease resistance in farmed *Haliotis midae* through probiotic treatment. Aquaculture, 245: 249-261.

Makridis, P., O. Bergh, J. Skjermo and O. Vadstein, 2001. Addition of bacteria bioencapsulated in *Artemia metanauplii* to a rearing system for halibut larvae. Aqua. Int., 98: 225-235.

Merrifield, D.L., A. Dimitroglou, A. Foey, S.J. Davies and R.T.M. Baker *et al*., 2010. The current status and future focus of probiotic and prebiotic applications for salmonids. Aquaculture, 302: 1-18.

Mohanty, S.N., S.K. Swain and S.D. Tripathi, 1993. Growth and survival of rohu spawn fed on a liver based diet. J. Inland Fish. Soc. India, 25: 41-45.

Moriarty, D.J.W., 1998. Control of luminous *Vibrio* species in penaeid aquaculture pond. Aquaculture, 164: 351-358.

Noh, S.H., K. Han, T.H. Won and Y.J. Choi, 1994. Effect of antibiotics, enzyme, yeast culture and probiotics on the growth performance of Israeli carp. Korean J. Anim. Sci., 36: 480-486.

Olafsen, J.A., 2001. Interactions between fish larvae and bacteria in marine aquaculture. Aquacult., 200: 223-247.

Picchietti, S., M. Mazzini, A.R. Taddei, R. Renna, M.F. Anna, V. Mulero, O. Carnevali, A. Cresci and A. Abelli, 2007. Effects of administration of probiotic strains on GALT of larval gilthead seabream: Immunohistochemical and ultrastructural studies. Fish Shellfish Immunol., 22: 57-67.

Planas, M., M. Perez-Lorenzo, M. Hjelm, L. Gram, I.U. Fiksdal, Q. Bergh and J. Pintado, 2006. Robiotic effect *in vivo* of Rosebacter strain 27-4 against *Vibrio* (*Listonella anguillarum*) infections in turbot (*Scophthalmus maximus*) larvae. Aquaculture, 255: 323-333.

Rengpipat, S., W. Phianphak, S. Piyatiratitivorakul and P. Menasveta, 1998. Effects of a probiotic bacterium on black tiger shrimp *Penaeus monodon* survival and growth. Aquaculture, 167: 301-313.

Rengpipat, S., A. Tunyamum, A.W. Fast, S. Piyatiratitivoraku and P. Menasveta, 2003. Enhanced growth and resistance to *vibrio* challenge in pond-reared black tiger shrimp *Penaeus monodon* fed a *Bacillus* probiotic. Dis. Aquat. Organ., 55: 169-173.

Ringo, E., P.D. Sinclair, T.H. Birkebeck and A. Barbour, 1992. Production of ecosapentanoic acid (20:5n-3) by *Vibrio pelagius* isolated from turbot (*Scophthalmus maximus*) Larvae. Applied Environ. Microbiol., 58: 3777-3778.

Rollo, A., R. Sulpizio, M. Nardi, S. Silvi and C. Orpianesi *et al*., 2006. Live microbial feed supplement in aquaculture for improvement of stress tolerance. Fish Physiol. Biochem., 32: 167-177.

Sharma, O.P. and S.K.S. Bhukhar, 2000. Effect of Aquazyn-TM-1000, a probiotic on the water quality and growth of *Cyprinus carpio* var. communis (L.). Indian J. Fish., 47: 209-213.

Silvi, S., M. Nawji, R. Sulpizio, C. Orpianesi, M. Caggiano, O. Carnevali and A. Cresci, 2008. Effect of the addition of *Lactobacillus delbrueckii* subsp. delbruechii on the gut microbiota composition and contribution to the well-being of European sea bass (*Dicentrarchus labrax*, L.). Microb. Ecol. Health Dis., 20: 53-59.

Smith, P. and S. Davey, 1993. Evidence for the competitive exclusion of *Aeromonas salmonicida* from fish with stress-inducible furunculosis by a fluorescent pseudomonas. J. Fish Dis., 16: 521-524.

Sorgeloos, P., E. Bossuyt, E. Lavina, M. Baeza-Mesa and G. Persoone, 1977. Decapsulation of *Artemia* cysts: A simple technique for the improvement of the use of brine shrimp in aquaculture. Aquaculture, 12: 311-315.

Subasinghe, R.P., D. Curry, S.E. McGladdery and D. Bartley, 2003. Review of the state of world aquaculture. Recent technological Innovations in Aquaculture, FAO Fisheries Circular, pp: 59-74.

Swain, S.K., P.V. Rangacharyulu, S. Sarkar and K.M. Das, 1996. Effect of a probiotic supplement on growth, nutrient utilization and carcass composition in mrigal fry. J. Aquacult., 4: 29-35.

Tannock, G.W., 1988. The normal microflora: New concepts in health promotion. Microbiol. Sci., 5: 4-8.

Thompson, F.L., P.C. Abreu and R. Cavalli, 1999. The use of microorganisms as food source for *Penaeus paulensis* larvae. Aquaculture, 174: 139-153.

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.

Villamil, L., C. Tafalla, A. Figueras and B. Novoa, 2002. Evaluation of immunomodulatory effects of lactic acid bacteria in turbot (*Scophthalmus maximus*). Clin. Diagn. Lab. Immunol., 9: 1318-1323.

Villamil, L., A. Figueras, M. Planas and B. Novoa, 2003. Control of *Vibrio alginolyticus* in *Artemia* culture by treatment with bacterial probiotics. Aquaculture, 219: 43-56.

Walker, A.W. and L.C. Duffy, 1998. Diet and bacterial colonization: Role of probiotics and prebiotics. Rev. J. Nutr. Biochem., 9: 668-675.

Wang, Y.B., 2007. Effect of probiotics on growth performance and digestive enzyme activity of the shrimp *Penaeus vannamei*. Aquaculture, 269: 259-264.

Wang, Y.B., J. Li and J. Lin, 2008. Probiotics in aquaculture: Challenges and outlook. Aquaculture, 281: 1-4.

Wang, Y.B., Z.R. Xu and M.S. Xia, 2005. The effectiveness of commercial probiotics in northern white shrimp *Penaeus vannamei* ponds. Fish. Sci., 71: 1036-1041.

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

Zhou, X.X., Y.B. Wang and W.F. Li, 2009. Effect of probiotic on larvae shrimp (*Penaeus vannamei*) based on water quality, survival rate and digestive enzyme activities. Aquaculture, 287: 349-353.

Ziaei-Nejad, S., M.H. Rezaei, G.A. Takami, D.L. Lovett, A.R. Mirvaghefi and M. Shakouri, 2006. The effect of *Bacillus* spp. bacteria used as probiotics on digestive enzyme activity, survival and growth in the Indian white shrimp *Fenneropenaeus indicus*. Aquaculture, 252: 516-524.