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Influence of Commercial Probiotics on Digestive Enzyme Activities of Black Tiger Shrimp *Penaeus monodon* (Fabricius) Reared in Semi-Intensive Culture Ponds

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Abstract: ***The influence of commercial probiotics on digestive enzyme activities of the shrimp Penaeus monodon was investigated. The commercial water and feed probiotics (Wunapuo 15 and Aqualact) were used in shrimp culture ponds (~1 ha), water probiotic @ 30kg/ha every 15 days intervals up to 110 days of culture and feed probiotic @ 5 gm / kg feed every alternate day starting from 15th day of culture till harvest. The activities of the digestive enzymes amylase, protease and lipase on 30, 60 and 90 days of culture in P. monodon from control (CP: control pond) and probiotic treated (WPB: water probiotic treated; FPB: feed probiotic treated; WFPB: water + feed probiotic treated) culture ponds during the summer crop. The mean digestive enzyme activities of each treatment groups was significantly different (P < 0.05) from that of the control. The maximum percent increase in all three digestive enzyme activities was observed in WFPB groups followed by FPB and WPB groups.***

Keywords: Probiotics, Digestive enzymes, Amylase, Protease, Lipase, *Penaeus monodon*

1. Introduction

Currently aquaculture is facing many challenges in all over the world. Notable among them are combating disease and epizootics, brood stock improvement and domestication, development of appropriate feeds and feeding mechanisms, hatchery and grow-out technology, and water quality management which evidently present considerable scope for biotechnological interventions. Bioremediation is a novel biotechnological approach to maintain water quality in rearing environment and also reducing the disease problems in cultivable aquatic organisms.

Recent threats of disease outbreak in shrimp culture all through the world are mainly due to virus, bacteria, protozoan and fungi. Farmers use several antibiotics and chemicals for the prevention and control of these diseases. However, several farmers, of late, are using probiotics to improve water quality by balancing bacterial population and reducing pathogenic bacteria load. The word probiotic is often used as an opposite of antibiotic, i.e. as promoter of life. It is derived from a Greek word meaning "for life" (pro = for; bios = life).

Penaeus monodon, the black tiger shrimp, is widely cultured in India and other Southeast Asian countries. However, very few studies have been carried out on the effects of probiotics on survival, growth and performance of *P. monodon* in real time field conditions (Dalmin *et al.,* 2001; Balakrishnan *et al*., 2003). While in most cases only water or feed probiotic effects have been studied separately over short periods, synergistic effects of water and feed probiotics have not been studied in parallel over long periods exclusively under natural field conditions. Unfortunately most published work on the influence of probiotics on growth and disease resistance was confined only to a single beneficial bacterial strain rather than to multiple strains.

Consequently this study aims at studying the long term synergistic effects of commercially available water and feed probiotics on digestive enzyme activities (Amylase, Protease and Lipase) of *P. monodon* in natural field conditions.

2. Materials and Methods

The present work was carried out in a private shrimp farm (Sharat Sea Foods Industries Ltd.) near Venkannapalem Village (14°.2′E; 80°.5′N) of Nellore District, Andhra Pradesh, India during the summer crop. Modified extensive shrimp culture ponds (~1 ha) were adopted for this work. Culture ponds adopted for this study were uniformly prepared, following usual practices like ploughing, liming and other pre-stocking management methods. The ponds were filled with filtered, chlorinated (20 ppm) and dechlorinated sea water up to 1.2 m depth. This was followed by manuring and fertilization and water quality variables were maintained at optimum levels. After one week of preparation and maintenance all culture ponds were simultaneously stocked @ 12 / m^2 with *P. monodon* post larvae (PL20) obtained from Sharat Shrimp Hatchery (SSF industries Ltd.), Venkannapalem village near Nellore of Andhra Pradesh, India after PCR screening for White Spot Syndrome Virus (WSSV). The shrimp culture ponds were divided into four groups of each three, control (CP) and probiotic treated (WPB: water probiotic treated; FPB: feed probiotic treated; WFPB : water + feed probiotic treated) culture ponds.

Feeding and Feed Management: Feeding for the first 30 days is dependent on survival in hapas installed and

maintained in the culture ponds and regular observation of feed consumption and movement of post larvae in culture ponds. Generally 1-1.5 kg feed is applied on day one to a pond with stocking density of one lakh and increased @ 400-500 g/d for the same density till 30 days. Feed quantity from then on would be calculated depending upon the survival rate and average body weight (ABW). After 30 day period feed consumption is regularly monitored through check trays and depending on this feeding rate can be adjusted at regular intervals. The body weight of shrimp is measured every 7-10 days by random sampling. After stocking post larvae were fed with "ULTRA" shrimp feed (THE WATERBASE LTD, Nellore, India) for the first 60 days and with CP shrimp feed (CP Aquaculture India Ltd., Chennai, India) for the remaining days of culture.

Probiotics: Most widely used water and feed probiotics viz. "Wunapuo-15" (TEAM AQUA CORPORATION, TAIWAN) @ 30kg/ha every 15 day intervals up to 110 days of culture and "Aqualact" (WOCKHARDT, Mumbai India Pvt. Ltd.) @ 5 gm / kg feed every alternate day starting from 15th day of culture till harvest respectively were used in the present study.

Enzyme activity assay: The activity of amylase was estimated by the method of Dahlquist (1962) considering the amount of maltose liberated from the starch as a measure of amylolytic activity. Protease activity was estimated by the method of Moore and Stein (1954) considering the amount of free amino acids liberated from the substrate as a measure of proteolytic activity. Lipase activity was estimated by the method of Huggins and Lapides (1947) as described by Bier (1957).

Statistical Analysis:

Data were statistically analyzed and comparison among different treatments was done by one way analysis of variance (ANOVA) to find out any significant deference among the experimental groups and the comparison between treatments was done using Duncan's multiple range test (DMRT) at $P<0.05$ (Snedecor and Cochran, 1968) (SPSS; 14.0 version).

Results and Discussion

The activity levels of the digestive enzymes amylase, protease and lipase obtained from the intestine and hepatopancreas (with stomach) on 30, 60 and 90 days of culture (DOC) in *P. monodon* from control (CP: control pond) and probiotic treated (WPB: water probiotic treated; FPB: feed probiotic treated; WFPB : water + feed probiotic treated) culture ponds during the summer crop are showed in Table 1 and 2. The corresponding percent changes were showed in figures 1 to 6. It is evident from the results that the activity levels of amylase, protease and lipase were significantly higher in the hepatopancreas (with stomach) and intestine (DMRT; $P<0.05$) (Table-1) of probiotic treated *P. monodon* at different time intervals of culture. Although the enzyme activities in the hepatopancreas as well as intestine increased significantly (Two Way ANOVA; $P<0.01$) (Table-2) with increase in culture duration in both control and probiotic treated P. monodon, the magnitude of increase was more pronounced in probiotic treated groups than in control groups. Maximum percent increase in all the three digestive enzyme activities was observed in WFPB groups followed by FPB and WPB groups (Figs. 1to 6).

In aquaculture, probiotics can be administered either as food supplements or as additives to the water (Moriarty, 1998). Probiotics in aquaculture have been shown 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). Studies in *P. monodon* with *Bacillus* bacteria have shown that growth and survival were improved and immunity was enhanced (Rengpipat *et al*., 2000). However the nutritional effects of commercial probiotics, especially on digestive enzyme activities, have not been studied in aquaculture in natural field conditions (Saeed Ziaei-Nejad *et al*., 2006).

Digestive enzymes play an important role in the digestion process of an organism. The results obtained in this study show that there was a significant increase of percent changes in amylase (Figs. 1 and 2) protease (Figs. 3 and 4) and lipase (Figs. 5 and 6) activities (DMRT; $P<0.05$) (Table -1) in the intestine and hepatopancreas + stomach (HP+S) of *P. monodon* from probiotic treated ponds compared to those from control ponds suggesting that the addition of probiotics improved diet digestibility including protein, starch and fatty acid which might in turn explain the better growth performance and feed conversion efficiency. An increase in digestive enzyme activities as a result of probiotic treatment has been reported in the shrimp P.indicus (Sambhu and Jayaprakas, 2001), the freshwater prawn, *M. rosenbergii* (Venkat *et al*., 2004), the white shrimp, *Litopenaeus vannamei* (*Lin et al.,* 2004) and in the Indian white shrimp, *F. indicus* (Saeed Ziaei-Nejad *et al*., 2006). Similar results have also been reported in the common carp, *C. carpio* treated with Ba*cillus* bacteria (Wang Yanbo and Xu Zirong, 2006).

In general, probiotics are inoculated into the rearing water to improve culture conditions or incorporated in the food through diet (Yousuke Taoka *et al*., 2006). It is quite likely that water and feed probiotics colonize the gut of the host organisms and enhance digestibility of feeds by enhancing the secretion of digestive enzymes like amylase, protease and lipases (Gatesoupe, 1999; Verschuere *et al*., 2000). Probiotics have been shown to improve intestinal microbial balance leading to improved food absorption (Fuller, 1989) and digestive enzyme activities (Verschuere *et al*., 2000; Tovar Ramirez *et al*., 2004). Probiotics, which include gram positive bacteria, particularly members of the genus Bacillus, do secrete a wide range of exoenzymes (Moriarty, 1996; 1998) and, thus, it becomes difficult to distinguish between the activity due to enzymes synthesized by the shrimp and activity due to enzymes synthesized by probiotic bacteria. However, the low proportion of probiotic bacteria (Ex: *Bacillus* sps.) in the gut of shrimp (Saeed Ziaei-Nejad *et al*., 2006) suggests that the exogenous enzymes secreted by probiotics would contribute at most a small proportion to

the total enzyme activity of the gut. Instead, perhaps, the presence of probiotics might have stimulated the synthesis of digestive enzymes in the gut which in turn might have contributed to an increase in the activity levels of digestive enzymes as seen in this study. The observed increases in specific activities of digestive enzymes might have led to enhanced digestion and increased absorption of food which in turn might have enhanced growth and growth related indices in *P. monodon* treated with pobiotics. The correlation of higher bacterial count with higher digestive enzyme activity and improved survival and growth indices strongly suggests that periodical addition of probiotics at different farming stages is necessary to maximize survival and growth in the shrimp.

In the intestine as well as hepatopancreas with stomach, WFPB induced a highly significant (Two Way ANOVA; $P<0.01$) increase in amylase, protease and lipase activities followed by FPB and WPB. Usually probiotics are inoculated into the rearing water to improve water quality variables. Since marine animals are obliged to drink constantly to prevent water loss from the body (Gatesoupe, 1999), there is every possibility that the probiotics present in the ambient medium would enter into the gut and get added to the probiotic bacteria already present there. Therefore, the intestinal microbiota of aquatic animals may change rapidly with the intrusion of microbes coming from water and food (Gatesoupe, 1999).

Conclusion:

The influence of microbial flora from the rearing water on the gastrointestinal flora of the cultured animal is widely recognized. As water and feed probiotics are reported to beneficially affect the host organism, it is only logical that water and feed probiotics when applied simultaneously would have a synergistic effect. The percent changes recorded for amylase, protease and lipase activities in the intestine and hepatopancreas with stomach of the shrimp amply demonstrate that WFPB (simultaneous application of water and feed probiotics) was more effective in enhancing digestive enzyme activities than either feed or water probiotics applied separately.

Table 1: Group- wise mean and standard error (± SE) of Digestive enzyme activities in Intestine and Hepatopancreas along with stomach

Crop	Group	Intestine			Hepatopancreas + Stomach		
		Amylase	Lipase	Protease	Amylase	Lipase	Protease
Crop	CP	6.60±0.037^{a}	1.92±0.037^{a}	5.15±0.037 ^{a}	6.05±0.038 ^{a}	1.30±0.038 ^{a}	3.80±0.038 ^{a}
	WPB	7.68±0.035^{b}	1.98±0.035^{b}	5.53±0.035 ^{b}	7.14±0.045 ^{b}	1.48±0.045 ^{b}	4.17±0.045 ^{d}
	FPB	8.88±0.025^{c}	2.30±0.025^{c}	5.79±0.025 ^{c}	8.39±0.049 ^{c}	1.68±0.049 ^{c}	3.87±0.049 ^{b}
	WFPB	9.65±0.024^{d}	2.44±0.024^{d}	6.03±0.024 ^{d}	9.20±0.037 ^{d}	1.76±0.037 ^{d}	4.06±0.037 ^{c}

Means having the same superscript in each column do not differ significantly ($P< 0.05$) among themselves (Duncan's Multiple Range Test).

Table 2: Two factor ANOVA

Crop	Group	Intestine			Hepatopancreas + Stomach		
		Amylase	Lipase	Protease	Amylase	Lipase	Protease
	FGroup	768.664*	459.540*	448.587*	477.941*	82.162*	32.555*
	FDur	501.922*	73.165*	2591.845*	359.79*	93.065*	1186.353*

*1% level of significant ($P<0.01$); F_{Group}: F-value due to groups; F_{Dur}: F-value due to duration

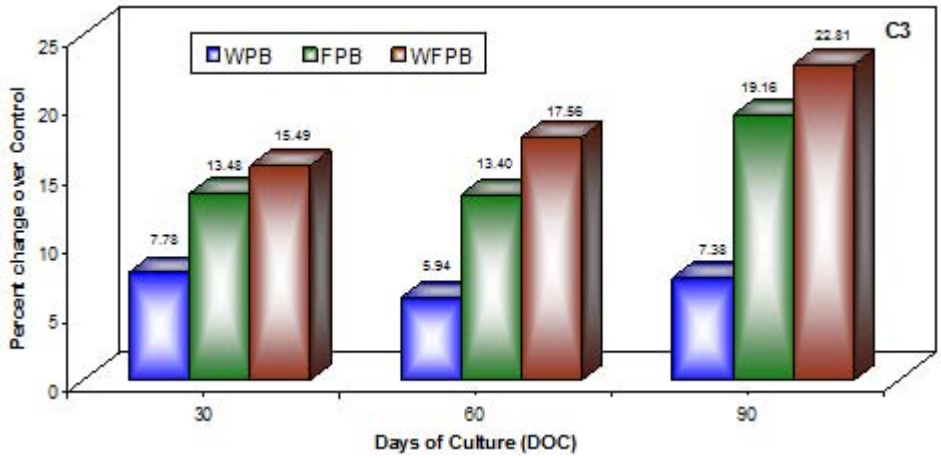

Figure 1: Percent change in amylase activity in HP+S (hepatopancreas + stomach) of *P. monodon* treated with probiotics (WPB: water probiotic; FPB: feed probiotic; WFPB: water + feed probiotic) from the successive summer crop.

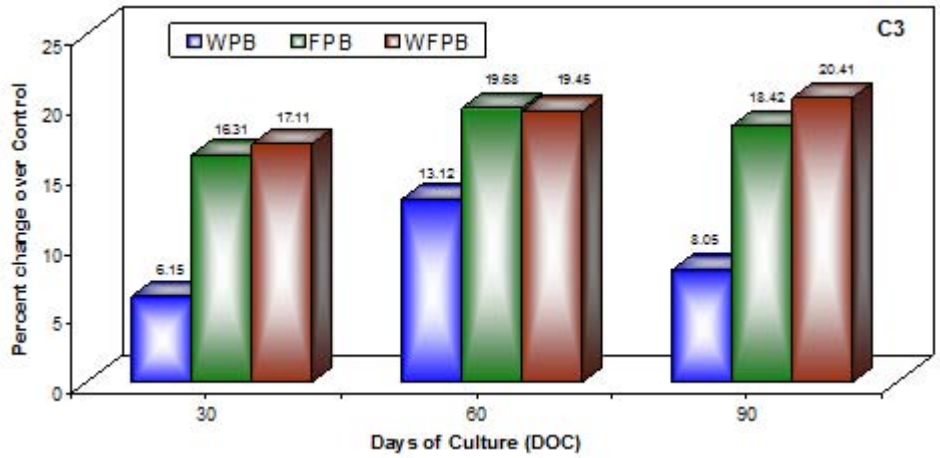

Figure 2: Percent change in amylase activity in intestine of *P. monodon* treated with probiotics (WPB: water probiotic; FPB: feed probiotic; WFPB: water + feed probiotic) from the successive summer crop.

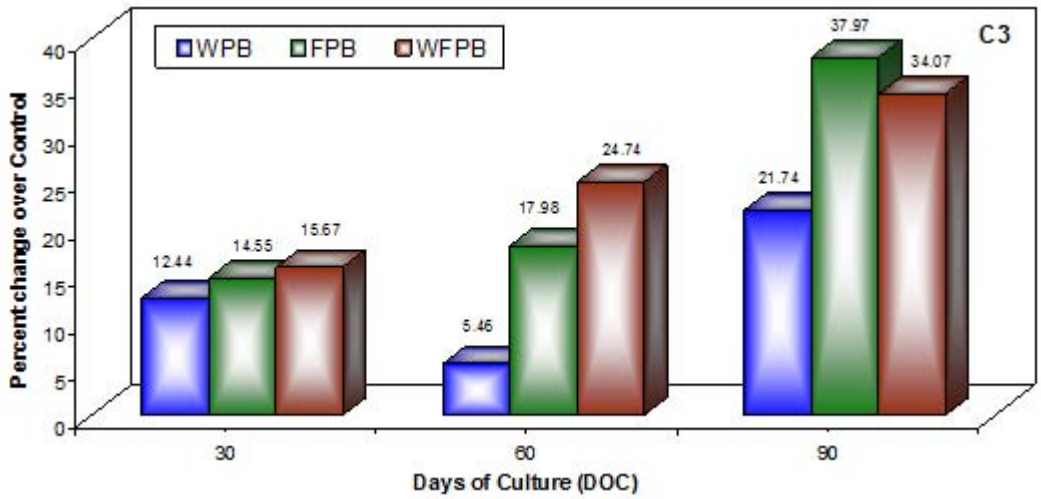

Figure 3: Percent change in protease activity in HP+S (hepatopancreas + stomach) of *P. monodon* treated with probiotics (WPB: water probiotic; FPB: feed probiotic; WFPB: water + feed probiotic) from the successive summer crop.

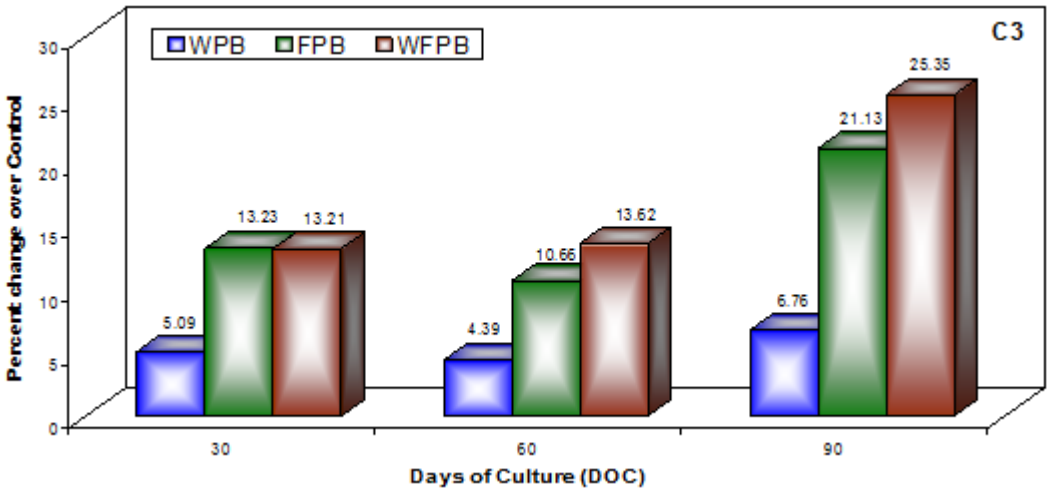

Figure 4: Percent change in protease activity in intestine of *P. monodon* treated with probiotics (WPB: water probiotic; FPB: feed probiotic; WFPB: water + feed probiotic) from the successive summer crop.

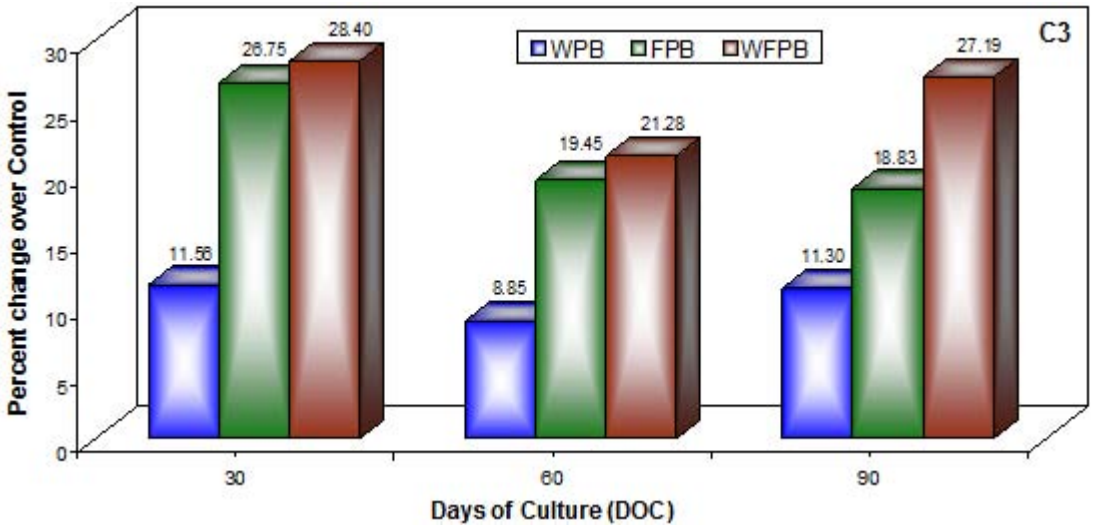

Figure 5: Percent change in lipase activity in HP+S (hepatopancreas + stomach) of *P. monodon* treated with probiotics (WPB: water probiotic; FPB: feed probiotic; WFPB: water + feed probiotic) from the successive summer crop.

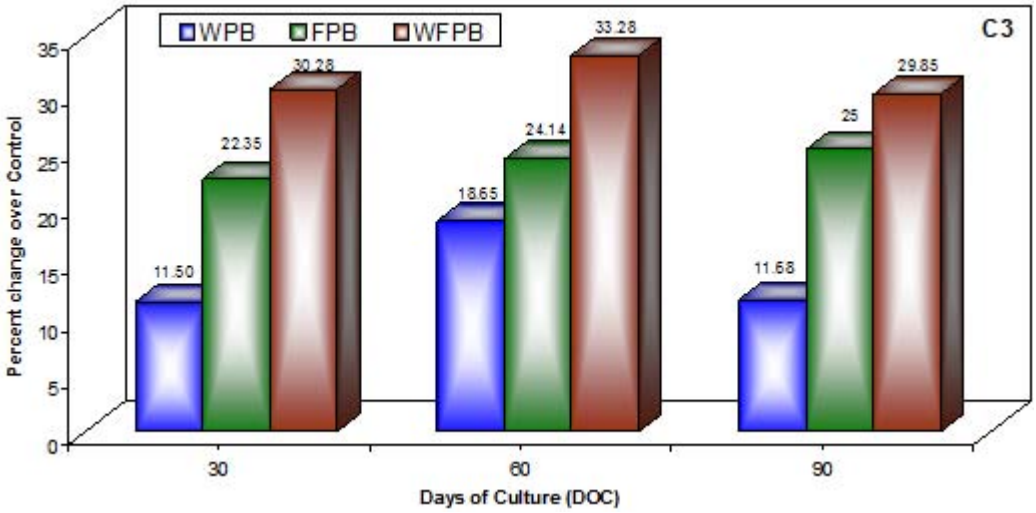

Figure 6: Percent change in lipase activity in intestine of *P. monodon* treated with probiotics (WPB: water probiotic; FPB: feed probiotic; WFPB: water + feed probiotic) from the successive summer crop

References

[1] Dalmin, G., Kathiresan, K. and Purushothaman, A. 2001. Effect of probiotics on bacterial population and health status of shrimp in culture pond ecosystem, Ind. J. Exp. Biol., 39: 939-942.

[2] Balakrishnan, S., John, K.R. and George, M.R. 2003. Antibiotic susceptibility of Bacillus spp. Isolated from shrimp (*Penaeus monodon*) culture ponds. Ind. J. Mar. Sci., 32 (1): 81-84.

[3] Dahlquist, 1962. Scand. J. Clin. Lab. Invest., 14: 145.

[4] Bier, M. 1957. Lipases. In : Methods in Enzymology, Vol. 1 (ed. Colowick, S.P. and N.O. Kapalson), Academic Press, New York, 631-634.

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

[6] Irianto, A. and Austin, B. 2002. Probiotics in aquaculture. J. fish Dis., 25: 633-642.

[7] Lin, H.Z., Guo, Z., Yingying, Y., Wenhui, Z. and Zhuojj, J. 2004. Effect of dietary probiotics on apparent digestibility coefficients of nutrients of white shrimp *Litopenaeus vannamei* (Boone). Aqua. Res., 35 (15): 1441.

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

[9] Rengpipat, S., Rukpratanporn, S., Pyyatiratitivorakul, S. and Menasveta, P. 2000. Immunity enhancement in black tiger shrimp (*Penaeus monodon*) by a probiont bacterium (*Bacillus,* S11). Aquaculture, 191: 271-288.

[10] Saeed Ziaei-Nejad, Mehran, H.R., Ghobad, A.T., Donald, L.L., Ali-Reza, M., Mehdi, S. 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.

[11] Verschuere, L., Rombaut, G. Sorgelous, P. and Verstraete. W. 2000. Probiotic bacteria as biological control agents in aquaculture. Microbiol. Mol. Biol. Rev., 64(4): 655-671.

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

[13] Sambhu, C. and Jayaprakas, V. 2001. Livol (IHF-1000), a new herbal growth promoter in white prawn, Penaeus indicus (Crustacea). Ind. J. Mar. Sci., 30: 38-43.
[14] Venkat, K. Himabindu., Narottam, P., Sahu and Kamal, K. Jain, 2004. Effect of feeding Lactobacillus-based probiotics on the gut microflora, growth and survival of postlarvae of *Macrobrachium rosenbergii* (de Man) Aquacult. Res., 35, 501-507.
[15] Wang, Y, Xu, Z. 2006. Effect of probiotics for common carp (*Cyprinus carpio*) based on growth performance and digestive enzyme activity. Ani. Feed. Sci. Tech., 127:283-292.
[16] Yousuke, T., Maeda, H., Jo, J.Y., Jeon, M.J., Bai, S.C., Lee, W.J., Yuge,K. and Koshio, S. 2006. Growth stress tolerance and non- specific immune response of Japanese flounder *Paralichthys olivaceus* to probiotics in a closed recirculating system. Fisher. Sci., 72: 310-321.
[17] Fuller, R. 1989. Probiotics in man and animals. J. Appl. Bacteriol., 66: 365-378.
[18] Moore, S. and Stein, W.H. 1954. A modified ninhydrin reagent for the photometric determination of aminoacids and related compounds. J. Biol. Chem., 211: 907-909.
[19] Moriarty, D.J.W. 1996. Microbial Biotechnology: A key to sustainable aquaculture. INFOFISH International, 4/96: 29-33.
[20] Moriarty, D.J.W. 1998. Control of luminous *Vibrio* species in penaeid aquaculture ponds. Aquaculture, 164: 351-358.
[21] Huggins, C. and Lapides, J. 1947. Chromogenic substrates IV esters of P-nitro-phenol as the substrate for the colorimetric determination of esterasterases. J. Biol. Chem., 170: 467-482.
[22] Tover-Ramirez, D., Zambonino, J., Cahu, C., Gatesoupe, F.J. and Vazquez juarez, R. 2004. Influence of dietary like yeast on European sea bass (*Dicentrarchus labrax)* Aquaculture, Vol. 234 (1-4): 415-427.