GAU Research Journal **37** (2) : 126-130 July 2012

Effect of probiotic *Lactobacillus acidophilus* on growth and survival of black tiger shrimp *Penaeus Monodon* (Fabricius) larvae

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Received : 15 January, 2011

ABSTRACT

The effect of probiotic *Lactobacillus acidophilus* was studied at the rate of $0.5X10^9$ to $1.5X10^9$ CFU/m^3 water on growth and survival of black tiger shrimp *Penaeus monodon* larvae and post larvae (Nauplii to PL-20). Use of *L. acidophilus* at the rate of $1.5X10^9$ CFU/m^3 water showed significantly ($P<0.05$) higher length and weight over the control. At PL-20 stage, highest length (14.05 ± 0.06 mm) and weight (3.27 ± 0.11 mg) was recorded in the treatment group $1.5X10^9$ CFU/m^3 probiotic in rearing water that was 5 % and 27 % higher as compared to the control respectively. Survival at PL-20 stage was also significantly ($P<0.05$) higher over the control. Highest survival was found 58.25 ± 3.62 % with *L. acidophilus* at the rate of $1.5X10^9$ CFU/m^3 that was 69.58 % higher as compared to the control (34.35 ± 1.33 %).

Key words : Probiotics, shrimp, larvae, growth

Rapid expansion of the aquaculture industry and potential for high profit resulted in over exploitation of the natural resources, poor water and sediment quality leading to disease outbreak and loss of production. Over the past few years, research efforts have gone into identification, treatment and control of shrimp diseases and as a result, a wide range of chemicals, therapeutants, vaccines and immunostimulants are manufactured and introduced into aquaculture practices.

For control of disease, the use of antibiotics is a good strategy in shrimp farming (Skjermo and Vadstein 1999). But it is not the ultimate solution to avoid diseases in shrimp hatcheries and culture systems because its massive use tends to select resistant bacteria strains (Baticados *et al.*, 1990; Holmstrom *et al.*, 2003) and prolonged use weakens them (Vasudevan 2000). Probiotics are nonpathogenic and nontoxic microorganisms without undesirable side-effects (Ali 2006). So, probiotics can be introduced into the culture environment to control and compete with pathogenic bacteria as well as to promote the growth of the cultured organisms.

Lactobacillus acidophilus is Gram-positive, rod-shaped and mesophilic bacteria (Altermann 2005) and is a suitable candidates for oral administration to shrimp to improve health (Ajitha 2004). Several bacteriocins had been associated with antibacterial activity of *L. acidophilus* such as lactocidin, acidophilin, heat stable lactocin-B, lactocin-F, acidocin-J1229 and heat labile acidophilin (Muriana and Luchansky 1993). *L. acidophilus* stimulates non specific immune response (Mohammed 1995; Uma 1995). Use of *L. plantarum* showed better growth in larval and post larval rearing of *P. monodon* (Anikumari 2004) and higher survival in *P. indicus* (Uma 1995). *Bacillus pumilus* and *Vibrio fluvialis* showed better survival for larval rearing of *P. japonicas* (El-Sersy *et al.*, 2006). *Bacillus* spp. showed increased growth rate of *Fenneropenaeus indicus* (Nejad *et al.*, 2006). Growth rate of gilthead sea bream *Sparus aurata* larvae increased with use *Lactobacillus* spp. (Suzer *et al.*, 2008). So, in order to know the effect of different doses of the probiotics *L. acidophilus* on growth, survival of the shrimp larva, the present study was taken up.

MATERIALS AND METHODS

Culture of Probiotic

Pure strain of *L. acidophilus* was procured from the Department of Dairy Microbiology, SMC College of

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Dairy, Anand Agricultural University, Anand which was inoculated at the rate of 2% to sterilized 10 % skimmed milk media and incubated overnight at 37 °C (APHA 1998). Five serial dilution (1/10) were carried out separately from probiotic culture and larval rearing water. Samples from these dilutions were inoculated in skimmed milk media and incubated at 37 °C for estimation of bacterial concentration and viability of the probiotic in rearing water. Concentration of the strain was 10^9 CFU/g (wet weight) and it was found to remain stationary under storage at -4 °C temperature for 48 h. Probiotic produced was used within 48 h. The population in rearing water did not vary significantly with applied concentration.

Water Treatment and Hygiene

Seawater filtered through quick sand filter then after it was chlorinated and dechlorinated (FAO 2007). Dechlorinated water filtered with 0.5 µ cartridge filter and then sterilized with UV sterilizer. All the equipments, plasticwares used were disinfected with 200 ppm chlorine water prior to use.

Live Feed Culture

The unicellular algae *Chaetoceros calcitrans* was cultured in treated seawater (salinity 30-32 ppt) using F/2 medium (Guillard 1972). Algal culture was produced in 70 l glass aquaria at 28 + 2 °C. Culture in the log phase was used for feeding. Cell density was estimated using a haemocytometer. *Artemia sanfranscicana* cysts were used to produce nauplii. *Artemia* hatching technique was followed as suggested by FAO, 2007.

Experimental Design and Details

L. acidophilus was applied daily at 16:00 h to the rearing media of *Penaeus monodon* larvae at the rate of 0.5×10^9, $10^9 \times 10^9$ and 1.5×10^9 CFU/m^3 and nil in treatments T1, T2, T3 and control respectively in four replicates. Accordingly *L. acidophilus* was weighed in electronic balance (PRECISA–ADAIR DUTT XB 120A, Swiss) having 0.01 mg sensitivity. Weighed probiotic diluted with distilled water and added to the larval rearing tanks.

P. monodon nauplii-V were stocked in half filled 100 l plastic drum at the rate of 100 nauplii/l and continuous oil free aeration was provided by using air blower. Water exchanged daily and larval feeding with live feed *Chaetoceros calcitrans* and *Artemia* nauplii as per feeding ptotocal (FAO 2007).

Larval survival was estimated by enumeration of larvae at various stages viz. Zoea-III, Mysis-III, PL-1, PL-6, PL-11, PL-15 and PL-20. The larval stages were determined using compound trinocular microscope (FOCUS–PHOTOPLUS 8000, India) and zoom stereo trinocular microscope (FOCUS- ZCXT-99, India). Growth of *P. monodon* larvae was measured in terms of total body length (mm) and wet weight (mg). 25 larvae from each tank were selected randomly for measurement of length at Mysis-III, PL-15 and PL-20 stages. Length was measured in computer using SCOPE Photo software (pre-calibrated with the help of micrometer slide, ERMA, Japan) attached to the microscope via digital camera. Weight of 25 larvae from each tank was measured at PL-15 and PL-20 stages in the electronic balance and average of that was recorded as average weight of the larvae.

Statistical Design

Recorded data were analyzed with single factor ANOVA for complete randomized design (Snedecor and Cochran 1967).

Stress Test

The quality of the post larvae was assessed by salinity stress test at suggested stage PL-15 (FAO 2007). Salinity stress test was performed by exposure of PL to 50 % of the ambient salinity (32 ppt). A sample of 30 PL was taken from the tank, placed into a beaker with seawater from PL tank. The water of the PL containing beaker was diluted with clean freshwater (1:1). After three hours the still active PL or which move when prodded with needle were counted and survival rate (%) calculated.

RESULTS AND DISCUSSION

The growth of the larvae and post larvae were measured in terms of length and weigh and total numbers of larvae were enumerated for survival rate. Detailed results are discussed hereunder. The larvae were also checked for quality by challenging them to the salinity stress. The treated group showed more resistance to salinity than the control. More than 75 % post larvae were active after salinity stress test while the control group 58 % post larvae were active.

Growth

Mean length of *P. monodon* larvae attained at various stages in different treatments are graphically illustrated in Fig. 1 Treatment T_3 showed highest length at Mysis-III (3.235 ± 0.01 mm), PL-15 (6.402 ± 0.01 mm) and PL-20 (14.050 ± 0.06 mm) stages. At Mysis-III stage, total length was at par in all the treatments while at PL-15 and PL-20 stages treatment T_3 showed

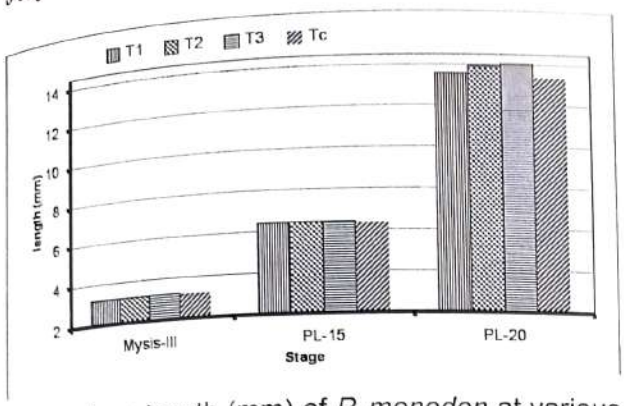

Fig. 1. Mean length (mm) of *P. monodon* at various stages obtained in different treatments of *L. acidophilus* along with control.

significantly highest length ($P<0.05$). Larvae in treatment T_3 attained 5 % more length as compared to the control. Mean weight of *P. monodon* larvae attained at various stages in different treatments are graphically illustrated in Fig. 2. Finally at PL-20 stage, treatment T_3 showed significantly highest body weight (3.270 ± 0.11 mg) than treatment T_1 and control, while it was at par with treatment T_2. The average body weight of the treatment T_3 was 27 % higher as compared to control. 27 % higher wet weight and 5 % higher total length obtained in the present study supports the results of several researchers. F_3 bacterial strain can enhance the growth of the *P. monodon* larvae (Maeda and Liao 1992), *Bacillus* S11 showed ability to increase weight of *P. monodon* post larvae (Rengipipat *et al.*, 1998). The results are also agreed with results of larval and post larval rearing of *P. monodon* with use of *L. plantarum* (Anikumari 2004). She obtained increased wet weight. The bacteria *Pediococcus*

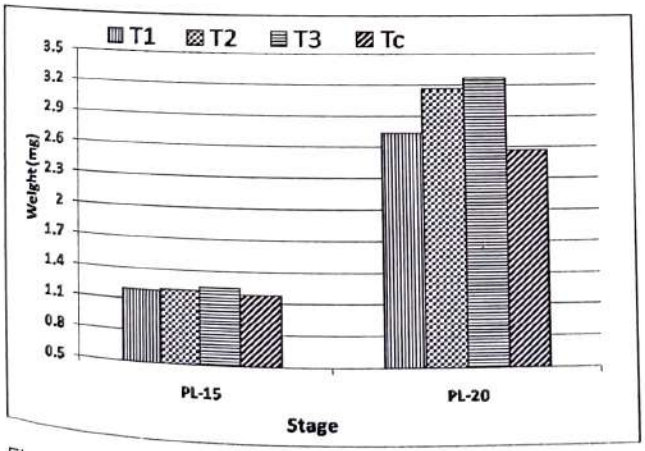

Fig. 2. Mean weight (mg) of P. *monodon* at various stages obtained in different treatments of *L. acidophilus* along with control

acidilacti as a probiotic feed additive in the post larvae of *P. monodon* showed improvement in growth (52 % higher weight and 15 % higher length) of the shrimp larvae (Tinha *et al.*, 2004). In the present study, the increment in length and weight was lower compared to this study may be due to sensitive nature of the larval stage as compared to post larval stage. Results are also comparable with improved wet weight *Fenneropenaeus indicus* larvae by adding of *Bacillus* spp in rearing water (Nejad *et al.*, 2006).

Survival Rate

Mean survival recorded at various stages in different treatments are graphically presented in Fig. 3. Survival rate was increased with increase in quantum of the *L. acidophilus* application to the rearing medium i.e. 0.5 X 10^9 to1.5X10^9 CFU/m^3 water. The higher mean survival at Zoea-III (98.15 ± 0.38 %), Mysis-III (96.25 ± 0.83 %), PL-1 (85.15 ± 0.64 %), PL-6 (78.45 ± 0.84 %), PL-11 (72.60 ± 1.14 %), PL-15 (62.10 ± 1.98 %) and PL-20 (58.25 ± 3.62 %) were recorded in treatment T_3 compared to other treatments and control. Finally at PL-20, treatment T_3 showed significantly higher ($P<0.05$) survival at PL-20 stage over the control and treatment T_1 while it was at par with treatment T_2. Survival in treatment T_3 was 69.58 % higher as compared to the control.

Uma (1995) recorded increased corresponding survival rate 72 ± 4 % to 94 ± 2 % by use of *L. plantarum* at the rate of 10^3 to 10^6 CFU/ml to the larval (PL-15 to PL-30) rearing medium of *P. indicus*, while in the present study; the highest survival rate observed was 58.25 ± 3.62 % at 1.5 X 10^9 CFU/m^3 concentration of *L. acidophilus*. The low survival rate obtained in the present study may be due to the sensitive nature of larval stages to change in quality of rearing medium compared to the post larvae from PL-15 to PL-30. This result supports the higher survival of *P. monodon* larval rearing with immobile cells of *Alteromonas* spp. BY-9 (Haryanti *et al.*, 2003) and with use of *L. plantarum* (Anikumari 2004). Use of the bacteria *Pediococcus acidilacti* as a probiotic feed additive in post larvae and juveniles of *P. monodon* showed 30 % higher survival of the shrimp larvae than the control (Tinha *et al.*, 2004). *B. pumilus* and *V. fluvialis* for larval rearing of *P. japonicus* increased survival rate by 94.9 % as compare to control (El-Sersy *et al.*, 2006). Results obtained by above scientists are comparable with present study. In the present study survival rate increased by 69.58 % as compared to the control.

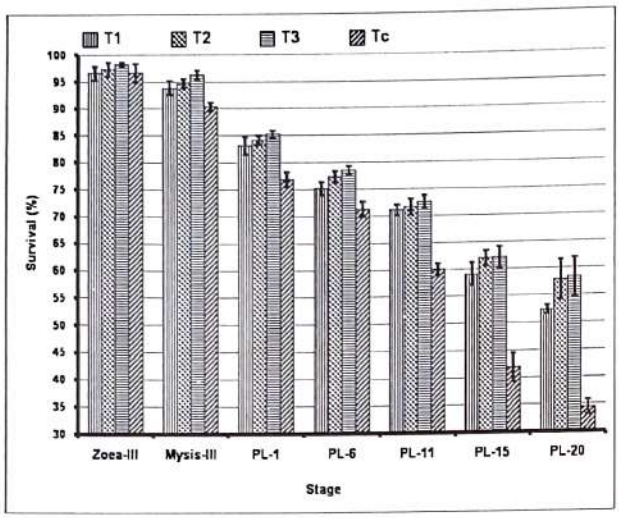

Fig. 3. The survival (%) of *P. monodon* at various stages obtained in different treatments of *L. acidophilus* along with control (mean ± SD, n=4)

Physico-Chemical Parameters

During the experiment, air and water temperature varied from 24-30 °C and 26-28 °C respectively. Salinity of the rearing water varied from 33-34 ppt. While the range of pH was found between 7.9-8.5. There was no significant variation in the pH value of rearing water. Dissolved oxygen fluctuated between 6.4-7.2 ppm. There was no significant variation of dissolved oxygen.

ACKNOWLEDGEMENTS

Authors are grateful to Dr. B. K. Kikani, Vice chancellor and Dr. D. B. Kuchchadiya, Director of Research, Junagadh Agril. University. Thanks are also due to Dr. J. B. Prajapati, Professor, SMC College of Dairy, Anand Agricultural University, Anand for providing *L. acidophilus* strain. We are also thankful to Mr. M. P. Patel and Mr. P. P. Patel for kind support in live feed production. Authors are also grateful to all staff directly or indirectly involved in work.

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