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RESEARCH ARTICLE

PROBIOTIC FOR LARVICULTURE OF *PORTUNUS PELAGICUS* (LINNAEUS, 1758)

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ABSTRACT

In aquaculture, probiotics can be considered a valid alternative to the use of antibiotics and in particular, in fish larviculture, to prevent high mortality owing to microbes and to improve survival rate. Many researchers reported promising results using a single beneficial bacterial strain in the culture of many finfish species. Therefore, *L. salivarius* isolated from the gut of female *Portunus pelagicus* and its influence on larviculture was examined. Four trial experiments were triplicate and designed in three different administrations of probiotic 1.0×10^6 cfu mL^{-1} (T-1), 5.0×10^6 cfu mL^{-1} (T-2) and 1.0×10^7 cfu mL^{-1} (T-3) and one control without any probiotic, and were added to rearing water every day. Addition of the probiotic (to first day hatch larvae) significantly increased survival rate ($p<0.05$) for all treatments over controls. Probiotic concentration 5.0×10^6 cfu mL^{-1} (T-2) did produce highest survival rate of larvae. The microbial load particularly *Vibrio* sp. was dominant in the control tanks than in experimental tanks. The added probiotic affected pH in treated groups and the less nitrogen contents were observed in the experimental tanks mainly due to the effect of probiotic. The specific activities of protease and amylase enzymes were significantly higher ($p<0.05$) in all treated groups over the control and highest were determined in treated group T-2. The highest concentration of probiotic did not increase the survival and the activity of the digestive enzymes. The results of present study suggest that that treatment with probiotics, *L. salivarius*, at a certain concentration as water additives could be used to improve survival rate and water quality, thereby convalescing digestive enzymes activity of *P. pelagicus*.

INTRODUCTION

The blue swimmer crab, *Portunus pelagicus* (Portunidae), is widely distributed throughout the coastal and estuarine areas of the tropical Western Pacific and Eastern Indian oceans (Xiao and Kumar, 2004). Currently, blue swimmer crabs are largely sourced from fisheries that are unreliable and seasonal (Otto and Jamieson, 2003). Driven by the increasing demands, the interest in *P. pelagicus* as a candidate species for aquaculture has increased. Conversely, the hatchery production of crab larvae is more often challenging owing to low or no survival rates during early stages. The intensive rearing conditions for marine fish larvae provide an appropriate environment for opportunistic bacteria (Skjermo *et al.,* 1997) that may cause microbial problems and high larval mortality. Routes of lethal pathogens entry into hatchery includes seawater, faecal matter, and exoskeleton of spawner and feeds (Lavilla-Pitogo *et al.,* 1990). Mass mortalities in crab occurred frequently in zoeal larvae, which were infected with the bacteria proliferated inside the larvae and almost all larvae died (Muroga *et al.*, 1989), *Vibrio* sp. transmit into hatching tanks through faeces discharge of spawner female (Talpur *et al.,* 2011). Due to the high mortality and contagious nature of disease, large amounts of antibiotics are often used for cure, and frequent use of antibiotics has led a growing concern due to the emergence and spread of antibiotic resistance bacteria that are becoming increasingly difficult to control and eradicate (Esiobu *et al.,* 2002; Nomoto 2005). Thus, the need for alternative approach is mounting and the application of probiotics may be considerable.

According to his original definition, probiotics are ‘‘organisms and substances which contribute to intestinal microbial balance.’’ Although application of probiotics in aquaculture seems to be increasing rapidly owing to their environment friendly role and proposed to extend the definition of probiotics in aquaculture to microbial “water additives.” The function of probiotics bacteria in crab aquaculture had not been worked out extensively, only first studies pertaining to swimming crab, *Portunus trituberculatus*, reported with addition of PM-4 bacteria (Nogami and Maeda 1992; Nogami *et al.,* (1997), which improved growth and survival of crab larvae.

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A growing number of studies have dealt clearly with probiotics in aquaculture, and it is now possible to survey its state of the art, from empirical use to the scientific approach (Ajitha *et al.,* 2004, Balcázar *et al.,* 2006, Irianto and Austin 2002a, Kesarcodi-Watson et al.2008; Merrifield *et al.,* 2010; Park *et al.,* 2000; Swain *et al.,* 2009; Vine *et al.,* 2006, Wang, *et al.,* 2008). Now days, Lactic acid bacteria (LAB) are gaining importance as aquaculture probiotics due to their environment and health friendly role. Focus for the selection of probiotic has been diverted to isolate those originating from the respective aquatic animals (Abd El-Rhman *et al.,* 2009; Aly *et al.,* 2008; Bjornsdottir *et al.,* 2010; Caipang *et al.,* 2010; Chabrillón *et al.,* 2006; Fjellheim *et al.,* 2010; Hjelm *et al.,* 2004; Irianto and Austin, 2002b). In aquaculture, lactic acid bacteria (LAB) have been evaluated as probiotics for survivorship and growth promoter in fish and shellfish (Aly *et al.,* 2008; Gatesoupe 1999; Avella *et al.,* 2010; Irianto and Austin, 2002a,b; Nikoskelainen *et al.,* 2001; Kesarcodi-Watson *et al.,* 2008; Vendrell *et al.,* 2008; Vine *et al.,* 2006), (Balca´zar *et al.,* 2007) and larval fish welfare (Rollo *et al.,* 2006). Selecting and testing of indigenous LAB from the gastro intestine of aquatic animals has been used in aquaculture by various researchers (Bairagi *et al.,* 2004; Balcázar *et al.,* 2008; Gildberg *et al.,* 1997; Jatoba *et al.,* 2008; Shiri-Harzevili *et al.,* 1998; Stoffels *et al.,* 1992). Therefore, the present study was aimed to evaluate the effect of indigenous *L. salivarius* (previously isolated from the gut of female crab, *P. pelagicus*) as water additive in larviculture of crab, *P. pelagicus*.

MATERIALS AND METHODS

Seawater for broodstock and larviculture

Ultraviolet (UV) treated seawater for broodstock and larviculture was filtered through a 10 µm net and sterilise/disinfected with sodium hypochlorite for 24 hours (h). This procedure was followed by supplemented with Ethylenediaminetetraacetic acid (EDTA) 100 gm per ton and neutralization with sodium thiosulphate (at same concentrations of sodium hypochlorite) at the beginning of the experiment for broodstock and larviculture use. The culture water exchange began from the day second, using disinfected seawater (28 ppt).

Broodstock and experimental larvae

Gravid females were collected from Strait of Tebrau (1° 22' N and 103° 38' E), Johor, West Malaysia and transported to marine hatchery of Institute of Tropical Aquaculture, Universiti Malaysia Terengganu, Malaysia for breeding. Females were disinfected according Talpur *et al.,* (2011) and kept in a 300 L capacity hatching tanks filled with disinfected seawater (28 ppt), sand mat, with mild aeration. Temperature of hatching tanks was maintained constant to 28°C using submersible heaters. Hatching tanks were siphoned and cleaned daily accompanied with about a 50-100% water exchange (treated). Upon breeding females were removed from the hatching tanks. Newly one day hatches Zoea 1 (Z1) actively moving at surface water column were collected and transferred to other tank filled with disinfected seawater (28 ppt) and used as experimental larvae in this study.

Probiotic bacteria and culture

The bacterial strain *L. salivarius* (*LS1*) used in this study was previously isolated from the cultivable gut microflora of female *P. pelagicus*. Probiotic was selected based on results showed *in vitro* inhibition against three shellfish pathogens (*V. harveyi*, *V. parahaemolyticus* and *Pseudoalteromonas piscicida*), validation as putative probiotic through a model of small scale *in vivo* experiments and probiotic pathogen bioassay (Talpur *et al.,* 2012a,b) . Probiotic bacteria to be added to crab rearing water was cultured in MRS Broth (Fluka) prepared in seawater (28 ppt) on an orbital shaker at 150 rpm for 24-28 h at 37°C.

Experimental design

Probiotic was inoculated to culture water according to method of Zhou *et al.,* (2009) with slight modification of concentration. Four trials were carried out with larvae (first day hatch larvae) of *P. pelagicus* in twelve aerated 10 litre transparent aquaria tanks filled with disinfected natural seawater (28 pp) at a density of 20 larvae L^{-1} (200/ tank) and control with seawater, larvae and without any probiotic bacteria. The probiotic, *L. salivarius* was tested at three concentrations level of $1x0^6$ colony forming units per millilitre (cfu mL^{-1}) (T-1), 5.0×10^6 cfu mL^{-1} (T-2), and 1.0×10^7 cfu mL^{-1} (T-3) and control without any probiotic were conducted in three replicate. After incubation, the probiotic bacterial cells were harvested by centrifugation 12,000 x g for 10 minutes. Cells washed three times with sterilise seawater (28 ppt) and re-suspended in the sterilise seawater before use. Cell density was measured to OD_{630nm}. Aquaria were inoculated until day 13 and the trials were terminated on day 14. The temperature was maintained at 28±1°C and the salinity range of sea water in the tanks was 28±0.5 ppt. Aquaria were confined in a water bath (3' x 5' x 1.5' flat bottom tank), which was filled with fresh tap water and supplied with submerged heater in order to maintain constant temperature to 28°C of aquaria. A 12 h dark and 12 h light photoperiod was maintained during the entire trial. Dead larvae, debris were siphoned out daily and water amount discharged during cleaning of aquaria, same quantity was re-filled. Any live larva found was transferred to respective tank with big bore pipette tip. To avoid the wash out of probiotics, water was changed at the rate of 10 -12 % daily in the probiotics treated tanks. In controls, to minimise the bacterial load either multiplying in the aquaria tanks or influx via live feed, water was changed 30-40 % daily from day two of the experiment. At the end of the experiment, the percent survival of larvae determined by direct counting of larvae or using formula: Survival rate % = Total number of larvae survived/ Initial number of larvae stocked x 100.

Feeding regime

Throughout the experiment, the larvae were fed with a mixture of live prey composed of 30-40 rotifers mL^{-1} (*Brachionus* sp.), microalgae *Nannochloropsis* sp ($8x10^5$ cells mL^{-1}) daily and five *Artemia* sp. nauplii mL^{-1} from day 9 to day 13. Rotifers were enriched with live microalgae (*Nannochloropsis* sp.) for 24 hours before to feed larvae, whilst *Artemia* nauplii were hatched daily from cysts (Great Lake *Artemia*, Salt Lake City, Utah, U.S.A) fed to larvae without enrichment. Rotifers and *Artemia* were harvested from culture tanks every morning during the experiment and 3×1 mL samples of harvested

stocks were taken and counted using a Sedgewick-Rafter counter under a microscope to obtain the average. The volumes of rotifer and *Artemia* stocks required to achieve designated densities were calculated and added to larviculture aquaria.

Sampling and analytical methods

Water samples from larvae rearing tanks (LRT) were collected on day 2, 6, 10, and 14 in sterile test tubes for bacteriological study. Evident of probiotic isolate from culture water was performed following the serial dilution of samples by plating on MRS agar plates. Marine agar (MA) was used for total viable bacteria counts (TVBC); specified TCBS agar was used to evaluate the total *Vibrio* counts (TVC). The growth media was prepared in seawater (28 ppt). Marine agar, TCBS plates incubated for 24-48 h and MRS plates were incubated for three days at 37^{o}C.

Water parameters

During the study period, water parameters such as temperature, salinity, dissolved oxygen (DO) and pH monitored using YSI 556 MPS multi probe meter (USA and were measured daily on site between 10.0 AM - 11.0 AM. The concentrations of NH_4–N and NO_2–N of water in the tanks were measured using ammonia and nitrite test kits (Merck chemicals).

Enzyme activities

Five to 15 larvae (Megalopa) before one day of termination of experiment (13DAH) were collected from each treatment and control groups for enzyme assay. All samples were collected after 6 h feeding between 09:00 AM and 11:00 AM. Larvae were washed with sterilise distilled water and immediately frozen at −80 °C in 2.0 mL Eppendorf tubes until enzyme assays were done. Frozen samples were homogenate in 500 µL buffer solution (10Mm sodium citrate/0.1M NaCl, pH 7.0). Homogenates were centrifuged at 13,000 ×g for 10 minutes on room temperature. Homogenates (crude or diluted) either used immediately for enzyme analysis or stored at -80^{o}C until procedure start. Enzyme determination assay was determined using assay kits. Protein contents determinations were performed using Bio Rad protein assay Kit (Bio Rad, USA). Protease determination was performed using Protease kit (Megazyme, Ireland). Amylase was determined using Amylase Assay Kit (Megazyme, Ireland). Enzyme assay was performed with whole larvae taken from each treatment and control. Enzyme activities were measured at 590nm as the change in absorbance using a Shimadzu 1800-UV spectrophotometer and expressed as specific activity (U mg^{-1} protein).

Statistical analysis of data

Percentage survivals were arc sin square root transformed to approximate normality and treatment differences were analysed using ANOVA (p=0.05) and enzymatic activity data was followed by Newman–Keul's multiple range test.. All statistics were performed using SPSS version 16 for Windows.

RESULTS

Water Quality

The effect of probiotic on water pH in larviculture tanks was shown in Table 1. The final mean pH in treated tanks ranged from 7.74±0.00 to 8.9±0.04 , while that of the control group 8.12±0.01 - to 8.15±0.02 . The pH across the experimental trials was statistically significantly different among groups (p<0.05). The NH_4–N concentrations of treated water and control (without probiotic) were presented in Table 2. No significant differences in NH_4–N concentrations were observed (p>0.05) throughout the experimental period at any stage among the treatments. The higher NO_4–N concentration was determined in the control and T-3 and T-2 had lower content. However, the NO_2–N concentrations, assays did show no difference (p>0.05) in groups treated, as compared with the control whereas NO_2–N concentration had a relatively lower content in T-3 followed by T-2 Table 3. However DO was observed >6mg L-1, Temperature and salinity were kept constant 28±1^{o}C and 28ppt respectively.

Survival of larvae

Application of the probiotic, *L. salivarius*, significantly increased survival rate (p<0.05) in all treatments and low survival was observed in controls was not significant different (p>0.05). Highest survival rate in treatments groups was observed in T-2 (9.3±0.3) and significantly low survival rate was found in T-1 Fig 1.

Bacteriology Study

Highest cfu mL^{-1} of probiotic in water samples were noticed on day 14 and lowest on day two of the experiment. Total viable bacteria counts (TVBC) and total *Vibrio* count (TVC) cfu mL^{-1} were highest on day two and decreased day by day and lowest on day 14 of the experiment. The occurrence of cfu mL^{-1} in treatments was not same as administrated**.** However, in control TVBC increased with time elapse, while TVC observed higher during day 6, 10 and 14of the larviculture but no LAB was detected in controls Table 5.

Enzyme activities

Specific enzyme activities for protease and amylase across all treatments were not performed at each zoeal stage of *P. pelagicus* larvae. Enzyme assays were performed at the end of experiment when larvae were reached to Megalopa stage (13DAH).

Protease activity

Specific enzyme activities for protease across all treatments were presented in Fig. 2. The highest specific protease activity in treated groups was observed in T-2 (0.16 ± 0.01 U mg^{-1} protein over the controls (0.12 ± 0.01 U mg^{-1} protein) and treatment T-3 relatively had low protease. Specific enzyme activities for protease across all treatments was statistically significantly different (p<0.05) and control was not significant different (p>0.05) Fig 2.

Amylase activity

Specific amylase activities of crab larvae across all treatments with or without LAB probiotic (control) were presented in Fig. 3. The highest specific amylase activity (0.35 ± 0.01 U mg^{-1} protein) was observed in T-2 over the control (0.27 ± 0.03 U mg−1 protein), there was statistically significant different (p<0.05) across the treatments including control.

DISCUSSION

In larviculture of marine species, probiotic supplementation has been successful via the rearing water (Avella *et al.,* 2010;

Table 1. Mean range of pH for control and probiotic treatments (Data represented as Mean ± SD)

Anima stage	Control	T-1	T-2	T-3
Z-1	8.14±0.05^{a}	8.08±0.03^{b}	8.9±0.04^{c}	8.07±0.05^{d}
Z-2	8.15±0.02^{a}	8.00±0.02^{b}	7.99±0.03^{c}	7.97±0.02^{d}
Z-3	8.13±0.04^{a}	7.95±0.02^{bc}	7.92±0.04^{a}	7.92±0.03^{b}
Z-4	8.12±0.01^{a}	7.80±0.02^{b}	7.81±0.03^{c}	7.80±0.03^{d}
M	8.13±0.00^{a}	7.76±0.00^{b}	7.74±0.00^{c}	7.75±0.00^{d}

Note: Z-1, Zoea-1 (day 3), Z-2, Zoea 2 (day 6), Z-3, Zoea 3 (day 9), Z-4, Zoea 4 (day 12) and M, Megalopa (day 13), S.D, standard deviation. Means in the same row with different superscript were significantly different (p<0.05).

Table 2. The NH_4–N concentrations (mg L^{-1}) of culture water treated with *L. salivarius* (T-1, T-2 and T-3) or without probiotic (control). Results were presented as means ± S.D of triplicate observations.

Anima stage	Control	T-1	T-2	T-3
Z-1	0.0241±0.00015^{a}	0.0236±0.00014^{a}	0.0233±0.00022^{a}	0.0232±0.00021^{a}
Z-2	0.0274±0.00041^{a}	0.0268±0.00020^{a}	0.0261±0.00016^{a}	0.0259±0.00025^{a}
Z-3	0.0292±0.00027^{a}	0.0287±0.00021^{a}	0.0289±0.00015^{a}	0.0278±0.00014^{a}
Z-4	0.0301±0.00017^{a}	0.0291±0.00019^{a}	0.0288±0.00022^{a}	0.0286±0.00016^{a}
M	0.0303±0.00009^{a}	0.0289±0.00017^{a}	0.0284±0.00040^{a}	0.0285±0.00014^{a}

Note: Z-1, Zoea-1 (day 3), Z-2, Zoea 2 (day 6), Z-3, Zoea 3 (day 9), Z-4, Zoea 4 (day 12) and M, Megalopa (day 13), S.D, standard deviation. Means in the same row with different superscript were significantly different (p<0.05).

Table 3. The NO_2–N concentrations (mg L^{-1}) of culture water treated with *L. salivarius* (T-1, T-2 and T-3) or without probiotic (control). Results were presented as means ± S.D of triplicate observations

Anima stage	Control	T-1	T-2	T-3
Z-1	0.0076±0.00022^{a}	0.0075±0.00020^{a}	0.0071±0.00006^{a}	0.0070±0.00010^{a}
Z-2	0.0102±0.00031^{a}	0.00100±0.00031^{a}	0.0096±0.00059^{a}	0.0096±0.00038^{a}
Z-3	0.00101±0.00011^{a}	0.0097±0.00059^{a}	0.0089±0.00041^{a}	0.0088±0.00050^{a}
Z-4	0.0091±0.00042^{a}	0.0086±0.00036^{a}	0.0081±0.00024^{a}	0.0080±0.00036^{a}
M	0.0090±0.00024^{a}	0.0084±0.00026^{a}	0.0080±0.00021^{a}	0.0080±0.00016^{a}

Note: Z-1, Zoea-1 (day 3), Z-2, Zoea 2 (day 6), Z-3, Zoea 3 (day 9), Z-4, Zoea 4 (day 12) and M, Megalopa (day 13), S.D, standard deviation. Means in the same row with different superscript were significantly different (p<0.05).

Table 4. Detection of probiotic, total viable bacteria counts (TVBC), total *Vibrio* counts (TBC) (cfu mL^{-1}) in water samples inoculated with probiotics bacteria at various concentrations and control (without probiotic) during larviculture of *P. pelagicus*

Isolate	Trial	cfu/ml	Day-2			Day-6			Day-10			Day-14		
			MRS	MA	TCBS	MRS	MA	TCBS	MRS	MA	TCBS	MRS	MA	TCBS
L. salivarius	T-1	$1x10^{6}$	$5.82x10^{3}$	$3.12x10^{3}$	$3.60x10^{2}$	$4.34x10^{4}$	$3.16x10^{2}$	$1.86x10^{2}$	$6.42x10^{4}$	$2.22x10^{2}$	$0.94x10^{2}$	$6.94x10^{4}$	$1.92x10^{2}$	$0.52x10^{2}$
	T-2	$5x10^{6}$	$2.96x10^{4}$	$2.34x10^{3}$	$3.46x10^{2}$	$5.62x10^{4}$	$4.18x10^{2}$	$1.62x10^{2}$	$6.92x10^{4}$	$3.1x10^{2}$	$0.72x10^{2}$	$7.94x10^{4}$	$1.12x10^{2}$	$0.44x10^{2}$
	T-3	$1x10^{7}$	$5.12x10^{4}$	$3.12x10^{3}$	$6.82x10^{2}$	$4.84x10^{4}$	$3.24x10^{2}$	$1.56x10^{2}$	$7.92x10^{5}$	$2.28x10^{2}$	$0.64x10^{2}$	$1.58x10^{5}$	$2.02x10^{2}$	$0.32x10^{2}$
Control			-	$6.51x10^{3}$	$5.73x10^{2}$	-	$5.22x10^{4}$	$5.82x10^{3}$	-	$1.12x10^{5}$	$5.33x10^{3}$	-	$6.98x10^{4}$	$5.11x10^{3}$

Note: MRS- deMan, Rogosa and Sharpe, M.A- marine agar, TCBS- thiosulphate citrate bile salts agar

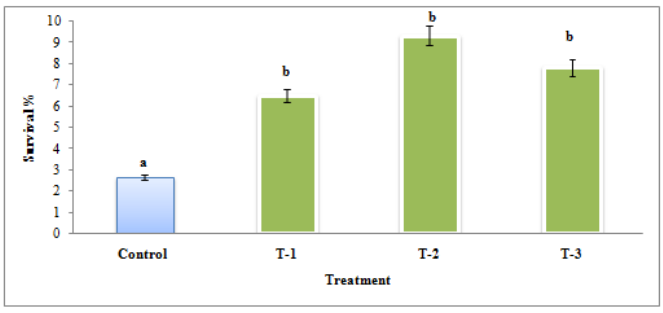

Figure 1. Mean survival of *P. pelagicus* larvae at different concentrations T-1 ($1x10^{6}$ cfu mL^{-1}), T-2 ($5x10^{6}$ cfu mL^{-1}) and T-3 ($1x10^{7}$ cfu mL-1) of *L. salivarius.* Error bars denote 95% confidence (p=0.05). Means with same letters on bars were significantly different (p<0.05).

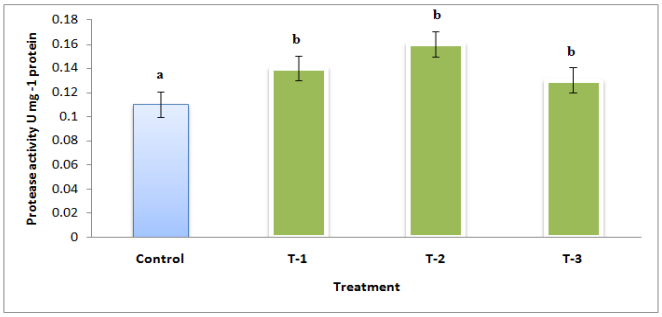

Figure 2. Specific activity of protease of *P. pelagicus* larvae (13DAH) treated with (T-1($1×10^{6}$ cfu mL^{-1}), T-2 ($5.0×10^{6}$ cfu mL^{-1}), T-3 ($1.0×10^{7}$ cfu mL^{-1}) and without probiotic (control), Error bars denote 95% confidence (p=0.05) intervals. Means with same letters on bars were significantly different (p<0.05)

Avella *et al.,* 2011; Makridis *et al.,* 2008; Nogami *et al.,* 1997; Nogami and Maeda, 1992; Plante *et al.,* 2007; Ringø and Vadstein, 1998; Xuxia *et al.,* 2010; Zhou *et al.,* 2009; Ziaei-Nejad *et al.,* 2006). Present study suggested that addition of *L. salivarius* via rearing water could significantly affect the nitrogen concentrations and pH of culture water compared with the control. Investigation done by Wang *et al.,* (2005) on water quality in shrimp, *P. vannamei*, ponds using commercial probiotics (Photosynthetic bacteria and *Bacillus* sp) significantly reduce the concentrations of nitrogen in pond water compared with the control. Similar type of results were observed by Zhou *et al.,* (2009) using *Bacillus sp.* in shrimp larvae (*P. vannamei*) as water additive could significantly improved the water quality by reducing the concentrations of

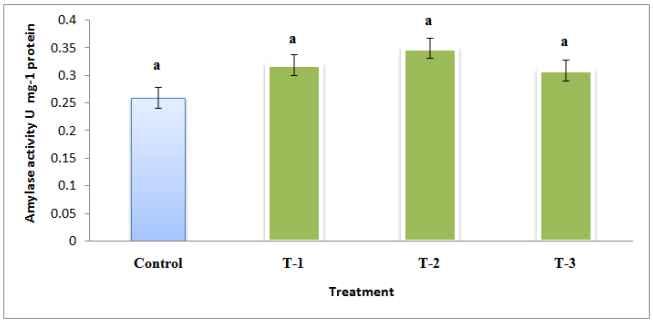

Figure 3. Specific activity of amylase of *P. pelagicus* larvae (13DAH) treated with (T-1(1×10^6 cfu mL^{-1}), T-2 (5.0×10^6 cfu mL^{-1}), T-3 (1.0×10^7 cfu mL^{-1}) and without probiotic (control), Error bars denote 95% confidence (*P*=0.05) intervals. Means with same letters on bars were significantly different ($p<0.05$).

ammonia and nitrite and affected the pH of culture water. The *Lactobacillus* isolates are competent to improve the water quality by reducing nitrogen contents in culture system (Ma *et al.,* 2009). In present study, the use of indigenous *L. salivarius* as water additive in *P. pelagicus* larviculture had shown inconsistent results (Zhou *et al.,* 2009) but perceptibly no adverse effect on the water quality was determined. The values of pH in treated groups, concentrations of ammonium and nitrite were relatively low compared to control as determined in this study were within suitable ranges (Boyd and Tucker, 1998, Zhou *et al.,* 2009). The less amount of ammonia and nitrite in the experimental tank indicates mainly due to the present of probiotic, which did initiate the nitrification. The results of this study suggest the good water quality *in vivo* for crab larviculture and the LAB strain used in this study was capable to improve the water quality providing suitable environment to rearing animal.

It was apparent from present study that the application of probiotic, *L. salivarius* via the water had beneficial effects on the survival rate of crab, *P. pelagicus* larvae. Previous study showed that LAB can act positively on cultured organisms by enhancing survival and growth in teleosts (Gomez-Gil *et al.,* 2000; Gildberg *et al.,* 1997; Nikoskelainen *et al.,* 2003; Gatesoupe, 1994, 2002; Suzer *et al.,* 2008), by stimulating the digestive (Soundarapandian and Babu, 2010; Ziaei-Nejad *et al.,* 2006: Suzer *et al.,* 2008), immune systems (Gatesoupe 1999) and by improving water quality (Kennedy *et al.,* 1998; Moriarty 1998; Soundarapandian and Babu, 2010; Ma *et al.,* 2009). Probiotics have been investigated as survival promoters in marine larviculture, when probiotics were used as water additive in rearing of larvae of green shell muscle *Perna canaliculus* significant survivals were obtained (Kesarcodi-Watson *et al.,* 2010). A similar finding was obtained by Nogami *et al.,* 1997**,** who used bacterial strain PM-4 (*Thalassobacter utilis*,) isolated from seawater and inoculated it into blue crab (*Portunus trituberculatus*) larval rearing tanks, observed a higher survival in the test tanks compared with the control tank, with no bacteria inoculated. It was hard to assess directly different studies using probiotics, because the effectiveness of a probiotic application depended on many factors (Gomez-Gil *et al.,* 2000) such as species composition, application level, frequency of application and environmental conditions. Furthermore, the results of present study demonstrated that addition of *L. salivarius* had increased larval survival was significant difference ($p<0.05$) in all treated groups, which specify that the dose of probiotics was only one of the aspects improving the survival rate of crab larvae. Moreover, the advantageous effect of the *L .salivarius* probiotic were clearly evident; in particular, enhanced survival and improved water quality to common rearing conditions. Probiotic application in aquaculture aims as therapeutic biotechnological tool to control the proliferation of harmful microorganisms. Addition of probiotics by live food and/or culture water decreased bacterial activity in some teleosts such as *Sparus aurata* (Salinas *et al.,* 2005, 2006; Diaz-Rosales *et al.,* 2006), *Paralichthys dentatus* (Eddy and Jones, 2002), *Scophthalmus maximus* (Planas *et al.,* 2006), *Salmo salar* (Robertson *et al.,* 2000) and in crustacean *Penaeus monodon* (Fabricius) (Soundarapandian and Babu 2010). *L. brevis*, was able to almost completely eliminate the *V. alginolyticus* load attached to *Artemia* while only high doses of the probiont managed to reduce *Vibrio* sp. occurring in the culture water (Villamil *et al.,* 2003). However, in the present study no total removal of bacteria achieved, nevertheless, *Vibrio* bacteria were observed in lower counts in experimental groups than control where increase in bacteria especially vibrios was high. The decrease in vibrios in treated tanks was owing to a vital probiotic property of LAB probiont to displace the pathogen, which is (Collado *et al.,* 2008). It could be explained that higher retention of delivering probiotic to rearing water suppressed the vibrios and created favourable environment for the crab larvae.

Probiotics in aquaculture organisms can produce a range of digestive enzymes (El-Haroun *et al.,* 2006; Soundarapandian and Babu 2010; Suzer et la. 2008; Wang *et al.,* 2006; Wang, 2007). The specific activity of measured digestive enzymes in the crab larvae was observed high in treated groups over the control. It can be explained that the digestive system of *P. pelagicus* larvae was activated where the probiotics would have the greatest effect because the genus LAB, do secrete a wide range of exoenzymes (Moriarty 1998). Administration of *Lactobacillus* probiotics could increase the specific activities of digestive enzymes in aquatic organisms (Soundarapandian and Babu, 2010; Suzer *et al.,* 2008). Enzymatic profile of experiment groups demonstrated remarkably better activities than the control groups. In the present study, different concentrations of probiotic had different effects on enzyme activity and specific activities of larval enzymes, protease and amylase were significantly different ($P<0.05$) in all experimental groups. Increased concentration of probiotic did not increase the activity of the digestive enzymes might be indigenous probiotic strains has a limit and the results of the present study were matching to Zhou *et al.,* (2009). The present study suggests that LAB can stimulate the specific activity of digestive enzymes in larvae due to its beneficial effect.

It could be concluded, the results of this study suggested that *L. salivarius* probiotic, supplemented as water additive at a certain concentration could significantly increase survival rate, improved water and some digestive enzyme activities of crab larvae. It is clear from the microbial load of rearing water that *Vibrio* sp. was dominant only in the control tanks not in experimental tanks strongly suggests that adding the probiotics restrained the growth of vibrios in treated groups and developed a suitable environment for larvae. Therefore, based on these results, the use of *L. salivarius* as a probiotic in larviculture of *P. pleagicus* as water additive was recommended.

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