Effects of heat-killed *Lactobacillus plantarum* strain L-137 on larvae quality and growth performance of white leg shrimp (*Litopenaeus vannamei*) juveniles

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Abstract- This study was conducted to determine the effects of supplemental LP20 on growth performance and immune response of postlarvae (PL) and juvenile stages of white leg shrimp, *Litopenaeus vannamei.* The LP20 contains 20% heat-killed *Lactobacillus plantarum* strain L-137 (HK L-137) and 80% dextrin in dried-weight basis. The study was conducted for two phases: Phase 1 (hatchery stage- PL_1 to PL_{15}) was carried out indoor with four treatments by supplementation of LP20 at 0.0 g (marked as LP20-0.0), 0.5 g (LP20-0.5) and 1.0 g (LP20-1.0) LP20 kg^{-1} of feed to the basal diet. The basal diet without LP20 was set up as a control that used in the whole experimental period (including Phase 1 and Phase 2). Initial PLs of *L. vannamei* were cultured at stocking density of 75,000 nauplii 0.5 m^3 $tank^{-1}$. The results showed that the final weight and length of PLs (at PL_{15}) fed feed containing 1.0 g of LP20 kg^{-1} of feed were significantly higher than those of PLs fed basal diet ($P<0.05$). Total bacteria count in PL_{15} was significantly different between LP20 supplemented groups (LP20-0.5 and LP20-1.0) and without LP20 supplemented groups (LP20-0.0 and control). Phase 2 (nursery stage- PL_{16} to PL_{45}) was conducted continuously from Phase 1, in which, similar-sized shrimps of each treatment, including LP20-0.0, LP20-0.5 and LP20-1.0 were selected and divided into two groups, fed with a reduction of bacterial doses (at 0.1 g and 0.25 g kg^{-1} of feed). The results showed that the highest specific growth rate (SGR) was found in the treatment LP20-1.0 that fed diet supplemented LP20 at 0.25 g kg^{-1} of feed (1.0LP20-0.25) and significantly differed compared with the control ($P<0.05$); however, there was no significant difference in SGR among the LP20 supplemented diets. The highest bacterial densities were observed in the hepatopancreas of shrimp of the 0.5LP20-0.1 treatment (in comparison with other treatments, $P<0.05$). Similarly, the lowest *Vibrio* spp. density (10×10^3 colony-forming units (cfu) $sample^{-1}$) was counted in the hepatopancreas of shrimp of the 0.5LP20-0.1 treatment. Bacterial challenge test showed that the significantly lowest cumulative mortality (7.5%) was found in the 0.5LP20-0.1 treatment when compared with other treatments ($P<0.05$). Thus, the findings of this study indicated that the supplementation of 0.5 g of LP20 kg^{-1} of feed to the diet used for white leg shrimp at PL stage and continuing feeding PL with 0.1 g of LP20 kg^{-1} of feed enhanced growth performance and improved immune system.

Keywords: Heat-killed bacteria, *Lactobacillus plantarum*, *Litopenaeus vannamei*, postlarvae, juvenile, growth performance, quality

I. Introduction

The requirement of white leg shrimp, *Litopenaeus vannamei*, production has been increased rapidly in the world. In which Asia countries accounted for 61% in 2011 and 85% in 2013 of global shrimp exports (FAO, 2015). It has contributed to the increase of white leg shrimp production as well as intensification culture system in many countries in Asia, including Viet Nam. However, it may due to high risk of diseases outbreak. Obviously, vibriosis is one of the major diseases occurred in cultured shrimp causing high mortality rate during practice period. Vibriosis has been reported related to a number of *Vibrio* species, including *V. harveyi*, *V. vulnificus*, *V. parahaemolyticus*, *V. alginolyticus* and *V. penaeicida*. Among these bacterial species, *V. harveyi* and *V. alginolyticus* are known as endemic opportunistic pathogens in cultured white leg shrimp. One of the serious diseases, namely early mortality syndrome or acute hepatopancreatic necrosis syndrome, has happened in cultured white shrimp in Southeast Asia since 2010; this resulted in thousands of farmer facing to lose their income every year.

Application of antibiotics in aquaculture is commonly to reduce the infection which might lead to overuse of antibiotic. Therefore, either reducing diseases outbreak (and/or mitigation of infections) or improving immunity system of cultured animals by antibiotic substitution should be considered in detail. In order to overcome these problems, many studies in stimulating and improving the immune system of shrimp, e.g. However, the development of genetically based host resistance is costly and impossible to attain the specific pathogen resistance populations. The use of antibiotic causes resistance in microbes for human health and environment pollution by its residues as well (Martines, 2009). Therefore, high qualities of shrimp larvae that are able to improve growth performance, immunity and prevention of pathogens are of primary concerns.

LP20 is a heat-killed *Lactobacillus plantarum* feed mixes containing 20% HK L-137, a new product manufactured by House Wellness Foods Corporation Japan, is considered to be a good nutrition additive to cultured white leg shrimp. We assume

that using LP20 may improve growth and quality of larvae white leg shrimp at postlarvae (PL) stage. Therefore, House Wellness Foods Corporation Japan has strongly supported for this current research.

Studies have shown that using LP20 boosts the immune system and enhances the health, resulting in improving growth performance of aquaculture. Previous study showed that addition of 0.1 or 1.0 g of LP20 kg^{-1} of feed have induced a good growth and survival rate of juvenile Kuruma shrimp (Tung *et al*., 2009). In addition, Duc *et al.* (2016) also found that supplementation of 0.1 g of LP20 kg^{-1} of feed enhanced good growth of white leg shrimp at the grow-out stage. However, there is no previous study on the effects of LP20 on growth performance of white leg shrimp at larvae and juvenile stages; thus, this present study was conducted.

II. MATERIALS AND METHODS

A. Experimental shrimp nauplii

Shrimp nauplii were obtained from a commercial hatchery (certification of SIS Hawaii, USA). Before commencement of the experiment, nauplii were slowly acclimated to the experimental conditions in the Wet-Lab, College of Aquaculture and Fisheries, Can Tho University, Viet Nam.

B. LP20 source and experimental design

LP20 (Itami, Japan) contains 20% heat-killed *L. plantarum* strain L-137 (HK L-137) and 80% dextrin in dried-weight basis. The concentration of LP20 in the dry completed product is 2×10^{11} colony-forming units (cfu) g^{-1}. The LP20 was prepared based on the previous method, described by Murosaki *et al.* (1998). The two doses of 0.5 g and 1.0 g of LP20 kg^{-1} of feed were supplemented to diets of white leg shrimp larvae and juveniles. Schematic design of these experiments is shown in Table 1.

Phase 1 (hatchery stage- PL_1 to PL_{15}) was carried out indoor with four treatments. The first diet contained 0.0 g of LP20 kg^{-1} of feed (marked as LP20-0.0), this treatment used as control and provided animal for the next phase (Phase 2). The second and the third treatments, LP20 was added to the diets at 0.5 g (LP20-0.5) and 1.0 g of LP20 kg^{-1} of feed (LP20-1.0), respectively. The fourth one is a control, which was fed diet without supplemented LP20, which used for the whole experimental period (including Phase 1 and Phase 2). Nauplii of shrimp were cultured in $0.5m^3$ tank at a stocking density of 75,000 nauplii $tank^{-1}$, for all treatments.

In the Phase 2 (nursery stage- PL_{16} to PL_{45}), after finishing the Phase 1, the similar size of shrimps from each treatment 1, 2 and 3 were introduced into two groups, with three replicates for each, at 1.0 g (Duc *et al*., 2016) and 0.25 g of LP20 kg^{-1} of feed, respectively. The treatment 0.0LP20-0.1 and 0.0LP20-0.25 indicated shrimp from LP20-0.0 treatment (Phase 1) were fed with diet containing 0.1 g and 0.25 g of LP20 kg^{-1} of feed, respectively. Treatment 0.5LP20-0.1 and 0.5LP20-0.25 indicated shrimp from LP20-0.5 treatment (Phase 1) were fed diet containing 0.1 g and 0.25 g of LP20 kg^{-1} of feed, respectively, and the similar marks set up for treatments 1.0LP20-0.1 and 1.0LP20-0.25.

At the end of Phase 1, the same sizes of PL (at PL_{15}) (10.1±0.24 mm in length and 0.0062±0.001 g in weight) were selected and used for Phase 2. The stocking density was 1,000 PL 0.5 m^3 $tank^{-1}$ (Tao *et al*., 2015); salinity was 15‰ with applying Biofloc technique. Bioflocs were created by using rice powder (73.4% carbohydrate and 0.26 % protein), at a C:N ratio of 15:1. Amount of rice powder was added into the tanks for every three day based on the amount of feed fed and protein level of the feed (Avnimelech, 1999). The experiment was randomly designed among treatments under shade condition (30% cover, using nylon netting).

Formalin stress test

The quality of PL_{15} at the end of Phase 1 was tested using formalin: at least ten PL_{15} were randomly selected from each of treatments and bathed with formalin at 150 ppm for one hour. Three replicates were done for each of treatments. The mortality of shrimp was recorded.

C. Visual criteria (size and colour of PL_{15})

Quality of PLs was evaluated daily based on visual observation criteria, described by FAO (2007).

D. Bacterial count

Three shrimps per tank were sampled at the stage of PL_{15} and PL_{45} for bacterial count using a standard plate count method. The whole shrimp of PL_{15} and the intestine (IN) and hepatopancreas (HP) of PL_{45} were dissected aseptically. The whole shrimp, IN and HP were weighed and homogenized in 1.0 mL of sterile saline solution (SSS) (0.85% NaCl). Serial 10-fold dilutions of the supernatant were performed; 0.1 mL of the solution was cultured on TCBS Agar (Himedia, India) and Nutrient Agar (Himedia, India) (1.5% NaCl) to enumerate the *Vibrio* spp. and total bacteria count, respectively. The treatments were carried out in three replicates. The plates were incubated at 30°C for 24 hours. The results are reported as cfu per larvae (for PL_{15}) and cfu per sample (for PL_{45}).

E. Bacterial challenge experiment

At the end of Phase 2, 15 shrimps from each replicate of each treatment were challenged by immersion with *Vibrio parahaemolyticus* at 1.1×10^8 cfu mL^{-1} (LD50). The challenge experiment was conducted as described by Tran *et al*. (2013) with modifications. Shrimps in the control group were used as negative treatment, immersed in sterilized Nutrient Broth plus 1.5% NaCl. Three replicates were performed for the challenge and negative treatments. The experiment was conducted for 14 days. Moribund shrimp were recorded every day and were collected for DNA extraction and PCR amplification as previously described (Sritunyalucksana *et al*., 2014).

Feed used for larvae and juveniles

At the larvae stage (Phase 1), larvae were fed commercial feed, including of Lansy and Frippak (Inve. Company, Belgium).

At the nursery stage (Phase 2), shrimps were fed commercial feed (Grobest Company, Viet Nam). Feeding behavior of shrimp was observed and the feed amount was adjusted by stages of shrimp according to the instruction of the manufacture and actual feeding level of shrimps (feeding regime was based on the sizes of PL, PL_{15} were fed with powder (42% protein) at 15% body

weight (BW). The amount of feed (crumble feed and pellet) was then reduced to 5-6% BW when the shrimp was weighed individually about 0.1-0.5 g up the end of the experiment. LP20 was mixed in water before adding to the feed; the feed was then coated by squid oil (1.5 mL 100 g^{-1} of feed). Animals were fed four times a day at 7:00, 11:00, 16:00 and 20:00.

F. Growth performance

The growth performance in weight (g) and length (mm) of PLs and juveniles were measured at the stages of PL_5, PL_{10}, PL_{15}, PL_{30} and PL_{45} at 5, 10, 15, 30 and 45 days post culture, respectively. At least 100 shrimps $tank^{-1}$ were weighed, three replicates for each treatment.

Specific growth rate (SGR,%) = {(lnWf-lnWi)/T} × 100

Daily weight gain (DWG) = (Wf-Wi)/T

Survival (%) = (final No. of shrimp/initial No. of shrimp) × 100

Where, Wf is final weight, Wi is initial weight and T is total day of the experiment

G. Water quality measurement

Temperature was measured using thermometer. pH value was measured every 3 days (at the morning and afternoon) using pH meter (HANA brand, USA). N-NO_2 and TAN (NH_3/NH_4) were checked every week using Test Sera (Germany).

H. Data analysis

The data were subjected to One-way ANOVA, followed by Duncan test to compare the weight gain (g), SGR (% body weight/day) and feed conversion ratio (FCR) (dry feed eaten/weight gain) among the treatments.

III. Results and Discussion

Water parameters

Table 2 and 3 showed the measurements of water parameters of all treatments. The results showed that water temperatures in the first phase were slightly higher, but stable and less fluctuated within the day compared with the second phase.

Water temperature in all treatments of Phase 2 ranged from 26-28^oC and slightly fluctuated between the morning and afternoon. The same trend for pH level, ranging from 7.9 to 8.2 within a day, was recorded.

Growth performance of shrimps

Phase 1 (PL stage- PL_1 to PL_{15}): Table 4 showed the growth performance of PL after 15 days. The results showed that both final weight and length of PL fed feed containing LP20 were significantly higher than those of PL fed feed without addition of LP20 ($P<0.05$); and the weight and length of PLs fed feed containing 1.0 g of LP20 kg^{-1} of feed was highest. Similarly, survival rates of PLs that fed feed containing LP20 were significantly higher than those of animals fed feed without LP20 ($P<0.05$). There was no significant difference in both shape and color of PLs in all treatments.

Formalin challenge test resulted in none of died PL_{15} was observed during the test, suggesting there was no significant difference in mortality of PL_{15} in all treatments.

Total bacteria count in PL_{15} was significantly different between the groups supplemented with LP20 (LP20-0.5 and LP20-1.0) and without LP20 (LP20-0.0 and controls). The highest density of total bacteria was observed in the LP20-1.0 treatment, showing significantly at $P<0.05$ (Figure 1). Total amount of *Vibrio* spp. in PL_{15} was lower in the treatments fed LP20. The lowest *Vibrio* spp. density was recorded in the treatment LP20-0.5 at the value of 0.74×10^5 cfu $larvae^{-1}$ in comparison to other treatments ($P<0.05$). It has been reported that total bacteria count was determined lower in the probiotic supplemented shrimp group than in the control shrimp group (Sivakumar *et al*., 2012). In this study, total bacteria count was increased, whereas the lower density of Vibrio count observed in the LP20 supplemented groups. It is possible that LP20 might increase intestinal probiotic bacteria and they inhibit the growth of pathogenic bacteria, *Vibrio* sp. The competition for similar nutrients and secretion of antimicrobial agents are among reported capacities of probiotics (e.g. *Bacillus* sp., *Lactobacillus* sp.) to control pathogenic bacteria in shrimp intestines (Moriarty 1998; Verschuere *et al*., 2000; Balcazar *et al*., 2006).

Phase 2 (rearing stage PL_{16} to PL_{45}): The growth performance of PLs in Phase 2 was shown in Table 5 and Table 6. The results revealed that no significant difference in the length of PLs among the treatments for the first 15 days after PL_{15} stage was observed ($P>0.05$); however, the body length of PLs fed feed supplemented with LP20 was significantly increased compared with such of PLs fed feed without LP20 after 45 days. The highest SGR (4.89±0.44% day^{-1}) of PLs was found for those in the treatment 0.5LP20-0.1 and significantly higher than that of PLs in the treatment 0.5LP20-0.25 and controls.

The highest SGR was found in PLs of the treatment 1.0LP20-0.25 that fed feed supplemented with 0.25 g of LP20 kg^{-1} of feed. There was a significant difference in SGR of PLs fed diet supplemented LP20 compared with the controls fed diet without LP20 ($P<0.05$); however, there was no significant difference in SGR among the PLs fed diets added LP20 ($P>0.05$).

After 30 days of rearing from PL_{15} to PL_{45}, the results showed that the highest survival rate was found in the PLs of the treatment 1.0LP20-0.1 and 1.0LP20-0.5; however, there was no significant difference among the PLs of treatments fed with different diets.

The highest FCR was found in the control treatment that differed significantly from the treatment fed with diet supplemented 0.25 g of LP20 kg^{-1} of feed ($P<0.05$), but not significantly differed from such fed with diet supplemented 0.1 g of LP20 kg^{-1} of feed ($P>0.05$). Both shape and color of juveniles were similar in all treatments after 45 days.

Quality of juveniles (PL_{45}) parameters

In the stage of PL_{45}, the highest amount of bacteria were determined in the HP of shrimp in the treatment 0.5LP20-0.1 ($P<0.05$). The lowest *Vibrio* spp. density was also observed in the HP of shrimp in the treatment 0.5LP20-0.1, with an amount of 1.0×10^4 cfu $sample^{-1}$ (Figure 2).

The density of *Vibrio* spp. in the IN samples was found to be at the lowest in shrimp of the treatment 0.5LP20-0.1 (accounted for

1.4×10^6 cfu sample^{-1}) in comparison to the control (accounted for 3.9×10^6 cfu sample^{-1}) (*P<0.05*) (Figure 3).

The effects of probiotic on microbiota was also examined in black tiger shrimp (*Penaeus monodon*). Shrimp were fed live-sprayed *Bacillus* (LS) or freeze-dried *Bacillus* (FD) for 120 days The result showed that shrimp supplemented with LS and FD contained significantly lower number of *Vibrio* count (P<0.05) in the hepatopancreas, intestine compared to those in the control group (Boonthai *et al*., 2011). Similarly, Li *et al.* (2007) reported that the number of Vibrio count in the digestive tract of *L. vannamei* treated with probiotic (*Bacillus licheniformis*) were significantly decreased compared to those in the control shrimp. Probiotics could prohibit the growth of pathogens by production of antimicrobial substance such as organic acids, hydrogen peroxide and bacteriocins (Reid *et al*. 2001). In case of *L. plantarum*, the bacteria has been reported to produce bacteriocin, plantaricin A (Nissen-Meyer *et al*. 1993).

LP20 contains HK L-137, heat-treated bacteria, and it might inhibit the growth of pathogens by increasing intestinal probiotic bacteria or by inducing antimicrobial substance to intestine indirectly.

Bacterial challenge experiment

The survival rate of the *V. parahaemolyticus* infected PLs in the treatments that supplemented with LP20 were significantly higher than those in the control (*P<0.05*). The highest cumulative mortality (62%) of the shrimps was recorded in the control treatment. The cumulative mortality in the treatments supplemented with LP20 exhibited in a range of 7.5% (in the treatment 0.5LP20-0.1) to 41.3% (1.0LP20-0.25). However, non-died shrimp was recorded in the negative treatment (Figure 4).

Similarly, several studies have been demonstrated with different *Lactobacillus* spp. harboring strong antimicrobial activity against pathogenic bacteria. Specifically, *P. monodon* were fed with supplemented *Lactobacillus acidophilus* food (10^5 CFU/g) for 1 month before challenging to *Vibrio alginolyticus.* The challenge resulted in 20% accumulative mortality in the probiotic supplemented group as compared to 86.7% in the control group (Sivakumar *et al*., 2012). Vaseeharan and Ramasamy (2003) documented that luminescent-pathogenic *Vibrio harveyi* could be inhibited by a probiotic bacteria *Bacillus subtilis* BT23 under in vitro and in vivo conditions. In this experiment, the result had demonstrated that the use of *L. plantarum* can reduce the shrimp mortality rate after challenging to *V. parahaemolyticus*.

Recently, we have shown that LP20 plays an important role in immune modulation of white leg shrimp by increasing THC, phenoloxidase activity, phagocytic activity, clearance efficiency and survival rates (Pham Minh Duc *et al*., 2016). It is possible that LP20 augments immune function and decreases the motality of the V. parahaemolyticus infected PLs.

Moribund shrimp were then randomly selected for testing the presence of *V. parahaemolyticus* in challenged shrimps using two step-PCR method. The results showed that the bacterium *V. parahaemolyticus* was detected in the challenged shrimps, showing a bright band at 230 base pair through agarose gel electrophoresis (Figure 5).

IV. CONCLUSION

Nauplii fed diet supplemented with 1.0 g of LP20 kg^{-1} of feed displayed an effective growth performance.

In the nursery stage (from PL_{16} to PL_{45}) that the PLs from LP20-1.0 treatment fed diet added with 0.25 g of LP20 kg^{-1} of feed showed a better growth performance; no significant difference in the SGR of PLs selected from LP20-0.5 fed with diet containing 0.1 g of LP20 kg^{-1} of feed.

The shrimps fed with diets supplemented with 0.5 g of LP20 kg^{-1} of feed at first and fed with such supplemented with 0.1 g of LP20 kg^{-1} of feed at later were enhanced growth performance and improved immune system.

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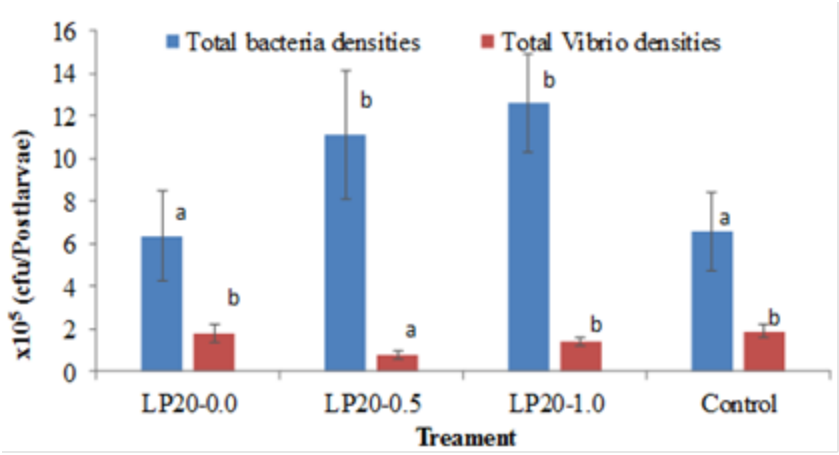

Fig. 1. Bacteria densities isolated from the shrimps at PL_{15} stage

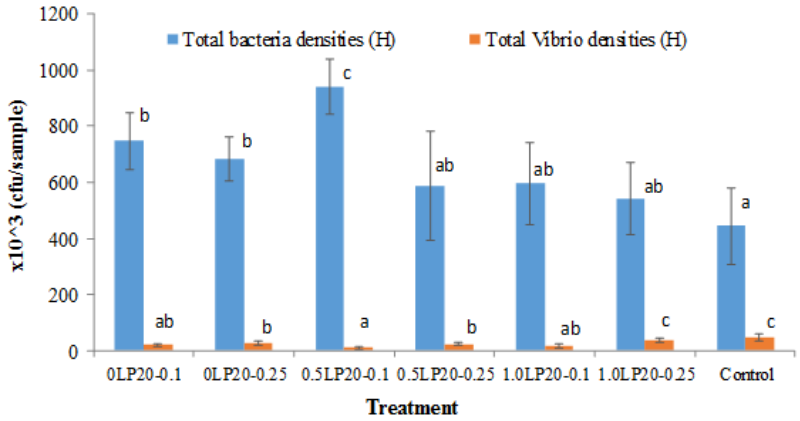

Fig. 2. Bacteria densities isolated from hepatopancreas of the shrimps at PL_{45} stage

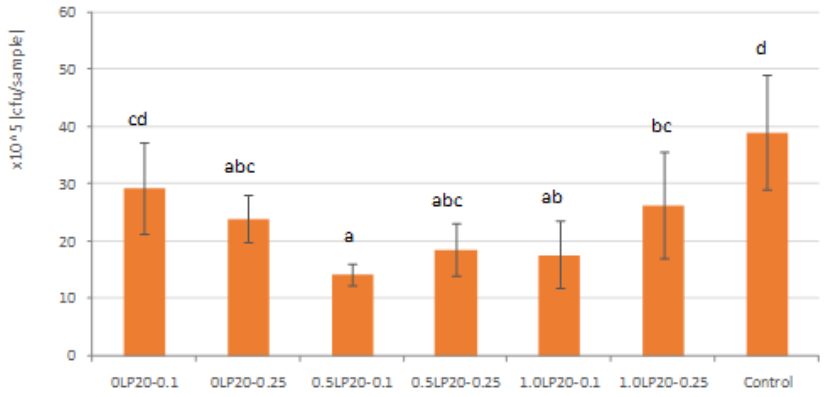

Fig. 3. *Vibrio* spp. densities isolated from intestine of the shrimps at PL_{45} stage

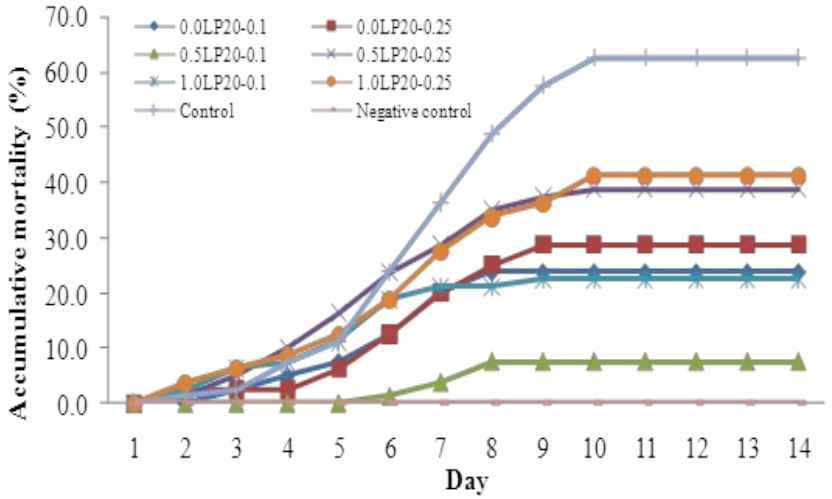

Fig. 4. Cumulative mortality in shrimps inoculated with *Vibrio parahaemolyticus* at 1.1×10^8 cfu mL^{-1} (LD50)

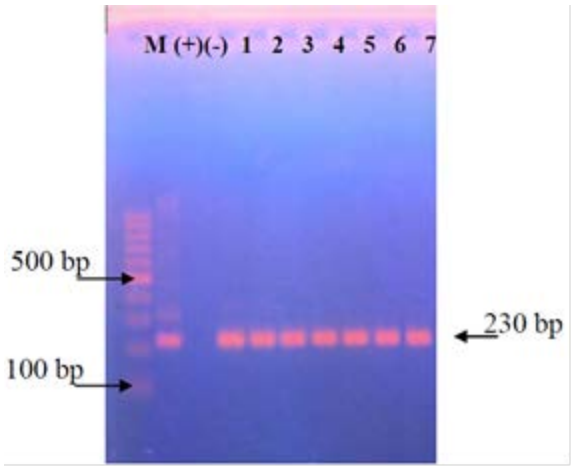

Fig. 5. PCR amplification results of detecting *Vibrio parahaemolyticus* from infected shrimp. Lane M indicates 100bp ladder; Lane (+) indicates positive control; Lane (-) indicates negative control; Lane 1,2,3,4,5,6,7 indicates the shrimp DNA isolated from treatment 0.0LP20-0.1, 00.LP20-0.25, 0.5LP20-0.1, 0.5LP20-0.25, 1.0LP20-0.1, 1.0LP20-0.25, respectively.

Table 1: Schematic design for the application of LP20 on postlarvae and nursery stages of *L. vannamei*

Treatment	Hatchery				Nursery	
	Nauplius	Zoea	Mysis	Post larvae (PL_{1-15})	PL_{16-45}	Bacterial challenge test
	No application of LP20			Application of LP20 (g/kg)		
				Phase 1	Phase 2	
1	No LP20			0.00	0.10	Selected 2 good result from phase 2
					0.25	
2				0.50	0.10	
					0.25	
3				1.00	0.10	
					0.25	
4				0.00		Control

Table 2: Measurements of water parameter in all treatments of Phase 1

Parameter		Treatment		
		0.0LP20	0.25LP20	1.0LP20
Temp. (^oC)	AM	28.8±1.9	28.5±1.6	28.3±1.3
	PM	29.3±1.5	29.0±1.3	28.8±1.1
pH	AM	8.18±0.19	8.20±0.19	8.17±0.21
	PM	8.18±0.20	8.18±0.20	8.18±0.19
Alkalinity (mg/L)		116.4±10.3	117.8±12.2	117.8±12.0
TAN (mg/L)		0.2±0.2	0.5±0.6	0.3±0.3
$N\text{-}NO_2^-$ (mg/L)		1.3±1.2	1.4±1.2	1.1±1.0

Table 3: Measurements of water parameter in all treatment of Phase 2

Parameter		Treatment						
		0.0LP20		0.5LP20		1.0LP20		Control
		0.1	0.25	0.1	0.25	0.1	0.25	
Temp. (^oC)	AM	26.4±0.9	26.4±0.8	26.4±0.8	26.4±0.8	26.5±0.8	26.4±0.8	26.4±0.9
	PM	28.0±1.2	27.9±1.2	27.8±1.2	28.0±1.2	27.1±3.0	27.8±1.1	27.8±1.2
pH	AM	7.9±0.3	8.0±0.4	8.0±0.4	8.0±0.4	8.0±0.4	8.0±0.4	8.0±0.4
	PM	8.1±0.3	8.2±0.3	8.2±0.3	8.2±0.3	8.1±0.3	8.2±0.3	8.1±0.3
Alkalinity (mg/L)		147.7±22.0	162.2±23.4	162.2±22.0	139.8±19.1	162.2±28.8	149.9±33.4	153.3 ±23.4
TAN (mg/L)		0.3±0.3	0.6±0.6	0.4±0.4	0.4±0.4	0.3±0.4	0.5±0.7	0.4±0.4
$N\text{-}NO_2^-$ (mg/L)		1.3±0.9	1.0±1.3	1.1±0.9	1.2±1.0	1.2±1.0	1.3±0.7	1.3±1.1

Table 4: Growth performance and survival rate of PL_{1-15} shrimp.

Parameter	0.0-LP20	0.5-LP20	1.0-LP20
Final Weight of PL_{15} (g)	$0.0048±0.0005^a$	$0.0060±0000^b$	$0.0068±0.0005^c$
Length of PL_{15} (mm)	$9.83±0.36^a$	$10.53±0.34^b$	$10.74±0.31^b$
Amount of feed (g)	$128.4±0.65^a$	$126.6±0.25^a$	$125.5±0.48^a$
Survival rate of PL_{15} (%)	$43.7±4.7^a$	$49.8±3.8^b$	$53.9±2.5^b$
Shape uniform of PL_{15} (%)	100	100	100
Colour uniform of PL_{15} (%)	100	100	100

Table 5: The growth performance in length (mm) of shrimp PL_{16-45}

Treatment	Intial	30 days	45 days	DLG (mm/day)	SGR_L (%/day)
0.0LP20-0.1	10.1±0.24[a]	20.9±1.6[a]	37.4±0.57[b]	0.98±0.12[abc]	4.60±0.29[abc]
0.0LP20-0.25	10.1±0.24[a]	23.1±2.7[a]	38.5±2.02[bc]	0.98±0.13[abc]	4.61±0.32[abc]
0.5LP20-0.1	10.1±0.24[a]	22.1±1.9[a]	37.6±0.31[bc]	1.105±0.19[c]	4.89±0.44[c]
0.5LP20-0.25	10.1±0.24[a]	20.4±1.6[a]	37.6±0.63[bc]	0.89±0.44[ab]	4.38±0.13[ab]
1.0LP20-0.1	10.1±0.24[a]	20.3±0.7[a]	37.6±0.30[bc]	1.03±0.07[bc]	4.73±0.17[bc]
1.0LP20-0.25	10.1±0.24[a]	20.6±1.7[a]	39.2±0.77[c]	0.97±0.02[abc]	4.58±0.05[abc]
Control	10.1±0.24[a]	20.9±1.6[a]	35.2±0.77[a]	0.85±0.04[a]	4.25±0.13[a]

Table 6: The growth performance in weight (g) of shrimp (PL_{16-45})

Treatment	Initial	30 days	45 days	DWG (g/day)	SGR (%/day)
0.0LP20-0.1	0.0062±0.001[a]	0.059±0.008[a]	0.47±0.02[ab]	0.018±0.005[a]	21.9±0.13[ab]
0.0LP20-0.25	0.0062±0.001[a]	0,089±0.12[b]	0.48±0.06[ab]	0.018±0.005[a]	22.0±0.40[ab]
0.5LP20-0.1	0.0062±0.001[a]	0.06±0.015[a]	0.45±0.04[ab]	0.015±0.006[a]	21.7±0.29[ab]
0.5LP20-0.25	0.0062±0.001[a]	0.07±0.02[ab]	0.45±0.05[ab]	0.015±0.006[a]	21.8±0.39[ab]
1.0LP20-0.1	0.0062±0.001[a]	0.06±0.01[a]	0.45±0.08[ab]	0.015±0.006[a]	21.9±0.55[ab]
1.0LP20-0.25	0.0062±0.001[a]	0.077±0.007[ab]	0.52±0.05[b]	0.02±0.001[a]	22.3±0.30[b]
Control	0.0062±0.001[a]	0.056±0.005[a]	0.403±0.05[a]	0.013±0.005[a]	21.5±0,38[a]

Table 7: Survival rate, feed conversion ratio and shrimp quality at the stages of PL_{16} to PL_{45}

Treatment	Survival rate PL_{45} (%)	FCR	Size uniform of PL_{45} (%)	Color of PL_{45}
0.0LP20-0.1	93.6±4.5[a]	1.41±0.62[ab]	90	Normal
0.0LP20-0.25	95.1±3.3[a]	1.37±0.21[a]	90	Normal
0.5LP20-0.1	95.6±2.7[a]	1.46±0.14[ab]	90	Normal
0.5LP20-0.25	94.4±4.4[a]	1.48±0.23[ab]	90	Normal
1.0LP20-0.1	97.3±3.4[a]	1.44±0.22[ab]	90	Normal
1.0LP20-0.25	97.6±0.9[a]	1.23±0.11[a]	90	Normal
Control	93.9±3.5[a]	1.66±0.17[b]	90	Normal