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Full Length Research Paper

Effect of *Bacillus subtilis* on the growth and survival rate of shrimp (*Litopenaeus vannamei*)

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The effect of *Bacillus subtilis*, isolated from digestive tract of *Macrobrachium rosenbergii* was investigated on growth and survival rate of *Litopenaeus vannamei* during 60 days of culture. Sixteen aquaria with four replicates were used for treatments and controls. Treatment groups were consisted of i) shrimp fed diet with *B. subtilis* (T1), and ii) shrimp fed diet mixed with *B. subtilis* and commercial probiotic (T2). Control groups were consisted of i) shrimp fed diet with commercial probiotic as positive control, and ii) shrimp fed unaltered diet as negative control. Results showed that *B. subtilis* was proliferated in digestive tract of treated shrimps, and the number of *Vibrio* spp. was reduced in digestive tract during the cultural period. Survival rate, 75.5± 4.62 %, and yields of shrimps, 190.00 ± 13.13 g, treated with *B. subtilis* were significantly greater (P<0.05) than the other treated and control groups. Also population density of total viable bacteria and *B. subtilis* counted in digestive tract of shrimps treated with *B. subtilis* were significantly higher (P<0.05) than the other treated groups. Results of this study indicate that the addition of *B. subtilis* can improve shrimp (*L. vannamei*) survival rate and yield.

Key words: *Litopenaeus vannamei*, probiotic, *Bacillus subtilis*.

INTRODUCTION

Shrimp aquaculture, as well as other industries, constantly requires new techniques due to increasing production yield. Economically, preparation of commercial pellet feed to get an appropriate and fast growth is one of the major problems in aquaculture in order to be most cost effectiveness of pellet feed during the cultural period. Having an appropriate growth, higher survival rate, healthy shrimp, better feed conversion ratio (FCR), and also reducing cultural period are the major important goals in this industry.

Probiotics are defined as a live microbial adjunct which has a beneficial effect on the host by modifying the host-associated or ambient microbial community, by ensuring improved use of the feed or enhancing its nutritional value, by enhancing the host response towards disease, or by improving the quality of its ambient environment (Verschuere, 2000). Probiotic bacteria could produce digestive enzymes and essential growth nutrients such as vitamins and amino acids, which are benefit for enhancing the best growth, also they could benefit to their invertebrate host by competitive exclusion against pathogens (Austin et al., 1995; Gomez-Gil et al., 2000) or by increasing the host resistance and immunity (Uma et al., 1999) which are benefit to achieve the higher survival rate and healthier animals.

Appropriate uses of probiotic in aquaculture industry were shown to improve intestinal microbial balance, and also to improve feed absorption, thus leading to increased growth rate (Parker, 1974; Fuller, 1989; Rengpipat et al., 1998) and also reduced feed conversion ratio (FCR) during the cultural period (Wang, 2005).

The genus *Bacillus* has been isolated from crustacean intestine (Rengpipat et al., 2000). Some species of this genus have shown inhibitory activity against various pathogens and also increase of mean weight and survival rate of *Penaeus monodon* larvae and postlarvae (Rengpi-

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pat et al., 1998; Sugita et al., 1998). The effect of probiotic, *Bacillus coagulans* SC8168, as water additive on larvae shrimp (*Penaeus vannamei*) based on water quality, survival rate and digestive enzyme activities was investigated at different larvae stage and different concentration (Zhou et al., 2009). The results of this study suggested that *B. coagulans* SC8168 supplemented at a certain concentration could significantly increase survival rate and some digestive enzyme activities of *L. vannamei* larvae. Ziaei-Nejad et al. (2006) examined the effect of commercial *Bacillus* probiotic by three experiments on the digestive enzyme activity, survival and growth of *Fenneropenaeus indicus* at various ontogenetic stages. Considerable suggestions were resulted from this study. Avakh (2006) tested *Bacillus subtilis* isolated from digestive tract of *Macrobrachium rosenbergii* on culture with this species. The study showed promising result on growth, survival rate and also inhibitory role against infection pathogen, *Aeromonas hydrophila*.

The objective of this study was to investigate the role of probiotic *B. subtillis* isolated (Avakh, 2006) from digestive tract of *M. rosenbergii* (freshwater prawn) on growth and survival rate of *L. vannamei*, and also the stability of this species in digestive tract and rearing water of the *L. vannamei*.

MATERIALS AND METHODS

Bacterial strain

The putative bacteria flora, *B. subtilis* strain, was isolated from digestive tracts of juvenile *M. rosenbergii* (Avakh, 2006) at Hatchery of Department Aquaculture Technology, Faculty of Agriculture at University Putra Malaysia.

A commercial probiotic (BIO-GOLD, Korea) which was available in Malaysia that was consisted of three species bacteria inside: i.) *L. acidophilus* (10^6 CFU/ml) ii.) *Bacillus subtilis* (10^6 CFU/ml) iii.) *L. plantarum* (10^6 CFU/ml).

Diet preparation

The *B. subtilis* which was isolated from digestive tract of juvenile *M. rosenbergii* (Avakh, 2006) was used for this study. Spraying method was used to prepare the diet according to Gildberg and Mikkelsen (1998). The bacteria of interest were revived by incubating a small amount of lyophilized stock in 250 ml of TSB for 48 h at 32ºC in incubator shaker. The revived bacteria were proliferated in four flasks containing 1000 ml of Tryptone Soy Broth, TSB (Difco, USA) at the same condition. Then the bacteria were centrifuge harvested at 4000 rpm for 15 min at 4ºC. The collected bacteria were resuspended in normal saline solution to 5×10^{13} CFU/ml. For feed preparation of T1, according to Avakh (2006), 1 kg of the commercial pellet feed from Vietnam, containing 38% crude protein, was sprayed by 3 ml of bacteria solution followed Gildberg and Mikkelsen (1998). Then the feed was oven-dried at 35ºC for 1 - 2 h. After spraying bacteria into the feed, samples were taken and cultured in MYP agar (Difco, USA) to determine the *B. subtilis* concentration in the feed. The final concentration of *B. subtilis* in commercial pellet feed was estimated at 2.7×10^{10} CFU/g. Finally, 200 g portions of the feed were packed in sealed plastic bags and stored at 4ºC for 2 weeks or -20ºC until used.

For feed preparation of T2, the prepared *B. subtilis* bacteria solution was diluted from 5×10^{13} to 1.6×10^{7} CFU/ml. Then 3 ml of the diluted solution was mixed to 5 ml of commercial probiotic according to manufacture's instructions and then applied to 1 kg of commercial pellet feed as mentioned. Samples for determination of *B. subtilis* concentration in the feed were taken and cultured in MYP agar. The final concentration of *B. subtilis* in T2 was estimated at 4.5×10^{10} CFU/ml. Because the bacteria inside the commercial probiotics were not well growing; therefore plates were counted after 72 h incubation.

Feed was prepared for T3, according to manufacture's instructtions (BIO-GOLD, Korea). To some extend 5 ml of commercial probiotic containing *L. acidophilus* (10^6 CFU/ml), *B. subtilis* (10^6 CFU/ml), and *L. plantarum* (10^6 CFU/ml) was added to 1 kg of pellet feed by spraying method as mentioned and then applied.

Experimental design and set up

One thousand and two hundred healthy, white shrimp leg, *L. vannamei*, PL 30 were obtain from UPM hatchery and were acclimated in a 100 m^3 fiberglass tank for one month. The larvae were fed with *Artemia* and commercial pellet feed at daily rate of 8% body weight, three times daily. Then eight hundred shrimps were individually weight and placed into the sixteen of 77L aquaria ($0.85 \times 0.38 \times 0.24$ m^3). Juvenile shrimps were acclimatized in 16 aquaria for one week, and during this time, were fed with unaltered commercial pellet feed, from Vietnam three times daily.

An experiment was conducted in completely randomized design (CRD), four treated groups were consisted of: T1, commercial pellet feed was mixed with *B. subtilis* (10^{10} CFU/g), T2, commercial shrimp feed diet, mixed with *B. subtilis* (10^4 CFU/g) and a commercial probiotic (*L. acidophilus* at 10^6 CFU/ml, *B. subtilis* at 10^6 CFU/ml, and *L. plantarum* at 10^6 CFU/ml), T3, commercial shrimp feed diet, mixed with commercial probiotic (positive control), T4, commercial shrimp feed unaltered diet, without probiotic supplement (negative control). Each treatment had four replicates each contained of 50 individuals. Shrimp were fed three times daily at 8:00, 12:00 and 16:00 hrs

Every two weeks, 20 shrimps were scoped out randomly for body weight measurement and shrimp survival was measured in each aquarium. The growth parameters were calculated according to Robertson et al. (2000), Felix and Sudharsan (2004) and Venkat et al. (2004):

$$\text{Weight gain (g/shrimp)} = \text{Final weight (g)} - \text{Initial weight (g)}$$

$$\text{Weight gain (\%)} = \frac{\text{Final weight (g)} - \text{initial weight (g)}}{\text{Initial weight (g)}} \times 100$$

$$\text{Food conversion ratio (FCR)} = \frac{\text{Total feed given (g)}}{\text{Wet weight gain (g)}}$$

$$\text{Specific Growth Rate (SGR; \%/day)} = \frac{\text{In final wt} - \text{In initial wt}}{\text{Duration (days)}} \times 100$$

$$\text{Daily weight gain (DWG; g/days)} = \frac{\text{Final weight (g) x initial weight (g)}}{\text{Days (60)}}$$

Yield of shrimps (g) = Mean body weigth (g) x Total viable shrimps at harvest

Table 1. Effect of basal diet supplemented with *B. subtilis* and commercial probiotics on growth performance of *L. vannamei* (Mean ± S.D.).

Diet	T1 B.S	T2 M	T3 +ve con.	T4 -ve con.
Initial mean weight (g)	4.04± 0.37[a]	3.89± 0.44[a]	3.97± 0.48[a]	4.01± 0.42[a]
Final mean weight (g)	9.07± 0.3[a]	8.38± 0.77[a]	7.39± 1.02[a]	8.49± 0.76[a]
Mean weight gain	5.03± 0.12[a]	4.49± 0.69[ab]	3.42± 0.86[b]	4.48± 0.85[ab]
Mean weight gain (%)	124.88± 8.1[a]	115.98± 18.67[ab]	86.01± 21.53[b]	112.78± 27.45[ab]
FCR	2.61± 0.14[a]	3.16± 0.52[a]	3.80± 1.17[a]	3.06± 0.8[a]
DG	0.61± 0.03[a]	0.55± 0.09[a]	0.49± 0.12[a]	0.57± 0.08[a]
SGR (%)	1.35±0.06[a]	1.28±0.12[a]	1.02±0.2[b]	1.24±0.21[ab]
Yield of shrimp	190.00± 13.13[a]	132.69± 17.54[b]	101.27± 20.72[b]	99.44± 20.48[b]
Survival (%)	75.5± 4.62[a]	59.5± 5.97[b]	60± 4.32[b]	45.5± 9.98[c]

Means in the same row with different superscripts are significantly different ($P < 0.05$).
FCR, feed conversion ratio; SGR, specific growth rate; DG, Daily gain.
B.S, *Bacillus subtilis*; M, Mixture of *Bacillus subtilis* and commercial probiotic; +ve con., Positive control; -ve con., Negative control.

Bacteriological study

Water samples and shrimp were collected from each aquarium, once every two weeks for bacteriological study. One gram of shrimp digestive tract of each treatment removed aseptically, pulled and homogenized in normal saline solution before being serial diluted in the same solution at 10 fold. Followed by spread plating on nutrient agar, NA (Difco, USA) and Tryptone Soy Agar, TSA (Difco, USA) for total viable bacteria count, and Mannitol-Egg Yolk-Polymyxin agar, MYP Agar (Difco, USA) for enumeration of *Bacillus* spp. and Thiosulphate citrate bile salt agar, TCBS (Difco, USA) for *Vibrio* count which were supplemented with 2% NaCl plate were incubated for 24 - 48 h at 32ºC before colonies being counted.

Water quality management

Water quality was monitored weekly, temperature and pH was measured using an YSI, pH and temperature meters (YSI, USA), respectively. Dissolved oxygen was estimated by a YSI, DO and temperature meter (YSI, model 57, USA) and ammonium and nitrite was estimated by an ammonia meter, HANNA instrument (HI 93715 m) respectively.

The shrimp aquaria were daily cleaned by siphoning out the material and unconsumed feed. Water exchanging was done 30% daily. All of the aquaria were supported by perfect and strong aeration system.

Statistical analysis

Data on growth parameters, bacteria count from digestive tract and rearing water between replicates and treatments were analyzed by using one-way analysis of variance and significance of differences between treatments were assessed by Duncan multiple range test (Zar, 1984; Sokal and Rohlf, 1995). The level of significance was accepted at $P < 0.05$. All statistical analysis was performed using SPSS, Released 15, software (SPSS Inc., USA).

RESULTS

Water quality parameters

During the experimental period, the temperature was maintained at 26 – 28°C and the range of salinity of the water was 25 – 28‰. There was no obvious effect of probiotics on the water quality in the treatment groups. Total ammonium (0 – 0.1 mgl^{-1}), nitrite (0 – 0.05 mgl^{-1}) and pH (7.0 – 7.6) were stable.

Growth parameters

At the beginning of study there were no significant differences ($P > 0.05$) for mean weight of shrimps calculated between all experimental groups (Table 1). After 60 days of culture, there were significance differences ($P < 0.05$) for survival rate between T1 (75.5 ± 4.62%) and the other treated and control groups (Table 1), and also the mean yield of shrimp for T1 (190 ± 13.13 g) indicated the greatest ($P < 0.05$) shrimp yield, among the treated and control groups (Table 1). After the cultural period of *L. vannamei* with variety of diet, however, the two treated groups (T1 and T2) and the positive control group (T3), showed great survival rate compared to negative control (T4), shrimps treated with feed which was inoculated by *B. subtilis* (T1) showed the greatest performance on survival rate and shrimp yield confirmed by significance differences ($P < 0.05$). Clearly, feed supplemented with *B. subtilis*, isolated from digestive tract of *M. rosenbergii*, appeared to enhance final survival rate (Figure 1b) and yield of product of *L. vannamei* (Figure 1a), while no significance differences ($P > 0.05$) was found between aquaria with or without the addition of bacterial supplement for the other parameters; final mean weight, SGR, FCR, weight gain, and daily gain (Table 1). It seemed that shrimps treated in T1 sacrificed some of the growth performance due to be higher density ($P < 0.05$) compared to other groups or perhaps it was due to decreasing of survival rate of T2, T3, and T4 during the first two weeks of experiment, because according to the general regulation of aquaculture it was expected that the treatments with lower population densities (T2, T3 and T4) ex-

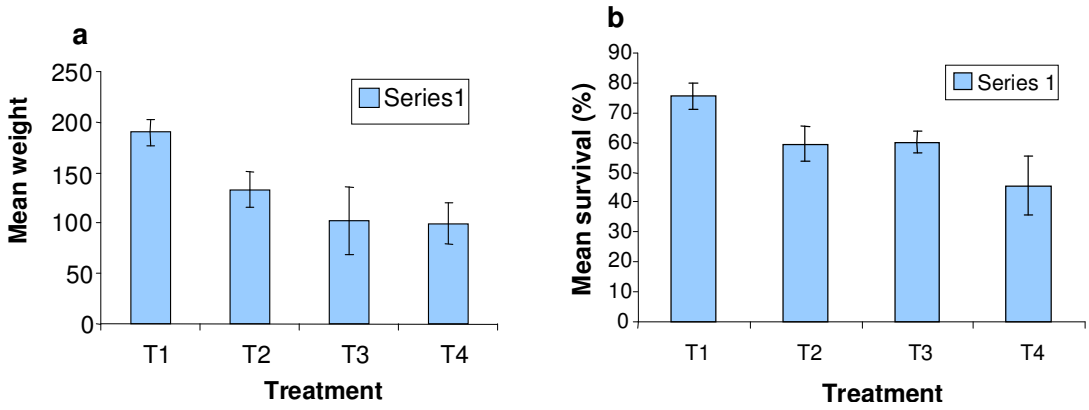

Figure 1. The mean yield of shrimp (a), and mean survival rate (%) (b), of *L. vannamei* by using diets supplemented with *B. subtilis* (T1), commercial probiotic and *B. subtilis* (T2), positive control (T3), and negative control (T4).

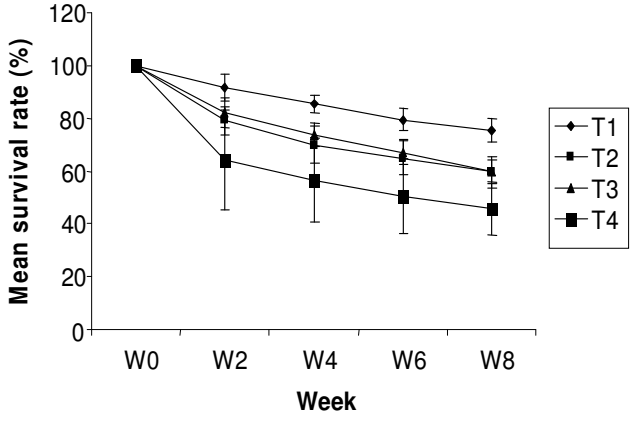

Figure 2. Evaluation of survival rate during cultural period for treatment groups (*B. subtilis*; T1, commercial probiotic and *B. subtilis*; T2), and control groups (positive control; T3, and negative control; T4).

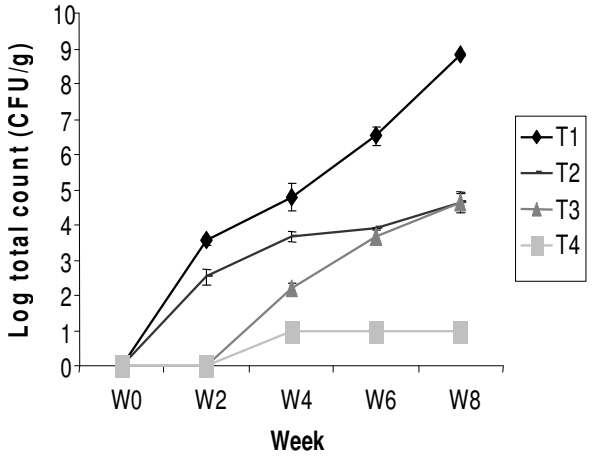

Figure 3. Log total *B. subtilis* count (CFU/g) in shrimp digestive tract during 60 days of culture with or without feed supplemented. *B. subtilis* (T1), commercial probiotic and *B. subtilis* (T2), positive control (T3), and negative control (T4).

hibits higher individual growth compared to those with higher population densities (T1). Therefore, by this actual hypothesis, parameters of final mean weight, SGR, FCR, weight gain, daily gain of T2, T3, and T4 should have shown greater results compared to T1, while it is interesting to note that the obtained results were indicated that there is no significant differences ($P > 0.05$) for these parameters between all the treated and control groups. It is highly recommended that this similarity of growth parameter results which is in contrast with the expectation is due to the T1 efficiency, otherwise, at least the final mean weight of the two treatments (T1 and T4) with 30% difference in density would be alter.

Bacteriological study

Total bacterial count in digestive tract of T1 ($2.9 \pm 0.59 \times 10^{11}$ CFU/g), was significantly higher than the other groups (Figure 8a). This significant difference started from the fourth week of experiment and it was continued until the end of this study. In the beginning of the experiment there were no observed *Bacillus* spp. in digestive tract of shrimps, but they were appeared in all treated shrimps with different concentration and finally, there were significant differences for *B. subtilis* counted between T1 ($6.8 \pm 0.05 \times 10^{8}$ CFU/g) and the other treated groups; T2 ($4.5 \pm 0.23 \times 10^{4}$ CFU/g) and T3 ($4.2 \pm 0.29 \times 10^{4}$ CFU/g), (Figure 3). No significance differences ($P > 0.05$) were observed for *Vibrio* spp. counted between all treated groups and the control groups (Figures 4 and 5).

Total bacterial count in digestive tract of T1 ranged from 5.9×10^{4} to $2.9 \pm 0.59 \times 10^{11}$ CFU/g while this concentration for T2, T3 and T4 ranged from 5.9×10^{4} to 9.7

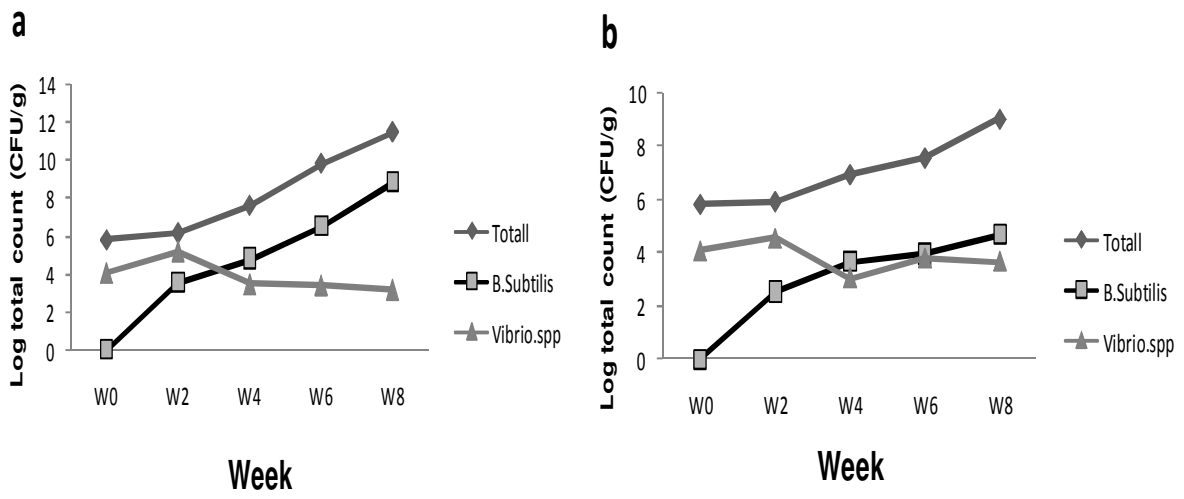

Figure 4. Log total viable bacteria, *B. subtilis*, *Vibrio* spp. CFU/g in shrimp digestive tracts during 60 days of culture with different feed diet supplement (a: T1, b: T2).

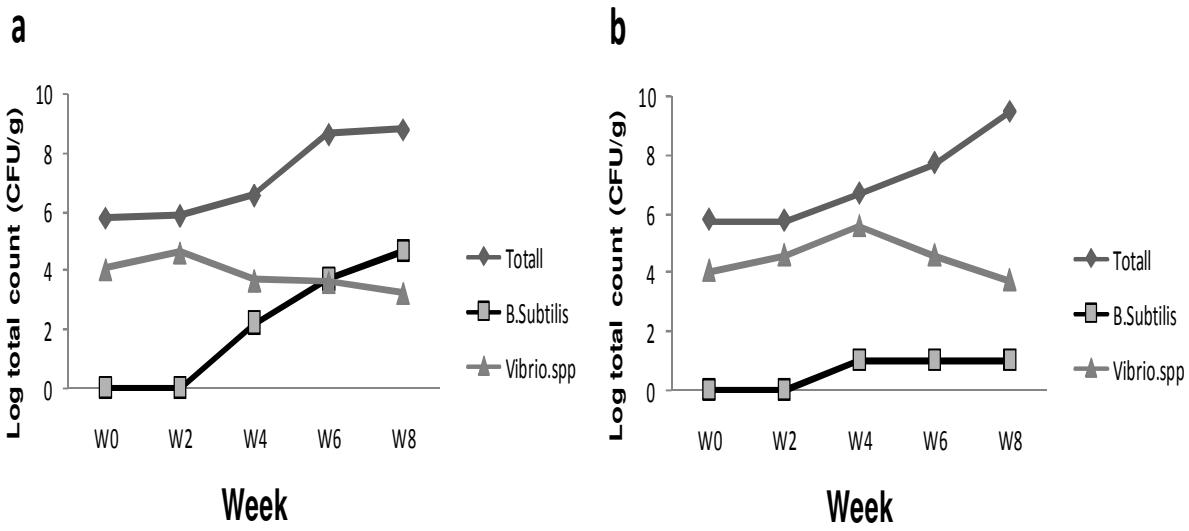

Figure 5. Log total viable bacteria, *B. subtilis*, *Vibrio* spp. CFU/g in shrimp digestive tracts during 60 days of culture with different feed diet supplement (a: T3, b: T4).

$\pm 0.18 \times 10^8$, 5.9×10^4 to $5.9 \pm 0.16 \times 10^8$ and 5.9×10^4 to $2.9 \pm 0.16 \times 10^9$ CFU/g, respectively (Figures 4, 5 and 8a). Mean concentration of *Vibrio* spp. in digestive tract for all the treatments and control groups ranged from 1.1×10^4 to $2.8 \pm 0.28 \times 10^3$ CFU/g (Figures 4 and 5). As indicated in figures, the number of *Vibrio* spp. was decreasing in digestive tract of all the treated and control groups.

Total bacterial counts in rearing waters aquaria were ranged from 2.8×10^4 to $8.4 \pm 0.16 \times 10^9$, 2.8×10^4 to $1.9 \pm 0.31x\ 10^9$, $2.8x\ 10^4$ to $3.9 \pm 0.36 \times 10^8$ and 2.8×10^4 to $4.7 \pm 0.24 \times 10^8$ CFU/ml for T1, T2, T3 and T4, respectively (Figures 6 and 7). There were significance differences for total viable bacterial counted in rearing water of T1 and the other groups from day 45 to 60 (Figure 8b). During the fourth and sixth week of culture, *Vibrio* spp. concentrations in the shrimp rearing water of T3 and T4 were significantly higher than T1 and T2 and in the end of experiment it was significantly higher for T3 ($3.1 \pm 0.25 \times 10^4$ CFU/ml) compared to other groups. The number of *Vibrio* spp. decreased in rearing tank waters of all the treated and control groups (Figures 6 and 7). *B. subtilis* was only found in rearing water of probiotic treatments from day 30 to 60, and ranged from $4.4 \pm 0.22 \times 10^3$ to $6.2 \pm 0.04 \times 10^4$, $1.5 \pm 0.21 \times 10^3$ to $5.6 \pm 0.15 \times 10^3$ and $3.5 \pm 0.29 \times 10^3$ to $3.3 \pm 0.24 \times 10^3$ for T1, T2 and T3, respectively (Figures 6 and 7). Low CFU number of *B. subtilis* in the rearing water tanks of T4 was counted (>30

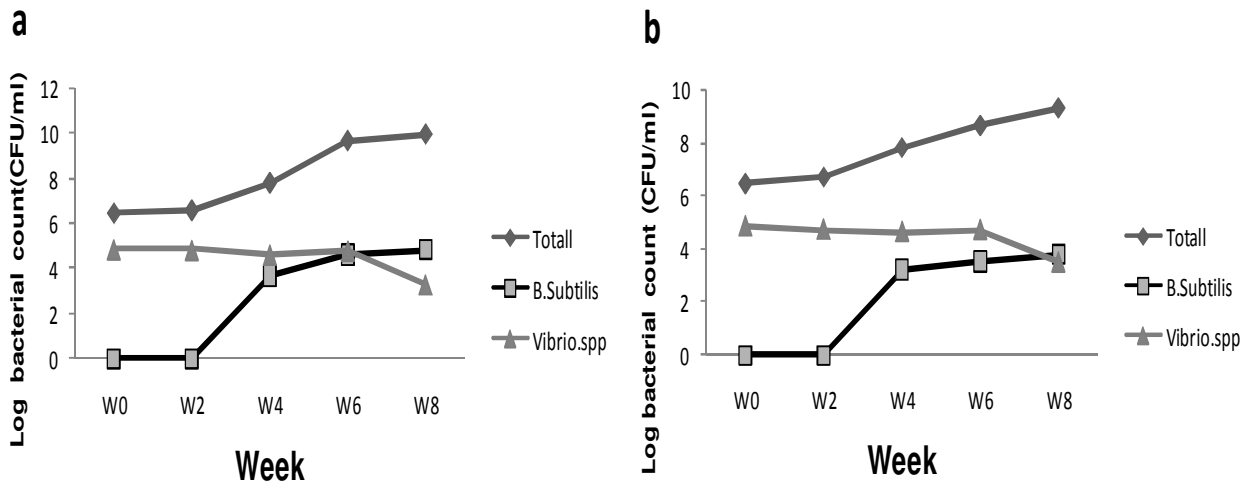

Figure 6. Log total bacteria, *B. subtilis*, *Vibrio* spp. CFU/ml in shrimp rearing water during 60 days of culture with different feed diet supplement (a: T1, b: T2).

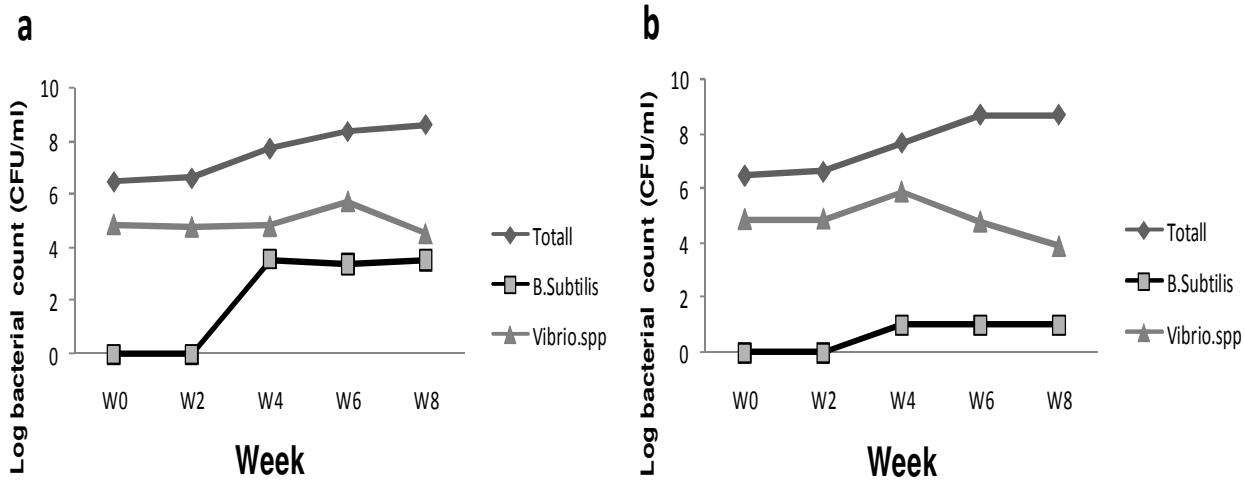

Figure 7. Log total bacteria, *B. subtilis*, *Vibrio* spp. CFU/ml in shrimp rearing water during 60 days of culture with different feed diet supplement (a: T3, b: T4).

CFU/plate) (Figure 7b).

DISCUSSION

It is important to provide healthy shrimp with higher production and probiotics has a great deal of potential (Gomez-Gil et al., 2000). Moriarty (1995, 1998) added *Bacillus* spp. as probiotic in the penaeid shrimp ponds; the result of this study shows increasing survival rate and decreasing of luminous *Vibrio* densities in the pond water. In *P. monodon*, *Bacillus* used as a probiotic was able to colonize both the culture water and the shrimp digestive tract; the *Bacillus* also was able to replace *Vibrio* spp. in the gut of the shrimp, thereby increasing shrimp survival (Rengpipat et al., 1998a). Similar results were obtained in the present study. Significant differences for survival rate ($P < 0.05$) in this study were observed in shrimps treated with *B. subtilis* (T1) compared to the other groups. Also significant differences ($P < 0.05$) for shrimp yield between T1 and the other experimental groups could be a great deal to determine the positive effect of bacteria *B. subtilis* on growth rate of treated shrimp (T1), however, there were no observed significant differences ($P > 0.05$) for growth parameters according to the results (Table 1). Therefore in competition on increasing growth between all the experimental groups, T1 had the greatest growth performance by considering the significant differences of survival rate. According to results of this study it is highly recommended that constantly application of *B. subtilis* to shrimp culture with the optimal dose had influenced them to

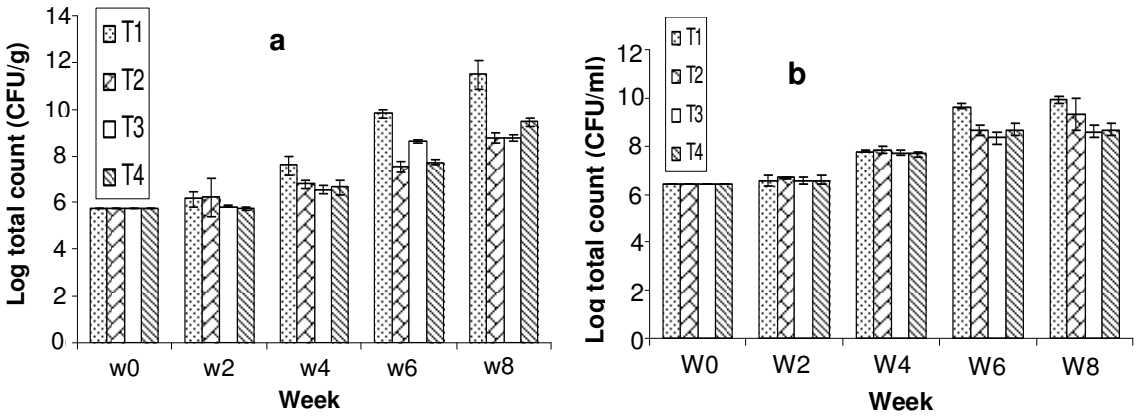

Figure 8. Log total viable bacterial count in (a) shrimp digestive tract (CFU/g), and (b) shrimp rearing water (CFU/ml), during 60 days of culture with or without feed supplemented. *B. subtilis* (T1), commercial probiotic and *B. subtilis* (T2), positive control (T3), and negative control (T4).

achieve the optimal growth rate and the greatest survival rate compared to the other experimental groups, and finally the greatest shrimp yield was obtained, which is the most important concern in the shrimp culture industry.

Effects of commercial probiotic on aquaculture has been investigated by researchers, and some of these research have not shown any positive effects on growth parameters or survival rate or any promising result on the cultural condition. For instance, Shariff et al. (2001) found that treatment of *P. monodon* with a commercial *Bacillus* probiotic did not significantly increase survival. Dennis et al. (2000) used a commercial bacteria supplement to culture on *L. vannamei* in outdoor tanks with no water exchanging. This study did not improve shrimp survival rate, mean final weight and FCR, or the quality of the shrimp tank water. Samocha et al. (1998a,b) tested a commercial probiotic with *P. setiferus*, and no promising data for growth parameters or water quality was obtained. Commercial probiotic as positive control, applied in the present study, did not improved growth performance of the related shrimp (T2 and T3) compared to T4, while it was expected that commercial probiotic used in this study should had shown a great promotion on *L. vannamei* culture due to be high density of bacteria inside [*L. acidophilus* (10^6 CFU/ml) *B. subtilis* (10^6 CFU/ml) *L.plantarum* (10^6 CFU/ml)]. It is difficult to directly assess different studies using probiotics, because the efficacy 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. This indicates that the quantity of probiotics was only one of the factors promoting the growth or survival rate of shrimp larvae (Zhou, 2009).

Bacillus bacteria are able to out-compete other bacteria for nutrients and space and can exclude other bacteria through the production of antibiotics (Moriarty, 1998; Verschuere et al., 2000). Many different antibiotic compounds are produced naturally by a range of *Bacillus* species, and it appears that other bacteria would be unlikely to have resistance genes to all of the antibiotics produced by the *Bacillus* probionts (Moriarty, 1998). *Bacillus* administration also has been shown to increase shrimp survival by enhancing resistance to pathogens by activating both cellular and humoral immune defenses in shrimp (Rengpipat et al., 2000).

Result of total viable bacteria and *B. subtilis* bacteria counted in digestive tract and rearing water showed that there were significance differences ($P < 0.05$) between bacterial counted in T1 and the other groups. It seemed *B. subtilis* was proliferated in digestive tract of shrimp in T1. *B. subtilis* has been shown to produce a wide variety of antibacterial and antifungal compounds in culture media (Alexander, 1977; Katz and Demain, 1977; Korzybski et al., 1978). It produce novel antibiotics such as difficidin and oxydifficidin that have activity against a wide spectrum of aerobic and anaerobic bacteria (Zimmerman et al., 1987) as well as more common antibiotics such as bacitracin, bacillin, and bacillomycin B (Parry et al., 1983). It is expected that gram-negative bacteria were replaced with *Bacillus* probiont (Austin et al., 1995). Rengpipat et al. (1998) showed that *Bacillus* S11 is harmless for shrimp and culture system, while providing greater survival during normal culture period and following disease challenge by luminescent bacterial challenges. This suggests that probiotic treatment is an effective alternative for enhancing shrimp health.

The findings of this study show the presence of viable *B. subtilis* in digestive tract and rearing water of shrimp fed with it (Figures 4, 5, 6, and 7). *B. subtilis* was colonized in rearing water while water quality was not affected by feed additives. However, as indicated in figures, there was a competitive exclusion between *B. subtilis* and *Vibrio* spp. in rearing water and digestive tract during the cultural period (Figures 4, 5, 6, and 7). It is clear that *B. subtilis* almost eliminated *Vibrio* spp. from digestive tract by action of competitive exclusion. Perhaps *B. subtilis*

proliferated in digestive tract had promoted the wellbeing of shrimps or contributed to the direct or indirect protection of shrimp against harmful bacteria and provided the well growth performance and also the great survival rate. However using of probiotics in aquaculture is still on perspective but this study suggests the using of *B. subtilis* as probiotic in culture with *L. vannamei*, while there is still need to consider all parameters which might affect and lead to loss of this bacteria during the cultural period.

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