Effect of probiotics on growth performance and digestive enzyme activity of the shrimp *Penaeus vannamei*

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**Abstract**

The effect of probiotics on growth performance and digestive enzyme activity of the shrimp *Penaeus vannamei* was investigated. Photosynthetic bacteria and *Bacillus* sp. were added to shrimp basal diets as probiotics at three concentrations: T-1, 2 g kg\(^{-1}\) (1 g kg\(^{-1}\) lyophilized photosynthetic bacteria cells (PSB) and 1 g kg\(^{-1}\) lyophilized *Bacillus* sp. (BS)); T-2, 10 g kg\(^{-1}\) (5 g kg\(^{-1}\) PSB and 5 g kg\(^{-1}\) BS); and T-3, 20 g kg\(^{-1}\) (10 g kg\(^{-1}\) PSB and 10 g kg\(^{-1}\) BS). Twelve aquaria with three replicates for each treatment group and the Control group were used. After 28 days, shrimp receiving the diets supplemented with probiotics showed significantly better growth performance than those fed the basal diet (Control). The mean digestive enzyme activity of each treatment groups was significantly different (\(P<0.05\)) from that of the Control. The protease activity of T-2 and T-3 was significantly higher compared with T-1 and the Control. However, there was no significant difference between T-2 and T-3. The amylase activity of T-2 was highest and significantly different (\(P<0.05\)) from that of the Control and T-1. Both treatment groups had significantly higher lipase and cellulase activity compared to the Control.

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**Keywords:** Probiotics; *Penaeus vannamei*; Growth performance; Digestive enzyme

1. **Introduction**

The demand for animal protein for human consumption is increasing and is met largely by terrestrial farm animals. Aquaculture is an increasingly important source of animal protein (Lara-Flores et al., 2003); however, the abuse of antimicrobial drugs, pesticides, and disinfectants in aquacultural disease prevention and growth promotion has led to the evolution of resistant strains of bacteria and questions of safety (Esiobu et al., 2002; Boyd and Massaaut, 1999). Thus, the research into the use of probiotics for aquatic animals is increasing with the demand for environment-friendly sustainable aquaculture (Gatesoupe, 1999). Probiotics have been defined as microbial dietary supplements of benefit to the host (Fuller, 1989). The benefits of such supplements include improved feed value, enzymatic contribution to digestion, inhibition of pathogenic microorganisms, antimutagenic and anticarcinogenic activity, growth-promoting factors, and increased immune response (Verschuere et al., 2000). The effect of probiotics for fish and shrimp has been reported (Mohanty et al., 1993, 1996; Sharma and Bhukhar, 2000; Wang et al., 2005; Wang and Xu, 2006).

Several microbial compounds and probiotics, such as \(\beta\)-glucans, lipopolysaccharides, peptidoglycans and...
probiotic bacteria, have been reported as stimulants of cellular functions in shrimp (Vargas-Albores et al., 1998; Gullian et al., 2004). These compounds and probiotics have been investigated to evaluate their usefulness as supplements against Vibrio spp. and white spot syndrome virus (WSSV) and for their immunostimulatory effect (Itami et al., 1998; Gullian et al., 2004). However, there has been little study of the effect of probiotics on growth performance and digestive enzyme activity in the shrimp Penaeus vannamei. Therefore, this study attempted to investigate the effect of probiotics, photosynthetic bacteria and Bacillus sp., on growth performances and digestive enzyme activity of P. vannamei, which was one of the most valuable shrimp species cultured in China.

2. Materials and methods

2.1. Bacteria

The photosynthetic bacteria (Rhodobacter sphaeroides) and Bacillus sp. (B. coagulans) were isolated from a carp (Cyprinus carpio) pond in Hai-Ning, China according to Wang and Xu (2006). They were cultured in the laboratory and checked routinely for purity. Three different concentrations of probiotics were included in three dietary formulations. Fresh cells, including photosynthetic and Bacillus sp. (∼10^8 and 10^9 colony-forming units (cfu) per 1 g of cells wet weight, respectively), were harvested and maintained at −20 °C. Aliquots of cells were freeze-dried (Virtis Advantage EL, lyophilizer, SP Industries Company, USA) and kept in a sterilized container at −20 °C.

2.2. Diets and experimental design

Four trials were carried out with the shrimp P. vannamei: 12 aquaria (300 l of water in each) with three replicates for each treatment and the Control were used. The ingredients and chemical composition of the basal diets used in the experiment were as described by Deshimaru and Kuroki (1974) and Wang et al. (2002); the basal diet formulation is given in Table 1. The ingredients were mixed, extruded and air-dried at room temperature, and kept at −20 °C.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Ingredients</th>
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<tbody>
<tr>
<td>Fish meal (%)</td>
<td>36</td>
</tr>
<tr>
<td>Soybean meal (%)</td>
<td>20</td>
</tr>
<tr>
<td>Peanut meal (%)</td>
<td>9</td>
</tr>
<tr>
<td>Wheat flour (%)</td>
<td>22</td>
</tr>
<tr>
<td>Corn flour (%)</td>
<td>5</td>
</tr>
</tbody>
</table>

^a Mineral premix to supply the following elements (mg kg⁻¹ diet): zinc (as sulfate) 72, iron (as sulfate) 36, manganese (as sulfate) 12, copper (as sulfate) 24, cobalt (as chloride) 0.6, iodine (as iodate) 1.2, chromium (trivalent, as chloride) 0.8, selenium (as selenate) 0.2, and molybdenum (as molybdate) 0.2.

^b Vitamin premix: (mg kg⁻¹) vitamin B₁₂, 0.1; nicotinic acid, 80.0; riboflavine, 50; pantothenic acid, 180; menadione, 40; folic acid, 6.0; biotin, 0.6; thiamine hydrochloride, 15; pyridoxine, 60; thiamin, 40; inositol, 400; astaxanthin, 60; choline chloride, 20.0; vitamin C, 250, and (IU) vitamin A, 6000; vitamin D₃, 2000; vitamin E, 6000 IU.

Treatment 1 (T-1), the experimental diet in each of three aquaria consisted of 2 g kg⁻¹ (wet weight) bacteria (1 g kg⁻¹ lyophilized photosynthetic bacteria cells (PSB) and 1 g kg⁻¹ lyophilized Bacillus sp. (BS)) added to the basal diet as probiotics. Treatment 2 (T-2) the experimental diet in each of three aquaria consisted of 10 g kg⁻¹ (wet weight) bacteria (5 g kg⁻¹ PSB and 5 g kg⁻¹ BS) added to the basal diet as probiotics. Treatment 3 (T-3), the experimental diet in each of three aquaria consisted of 20 g kg⁻¹ (wet weight) bacteria (10 g kg⁻¹ PSB and 10 g kg⁻¹ BS) added to the basal diet as probiotics.

Photosynthetic bacteria on malate basal medium and Bacillus sp. on normal nutrient agar were counted by spore staining with the spread plate technique (Van Niel, 1971; Austin, 1988) before mixing; the viable count was 4.5 × 10^8 and 5.6 × 10^9 cfu/1 g of cells (wet weight), respectively. The basal diet and probiotics were mixed immediately before feeding and the probiotics were mixed into the feeds by surface coating. The other three aquaria served as the Control and contained only the basal diet.

Healthy shrimp (P. vannamei) provided by the Shrimp Hatchery of Xiaoshan, China were acclimated in two concrete tanks (each measuring 400 cm × 150 cm × 100 cm), fed the basal diet three times daily for 2 weeks, then distributed into 12 aquaria at an initial density of 80 shrimps per aquarium. All shrimps had similar initial weights (0.66 ± 0.04) g. The experimental protocol was a completely randomized design with four treatments (T₁–T₃ and Control). Each treatment had three replicates, and culture was for 28 days.

All shrimps were fed daily at 6:00 h, 14:00 h and 20:00 h. Each day, any remaining diets were collected by siphoning before feeding. Every third day, each aquarium was partially cleaned and the water was partially changed (about 50%). The aquaria were supplied with flow-through water that had been filtered through a special cotton filter.
(flow-rate 1 l min\(^{-1}\)), then passed successively through a tungsten heater and a degassing column packed with plastic rings (Zhenhua Electric Industrtraitment Co., Ltd., China). For water quality control, temperature and dissolved oxygen (DO) were measured daily, and weekly measurements were made of total ammonium, nitrite and pH levels using the Hach kit model DREL 2400 (Hach Company, Colorado, USA). The level of dissolved oxygen was maintained above 6 mg l\(^{-1}\) by an air pump (ADP-2200, Jinlai Pump Factory, China).

2.3. Sampling and analytical methods

The weights of all shrimps were determined at the start (Initial Weight) and at the end (Final Weight) of the 28 day experiment. The Daily Weight Gain (DWG; g d\(^{-1}\)) was calculated as:

\[
\text{Final Weight (g)} \times \frac{\text{Initial weight (g)}}{28d}
\]

The Relative Gain Rate (RGR; \%) was calculated as:

\[
\frac{\text{Final weight (g)} \times \text{Initial weight (g)}}{\text{Initial weight (g)}} \times 100\%.
\]

In order to study the effect of probiotics on digestive enzyme activity, six shrimps were collected from each aquarium at the end of the 28 day study and crude enzymatic extracts was prepared as described by Ding et al. (2004). A crude mixture of intestine was obtained by dissection at 4 °C, and rinsed with cold distilled water. The total intestine content was homogenized at 4 °C in phosphate-buffered saline (PBS pH 7.5) (1 g/10 ml) in a hand-held glass homogenizer. The homogenate was centrifuged at 5000 g for 20 min at 4 °C. The supernatant was recovered and kept at 4 °C; all enzymatic assays were conducted within 24 h after extraction.

Protein activity was evaluated according to Lowry et al. (1951) using a Folin-phenol reagent and protease activity was determined according to Jin (1995). Amylase activity was measured according to Jiang (1982) and Worthington (1993) using iodine to reveal non-hydrolyzed starch. Lipase activity was determined by measurement of fatty acids released by enzymatic hydrolysis of triglycerides in a stabilized emulsion of olive oil (Borlongan, 1990; Jin, 1995). Cellulase activity was measured according to Jin (1995). Protease, amylase, lipase and cellulase activities are expressed as specific activity (U g\(^{-1}\) intestine content).

Statistical analysis using one-way analysis of variance (ANOVA; Statistical Analysis System, SAS, version 6.03) was used to find any significant difference between various parameters for the treatment and Control groups. A significance level of \(P<0.05\) was used.

3. Results

3.1. Growth performances

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 quality of water in the treatment groups. Total ammonium (0–0.1 mg l\(^{-1}\)), nitrite (0–0.05 mg l\(^{-1}\)) and pH (7.0–7.6) were stable.

![Fig. 1. Specific activity of intestinal protease from shrimp fed a basal diet (Control) and three diets containing different concentrations of probiotics (T1–T3) at the end of 28 days culture. Means with different superscripts are significantly different (\(P<0.05\)).](image-url)
and within acceptable ranges (Boyd and Tucker, 1998). During the first week, however, the concentration of nitrite increased to 0.05 mg l$^{-1}$ but later decreased to near zero.

No significant difference was observed for Initial Weight between the treatment groups (T1–T3) and the Control. After 28 days, there was no significant difference between the mean weights of groups T1–T3 (average overall 1.71 (±0.06) g), although the mean weight in each group increased with increasing concentration of probiotics. The mean weight of each treatment group was significantly higher than that of the Control (1.57 (±0.05) g) (Table 2).

The values of Daily Gain (DWG) and Relative Gain Rate (RGR) in all groups treated with probiotics at all concentrations were significantly higher than those of the Control. However, mean values of DWG and RGR were not significantly different among treatment groups. These results show that probiotics composed of equal

weight of photosynthetic bacteria and Bacillus sp. increased growth performance in shrimps.

3.2. Enzyme activity

After culture for 28 days, the mean digestive enzyme activities of all treatment groups were significantly different from that of the Control (Figs. 1–4).

The protease activity was significantly higher in T-3 (979.83 (±42.46) U g$^{-1}$) and T-2 (938.50 (±52.72) U g$^{-1}$) compared with T-1 (851.67 (±39.67) U g$^{-1}$) and the Control (515.50 (±31.56) U g$^{-1}$) but there was no significant difference between T-3 and T-2 (Fig. 1). Amylase activity was significantly higher for T-2 compared to T-1 and the Control (Fig. 2); however, there was no significant difference between T-2 and T-3 (Fig. 2). The average values of lipase and cellulase activity of shrimp intestines for all treatment groups differed with different concentrations of probiotics, and were significantly different from those of the Control (Figs. 3 and 4).

4. Discussion

Since the first use of probiotics in aquaculture, a growing number of studies have demonstrated their ability to control potential pathogens and to increase the growth rates and welfare of farmed aquatic animals (Gatesoupe, 1991; Lara-Flores et al., 2003; Carnevali et al., 2004; Macey and Coyne, 2005; Wang et al., 2005; Wang and Xu, 2006). Here, we report, for the first time, an enhancement of the growth rate of the shrimp P. vannamei, one of the most important farmed species for the Chinese, and even the world, shrimp market, as a result of supplementing their basal feed with probiotics consisting of equal amounts of photosynthetic bacteria and Bacillus sp.
All the probiotic-supplemented diets resulted in an increase of Final Weight, DWG and RGR, showing that the addition of probiotics increased the growth performance of shrimps. Similar results were reported by Ghosh et al. (2003) and Swain et al. (1996) for Indian carp (Labeo rohita). Noh et al. (1994) and Bogut et al. (1998) showed that a commercial probiotic preparation of Streptococcus faecium improved the growth and feed efficiency of Israeli carp (C. carpio). These effects have been demonstrated also in the Indian white shrimp Fenneropenaeus indicus (Ziaei-Nejad et al., 2006).

It is important to consider the possibility of using different species, as suggested by Noh et al. (1994) and Bogut et al. (1998). According to our earlier study (Wang and Xu, 2006), mixed probiotics (photosynthetic bacteria and Bacillus sp. isolated from carp ponds) induced the best growth performance compared with individual probiotics, and showed that each could increase the effect of the other. Here, we studied the effects of a combination of photosynthetic bacteria and Bacillus sp. at different concentrations on growth performance in shrimp. There was no significant difference among the treatment groups with different concentrations of probiotics (Table 2). This indicated that the quantity of probiotics is only one of the factors promoting the growth performance of shrimps.

The enhanced growth performance of shrimp might be due to increasing digestive enzyme activity induced by the probiotics. The shrimp digestive system is activated particularly in the larval and early post-larval stages, where the probiotics would have the greatest effect (Lovett and Felder, 1990; Kamarudin et al., 1994). Furthermore, bacteria, particularly members of the genus Bacillus secrete a wide range of exoenzymes (Moriarty, 1996, 1998). We could not distinguish between activity due to enzymes synthesized by the shrimp and that due to enzymes synthesized by the probiotics. However, the exogenous enzymes produced by the probiotics would represent, at most, only a small contribution to the total enzyme activity of the gut (Ding et al., 2004; Ziaei-Nejad et al., 2006), and the presence of the probiotics might stimulate the production of endogenous enzymes by the shrimp.

The higher level of enzyme activity obtained with diets containing probiotics improved the digestion of protein, starch, fat and cellulose, which might in turn explain the better growth observed with the probiotic-supplemented diets. Similar effects have been reported for fish and shrimp, in which digestion was shown to increase considerably in response to probiotics in the diet (Lara-Flores et al., 2003; Tovar-Ramirez et al., 2004; Ziaei-Nejad et al., 2006).

In the present study, different concentrations of probiotics had different effects on enzyme activity, especially those of protease and amylase (Figs. 1–4). However, the activity of the digestive enzymes did not increase with increased concentration of probiotics. The enzyme activity of the T-3 group, which was given the highest concentration of probiotics used in this study (20 g kg⁻¹), was not significantly greater than that of the other treatment groups (Figs. 1–4), suggesting that the increased activity of the digestive enzymes in the shrimp intestine induced by extrinsic probiotic strains has an inherent limit. Based on these results, use of a 10 g kg⁻¹ (wet weight) supplement of probiotics (5 g kg⁻¹ PSB and 5 g kg⁻¹ BS) in shrimp P. vannamei diet was recommended to stimulate productive performance.

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References


