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Effect of probiotics on alkaline phosphatase activity and nutrient level in sediment of shrimp, *Penaeus vannamei*, ponds

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A B S T R A C T

The effect of probiotics on alkaline phosphatase activity (APA) and nutrient concentrations (total phosphorus, (TP); total inorganic phosphorus, (TIP); total organic phosphorus, (TOP); total organic carbon (TOC) and total nitrogen (TN)) in sediment of shrimp, *Penaeus vannamei*, cultural pond was investigated. Three ponds were treated with commercial probiotics and three were used as the control (without any probiotics). TP was significantly lower ($P<0.05$) in the treatment group compared with the control group at 20, 40 and 60 days post treatment. However, the difference of TP content was reduced to less significant after 80 days. The TIP concentrations of the treatment in sediment was lower ($P<0.05$) than that of the control on day 20, 40 and 80. No significant difference ($P>0.05$) was found in TOP content. The amount of total N and TOC contents at day 0 of the experiment were not significantly between treatment and control ponds ($P>0.05$). However, the probiotic supplementation remarkably decreased TN and TOC ($P<0.05$) in the treatment group after day 20. APA was no significant difference ($P>0.05$) between treatment and the control groups. The seasonal APA followed a similar trend for all the ponds, low at the beginning, peaked on day 20, and then showed a second peak on day 100. The data showed that the application of probiotics would mitigate the nitrogen and phosphate pollution in ponds sediments.

1. Introduction

The increasing use of probiotics in shrimp ponds was reported with the demand for environment-friendly aquaculture (Wang et al., 2005; Vine et al., 2006; Wang, 2007; Balcázar et al., 2007; Hai et al., 2007; Kesarcodi-Watson et al., 2008). The potential benefits of probiotics in aquaculture ponds include: enhanced decomposition of organic matter; reduction in nitrogen and phosphorus concentrations; control of ammonia, nitrite, and hydrogen sulfide; lower incidence of diseases and greater survival; and increasing shrimp and fish production (Boyd and Massaaut, 1999).

Extracellular enzymes are important in the environment for degradation of macromolecular compounds and for providing food substrates for algae and bacteria (Nausch, 2000). In general, they are substrate inducible and product repressible catalysts (Martinez et al., 1996). It was reported that extracellular enzymes are directly related to available organic matter (Karner et al., 1995; Martinez et al., 1996). Alkaline phosphatase (AP; EC 3.1.3.1) is one of extracellular enzymes. It hydrolyses a wide range of organic *P* compounds due to its low specificity for organic moiety compared to more specific phosphatases such as 5′-nucleotidases (Ammerman and Azam, 1985). In addition, alkaline phosphatase activity (APA) is sensitive to phosphate availability and particularly to the intracellular phosphate pool. As a result, it has often been used as an indicator of the phosphorus nutritional status (Labry et al., 2005), particularly in lake waters where phosphorus was generally the limiting factor (Berman, 1970; Pettersson and Jansson, 1978; Zhou et al., 2000; Zhang et al., 2007) and in marine waters (Li et al., 1998; Nausch, 1998; Hoppe and Ullrich, 1999; Hoppe, 2003; Sebastian and Niell, 2004).

The purpose of this study was to investigate the effect of probiotics on alkaline phosphatase activity and concentrations of *P* fractions, total organic carbon (TOC) and total nitrogen (TN) in shrimp, *Penaeus vannamei*, pond sediment. At the same time, the dynamic change of these properties after treatment was also determined in the present research.

2. Materials and methods

2.1. Experimental design

The study was conducted from May 2, 2007 to August 29, 2007 at Ningbo shrimp ponds, located in the west coast of the East China Sea. Six shrimp ponds were selected with three treatments and three controls. The commercial probiotics (Huzhou Rongqia Biotechnology Co., China) were added into the treatment ponds and not into the

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Table 1
Concentrations of total phosphorus (TP) in shrimp pond sediment with and without probiotics

Days of culture (d)	Control (mmol g^{-1})	Treatment (mmol g^{-1})
0	0.036±0.001	0.038±0.002
20	0.032±0.003*	0.026±0.002
40	0.021±0.001*	0.018±0.001
60	0.027±0.002*	0.023±0.002
80	0.034±0.002	0.030±0.003
100	0.035±0.002	0.033±0.002
120	0.045±0.003	0.042±0.003

Results were presented as means±S.E. of triplicate observations. Means in the same row with asterisk were significantly different ($P<0.05$).

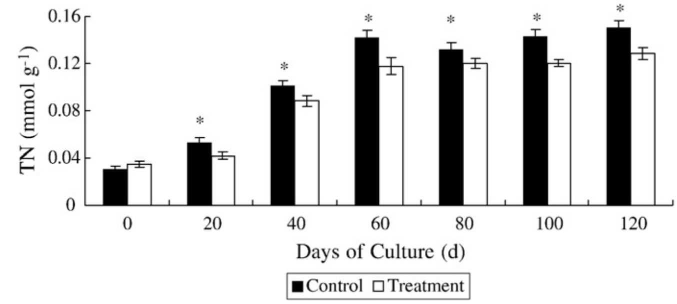

Fig. 1. Total nitrogen (TN) concentration in shrimp pond sediment with and without probiotics at end of 120 days culture. Means with asterisk are significantly different ($P<0.05$).

control ponds. The maximum depth from 120 to 130 cm with similar morphometric and size features (0.33–0.36 ha). The ponds had been used for six culture cycles and therefore, were considered aged ponds. The management and husbandry process was similar to the commercial producer. The pond bottom was disinfected using calcium oxide prior to stocking. All of the ponds were filled with sand-filtered seawater with approximately 35‰ salinity after 15 days solarization.

Each pond was stocked at a density of 600,000/ha healthy shrimp juveniles, *Penaeus vannamei*, from the hatchery. Shrimps were fed with commercial pellets (made in Huangguan Company, China) twice a day for the first month at a rate of 6–10% of the shrimp body weight and three times a day until harvest at 4–5% the body weight. The pair of paddlewheel aerators was used 6–12 h daily. Water was added to compensate for evaporative water losses.

2.2. Probiotics and application

The commercial probiotics in the form of solid packed in airtight bottles (Huzhou Rongqia Biotechnology Co., Zhejiang province, China) was obtained from a local distributor. The product had bacterial cell densities of 10^{10} cfu (colony-forming units) g^{-1} and contained *Bacillus sp.*, *Nitrosomonas sp.*, *Nitribacter sp.* and *Lactobacillus*. The rate and frequency of application of the probiotics in shrimp treatment ponds was carried out according to the manufacture's instruction. The probiotics was diluted in treatment pond water (w/v=1 g/100 ml) and left for 2 h under aeration. Initial application was carried out at 10.0 mg dm^{-3}/pond on the day before stocking the juveniles of the shrimp. A subsequent weekly reapplication was 5.0 mg dm^{-3} for 12 weeks. After that, the application dose was increased to 10.0 mg dm^{-3} until the end of culture cycle.

2.3. Sampling

Five replicate sediment samples were obtained from each pond randomly using Ekman grab at 20 days interval from May 2 to August 29 and transported in polythene bags to a laboratory for chemical analyses. The sediment samples were homogenized in a grinder after removal of any visible plant material, oven dried (80 °C, 48 h), and sieved to <2 mm for the analyses of *P* fractions, TOC and TN in our laboratory according to the standard method of China. A portion of the collected samples were also transferred on ice hermetically to a laboratory describe and stored in the dark at −70 °C freezer (Forma 702, Thermo, USA) until enzyme analysis. Water temperature and salinity in each pond were measured in field using the Hach kit (Model DREL 2400, Hach Company, Colorado, USA).

2.4. Chemical analysis and AP assay

Concentration of TP in sediment samples was determined according to Menzel and Corwin (1965) based on the liberation of organically bound fractions by persulfate oxidation. Total inorganic phosphorus (TIP) content was determined following the method of Chang and Jackson (1957) with ammonium fluoride as a selective extractant. The concentration of total organic phosphorus (TOP) was calculated by subtracting TIP from TP. Total nitrogen was determined using a Carlo-Erba NA 1500 CNS Analyzer (Haak-Buchler Instruments, Saddlebrook NJ). The content of TOC in sediment sample was measured using a TOC analyzer (TOC-5000, Shimadzu, Japan).

The activity of AP was assayed spectrophotometrically as the release of *p*-nitrophenol from the model substrate *p*-nitrophenyl phosphate (*p*NPP) according to Hadas and Pinkas (1997). The reaction mixture contained 1.0 g sediment, 2.6 ml 0.05 mol L^{-1} Tris buffer (pH 8.4) 0.03 ml 0.1 mol L^{-1} $MgCl_2$ and 0.1 ml 10.0 mmol L^{-1} *p*NPP. Samples were incubated at 37 °C for 1 h and the reaction was terminated by addition of 0.3 ml NaOH (1.0 mol L^{-1}). The spectrophotometric reading was taken at 410 nm (SP-2100PC, Spectrum Co., Shanghai, China) and the results of specific APA were expressed as mg *p*-nitrophenol (kg dry wt)$^{-1}$ h^{-1}. For all samples, triplicates were analyzed and the data were reported as the average in this study.

Table 2
Concentrations of total inorganic phosphorus (TIP) and total organic phosphorus (TOP) in shrimp (*Penaeus vannamei*) pond sediment with and without probiotics

Days of culture	Control (mmol g^{-1})		Treatment (mmol g^{-1})	
(d)	TIP	TOP	TIP	TOP
0	0.027±0.001	0.009±0.001	0.028±0.002	0.010±0.001
20	0.024±0.002*	0.008±0.001	0.019±0.002	0.007±0.000
40	0.019±0.001*	0.002±0.001	0.015±0.002	0.003±0.001
60	0.023±0.003	0.004±0.001	0.019±0.002	0.004±0.001
80	0.027±0.002*	0.007±0.001	0.022±0.002	0.008±0.001
100	0.026±0.002	0.009±0.001	0.023±0.002	0.010±0.001
120	0.037±0.002	0.008±0.001	0.036±0.002	0.006±0.003

Results were presented as means±S.E. of triplicate observations. Means of each indicator in the same row with asterisk were significantly different ($P<0.05$).

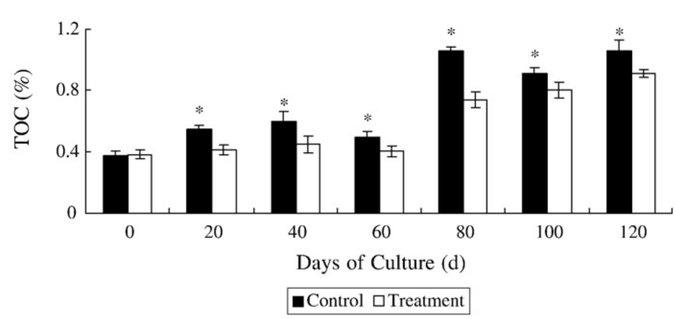

Fig. 2. Total organic carbon (TOC) concentration in shrimp pond sediment with and without probiotic at end of 120 days culture. Means with asterisk are significantly different ($P<0.05$).

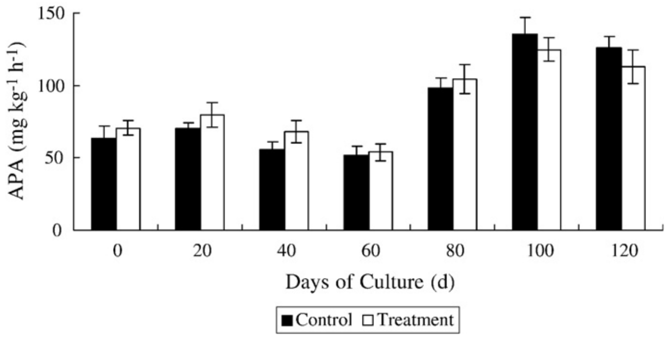

Fig. 3. Alkaline phosphatase activity (APA) in shrimp pond sediment with and without probiotic at end of 120 days culture. Means with asterisk are significantly different ($P<0.05$).

Analysis of variance (ANOVA) was used to determine the significant ($P<0.05$) difference between the tested groups. All statistics were performed using SPSS for Windows version 11.5 (SPSS, Chicago, USA).

3. Results

Water temperatures in shrimp ponds were ranged from 23.2 °C and 28.4 °C and no difference between the treated and the control ponds. The salinity ranged from 10 to 35 parts per thousand (ppt), and there were no significant differences between treated and the control ponds.

The amount of TP in treated ponds ranged from 0.021 ± 0.001 mmol g^{-1} to 0.045 ± 0.003 mmol g^{-1}, while that of the control ponds ranged from 0.018 ± 0.001 mmol g^{-1} to 0.042 ± 0.003 mmol g^{-1} (Table 1). Significant differences of TP ($P<0.05$) were observed on the 20th day, 40th day and 60th day between the treatment groups (0.032 ± 0.003 mmol g^{-1}, 0.021 ± 0.001 mmol g^{-1} and 0.027 ± 0.002 mmol g^{-1}, respectively) and the control groups (0.026 ± 0.002 mmol g^{-1}, 0.018 ± 0.001 mmol g^{-1} and 0.023 ± 0.002 mmol g^{-1}, respectively). There were no significant differences of TP ($P>0.05$) in other sampling date (day 0, 80, 100 and 120).

The concentration of TIP on the 20th day, 40th day and 80th day in ponds sediment treated with probiotics was significantly ($P<0.05$) lower than that of the controls (Table 2). However, TOP was not significantly difference ($P>0.05$) in the sediment of treated and control ponds during the entire 120 days culture.

The concentrations of TN and TOC in probiotic treated ponds were significantly decreased ($P<0.05$) after 20 days of the experiment (Figs. 1 and 2). TN in treated ponds ranged from 0.034 ± 0.003 mmol g^{-1} to 0.128 ± 0.005 mmol g^{-1}, while it control ponds ranged from 0.031 ± 0.002 mmol g^{-1} to 0.150 ± 0.006 mmol g^{-1}. The maximum TOC content in sediments was 1.06% at 80 days in control ponds and the minimum was 0.91% at 120 days in treated.

The AP activity was similar in both treatment and control groups during the entire study period. Even though the treatment group showed a slightly increasing the activity before the 40 days, no statistical difference ($P>0.05$) was found. In addition, the AP activities in sediment showed a similar trend in both treated and control ponds, e.g., low in the beginning, peaking on day 20 (79.48 ± 8.30 mg kg^{-1} h^{-1} and 70.15 ± 4.35 mg kg^{-1} h^{-1}, respectively), with a second peak on day 100 (124.81 ± 8.38 mg kg^{-1} h^{-1} and 135.44 ± 11.70 mg kg^{-1} h^{-1}, respectively) (Fig. 3).

4. Discussion

Ponds sediment plays an important role in nutrients cycling by retaining or releasing nutrients. Moriarty (1996) strongly advocated the use of probiotics amendments in aquacultural pond. Suhendra et al. (1997) found that routine use of commercial probiotics in a shrimp farm in West Java resulted in reduced organic matter accumulation, improved water quality and enhanced environmental conditions. Our data showed decreased concentrations of TP, TIP, TN and TOC in sediment after the ponds treated with commercial probiotics. We concluded that the probiotics played an important role of nutrient cycling and improved the shrimp pond environment.

Reducing sediment nutrient level in our shrimp ponds was in agreement with a previous study that nitrogen level in water was significantly ($P<0.05$) decreased after the probiotic additions (Wang et al., 2005). A lower amount of TP was observed in the early phase of the culture period (20–60 d). A similar finding was reported by Matias et al. (2002), who reported the improved initial water quality by addition of commercial microbial products in tropical shrimp (*Penaeus monodon*) cultural ponds. Our data also showed that the addition of the probiotics to shrimp ponds did not result in significant improvement of the amount of TP between 80–120 days.

During intensive shrimp culture process, it was common to accumulate high density of organic material in the pond bottom originated from unused feed, feces and plankton die-offs (Avnimelech et al., 1995). As a consequence, nutrient (N, P and C) level in pond sediment are usually higher in the final phase compared with the starting phase through the accumulation. Our findings were similar to that of Green and Boyd (1995), who reported that significantly greater N, P and organic matter concentrations were in pre-drain samples, indicating pond sediment was a major nutrient sink.

Zhou et al. (2001) reported that the fish feces in different sites of sediment associated with caged culture of *Oreochromis niloticus* exhibited a remarkable APA as compared with the control in a shallow Chinese freshwater lake (Lake Donghu). In contrast, no significant APA differences were detected between the treatment and control ponds in our study. This discrepancy may be resulted from the difference amount of the supplemented materials. In addition, the APA was increased following time, and relative higher activities were found in the final phase (80–120 days), which may due to the nutrient accumulation in the pond sediment.

In summary, the probiotics application significantly decreased the amount of TN and TOC in pond sediment. Total P and TIP in sediment were also reduced at certain periods of the culture. However, no significant difference was detected in sediment APA and TOP between treatment and the control. Although more research for the application technologies and the optimization of the commercial products are still needed, proper application of probiotics will improve sediment environment for shrimp culture and yield in ponds.

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