Aquaculture 287 (2009) 94–97

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

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

Yanbo Wang [a,*], Zhenli He [b]

[a] Key Laboratory of Food Safety of Zhejiang Province, Food Science and Biotechnology College, Zhejiang Gongshang University, 149, Jiaogong Road, Hangzhou (310035), China
[b] University of Florida, Institute of Food and Agricultural Sciences, Indian River Research and Education Center, 2199 S., Rock Road, Fort Pierce, FL34945, USA

ARTICLE INFO

Article history:
Received 1 August 2008
Received in revised form 4 October 2008
Accepted 6 October 2008

Keywords:
Alkaline phosphatase activity
Nutrient sediment
Shrimp
Pond

ABSTRACT

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

* Corresponding author. Food Quality and Safety Department, Food Science and Biotechnology College, Zhejiang Gongshang University, 149, Jiaogong Road, Hangzhou, 310035, China. Tel.: +86 571 88071024 8595.
E-mail address: wangyb@mail.zjgsu.edu.cn (Y. Wang).

0044-8486/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquaculture.2008.10.022

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 ($P<0.05$).

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).

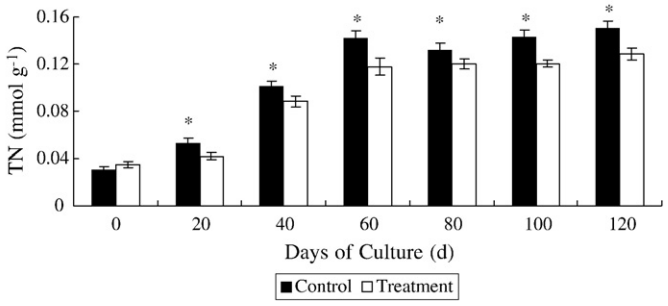

Fig. 1. Total nitrogen (TN) concentration in shrimp pond sediment with and without probiotics at end of 120 days culture. Means with asterisk are significantly different ($P<0.05$).

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 ($P<0.05$).

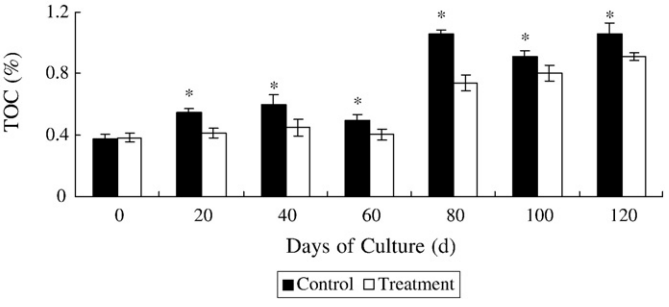

Fig. 2. Total organic carbon (TOC) concentration in shrimp pond sediment with and without probiotic at end of 120 days culture. Means with asterisk are significantly different ($P<0.05$).