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The effects of commercial microbial agents (probiotics) on phytoplankton community structure in intensive white shrimp (*Litopenaeus vannamei*) aquaculture ponds

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Abstract A mesocosm experiment was conducted to study the effects of commercial microbial agents (probiotics) on the phytoplankton community structure in a shrimp (*Litopenaeus vannamei*) farm located in Yinzhou-Ningbo, Zhejiang Province, China. Qualitative and quantitative analyses of the phytoplankton were examined along with physico-chemical parameters in the ponds treated with microbial agents and in the untreated ponds without microbial agents. A total of 18 well-diversified species of phytoplankton belonging to Bacillariophyta, Dinoflagellata, Cyanophyta and Chlorophyta were investigated during the study period. The average phytoplankton abundance in the treated ponds (6.08×10^5 cells L^{-1} in HJW ponds and 7.11×10^5 cells L^{-1} in JK27 ponds) was significantly less than that in the control ponds (1.27×10^6 cells L^{-1}, $P < 0.05$). The dominant group in both the treated ponds was Bacillariophyta (70.84 % in HJW and 64.36 % in JK27), whereas the dominant group in the control ponds was Cyanobacteria (37.05 %). The analysis showed that the addition of probiotics significantly increased ($P < 0.05$) the concentration of *Coscinodiscus* species from Bacillariophyta in the treated ponds (HJW and JK27) and significantly decreased ($P < 0.05$) the concentration of *Oscillatoria* species compared with the control ponds. None of the water quality parameter results differed significantly between the treatments ($P > 0.05$). The findings of the present study suggest that the application of commercial probiotics in shrimp farms could positively influence the growth of beneficial algae, such as Bacillariophyta rather than harmful algae, such as Cyanobacteria, and thus improves the water quality, the health of the shrimp and increases production.

Keywords Probiotics · Shrimp culture · *Litopenaeus vannamei* · Succession of phytoplankton community structure

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Introduction

Shrimp production has developed rapidly over the last three decades in China; however, the enormous expansion has been accompanied by strong controversies on the environmental, economic and social impacts of shrimp culture. The tremendous expansion of shrimp culture has focused attention on the need for effective management strategies to develop sustainable shrimp production (Rocha et al. 2004; Samocha et al. 2004). One of the newest approaches for cost-effective and environment-friendly water quality control in shrimp production is the application of probiotics to the ponds (Hong et al. 2005; Gomez et al. 2009). The application of probiotics involves the manipulation of microorganisms in the ponds to enhance the mineralization of organic matter and remove undesirable waste compounds (Wang et al. 2005; Farzanfar 2006; Zhang et al. 2011). Probiotics diminish the growth of pathogens and increase the growth of beneficial bacteria, leading to improved water quality and healthier fish or shrimp (Ninawe and Selvin 2009; Chen and Hu 2011; Silva et al. 2012).

The biological profile of an aquatic ecosystem depends on the biomass of phytoplankton. Knowledge of the abundance, composition and succession of the phytoplankton is a prerequisite for the successful management of an aquatic ecosystem. Phytoplanktons are primary producers for the entire aquatic body and comprise the major portion in the ecological pyramids (Field et al. 1998; Chisti 2007). Phytoplanktons are excellent indicators of the environmental conditions and aquatic health within ponds, because they are sensitive to changes in water quality. They respond to low dissolved oxygen levels, high nutrient levels, toxic contaminants, poor food quality and predation (Casé et al. 2008).

Various studies on phytoplankton community structure have been reported (e.g., Luan et al. 2006; Casé et al. 2008; Huang et al. 2012 etc.); however, information on the effects of probiotics on the phytoplankton community structure in intensive shrimp ponds (*Litopenaeus vannamei*) is scanty and inadequate. Few studies have been documented so far (Yusoff et al. 2002; Paiva-Maia et al. 2013). This study was undertaken to illustrate the qualitative and quantitative changes of the phytoplankton community structure caused by adding commercial bacterial products (probiotics) to intensive shrimp ponds. The findings will provide knowledge on the potential roles of probiotics on phytoplankton and water management in shrimp aquaculture farm.

Materials and methods

Experimental design

The study was conducted in a shrimp farm at Yinzhou-Ningbo, Zhejiang province, eastern China (29°32′N, 121°31′E). Fifteen concrete ponds with the same management and size (3.5 m^3 each) were used in the experiment. The experiment was designed with two treatments and one control with five replicates each. In treatment 1, the ponds were treated with commercial bacterial agents named Huo-Jun-Wang (denoted HJW hereafter). In treatment 2, the ponds were treated with commercial bacterial agents named Jūn-kè-27 (denoted JK27 hereafter). The control ponds were not treated with any microbial agents. The main bacterial components and concentration administered in each pond are presented in Table 1.

Table 1 Description of microbes' agent products, their main components and concentration amounts applied in each pond

Treatment	Name of product	Main components	Concentration in each pond
HJW	Huo-Jun-Wang agents	*Bacillus subtilis, yeast, Streptococcus faecalis, Pediococcus and Actinomycetes*	4 ppm (14 g)
	Photosynthetic bacteria	CP1 and CP3 at low and high salinity level, respectively	20 ppm (70 ml)
JK27	Jūn-kè-27 agents	Bdellovibrio, psychrotrophic, Bacillus host strain, culture medium etc.	4 ppm (14 ml)
Control	–	–	–

CP1 photosynthetic bacteria suitable for growth at low salinity level, *CP3* photosynthetic bacteria suitable for growth at high salinity level

Experimental process

During the study, the experimental ponds were preliminarily disinfected with commercial disinfectant (chlorine dioxide); 100 ppm of ClO_2 were diluted with water and applied to the ponds. Ponds were filled with seawater up to a depth of 1.2 m. Approximately 400 individuals of *L. vannamei* shrimp of 6.0 cm length were added in each pond. The shrimp were fed with commercial pellets (Sino fish feed) three times per day at the rate of 2–8 % of their body weight. The commercial bacterial products (manufactured by Jiangsu Green Tech Co., Ltd., China) were in the form of tiny granules aseptically packed in airtight bags. The addition of probiotics was as follows: in HJW, each pond was treated with 14 g (4 ppm) of probiotics from Huo-Jun-Wang agents, followed by the addition of photosynthetic bacteria CP1 and CP3, pouring 70 mL (20 ppm) into each pond, respectively. In JK27, 14 mL (4 ppm) of probiotics from Jūn-kè-27 agents were poured into the pond (Table 1). The first application of probiotics was carried out a day prior to sampling; the second application was done 1 week later. Supplementary application of the same concentration of probiotics was performed every four consecutive days until the end of the experiment. Water exchange in the ponds was kept to a minimum (by replacing only the water lost through evaporation and seepage). Aeration was provided to the mesocosm. The experiment started on September 27, 2013, and ended on October 25, 2013.

Phytoplankton sampling

Phytoplankton were sampled with a 5-L bucket and were collected into a 500-mL plastic container by filtering with phytoplankton net of mesh size 25 μm, and then, they were preserved using 4 % formalin for fixing and 1 % Lugol's iodine solution for staining. In the laboratory, 1 mL of the solution was transferred from the concentrated sample for identification and counting using the method described by Utermohl (1958). Phytoplankton species identification and counting were done using the methods of Yamaji (1991) and Tomas (1997).

The phytoplankton numerical diversity (H') was calculated using a version of Shannon–Wiener index (Shannon and Weaver 1963)

$$H' = -\sum_{i=1}^{s} P_i \log_2 P_i$$

where P_i is relative species biomass (n/N), n is the individual amount of the species organism, N is the total individual amount and S is the number of species in the sample.

Evenness (J) was calculated according to Pielou (1966)

$$J = \frac{H'}{\log_2 S}$$

where H' is the Shannon–Wiener index in a sample and S is the number of species in a sample.

Water quality analysis

The temperature, pH and dissolved oxygen were measured daily with a digital oximeter (YSI model 550, Yellow Springs, Ohio, USA) in situ. Water samples were filtered using a 0.45 μm GF/F Whatman glass fiber filter prior to analysis. Other water samples for ammonium, phosphate and nitrate–nitrite were stored frozen at −20 °C in HCl-washed polypropylene cryovial tubes and were measured with a Smart Chem discrete auto analyzer (Smart Chem200, Alliance, France).

In the analysis, the indophenol blue method was used to determine ammonia, nitrate–nitrite was measured with the cadmium–copper reduction method and the phosphate concentration was determined using the ammonium molybdate method (Koroleff 1983).

Dissolved inorganic nutrients (NH_4^+, PO_4^{3-} and $NO_3^+ + NO_2^-$) were calculated from the slope of a linear regression of concentration against time (Michaud et al. 2006). Chlorophyll *a* was determined following the methods of the Turner Designs Trilogy fluorometer for the fluorometric analysis of pigment (Strickland and Parsons 1972).

Statistical data analysis

Phytoplankton abundance, species composition and water quality parameters were analyzed using SPSS Software (SPSS 16.0). Phytoplankton abundance data were first transformed into logarithm x, then were analyzed by one-way ANOVA followed by a Duncan's multiple range test for post hoc comparisons of means. The normal distribution of the data and the homogeneity of variances among treatments were verified before the ANOVA was performed. Pearson's correlation analysis was conducted using SPPS software to establish the relationship among various environmental variables with phytoplankton density ($P < 0.01$ and $P < 0.05$). The figures were drawn with Origin Pro 8.0 software.

Results

Physico-chemical parameters of water

The average recorded pH ranged from 7.12 to 7.93, whereas the average dissolved oxygen (DO) and water temperature varied from 4.02–5.89 mg L^{-1} to 24.5–27.1 °C, respectively, in both treatments. None of the physical variables differed significantly within treatments ($P > 0.05$) during the experiment. The chemical water quality parameters, including ammonia (NH_4^+), phosphate (PO_4^{3-}), total phosphorus (TP), nitrate–nitrite ($NO_3^- + NO_2^-$) and Chl *a* results are presented in Table 2; none of them differed significantly within treatments ($P > 0.05$); however, their concentration increased non-significantly with time. The nitrate–nitrite ($NO_3^- + NO_2^-$) and ammonia (NH_4^+) concentrations were lower for

Table 2 Ammonia (NH_4^+), phosphate (PO_4^{3-}), nitrate–nitrite ($NO_3^- + NO_2^-$) and chlorophyll *a*, at the beginning, mid-phase and at the end of experiment (mean ± SD, $n = 5$) of the shrimp ponds in treated and untreated ponds with probiotics

Treatment	NH_4^+ mg L^{-1}	PO_4^{3-} mg L^{-1}	$NO_3^- + NO_2^-$ mg L^{-1}	Chl *a* μg L^{-1}
Beginning of experiment (day 1–9)				
HJW	0.63 ± 0.16	0.15 ± 0.01	0.027 ± 0.01	27.86 ± 19.24
JK27	0.76 ± 0.15	0.12 ± 0.02	0.085 ± 0.06	29.99 ± 12.50
Control	0.84 ± 0.18	0.16 ± 0.03	0.110 ± 0.05	41.52 ± 10.95
Middle of experiment (day 12–16)				
HJW	3.51 ± 0.95	0.35 ± 0.13	0.098 ± 0.11	178.56 ± 94.40
JK27	3.56 ± 0.15	0.27 ± 0.10	0.13 ± 0.07	134.66 ± 51.07
Control	3.81 ± 0.90	0.18 ± 0.05	0.17 ± 0.07	179.86 ± 51.13
End of experiment (day 20–24)				
HJW	8.33 ± 2.56	1.49 ± 0.11	0.366 ± 0.48	225.43 ± 42.04
JK27	8.70 ± 0.60	1.57 ± 0.27	0.533 ± 0.42	249.43 ± 51.29
Control	9.68 ± 0.87	1.68 ± 0.29	0.640 ± 0.82	198.09 ± 31.99

HJW, a probiotics from Huo-Jun-Wang agents; JK27, a probiotics from Jūn-kè-27 agents; Control: without the use of probiotics

both treatments (HJW and JK27) compared with the control. However, these differences were not statistically significant (one-way ANOVA; Table 2, $P > 0.05$).

Phytoplankton abundance

Major changes of phytoplankton cell abundance over time for the treated ponds, and the control ponds are shown in Fig. 1. ANOVA test results showed that there were no significant differences in the mean phytoplankton abundance within the treated ponds (HJW and JK27) on most sampling days ($P > 0.05$). The control ponds showed significant difference by having greater species abundance than the treated ponds from day 4 to the end of the experiment ($P < 0.05$). The mean phytoplankton abundance in the treated ponds (HJW and JK27) measured from day 1 to day 12 increased slowly over time ($P < 0.05$, Fig. 1) until day 16, when the mean phytoplankton abundance reached its peak values. However, in the control ponds, the mean algae abundance increased throughout the experiment (Fig. 1). Toward the end of the culture cycle, the phytoplankton abundance in the control grew to as much as two times greater than the abundance in the treated ponds (HJW and JK27); for example, on day 24, HJW had a total phytoplankton population of 1.08×10^5 cells L^{-1} opposed to 2.25×10^5 cells L^{-1} in the control group. The total average cell abundance of phytoplankton in the treated ponds was 6.08×10^5 and 7.11×10^5 cells L^{-1} in HJW and JK27, respectively, whereas in the control, it was 1.27×10^6 cells L^{-1}.

Diversity index (H') and evenness (J)

The species diversity index values (H') and evenness (J) of the phytoplankton community structure in the treated ponds (HJW and JK27) were relatively lower than that in the control ponds (Fig. 2). The treated ponds had a peak diversity index of 1.697 and 1.758, respectively on day 12, whereas the lowest value for the diversity index was noted on day 9 for

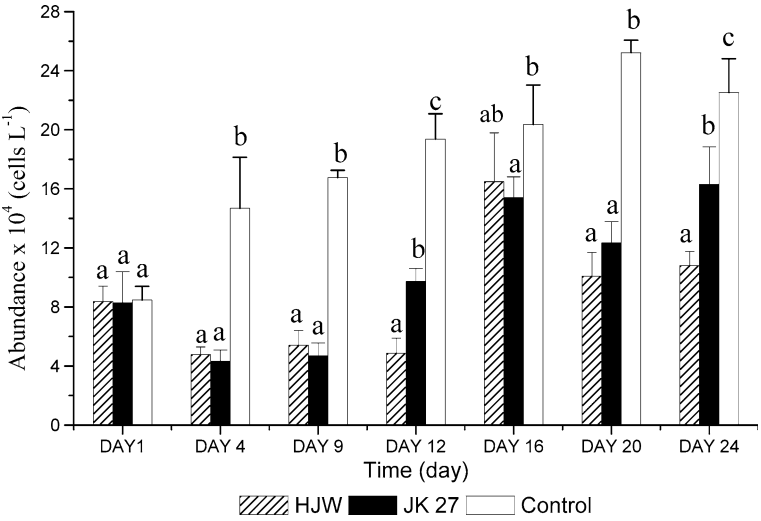

Fig. 1 Average changes of phytoplankton cell abundance over time for ponds treated with: HJW, a probiotics from Huo-Jun-Wang agents; JK27, a probiotics from Jūn-kè-27 agents; Control: without the use of probiotics. The *bars* denote SD ($n = 5$). *Letters* indicate the differences between treatments within the same experimental days. Means not sharing a common letter are significantly different ($P < 0.05$)

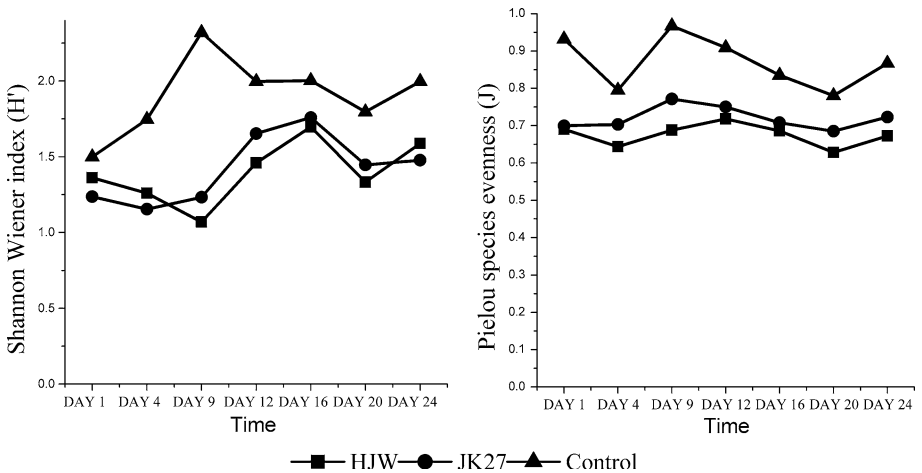

Fig. 2 Shannon–Wiener index (H') and evenness index (J) in ponds treated with: HJW, a probiotics from Huo-Jun-Wang agents; JK27, a probiotics from Jūn-kè-27 agents; Control: without the use of probiotics

HJW and day 4 for JK27. The highest diversity index (H') in the control ponds was 2.319 on day 9 (Fig. 2).

Phytoplankton community structure

Four major groups of phytoplankton, including Bacillariophyta, Dinoflagellata, Cyanophyta and Chlorophyta, were distinguished in the ponds with a total of 18 phytoplankton species.

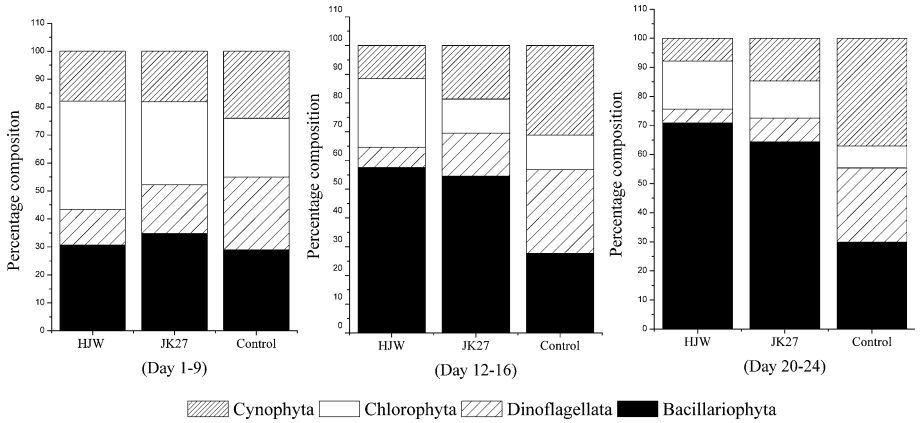

Fig. 3 Percentage composition of the major phytoplankton groups in the ponds treated with: HJW, a probiotics from Huo-Jun-Wang agents; JK27, a probiotics from Jūn-kè-27 agents; Control: without the use of probiotics

Nine species belonged to Bacillariophyta (diatoms), four species belonged to Dinoflagellata, three species belonged to Cyanophyta and two species belonged to Chlorophyta. During the initial culture days (day 1–day 9), the Chlorophyta (38.70 %) and Bacillariophyta (34.78 %) were the dominant groups in the ponds treated with HJW and JK27 probiotics, respectively (Fig. 3). During the final culture days, HJW and JK27 had one peak of algal dominance (Bacillariophyta bloom) that was succeeded by *Coscinodiscus* species and *Navicula* species. The control ponds exhibited three episodes of algal blooms, constituted mostly by Cyanophyta, Bacillariophyta and Dinoflagellata. However, the Cyanophyta were noted to be dominant in terms of species composition. The Cyanophyta bloom was preceded by *Oscillatoria erythraea, Spirulina* species and *Anabaena* species.

Selection of the principal dominant species that showed a contribution rate of more than 2 % of the phytoplankton species was conducted (Table 3). Five algal species showed high percentage compositions throughout the culture period, which could mean that these algae

Table 3 Summary of dominant species in the ponds, their mean cells abundance (cells L^{-1}) and percentage composition (mean ± SD, $n = 7$)

Dominant species	HJW	JK27	Control
Consinodicus (cells L^{-1})	20,493 ± 3621.8a (23.59)	15,595 ± 5460.8a (15.36)	15,219 ± 2882.4b (8.36)
Navicula (cells L^{-1})	12,429.71 ± 2427.2a (14.31)	2132.57 ± 3427.8a (21.01)	11,212.71 ± 7234.2b (6.16)
Skeletonema (cells L^{-1})	13,953.14 ± 2713.1 (16.06)	14,205.71 ± 726.7 (13.99)	8551 ± 4402.1 (4.70)
Oscillatoria (cells L^{-1})	4097.143 ± 1513.2a (4.72)	4922.429 ± 1868.9a (4.85)	34,782 ± 7763.5b (19.11)
Spirulina (cells L^{-1})	2683.429 ± 1212.7a (3.09)	3884.571 ± 1518.3a (4.20)	26,185 ± 5772.8b (14.39)

The values in parenthesis are the percentage composition. *Letters* indicate the differences between treatments. Means not sharing a common letter are significantly different at ($P < 0.05$)

had relatively high abundance values. The main features of HJW consist of species from Bacillariophyta, mostly *Coscinodiscus* spp., which accounts for 23.59 %, with the mean density of 2.05×10^4 cells L^{-1}, which was significantly higher ($P < 0.05$) than that recorded in the control ponds (1.52×10^4 cells L^{-1}). In the JK27 probiotics group, the dominant species were *Navicula* species (21.01 %) with the mean density of 2.13×10^3 cells L^{-1}, which was also significant ($P < 0.05$) compared with that in the control ponds (1.12×10^3 cells L^{-1}). In the control ponds, *O. erythraea* (19.11 %) from Cyanophyta became the key species with a mean concentration of 3.47×10^4 cells L^{-1} (Table 3).

Correlation of water parameters and phytoplankton abundance

Phytoplankton cell abundance exhibited significant positive correlation ($P < 0.01$) with NH_4^+, TP and Chl *a*, in the HJW treated ponds ($R = 0.468$, 0.868, 0.836, respectively), whereas NH_4^+, PO_4^{3-}, $NO_2^- + NO_3^-$, TP, TOC and Chl *a* showed highly significant positive correlations with phytoplankton abundance in both JK27 and the control ponds ($P < 0.05$ and $P < 0.01$). However, other parameters, such as WT, pH, DO and COD, did not show strong relationships with phytoplankton cell abundance during the study (Table 4).

Shrimp production at the end of the experiment is shown in Table 5. There was no significant difference in shrimp's final length and weights between the control and treated ponds ($P > 0.05$). The survival rate of the treatment ponds with HJW was 75.21 % and for JK27 it was 74.40 %, whereas the survival rate of the control was 73.69 %. The results showed that the microbial agents did not improve the growth, survival rate of shrimp and the feed conversion ratio ($P > 0.05$).

Table 4 Correlation coefficient (*R*) and significant between physico-chemical parameters and phytoplankton density in intensive shrimps pond ($n = 21$)

Parameters	(*R*)		
	HJW	JK27	Control
WT	−0.744	−0.492	−0.407
pH	−0.698	−0.656	−0.387
DO	−0.621	−0.587	−0.319
NH_4^+	0.468*	0.787*	0.464*
PO_4^{3-}	0.838	0.780*	0.515*
$NO_3^- + NO_2^-$	0.577	0.377*	0.309*
TP	0.868*	0.807*	0.586**
TOC	0.503	0.796*	0.557**
Chlorophyll *a*	0.836*	0.779*	0.437*

(*R*), correlation coefficient, WT, water temperature, DO, dissolved oxygen, NH_4^+, ammonium N; PO_4^{3-}, phosphorus; $NO_3^- + NO_2^-$, nitrate–nitrite; TP, total phosphorus; TOC, total organic carbon; Chl-*a*, chlorophyll *a*

* Correlation is significant at 0.05 levels

** Correlation is significant at 0.01 levels

Table 5 Average survival rate, feed conversion ratio, individual weight and length of shrimps at the end of experiments in experimental ponds (mean ± SD)

	HJW	JK27	Control
Survival rate (%)	75.21 ± 7.67	74.40 ± 11.14	73.69 ± 3.70
Feed coefficient	1.80 ± 0.19	1.69 ± 0.27	2.01 ± 0.37
Weight (g/ind)	6.32 ± 0.86	7.02 ± 0.63	6.35 ± 1.23
Length (cm)	8.62 ± 0.80	9.12 ± 0.84	8.61 ± 0.94

Discussion

Phytoplankton plays a pivotal role in maintaining ecological functions, including the ways to balance aquatic productivity for higher performance and sustainability of aquatic resources such as fish, shrimp and other organisms (Burford 1997; Lorenzen et al. 1997). The increase in uneaten feed at the bottom of shrimp ponds, which contributes to the production of excess algal blooms, spurred the introduction of the use of probiotics as a solution to restrain pathogenic algae. Yusoff et al. (2002) studied the effects of commercial bacterial products in shrimp ponds raising *P. monodon* and found that the ponds treated with the products containing *Bacillus* spp. and *Saccharomyces* spp. had a significantly lower concentration of total phytoplankton abundance compared with untreated ponds. A similar pattern of development was noted in the present study, where the mean phytoplankton cell abundance and species composition in the treated ponds (HJW and JK27) were significantly lower than in the untreated ponds (Fig. 1). The low phytoplankton cell density in the treated ponds suggests that the bacteria strain (*Bacillus* spp.) presumably limited the stability and growth of the harmful bacteria species, such as *Vibrio* (Rajinikanth et al. 2010; Paiva-Maia et al. 2013) and nuisance algae species from Cyanobacteria and Dinoflagellata in the ponds treated with probiotics.

Bacillus species are important candidates for developing commercial biological agents for nitrogen removal and water quality enhancement (Hong et al. 2005). Previously, some studies reported that the bacteria strains *B. subtilis* and *B. licheniformis* exhibited strong nitrite removal ability. *Bacillus* species could utilize nitrate and nitrite as alternative electron acceptors (Meng et al. 2009; Chen and Hu 2011). In the present study, despite the lack of significant difference in phytoplankton abundance in HJW and JK27 treated ponds, HJW showed a reduction in the number of Cyanophyta and Dinoflagellata species composition compared with JK27 (Fig. 2). This might be related to the types of bacteria strains present, and the amount of concentration applied in HJW. These results suggest that the probiotics composed of *B. subtilis, Streptococcus faecalis, Pediococcus, Actinomycetes,* yeast with additional of photosynthetic bacteria (CP1 and CP3) in HJW most likely showed strong ability to reduce harmful algae compared with the probiotics applied in JK27. In addition, the results were supported by Zhang and Chen (2004) who reported that the use of photosynthetic bacteria for bioremediation in aquaculture ponds can remove organic matter, NH_4^+, $NO_2^- + NO_3^-$, COD and other harmful wastes. However, further studies are necessary on selecting the precise concentrations and the most effectively bacterial strains to control phytoplankton community structure in shrimp aquaculture farms.

According to Boyd (1989), Bacillariophyta enhance shrimp growth better than Cyanophyta, and most shrimp farm managers prefer a high ratio of diatoms and green algae in a phytoplankton community. Diatoms and green algae are considered as beneficial algae because they act as the food for aquatic invertebrates and fish, whereas

Cyanobacteria and Dinoflagellata are associated with poor water quality and eutrophication (Paerl 1988; Jú et al. 2008). In the present study, Bacillariophyta was the most abundant and was dominated by *Coscinodiscus, Navicula* and *Skeletonema* species. The Cyanobacteria were the second most abundant and were dominated by *Oscillatoria* and *Spirulina* species at the end of the experiment (Fig. 3; Table 3). Some authors (e.g., Huang et al. 2004; Luan et al. 2006; Shaari et al. 2011; Guo et al. 2012) showed comparable results of dominance of Bacillariophyta in their studies. The trends showed that Bacillariophyta community structure has been maintained in ponds treated with probiotics from the beginning until the end, whereas in the control the structural stability of Bacillariophyta dropped and was replaced by Cyanophyta at the end (Fig. 3). In our study, there were more Coscinodiscus species and less *Oscillatoria* species in the treated ponds at the end of the experiment compared with the control ponds (Table 3). The presence of stable water quality after the addition of probiotics in the treated ponds may be one of the principal factors responsible for these effects. The different species composition among treatments gradually revealed that probiotics can effectively reduce the number of *Oscillatoria, Spirulina* and *Anabaena* species and can promote the growth of beneficial algae species such as *Coscinodiscus* spp. *Navicula* spp. *and Skeletonema* spp. (Table 3).

Abiotic factors in the environment, on the other hand, e.g., nitrogen, phosphorus and other nutrients, affect the succession of dominant species in the phytoplankton community and the quality of farmed animals, such as shrimp (Zhao et al. 2004; Cremen et al. 2007). Compared with the data obtained in the present study, the sequence of dominance and abundance of the phytoplankton changed with the variation of environmental factors. NH_4^+–N, TP and Chl *a* concentration appeared to correlate with phytoplankton abundance in the ponds treated with probiotics, whereas NH_4^+, PO_4^{3-}, TP, $NO_2^- + NO_3^-$, TOC and Chl *a* influence the growth of phytoplankton in the untreated ponds (Table 4). This suggested that the nutrient concentrations across the treatment might have a profound impact on the phytoplankton community structure.

Vanni and Findlay (1990) and Clifford (1992) agreed that high phosphate concentrations usually encouraged the growth of Cyanophyta, whereas high nitrate concentration encourages the growth of diatoms. Cremen et al. (2007) revealed that high ammonium and nitrite levels that result in high N:P ratio will promote diatom blooms. In addition, Smith (1983) reported that some shrimp ponds with high nitrogen loading rates could cause the absence or rare occurrence of Cyanophyta. In the present study, the high PO_4^{3-} concentrations at the end phase of shrimp cultivation significantly coincided with the abundance of Cyanophyta, whereas the high $NO_2^- + NO_3^-$ concentrations at the mid-phase and the final phase might be related to diatom dominance (Table 2).

Major problems related to water quality in aquaculture systems are due to the inadequate production and management of plankton. With the onset of eutrophication of the water bodies, the Bacillariophyta population decreases and Cyanobacteria and Dinoflagellata persist (Yusoff et al. 2010). In the present study, different factors working together contributed to the dominance and prevalence of Cyanobacteria and Dinoflagellata blooms in the untreated ponds. This effect could be the result of the following factors: infrequent water exchange, the increase in nutrients, (phosphate, ammonia and nitrogen), the competition of microorganisms and adverse environmental conditions (e.g., a high degree of turbidity, increased salinity and reduction in temperature).

According to Pérez-Linares et al. (2003) and Zimba et al. (2006), the dominant Cyanobacteria that form harmful blooms, such as *Schizothrix calcicola*, *Microcystis*, *Oscillatoria* and *Anabaena*, are relatively poor oxygen producers. In turn, they can generate compounds that are toxic to the farmed animals. However, the Cyanobacteria bloom in the

present study was composed of heterogeneous species of *Oscillatoria, Anabaena* and *Spirulina* that helped to stabilize the algae community structure, which may explain why the diversity index did not decrease in the control ponds (Fig. 2). The stability of the algal blooms prevented the algal collapses that could have otherwise caused anoxia and substantial release of sulfides, toxic gases and other toxins (Alonso-Rodríguez and Páez-Osuna 2003). Such conditions could have caused severe stress to the shrimp and made them more susceptible to disease (Corre et al. 2005).

The positive effects of probiotics on the phytoplankton community were expected to be favorable for the growth of the shrimp. No significant difference was found between the treated and the control ponds in the survival rate and growth rate (Table 5), which may be due to the short period of the experiment. However, the survival rate in this study was higher than that reported earlier (average 56 %) by Cremen et al. (2007).

Conclusions

The application of probiotics significantly changes the phytoplankton community structure in aquaculture ponds. The increase in the abundance of beneficial algae such as diatoms, the sustained presence of dominant species, especially the *Coscinodiscus* species, and the inhibition of harmful algae were considered to be a result of the active working of the probiotics. However, further studies are necessary on selecting autochthonous bacterial strains and applying adequate concentrations of this macrobiotic to improve the ecological conditions and productivity of shrimp farms.

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