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The Effectiveness on Water, Sediment Quality and Shrimp Production (*Penaeus monodon*) at Ponds Treated with *Streptomyces* sp. A1 Probiotic in Thua Thien Hue– Viet Nam

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The use of probiotics has increasingly become popular in shrimp culture not only for disease prevention and nutrition improvement but also because of an increasing demand for environment– friendly aquaculture. However, in Viet Nam, most of probiotics were imported with unknown origin so that they can pose a threat of spreading pathogenic or genetically modified microorganisms, resulting in the broken safe biology. Also, the beneficial effect of using such microbial products in aquaculture is still debatable as their efficacy is yet unclear. The objectives of this paper is to assess the effectiveness on water, sediment quality and shrimp production in ponds treated with a *Streptomyces* sp. A1 probiotic in Phu Loc district, Thua Thien Hue province (Viet Nam). Results showed that the probiotic could significantly increase pH, salinity, dissolved oxygen and reduce biochemical oxygen demand, chemical oxygen demand, concentrations of NH_4–N, NO_2–N, NO_3–N, presumptive *Vibrio* count and total coliforms of shrimp pond water. Simultaneously, the probiotic was likely to maintain pH, moisture, concentrations of total organic carbon, total nitrogen, total phosphorus and total sulfur suitable for shrimp pond sediment, especially reduce total carbon. Moreover, an average of 364.38 kg shrimp/ha was obtained in treated ponds with a feed conversion ratio of 1.10, per day growth of 0.21 g and survival rate of 85.43% compared with 240 kg shrimp/ha, 1.67, 0.13 and 84.97, respectively, in control ponds. This indicates that the addition of the probiotic had a noticeable influence on water and sediment quality of shrimp ponds and shrimp production. The finding of this study will facilitate to apply effectively a novel and safe indigenous probiotic into shrimp aquaculture, thereby improving shrimp aquaculture environment, increasing shrimp production, limiting to use antibiotics and chemicals, ensuring safe products and reaching for the sustainable shrimp aquaculture development of Thua Thien Hue in particular and Viet Nam in general.

Key words: *Penaeus monodon*, probiotic, shrimp pond sediment, shrimp pond water, *Streptomyces* sp. A1

INTRODUCTION

Tam Giang – Cau Hai is largest and most typical lagoon of Vietnam, with area of water surface is about 22.000 ha, or 48.2% of total area of all coastal lagoons of Vietnam. With this advantage, Thua Thien Hue province (Viet Nam) has many favorable conditions for development of shrimp culture (IMOLA, 2005). In fact, remarkable increase in shrimp culture helps to restructure the local rural economy in a positive direction (Tuan, 2010). However, a rapid, large– scale and often unplanned increase in brackish water shrimp culture ponds in recent years have resulted in shrimp epidemic diseases (Thua Thien Hue Department of Fisheries, 2010).

The shrimp diseases have mainly accounted for polluted pond environment. The addition of substantial amounts of antibiotics is still the choice for control the disease. Yet the abuse of antibiotics has resulted in the development of multi–resistant bacterial strains and antibiotic residues in shrimp products (Tendencia *et al*., 2001; Molina *et al*., 2002; Jayasree *et al*., 2006; Nakayama *et al*., 2006). Given the world–wide trade in aquaculture products, health problems related to antibiotic use in aquaculture are not limited to producing countries, but are also relevant to importing countries. Actually, there were many events of returning consignments to Viet Nam from Japan for not maintaining the prescribed standards on residual antibiotics of the products. Therefore, to make the shrimp aquaculture industry more sustainable, alternative strategies to control infections are urgently needed. One of the most successful alternative methods has been reported to be the use of probiotics as biological control agents in shrimp aquaculture (Verschuere *et al*., 2000; Vine *et al*., 2006; Tom *et al*., 2007). However, most such probiotics in Viet Nam are either imports or domestic products with unknown sources (Ministry of Fisheries, 2005), resulting in a threat of spreading pathogenic and genetically modified strains. Meanwhile, a actinomycetes strain *Streptomyces* sp. A1, which we isolated from shrimp culture pond sediments in Thua Thien Hue (Viet Nam), showed the activity against pathogenic strains *Vibrio* spp. through production of inhibitory compounds and siderophore, the ability to produce extracellular enzymes to decompose organic compounds, the formation of heat– and desiccation– resistant spores and the stability in preservation. Besides, *Streptomyces* sp. A1 can be mass–cultured, harvested, and fortified to feed (Chau *et al*., 2011).

This paper gives an account of the effectiveness on water, sediment quality and shrimp production (*Penaeus*

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monodon) at ponds treated with *Streptomyces* sp. A1 probiotic in Phu Loc district, Thua Thien Hue province (Viet Nam). The results will facilitate to offer the effective application of actinomycetes for improving the shrimp aquaculture in Thua Thien Hue and Viet Nam.

MATERIALS AND METHODS

Preparation of *Streptomyces* sp. A1 probiotic

Streptomyces sp. A1 was cultivated in optimal starch casein broth (SCB) sterilized for 15 min at 121°C in 250 mL Erlenmeyer flasks, on an incubated shaker (Jeiotech SI–600R, Korea) at 150 rpm, 30°C for 2 days. Then, this suspension were transferred to sterilized SCB in a 10–L fermentor (Infors Labfors HT, Switzerland) with a ratio of 1: 10 (v/v) for 3 days. After that, the fementor was left to stand for 30 min to allow the vegetative biomass (micro–colonies) of the *Streptomyces* sp. A1 to settle. The biomass was harvested using sterile filter papers and a vacuum filter, washed with sterile distilled water at least three times, dried at 35°C for 30 min, packaged and stored at 4°C.

Treated design

Tiger shrimp (*Penaeus monodon*) were cultured in high tide ponds in Phu Loc district, Thua Thien Hue province, Viet Nam (16°16'20.39"N, 107°52'29.77"E), during from Jun 28th to September 6th 2012, following the local farmer's experiences. The larvae of *P. monodon* (stage PL 9) were put into the ponds with each area of 1000 m^2 and stocking density of 30 shrimp/m^2. Shrimps were fed commercial feed with amounts equal to 1% of body weight divided into three times per day. After a month, with a lower density of 20 shrimp/m^2, *Streptomyces* sp. A1 probiotic was added directly with a density of 10^5 CFU per liter into water of the treated pond at intervals of 10 days during shrimp culture period. The ponds without adding the probiotic served as control ones. There were 6 phases to collect water and sediment samples referred as I to VI.

Water analysis

Temperature, conductivity, turbidity, salinity, pH, dissolved oxygen (DO) and total dissolve solid (TDS) of water samples were measured in the field using the Horiba U–52 (Japan). Water samples were mixtures collected near the surface of the center and sides of each pond by filling a sterile hand–held bottles. Water samples were stored in pre–sterilized plastic bottles and chilled immediately with ice and brought back aseptically to the laboratory for analysis. The concentrations of total suspended solid (TSS), inorganic nitrogen compounds (NH_4–N, NO_2–N, and NO_3–N), soluble reactive phosphorus (SRP), chemical oxygen demand (COD), biochemical oxygen demand (BOD) and presumptive *Vibrio* count on thiosulfate citrate bile salt sucrose agar (TCBS) and total coliforms in water samples were measured according to the procedures of APHA (2002).

Sediment analysis

Sediment samples were mixtures collected randomly on the surface of pond bottom (0–10 cm). The samples were stored in Pyrex bottles and transported aseptically to the laboratory and processed immediately for analyses. The pH (H_2O) and pH (KCl) were measured by the glass electrode method using a Hach–C535 (Hach, USA). Organic carbon (TOC) and total carbon (TC) were determined after dry combustion (ISO 10694: 1995). Total nitrogen (TN) was determined by modified Kjeldahl method (ISO 11261:1995). Total phosphorus (TP) was determined by Ascorbic acid method (10 TCN 373–99). Total sulfur (TS) was determined by dry combustion (ISO 15178:2000).

Growth analysis

The growth parameters were calculated by using the following formulae (Felix and Sudharsan, 2004)

$$\text{Per day growth (g)} = \frac{\text{Mean weight gain (g)}}{\text{Number of days}}$$

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

Survival (%)

$$= \frac{\text{Number of shrimps survived at the end of the experiment}}{\text{Number of shrimps stocked at the start of the experiment}} \times 100$$

Final production (kg/ha)

$$= \frac{\text{Total shrimp weight harvested (kg)}}{\text{Area of pond (ha)}}$$

Statistical analysis

Data were analysed using one–way ANOVA (SAS, version 6.03) to find significant difference on various parameters between treated and control ponds. A significance level of $P<0.05$ was used.

RESULTS

Proliferation of *Streptomyces* sp. A1

A large amount of actinomycete biomass (17.0 g/l) was obtained, meeting the requirement for further experiments.

Water quality

The results of water quality analysis were shown in Tables 1 and 2. Water temperatures were nearly the same in the treated ponds at sampling phases, and there was no considerable difference in water temperature between treated and control ponds. pH values of the water sam-

Table 1. Water quality in the treated ponds at various phases (from I to VI)

Parameters	I	II	III	IV	V	VI	Average
Temperature (˚C)	29.2	30.5	29.0	29.0	31.0	32.0	29.9
pH	7.0	6.8	6.9	6.8	6.8	6.9	6.9
Salinity (‰)	12.9	15.0	16.7	19.9	23.1	15.4	17.2
Conductivity (mS/cm)	21.6	24.8	27.2	32.0	36.6	25.4	27.9
TDS (g/l)	13.4	15.4	16.9	19.5	22.3	15.8	17.2
DO (mg/l)	19.8	19.1	12.7	11.2	7.8	12.2	13.8
Turbidity (NTU)	13.0	50.0	111	81.2	84.8	136	79.3
TSS (mg/l)	6	28	51	38	36	58	36
BOD_5 (mg/l)	8	3	4	6	6	12	7
COD (mg/l)	83	85	75	83	31	69	71
NH_4^+–N (mg/l)	ND	ND	ND	ND	ND	ND	ND
NO_2^-–N (mg/l)	ND	0.01	0.02	ND	0.00	ND	0.01
NO_3^-–N (mg/l)	ND	0.11	0.05	0.14	0.12	0.02	0.09
SRP (mg/l)	0.1	0.2	0.1	0.2	0.4	ND	0.2
Presumptive *Vibrio* (10^4 CFU/ml)	NT	NT	4.2	2.6	35	6	8.0
Total coliform (10^3 CFU/100 ml)	0.17	0.7	1.75	4.8	10.8	46	10.7

ND: non–detected; NT: non–tested

Table 2. Water quality in the control ponds at various phases (from I to VI)

Parameters	I	II	III	IV	V	VI	Average
Temperature (˚C)	29.2	30.5	28.9	29.9	30.9	31.7	30.2
pH	6.5	6.7	6.7	6.9	6.8	7.1	6.8
Salinity (‰)	13.1	14.4	16.2	19.0	21.8	14.2	16.5
Conductivity (mS/cm)	19.0	24.4	26.4	30.6	34.7	23.5	26.4
TDS (g/l)	13.5	15.1	16.4	18.7	21.1	14.6	16.6
DO (mg/l)	7.3	7.4	6.2	6.9	12.4	16.4	9.4
Turbidity (NTU)	23	91.5	114	157	129	43.7	93
TSS (mg/l)	13	58	87	85	69	60	62
BOD_5 (mg/l)	8	5	2	6	6	18	7.5
COD (mg/l)	81	87	74	79	37	71	72
NH_4^+–N (mg/l)	ND	0.02	ND	0.01	0.01	0.20	0.06
NO_2^-–N (mg/l)	ND	0.01	0.02	0.03	ND	ND	0.02
NO_3^-–N (mg/l)	0.01	0.06	0.01	ND	0.08	1.94	0.35
SRP (mg/l)	0.1	0.1	0.2	0.2	0.1	ND	0.1
Presumptive *Vibrio* (10^4 CFU/ml)	NT	NT	15	23	188	39	44.2
Total coliform (10^3 CFU/100 ml)	0.14	0.65	1.75	3.4	10.8	54	11.79

ND: non–detected; NT: non–tested

ples were not considerably changed during shrimp culture period. The water salinity of all ponds increased linearly from I to V phases. COD of water samples in the treated and control ponds were remarkably changed through survey phases with average values of 71 and 71.5 mg/l, respectively. Also, average BOD of water samples in the treated ponds (7.0 mg/l) was lower than that in control ponds (7.5 mg/l). The concentrations of NH_4–N in all water samples collected from the treated ponds were did not detected whereas those from the control ponds ranged from 0.01 to 0.2 mg/l with average value of 0.06 mg/l. Following the same trend with BOD and COD, the average value of NO_3–N of water samples in treated ponds (0.09 mg/l) was lower than that in control ponds (0.35 mg/l). According to Boyd (1990), the suitable concentrations of NO_3–N for shrimp aquaculture ranged from 0.1 to 10 mg/l, then concentration of NO_3–N detected in all water samples in this study appeared to be reasonable. However, the average concentration of RSP in water samples of treated ponds (0.2 mg/l) was higher than that of control ponds (0.1 mg/l). Total coliform of water samples in ponds increased linearly through phases in respond to increased accumulation of shrimp feces during shrimp growth. The average total coliform in treated ponds was lower than that in control ponds. Especially, presumptive *Vibrio* count on TCBS agar was significantly reduced in treated ponds. In comparision to the National Technical Regulation on Coastal Water Quality (QCVN10: 2008/BTNMT) and National Standard on Coastal Water Quality (TCVN 5943–1995) or the procedure for intensive culture of tiger shrimp (28 TCN 171: 2001), the average temperature, pH, salinity and NO_2–N of water samples in all ponds generally were within the limits of the coastal water used for aquaculture purposes. However, COD was over the limit of the coastal water used for aquaculture purposes in QCVN10:2008/BTNMT. Also, average total coliform was over the limit of the coastal water used for aquaculture purposes (10^3 CFU/100 ml) in QCVN10:2008/BTNMT and TCVN 5943–1995.

Sediment quality

Sediment samples were collected from ponds and their properties were determined (Tables 3 and 4). Over the study periods, in the treated ponds, sediment pH (H_2O) averaged 7.39 in a range of 7.2 and 7.61, and pH (KCl) averaged 7.22 in a range of 7.15 to 7.3. It is likely to suitable because shrimp pond bottom soils always need to have a pH of 7.5 or above to encourage decomposition of organic matter (Boyd, 2000). The high pH reflects the effect of the applications of liming materials into dry shrimp pond bottom and brackishwater buffers the soil so that acidic conditions usually do not develop in the surface layer of soil (Boyd, 2000). The sediment moisture in treated ponds significantly ranged from 32.11 to 70.22% and averaged 51.5%. It may be due to a long spell of heavy rain occured in this time. The optimum moisture content was about 20% for organic matter composition (Boyd, 2000). The average total carbon (TC) concentration in sediment samples of treated ponds (4.64%) was lower than that of control ponds (4.91%). Conversely, the average total organic carbon (TOC) concentration in sediment samples of treated ponds (2.78%) was higher than that of control ponds (2.57%). However, these concentrations match with suggestion that total organic carbon (TOC) concentrations ranged from 0.18% to 7.20% in many samples of the upper soil layer of shrimp ponds in some countries (Boyd, 1995). Total nitrogen (TN) concentration in sediment samples of treated ponds averaged 0.17% (ranging from 0.13 to 0.22 %) and of control ones averaged 0.18% (ranging from 0.15 to 0.24%) were not significantly different. Also, total phosphorus (TP) concentration in sediment samples of treated pond averaged 0.08% and of control ponds averaged 0.07% and there was no significant effect of the treatment by probiotic supplement on TP concentration. Whereas, the average total sulfur (TS) concentration in sediment samples of treated ponds (0.27%) was higher than that of control ponds (0.24%).

Table 3. Sediment quality in the treated ponds at various phases (from I to VI)

Parameters	I	II	III	IV	V	VI	Average
pH (H_2O)	7.45	7.20	7.38	7.32	7.38	7.61	7.39
pH (KCl)	7.22	7.15	7.19	7.23	7.21	7.30	7.22
Moisture (%)	50.72	46.03	32.11	60.47	70.22	50.63	51.50
TC (%)	5.10	4.18	4.36	4.87	4.58	4.75	4.64
TOC (%)	3.02	2.70	2.86	2.70	2.38	3.02	2.78
TN (%)	0.17	0.13	0.16	0.18	0.18	0.22	0.17
TS (%)	0.37	0.26	0.30	0.16	0.25	0.28	0.27
TP (%)	0.08	0.07	0.08	0.08	0.07	0.07	0.08

Table 4. Sediment quality in the control ponds at various phases (from I to VI)

Parameters	I	II	III	IV	V	VI	Average
pH (H_2O)	7.47	7.39	7.36	7.38	7.53	7.61	7.46
pH (KCl)	7.22	7.25	7.24	7.16	7.15	7.39	7.24
Moisture (%)	34.80	52.75	37.00	59.29	54.28	56.56	49.31
TC (%)	4.96	4.64	4.56	5.21	5.59	4.47	4.91
TOC (%)	2.86	2.54	2.70	2.54	2.70	2.07	2.57
TN (%)	0.16	0.16	0.16	0.15	0.18	0.24	0.18
TS (%)	0.18	0.21	0.29	0.22	0.26	0.27	0.24
TP (%)	0.10	0.05	0.05	0.09	0.07	0.07	0.07

Table 5. Growth parameters in treated and control ponds

Treatment	Per day growth (g)	FCR	Survival rate (%)	Production (kg/ha)
Treated ponds	0.21	1.10	85.43	364.38
Control ponds	0.13	1.67	84.97	240

Shrimp production

Shrimp from ponds treated with probiotic had significantly higher survival rate (85.43%), per day growth (0.21 g) and final production (365.38 kg/ha) with feed conversion rate (FCR, 1.67%) in compasition with those of control ponds (P<0.05) (Table 5). This indicates that the addition of the probiotic had a noticeable influence on shrimp production.

DISCUSSION

Microbial processes are intricately intertwined with water, sediment quality and production in shrimp ponds. Uneaten feed, shrimp feces, remains of shrimp settle to pond bottoms where microbial decomposition of this settled organic matter may deplete dissolved oxygen (DO) concentrations. Resultant anarobic decomposition depresses the redox potential and high concentration of nitrite, hydrogen sulfide and other reduced substances may result. In extensive ponds, shrimp obtain their nutrient by eating natural food organisms growing on the pond soil. In other words, excessive concentrations of organic matter in sediments may cause anaerobic conditions at the bottom which harm benthos and shrimp. Besides, the proliferation of opportunistically pathogenic *Vibrio* spp. commonly present in brackishwater and its sediments is considered as the major causation of the shrimp mortality in water rich in organic nutrients.

Despite the source of several novel antibiotics, marine actinobacteria has been given no attention for use a probiotic in aquaculture. However, it was recommended that marine actinomycetes are promising candidates to be utilized in marine aquaculture (You *et al.*, 2005, 2007). You *et al.* (2005) described the potential of actinomycetes against shrimp pathogenic *Vibrio* spp. and recommended marine actinomycetes as potential probiotic strains due to their ability to degrade macromolecules, such as starch and protein, in culture pond water; the production of antimicrobial agents; and the formation of heat– and desiccation–resistant spores. More recently, there were a few studies on the possible use of marine actinomycetes in disease prevention against aquatic pathogens. Surajit *et al.* (2006) reported a preliminary study on the use of *Streptomyces* on the growth of black tiger shrimp. Kumar *et al.* (2006) extracted the antibiotic product from marine actinomycetes and incorporated it into feed to observe the *in vivo* effect on white spot syndrome virus in black tiger shrimp. You *et al.* (2007) recommended the use of actinomycetes to prevent the disease caused by *Vibrio* spp. All of this research indicated the importance of marine actinobacteria in aquaculture.

The development of suitable probiotics requires empirical and fundamental experimental researches. It may be mentioned that a good number of probiotics, of foreign origin were tested in some hatcheries and culture ponds and was not found to be effective. This observation demands to discover new probiotics from the indigenous origin to be effective in local environment (Shafiqur *et al.*, 2009).

Given this circumstances, the addition of a indigenous actinomycetes strain, *Streptomyces* sp. A1, with the high activity against pathogenic strains *Vibrio* spp. through production of inhibitory compounds, the ability to produce extracellular enzymes to decompose organic compounds, the formation of heat– and desiccation–resistant spores and the stability in preservation mass–cultured, harvested, and fortified to feed appear to be an appropriate choice for shrimp aquaculture in Thua Thien Hue. In fact, findings of this study showed that the *Streptomyces* sp. A1 probiotic can be used to treat tiger shrimp (*Penaeus monodon*) ponds with ability to increase pH, salinity, DO and reduce BOD, COD, NH_4–N, NO_2–N, NO_3–N and total coliforms of shrimp pond water. Especially, presumptive *Vibrio* count on thiosulfate citrate bile salt sucrose (TCBS) agar was significantly reduced in treated ponds compared to control ponds. Also, the probiotic was likely to maintain suitable quality of shrimp pond sediment and improve shrimp production. The results may offer the potential application of actinomycetes for improving the sustainable shrimp aquaculture in Thua Thien Hue and Viet Nam.

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