Kasetsart J. (Nat. Sci.) 40 : 499 - 506 (2006)

Competition on Using Nutrient for Growth between *Bacillus* spp. and *Vibrio harveyi*

Watchariya Purivirojkul[1]*, Nontawith Areechon[2], Prapansak Srisapoome[2] and Monchan Maketon[1]

ABSTRACT

Competition, by using nutrient for growth, between *Bacillus pumilus* NW01, *B. sphaericus* NW02 and *B. subtilis* NW03 and *Vibrio harveyi in vitro* was studied by culturing each bacteria in Nutrient Broth (+ 1.5% NaCl). The initial concentration of 10^2 CFU/ml in monoculture and co-culture of *Bacillus* spp. and *V. harveyi* was used. Total *Bacillus* and *Vibrio* counts were conducted after 0, 24, 48, 72, 96 and 120 hours. *B. pumilus* NW01, *B. sphaericus* NW02 and *B. subtilis* NW03 decreased *V. harveyi* by 39.10, 43.62 and 34.46%, respectively. Antagonistic properties of *Bacillus* spp. against *V. harveyi in vivo* was tested by feeding shrimp with spores (10^{11}-10^{12} CFU/g) of each *Bacillus* and their mixture at 5 g/kg for 1 month. The amount of *Vibrio* spp. in the intestine of all *Bacillus* treated shrimp decreased by 20.97-32.45 % as compared with the control. The results showed that these *Bacillus* spp. could be applied as an effective probiotic in *Penaeus monodon* culture.

Key words: competition, nutrient, *Bacillus* spp., *Vibrio harveyi*

INTRODUCTION

A bacterium, *Vibrio harveyi*, has been reported as the most common pathogenic agent of *Penaeus monodon* (Lavilla-Pitogo and de la Pena, 1998). Control of bacterial problems in penaeid hatcheries and grow-out ponds have relied on the use of antibiotics, immunostimulants or probiotics (Gomez-Gil *et al*., 2000). There is an increasing interest within the industry on the control or elimination of antibiotic use because antibiotics can result in the development of resistant strains of bacteria (Weston, 1996).

Many genera of bacteria were used as probiotics such as *Vibrio* (Gullian *et al*., 2004), *Bacillus* spp. (Moriarty, 1998; Rengpipat *et al*., 2000; Gullian *et al*., 2004) and those bacteria isolated from the intestine of *Penaeus monodon* (Rengpipat *et al*., 2000). There are several mechanisms of probiotics including the production of inhibitory compounds, competition for chemicals or available energy, competition for adhesion sites, and/or the enhancement of the immune response and improvement of water quality (Verschuere *et al*., 2000).

A common method to screen potential probiotics is to perform *in vitro* antagonism tests (Verschuere *et al*., 2000). Probionts can be selected based on the production of inhibitory compounds or siderophores, or on the competition for nutrients (Dopazo *et al*., 1988). The pre-selection of probionts based on these *in vitro* antagonism tests

[1] Department of Zoology, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand.
[2] Department of Aquaculture, Faculty of Fisheries, Kasetsart University, Bangkok 10900, Thailand.
* Corresponding author, e-mail: fsciwyp@ku.ac.th

Received date : 21/09/05 Accepted date : 10/03/06

has often led to the finding of effective probiotics (Gibson *et al*., 1998). The next important step is the *in vivo* test which can be confirmed by continuous proliferation in the gut after being ingested. The possible modes of action require implicitly that the candidate probiotics are able to reach the location where their probiotic effect is most required (Verschuere *et al*., 2000).

In this study, potential probionts which were isolated from *P.enaeus monodon* intestine were tested for antagonistic activity against *Vibrio harveyi in vitro* and *in vivo* trials.

MATERIALS AND METHODS

1. Isolation and identification of *Bacillus* spp.

Bacillus spp. were isolated from the intestine of *Penaeus monodon* collected from shrimp farms in Chachoengsao province. Two hundred samples of shrimp were investigated. Intestines were rinsed in 5 ml of 1.5% NaCl per animal and heat shocked with water at 80 °C for 20 min followed by a cold shock with normal tap water (Purivirojkul *et al*., 2005). Then the solution was spread on plates using the spread plate technique on Nutrient Agar (NA) supplemented with 1.5% NaCl (w/v) and incubated at 35 °C for 24 h. Isolates were purified by streaking on NA supplemented with 1.5% NaCl (w/v). Catalase test was used for identifying *Bacillus* species. Species identification were done by VITEK 32 *Bacillus* and API 50CHB (Biomérieux).

2. Broth co-culture of *Bacillus* spp. with *Vibrio harveyi*

The method was modified from Hjelm *et al*. (2004). *Bacillus pumilus* NW01, *B. sphaericus* NW02 and *B. subtilis* NW03 (isolated from shrimp intestine in experiment 1) were tested for antagonistic activity against *V. harveyi* in a broth co-culture experiment. Bacteria were pre-cultured in 10 ml NB (Nutrient Broth) for 24 hours (110 rpm.) and transferred to test tubes containing 5 ml NB. These were inoculated with 10^2 CFU/ml *V. harveyi* together with 10^2 CFU/ml of *B. pumilus* NW01, *B. sphaericus* NW02 and *B. subtilis* NW03. Each bacterium strain had a control group to compare the bacterial concentration. Flasks were incubated at 35°C with shaking at 110 rpm. All combinations were tested in triplicate. Samples were collected after 0, 24, 48, 72, 96 and 120 hours for total *Bacillus* and total *Vibrio* counts by spread plate technique on NA supplemented with NaCl 1.5% (w/v) and TCBS agar (only *V. harveyi* can grow on TCBS agar).

3. Antagonistic activity properties of *Bacillus* spp. against *V. harveyi in vivo*

Penaeus monodon was obtained from shrimp farms in Chachoengsao Province, Thailand. Shrimp with a mean fresh weight of approximately 8-10 g per animal were used. They were acclimatized in an aerated aquarium filled with 25 ppt sea water and the water was changed every week before the start of the experiment. *Bacillus pumilus* NW01, *B. sphaericus* NW02 and *B. subtilis* NW03 spore were prepared at a concentration of 10^{11}-10^{12} CFU/g powder using clay as a filter. The experiment was designed as a CRD with 8 treatments and 3 replications each as shown in Table 1.

Each treatment was mixed with the shrimp feed, at ratio of 5 g: 1 kg feed and then fed at 3% of the body weight at four times per day.

Study for the bacterial concentration in shrimps intestine

The average concentration of both probiotic bacteria and *Vibrio* spp. in shrimp intestines was determined after 4 weeks of feeding. Shrimp intestines were rinsed in 1.5% NaCl and spreaded on NA supplemented with NaCl 1.5% (w/v) and TCBS. Plates were incubated at 35°C 24 hours. The number of bacteria was reported as CFU/g.

Table 1 Eight treatments of probiotic properties study.

Treatment	Species of *Bacillus* spp.
1	*B. pumilus* NW01
2	*B. sphaericus* NW02
3	*B. subtilis* NW03
4	*B. pumilus* NW01 + *B. sphaericus* NW02 (1:1)
5	*B. pumilus* NW01 + *B. subtilis* NW03 (1:1)
6	*B. sphaericus* NW02 + *B. subtilis* NW03 (1:1)
7	*B. pumilus* NW01+ *B. sphaericus* NW02+ *B. subtilis* NW03 (1:1:1)
8	No *Bacillus* (control)

RESULTS

1. Isolation and identification of *Bacillus* spp.

Out of 20 isolates from shrimp intestines, there were only 3 species belonging to the genus *Bacillus* which were identified as *B. pumilus* NW01, *B. sphaericus* NW02 and *B. subtilis* NW03.

2. Broth co-culture of *Bacillus* spp. with *V. harveyi*

Vibrio harveyi, grown as monoculture increased in concentration from 10^2-10^3 CFU/ml to 10^8 CFU/ml in one day (Table 2). The presence of *B. pumilus* NW01, *B. sphaericus* NW02 and *B. subtilis* NW03 (initial level of 10^2 - 10^3 CFU/ml) inhibited growth of *V. harveyi* during the first day from 1.40×10^7 CFU/ml of the control to 9.33×10^4, 2.47×10^4 and 4.60×10^4 CFU/ml, respectively (Table 2). A further reduction was seen during the following 120 hours, reducing *V. harveyi* from 3.6×10^5 CFU/ml to 9.83×10^2, 4.97×10^2 and 1.98×10^3 CFU/ml which caused a reduction of 39.10, 43.62 and 34.46% respectively (Table 3 and Figure 3). While *Bacillus* spp. concentrations in co-culture treatment increased to 10^9 CFU/ml in 96 hours and did not differ ($P>0.05$) from the control treatment (Table 4 and Figure 2-4).

3. Antagonistic activity properties of *Bacillus* spp. against *V. harveyi in vivo*, bacterial concentration in shrimp intestine

Bacillus spp.

The concentration of *Bacillus* spp. in shrimp intestine after being fed with *B. pumilus* NW01 ($552.00 \pm 213.45 \times 10^4$), *B. sphaericus* NW02 ($514.00 \pm 217.25 \times 10^4$), *B. subtilis* NW03 ($526.00 \pm 197.66 \times 10^4$), the mixture of *B. pumilus* NW01 + *B. sphaericus* NW02 ($565.33 \pm 200.07 \times 10^4$), the mixture of *B. pumilus* NW01+ *B. subtilis* NW03 ($724.67 \pm 174.14 \times 10^4$), the mixture of *B. sphaericus* NW02 + *B. subtilis* NW03 ($526.00 \pm 174.06 \times 10^4$) and the mixture of *B. pumilus* NW01 + *B. sphaericus* NW02 + *B. subtilis* NW03 ($536.67 \pm 168.59 \times 10^4$) for 4 weeks was significantly higher ($P<0.05$) than the control whose number of *Bacillus* spp. average was $6.67 \pm 5.25 \times 10^4$ CFU/g, as shown in Figure 5 and Table 5.

Vibrio spp.

The concentration of *Vibrio* spp. in shrimp intestine 4 weeks after fed with normal feed (control) ($426.13 \pm 164.73 \times 10^4$) was significantly higher ($P<0.05$) than shrimp fed with *B. pumilus* NW01, *B. sphaericus* NW02, *B. subtilis* NW03, the mixture of *B. pumilus* NW01 + *B. sphaericus* NW02, the mixture of *B. pumilus* NW01 + *B. subtilis* NW03, the mixture of *B. sphaericus* NW02 + *B. subtilis* NW03 and the mixture of *B. pumilus* NW01 + *B. sphaericus* NW02 + *B. subtilis* NW03 whose numbers of *Vibrio* spp. in intestine were $59.73 \pm 37.00 \times 10^4$, $77.80 \pm 48.03 \times 10^4$, $85.93 \pm 43.29 \times 10^4$, $92.73 \pm 51.87 \pm 10^4$, $76.00 \pm 46.53 \times$

Table 2 Concentration of *B. pumilus* NW01, *B. sphaericus* NW02, *B. subtilis* NW03 and *V. harveyi* in monocultures and co-culture (CFU/ml).

Time (hrs)	0	24	48	72	96	120
Bacteria						
B. pumilus NW01	$5.73\pm0.85 \times 10^2$	$2.0\pm0.77 \times 10^8$	$2.42\pm0.33 \times 10^8$	$1.33\pm0.52 \times 10^8$	$1.17\pm0.20 \times 10^9$	$1.24\pm0.15 \times 10^9$
B. sphaericus NW02	$4.3\pm0.40 \times 10^2$	$1.08\pm0.18 \times 10^8$	$6.53\pm0.20 \times 10^8$	$8.13\pm0.40 \times 10^8$	$1.09\pm0.15 \times 10^9$	$1.39\pm0.32 \times 10^9$
B. subtilis NW03	$6.07\pm0.76 \times 10^2$	$9.6\pm1.15 \times 10^7$	$3.14\pm1.57 \times 10^9$	$4.83\pm1.56 \times 10^9$	$1.03\pm0.09 \times 10^9$	$1.15\pm0.23 \times 10^9$
V. harveyi	$4.23\pm0.25 \times 10^2$	$1.40\pm0.21 \times 10^7$	$1.10\pm0.18 \times 10^7$	$4.40\pm0.40 \times 10^6$	$7.80\pm1.90 \times 10^5$	$3.60\pm1.11 \times 10^5$
B. pumilus NW01 co-culture	$3.97\pm0.90 \times 10^2$	$2.12\pm0.70 \times 10^8$	$2.43\pm0.78 \times 10^8$	$2.86\pm0.15 \times 10^8$	$2.51\pm0.53 \times 10^9$	$1.67\pm0.36 \times 10^9$
B. sphaericus NW02 co-culture	$3.87\pm0.23 \times 10^2$	$8.20\pm1.55 \times 10^7$	$8.56\pm0.76 \times 10^7$	$8.63\pm0.76 \times 10^7$	$1.08\pm0.24 \times 10^9$	$1.06\pm0.24 \times 10^9$
B. subtilis NW03 co-culture	$4.60\pm0.18 \times 10^2$	$2.50\pm0.14 \times 10^8$	$9.03\pm1.56 \times 10^8$	$2.37\pm0.68 \times 10^9$	$1.15\pm0.12 \times 10^9$	$1.05\pm0.14 \times 10^9$
V. harveyi in co-culture with *B. pumilus* NW01	$4.40\pm0.46 \times 10^2$	$9.33\pm1.23 \times 10^4$	$5.50\pm1.31 \times 10^4$	$8.07\pm1.52 \times 10^3$	$3.34\pm1.90 \times 10^3$	$9.83\pm1.37 \times 10^2$
V. harveyi in co-culture With *B. sphaericus* NW02	$4.50\pm0.56 \times 10^2$	$2.47\pm0.47 \times 10^4$	$5.57\pm1.14 \times 10^4$	$8.67\pm1.15 \times 10^3$	$2.96\pm1.47 \times 10^3$	$4.97\pm1.18 \times 10^2$
V. harveyi in co-culture With *B. subtilis* NW03	$4.47\pm0.27 \times 10^2$	$4.60\pm0.66 \times 10^4$	$7.93\pm0.67 \times 10^3$	$3.56\pm0.49 \times 10^3$	$2.23\pm0.30 \times 10^3$	$1.98\pm0.16 \times 10^3$

10^4, $82.93 \pm 35.06 \times 10^4$, $119.73 \pm 58.50 \times 10^4$ CFU/g, respectively, as shown in Figure 5 and Table 5.

DISCUSSION

Three species of *Bacillus* were isolated and identified from shrimp intestine, namely *B. pumilus*, *B. sphaericus* and *B. subtilis*. These bacteria might come from water, soil, food or normal flora in the intestine. Bonde (1981) reported that *Bacillus* in seawater was dominated by *B. licheniformis* followed by *B. subtilis* and *B. pumilus*. Other species encountered in low numbers included *B. brevis*, *B. firmus* and *B. sphaericus*, largely in nonpolluted areas. In a numerical study of North Sea sediments, Boeyé and Herts (1976) found that *B. subtilis*, *B. licheniformis* and *B. firmus* strains predominated. So, it was possible for *B. pumilus*, *B. sphaericus* and *B. subtilis* to contaminate the intestine of shrimp by sea water.

Tests of antagonism, adhesion or challenge are essential in selecting a potential probiont. Antagonism may be due to competition for nutrients that favour the growth of probionts, or the expression of their inhibitory effects (Gatesoupe, 1999). Competitive exclusion has been mentioned as a possible mechanism for probiotic effects. Iron is required by most organisms, and its availability in animal tissues may be a virulence factor for pathogens. Smith and Davey (1993) suggested that the growth inhibition of *Aeromonas salmonicida* by *Pseudomonas fluorescens* was due to competition for free iron.

From the results, *Bacillus* spp. from the intestine of the black tiger shrimp displayed *in vitro* nutrient competition against *V. harveyi* in liquid media. Hjelm *et al*. (2004) used an *in vitro* antagonism test as a selection

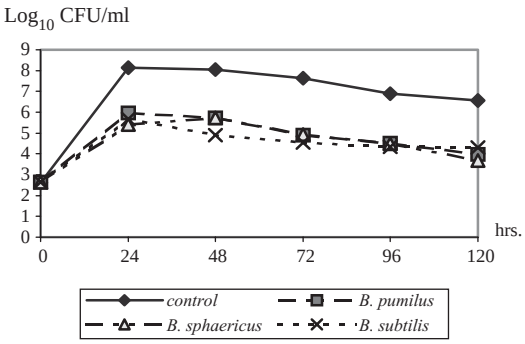


Figure 1 Growth of *V. harveyi* monoculture (control) and in co-culture with *Bacillus* spp.

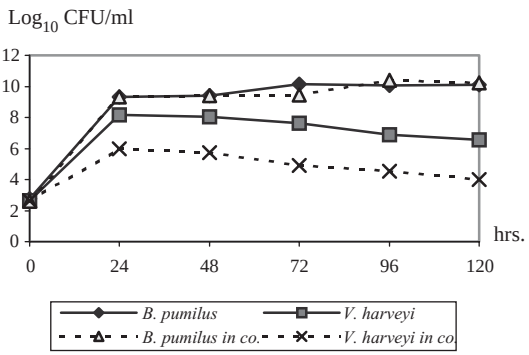


Figure 2 Growth of *B. pumilus* NW01 *V. harveyi* monoculture and in culture.

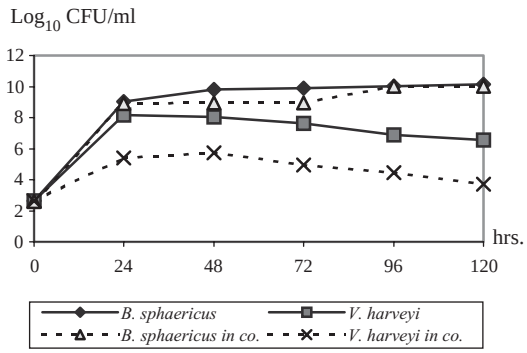


Figure 3 Growth of *B. sphaericus* NW02 and *V. harveyi* monoculture and in co-culture.

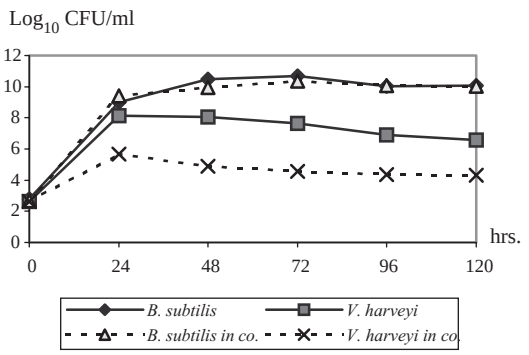


Figure 4 Growth of *B. subtilis* NW03 and *V. harveyi* monoculture and in co-culture.

Table 3 Percent changes of *V. harveyi* when co-culture with *Bacillus* spp. in 0-120 hrs.

Time (hrs) / Bacteria	0	24	48	72	96	120
Control	0	0	0	0	0	0
B. pumilus NW01	0.64 [a]	-26.70 [a]	-28.60 [a]	-35.81 [a]	-34.36 [a]	-39.10 [a]
B. sphaericus NW02	1.01 [a]	-33.80 [a]	-28.54 [a]	-35.40 [a]	-35.12 [a]	-43.62 [a]
B. subtilis NW03	0.89 [a]	-30.48 [a]	-39.06 [a]	-40.46 [a]	-36.90 [a]	-34.46 [a]

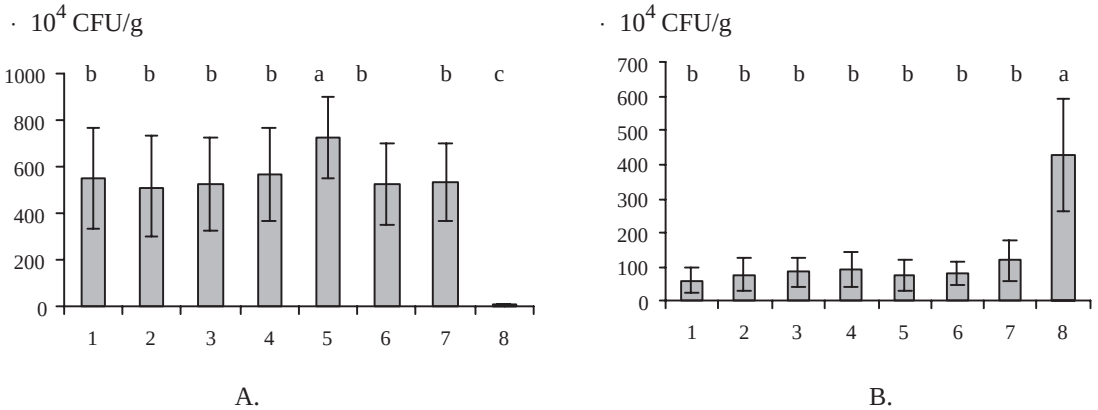
Means values within the same column sharing the same superscript are not significantly different at P=0.05

Table 4 Percent changes of *Bacillus* spp. when co-culture with *V. harveyi* in 0-120 hrs.

Time (hrs) / Bacteria	0	24	48	72	96	120
B. pumilus NW01	5.80	-0.28	-0.02	6.61	-3.29	-1.27
B. sphaericus NW02	1.75	1.31	8.99	9.83	0.01	1.16
B. subtilis NW03	4.32	-4.63	5.16	2.90	-0.46	0.41

Note " – " indicates the percent reductions
No sign of " - " indicate the percent increase

criterion for potential probionts and typical procedure when searching for probiotic or biocontrol strains in many environments. They reported that *Roseobacter* 27-4 which isolated from a range of marine and larval rearing samples, inhibited growth of *Vibrio anguillarum* 90-11-287 or *Vibrio splendidus* DMC-1 in broth-coculture experiments. For antagonist activity of *Bacillus*



A. B.

Figure 5 The concentration of *Bacillus* spp. (A) and *Vibrio* spp. (B) in *P. monodon* intestine after 4 weeks of culture when provided with 8 types of feeds (1 = *B. pumilus* NW01, 2 = *B. sphaericus* NW02, 3 = *B. subtilis* NW03, 4 = the mixture of *B. pumilus* NW01+ *B. sphaericus* NW02, 5 = the mixture of *B. pumilus* NW01 + *B. subtilis* NW03, 6 = the mixture of *B. sphaericus* NW02 + *B. subtilis* NW03, 7 = the mixture of *B. pumilus* NW01 + *B. sphaericus* NW02 + *B. subtilis* NW03, 8 = control).

Table 5 The concentration of *Bacillus* spp. and *Vibrio* spp. in *P. monodon* intestine after 4 weeks of feeding with *Bacillus* spp.

T	Feeds	*Bacillus* spp.		*Vibrio* spp.	
		(x 10^4 CFU/g)	% changed compare with control	(x 10^4 CFU/g)	% changed compare with control
1	*B. pumilus* NW01	552.00 ± 213.45^{b}	232.80	59.73 ± 37.00^{b}	-32.45
2	*B. sphaericus* NW02	514.00 ± 217.25^{b}	229.04	77.80 ± 48.03^{b}	-28.09
3	*B. subtilis* NW03	526.00 ± 197.66^{b}	230.25	85.93 ± 43.29^{b}	-26.44
4	*B. pumilus* NW01 + *B. sphaericus* NW02	565.33 ± 200.07^{b}	234.05	92.73 ± 51.87^{b}	-25.19
5	*B. pumilus* NW01 + *B. subtilis* NW03	724.67 ± 176.14^{a}	247.14	76.00 ± 46.53^{b}	-28.47
6	*B. sphaericus*NW02 + *B. subtilis* NW03	526.00 ± 174.06^{b}	230.25	82.93 ± 35.06^{b}	-27.03
7	*B. pumilus* NW01+ *B. sphaericus* NW02 + *B. subtilis* NW03	536.67 ± 168.59^{b}	231.31	119.73 ± 58.50^{b}	-20.97
8	Control	6.67 ± 5.25^{c}	0.00	426.13 ± 164.73^{a}	0.00

Means values within the same column sharing the same superscript are not significantly different at P=0.05

spp. in *in vitro* test, Maketon and Masawhang (2000) found that *Bacillus subtilis* AM-01, *B. licheniformis* AM-04 and *Nitrosomonas* sp. AM-11 showed good potential of competition and colonization activities against *Vibrio harveyi*.

In *in vivo* experiment, when shrimp were fed with spores of *Bacillus* spp., the number of *Vibrio* spp. in shrimp intestine decreased significantly. The results showed that spores of *Bacillus* spp. in feed might germinate in the gut and had a superiority in nutrient competition to *Vibrio* spp. Many strains of *Bacillus* spp. have antagonist activity to reduce pathogenic bacteria. For example, Moriarty (1998) reported an increase of prawn survival in ponds where some strains of *Bacillus* spp. were introduced. This treatment decreased the proportion of pathogenic luminous *Vibrio* spp. in the sediments and to a lesser extent in the water. *Bacillus* strain S11 could decrease the mortality of *Penaeus monodon* after challenging the pathogenic *V. harveyi* D331 (Rengpipat *et al.*, 2000). *Bacillus* strain IP5832 could decrease the mortality of turbot larvae when challenged with an opportunistic *Vibrionaceae* species (Gatesoupe, 1994). Moreover, other strains of bacteria had antagonistic activity such as *Vibrio alginolyticus* which isolated from Pacific ocean seawater decreased observation of *Vibrio parahaemolyticus* in the shrimps. *Lactobacillus* or *Carnobacterium* isolated from rotifers (*Brachionus plicatilis*) could decrease mortality of turbot larvae challenged with a pathogenic *Vibrio* sp. (Gatesoupe, 1994). *Aeromonas media* A 199 showed antagonist activity by decreasing the mortality and suppressing a pathogen of Pacific oyster larvae when challenged with a pathogenic *V. tubiashii* (Gibson *et al.*, 1998). *Lactobacillus plantarum* inhibited the growth of a strain of *Aeromonas salmonicida* in a rotifer culture (Gatesoupe, 1994).

CONCLUSIONS

Bacillus pumilus, B. sphaericus and *B. subtilis* exhibited competition with marine shrimp pathogenic bacteria for nutrients. They could reduce *V. harveyi*, when cultured together in *in vitro* by 39.10, 43.62 and 34.46%, respectively but there were no significant differences ($P<0.05$) amongst the *Bacillus* treatments. Furthermore, when fed shrimp with these *Bacillus* spp., they showed antagonistic activity against *V. harveyi* in the shrimp intestine. The concentration of *Vibrio* spp. in intestine decreased by 20.97-32.45% and there were no significant differences ($P<0.05$) amongst the *Bacillus* treatments. So, these *Bacillus* spp. could be applied as possible probiotics in shrimp culture.

ACKNOWLEDGMENTS

This research was supported by National Research Council of Thailand.

LITERATURE CITED

Boeyé, A. and M. Herts. 1976. Numerical taxonomy of Bacillus isolates from North Sea sediments. **Int. J. Syst. Bacteriol.** 26: 427-441.

Bonde, G.J. 1981. *Bacillus* from marine habitats: Allocation to phena established by numerical Techniques, pp. 181-215. *In* R.C.W. Berkeley and M. Goodfellow (eds.). **The Aerobic Endospore-Forming Bacteria: Classification and Identification.** Academic Press, London.

Dopazo, C.P., M.L. Lemos, C. Lodeiros, J. Bolinches, J.L. Barja and A.E. Toranzo. 1988. Inibitory activity of antibiotic-producing marine bacteria against fish pathogens. **J. Appl. Bacteriol.** 65: 97–101.

Gatesoupe, F.J. 1994. Lactic acid bacteria increase the resistance of turbot larvae, *Scophthalmus maximus*, against pathogenic *Vibrio*. **Aquat. Living Resour.** 7: 277–282.

Gatesoupe, F.J. 1999. The use of probiotics in aquaculture: review. **Aquaculture** 180:

147–165.

Gibson, L., J. Woodworth and A. George. 1998. Probiotic activity of *Aeromonas media* on the Pacific oyster, *Crassostrea gigas*, when challenged with *Vibrio tubiashii*. **Aquaculture** 169: 111–120.

Gomez-Gil, B., A. Roque and J.F. Turnbul. 2000. The use and selection of probiotic bacteria for use in the culture of larval aquatic organisms. **Aquaculture** 191: 259–270.

Gullian, M., F. Thompson and J. Rodriguez. 2004. Selection of probiotic bacteria and study on their immunostimulatory effect in *Penaeus vannamei*. **Aquaculture** 233: 1-14.

Hjelm, M., O. Bergh, A. Riaza, J. Nielsen, J. Melchiorsen, S. Jensen, H. Duncan, P. Ahrens, H. Birkbeck and L. Gram. 2004. Selection and Identification of Autochthonous Potential Probiotic Bacteria from Turbot Larvae *(Scophthalmus maximus)* Rearing Units. **System. Appl. Microbiol.** 27: 360–371.

Lavilla-Pitogo, C. R. and L.D. de la Pena. 1998. Bacterial disease in shrimp (*Penaeus monodon*) culture in Philippines. **Fish Pathol.** 33: 405–411.

Maketon, M. and K. Masawhang. 2000. Potential of some beneficial bacterias in colonizing *Vibrio harveyi*, a luminous bacteria causing disease in shrimp, pp. 259-268. *In* **The Proceedings of 38th Kasetsart University Annual Conference.** Kasetsart University, Bangkok.

Moriarty, D.J.W. 1998. Control of luminous *Vibrio* species in penaeid aquaculture ponds. **Aquaculture** 164: 351-358.

Purivirojkul, W., M. Maketon and N. Areechon. 2005. Probiotic Properties of *Bacillus pumilus*, *Bacillus sphaericus* and *Bacillus subtilis* in Black Tiger Shrimp (*Penaeus monodon* Fabricius) Culture. **Kasetsart J. (Nat. Sci.)** 39: 262-273.

Rengpipat, S., S. Rukpratanporn, S. Piyatiratitivarakul and P. Menasveta. 2000. Immunity enhancement in black tiger shrimp (*Penaeus monodon*) by a probiont bacterium (*Bacillus* S11). **Aquaculture** 191: 271-288.

Smith, P. and S. Davey. 1993. Evidence for the competitive exclusion of *Aeromonas salmonicida* from fish with stress-inducible furunculosis by a fluorescent pseudomonad. **J. Fish Dis.** 16: 521–524.

Weston, D.P. 1996. Environmental considerations in the use of antibacterial drugs in Aquaculture, pp. 140–165. *In* D. Baird, M.V.M. Beveridge, L.A. Kelly and J.F. Muir (eds.). **Aquaculture and Water Resource Management.** Blackwell, Oxford.

Verschuere, L., G. Rombaut, P. Sorgeloos and W. Verstraete. 2000. Probiotic bacteria as biological control agents in aquaculture. **Microbiol. Mol. Biol. Rev.** 64: 655-669.