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Interactions Between the Pathogenic Bacterium *Vibrio parahaemolyticus* and Red-tide Dinoflagellates

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Abstract – *Vibrio parahaemolyticus* is a common pathogenic bacterium in marine and estuarine waters. To investigate interactions between *V. parahaemolyticus* and co-occurring red-tide dinoflagellates, we monitored the daily abundance of 5 common red tide dinoflagellates in laboratory culture; *Amphidinium carterae*, *Cochlodinium ploykrikoides*, *Gymnodinium impudicum*, *Prorocentrum micans,* and *P. minimum*. Additionally, we measured the ingestion rate of each dinoflagellate on *V. parahaemolyticus* as a function of prey concentration. Each of the dinoflagellates responded differently to the abundance of *V. parahaemolyticus*. The abundances of *A. carterae* and *P. micans* were not lowered by *V. parahaemolyticus*, whereas that of *C. polykrikodes* was lowered considerably. The harmful effect depended on bacterial concentration and incubation time. Most *C. polykrikoides* cells died after 1 hour incubation when the *V. parahaemolyticus* concentration was 1.4×10^7 cells ml^{-1}, while cells died within 2 days of incubation when the bacterial concentration was 1.5×10^6 cells ml^{-1}. With increasing *V. parahaemolyticus* concentration, ingestion rates of *P. micans*, *P. minimum*, and *A. carterae* on the prey increased, whereas that on *C. polykrikoides* decreased. The maximum or highest ingestion rates of *P. micans*, *P. minimum*, and *A. carterae* on *V. parahaemolyticus* were 55, 5, and 2 cells $alga^{-1}$ h^{-1}, respectively. The results of the present study suggest that *V. parahaemolyticus* can be both the killer and prey for some red tide dinoflagellates.

Key words – algicidal bacteria, feeding, harmful algal bloom, ingestion, red tide

1. Introduction

Bacteria and red-tide dinoflagellates are major components of marine ecosystems (Azam 1998; Doucette et al. 1998). They usually co-occur, thus most studies have investigated their joint interactions (Lee 1990; Doucette et al. 1999; Mayali and Azam, 2004). Some bacteria are known to kill red tide dinoflagellates such as algicidal bacteria (Kitaguchi et al. 2001; Amaro et al. 2005). A number of algicidal bacteria have been reported since the 1990s (Imai et al. 1993, 2001; Doucette et al. 1998; Park et al. 1998; Kim et al. 1999; Byun et al. 2002; Mayali and Azam 2004; Imai and Kimura 2008). Lysis of algae by algicidal bacteria is known to play an important role in terminating red tides (Skerratt et al. 2002). On the contrary, bacteria have been revealed to be eaten by red-tide dinoflagellates (Nygaard and Tobiesen, 1993; Seong et al. 2006). Additionally, some bacteria are known to live inside red tide dinoflagellates in a symbiotic relationship (Green et al. 2004; Hackett et al. 2004; Jasti et al. 2005). Thus, interactions between bacteria and red tide dinoflagella can be complicated.

Vibrio parahaemolyticus is a common pathogenic bacterium in marine and estuarine waters (Hervio-Heath et al. 2002; Makino et al. 2003; Yeung and Boor 2004). This bacterium, when ingested, causes watery diarrhea often with abdominal cramping, nausea, vomiting, fever, and chills (Dadisman et al. 1972). *V. parahaemolyticus* can also cause an infection of the skin when an open wound is exposed to warm seawater (Wright et al. 2009). *V. parahaemolyticus* is sometimes abundant during red tides dominated by dinoflagellates (Romalde et al. 1990; Eiler et al. 2006). However, there are very few studies on interactions between *V. parahaemolyticus* and red-tide dinoflagellates (Bienfang et al. 2011), which makes the exploration of this topic worthwhile.

We isolated and established a clonal culture of *V.*

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parahaemolyticus from seawater to investigate interactions between *V. parahaemolyticus* and co-occurring red-tide dinoflagellates. In particular, we monitored the abundance of 5 common red tide dinoflagellates and measured the growth and ingestion rates of the dinoflagellates on *V. parahaemolyticus* as a function of the prey concentration on the daily basis. The results of the present study provide a basis for understanding interactions between *V. parahaemolyticus* and red tide dinoflagellates and dynamics of these two components in marine ecosystems.

2. Materials and Methods

Preparation of experimental organisms

Red-tide dinoflagellates were grown at 20 ºC in enriched f/2 seawater media (Guillard and Ryther, 1962) without silicate under a 14h light:10h dark cycle of 30 μE $m^{-2}s^{-1}$. The mean equivalent spherical diameter (ESD) ± standard deviation was measured by an electronic particle counter (Coulter Multisizer II, Coulter Corporation, Miami, Florida, USA) (Table 1).

For isolation of *Vibrio parahaemolyticus,* water samples were collected from surface waters of Shiwha Bay, Korea, in September 2005. Samples were immediately transferred to the laboratory with a temperature of below 4 ºC. Subsequently, 0.2 ml of each diluted sample was inoculated on to Marine agar plate (Difco 2216, Franklin lakes, NJ). Samples in the plate were incubated at 37 ºC for a week under dark condition. To isolate the colony separately, each colony was streaked on the new plate. Again, each colony was isolated and transferred to 50 ml of Na broth (Andersen et al. 1974). Isolated bacterial cultures were incubated until the stationary phase (approximately 2-3 d) on a shaker at 70 rpm at 37 ºC. *V. parahaemolyticus* was identified by analyzing the sequence of 16S rDNA. Its sequence shows 99% similarity with species of *V. parahaemolyticus*.

Harmful effects of *Vibrio parahaemolyticus*

Experiment 1 was designed to assess whether dense culture or filtrate of *V. parahaemolyticus* is able to kill red-tide dinoflagellates *Amphidinium carterae*, *Cochlodinium polykrikoides*, *Gymnodinium impudicum*, *Prorocentrum micans*, and *P. minimum* in laboratory culture.

A dense culture of the target dinoflagellate (20,000-400,000 cells) was added to each well of 6 well plate chambers. A dense culture of *V. parahaemolyticus* (ca, 8×10^5-8×10^8 cells) was added to each well of the chambers (Two final concentrations = 10^6 and 10^7 cells ml^{-1}). Triplicate experimental wells for each *V. parahaemolyticus* final concentration (mixture of target dinoflagellate and *V. parahaemolyticus*) and triplicate control wells (target dinoflagellate only) were set up. After 0.5, 1, 2, 4, and 6 h of incubation, swimming behaviors of target dinoflagellate cells in each well were examined under an inverted light

Table 1. Dinoflagellate species used as predators on and/or victims by target algicidal bacteria. Mean equivalent spherical diameter (ESD, μm) (± Standard deviation) was measured by an electronic particle counter measured before these experiment; n>2000 for each species. MIR: Maximum ingestion rate of the dinoflagellates on *Vibrio parahaemolyticus* (*Vp*, cells $alga^{-1}h^{-1}$). LCBKD: Lowest concentration of algicidal bacteria for killing the target dinoflagellate (in bacterial cells ml^{-1}). NHE: No Harmful Effect

Species	ESD (±SD)	MIR	Target bacterium	LCBKD	References
Amphidinium carterae	6.6 (1.5)	1.2	*Vibrio parahaemolyticus*	NHE	This study
Amphidinium carterae			*Vibrio harveyi* *V. alginolyticus* *V. parahaemolyticus*	NHE	Nayak et al. 2000
Prorocentrum minimum	12.1 (2.5)	5.1	*V. parahaemolyticus*	1.4×10^7	This study
Prorocentrum minimum			*Psedoalteromonas haloplanktis*	2.5×10^4 (cfu ml^{-1})	Kim et al. 2009
Prorocentrum minimum			*Shewanella IRI-160*	~10^9	Hare et al. 2005
Prorocentrum micans	26.6 (2.8)	55	*V. parahaemolyticus*	1.4×10^7	This study
Prorocentrum micans			*Psedomonas* sp. LG-2	1.3×10^6	Lee and Park, 1998
Gymnodinium impudicum	17.8 (2.6)	1.6	*V. parahaemolyticus*	1.4×10^7	This study
Gymnodinium nagasakiense			*Flavobacterium s*p.	> 10^6	Fukami et al. 1992
Cochlodinium polykrikoides	25.9 (2.9)	ND	*V. parahaemolyticus*	$8.8\text{x}10^5$	This study
Cochlodinium polykrikoides	25.9 (2.9)	ND	*Alteromonas* sp. *Psedoalteromonas* sp	<10^5~10^7	Imai and Kimura 2008
Cochlodinium polykrikoides	25.9 (2.9)	ND	*Alteromonas* sp.	9.0×10^5	Lee et al. 2008

microscope.

To test the harmful effects of the filtrate from *V. parahaemolyticus* culture on each dinoflagellate, dense cultures (10^6 and 10^7 cells ml^{-1}) of exponentially growing *V. parahaemolyticus* were transferred to centrifuge tubes. After 20 min of centrifugation at 20,000 g, the supernatant (suspended aliquot) was filtered through a 0.2 μm pore-sized filter (Whatmann, Polycarbonate, Maidstone, UK) to remove bacteria cells. Filtered supernatants (0.01-0.1 ml^{-1}) were transferred into each of the triplicate wells containing the target dinoflagellate. Additionally, triplicate control wells (target dinoflagellate only without added filtrate) were set up. After 0.5, 1, 2, 4, and 6 h of incubation, swimming behaviors of target dinoflagellate cells were examined as described above.

Numerical response by dinoflagellates to *Vibrio* concentration

Experiment 2 was designed to investigate numerical responses by red-tide dinoflagellates *Amphidinium carterae*, *Cochlodinium polykrikoides*, *Gymnodinium impudicum*, *P. minimum*, and *Prorocentrum micans* to the concentration of *V. parahaemolyticus* as a function of elapsed incubation time.

Dense cultures of each red tide alga (80,000-1,600,000 cells) and/or *V. parahaemolyticus* (ca, 8×10^5-8×10^8 cells) were transferred to 80 ml PC bottles. Triplicate experimental bottles (mixture of target dinoflagellate and *V. parahaemolyticus*) and triplicate control bottles (target dinoflagellate only) were established at each *V. parahaemolyticus* concentration. The initial concentrations of *V. parahaemolyticus* were 1×10^4, 1×10^5, 8×10^5, 1×10^6, 1×10^7 cells ml^{-1}. The bottles were filled to capacity with freshly filtered seawater, and then placed on the shelf (30 μE $m^{-2}s^{-1}$). From day 0 to day 6, a 4 ml aliquot was removed from each bottle everyday and fixed with 5% Lugol's solution, while another 4 ml aliquot were fixed with 4% formalin. All or >300 predator cells, fixed in Lugol's solution, in three 1 ml Sedqwick-Rafter counting chambers were enumerated. The aliquots fixed with formalin were filtered onto 0.2 μm pore sized, 25 mm PC black membrane filters and then the concentrated cells on the membranes were observed under an epifluorescence microscope (Olympus BX51) with UV-light excitation at a magnification of 1000x to determine the concentration of bacteria stained using 4'6'-diamidino-2-phenylindole (DAPI. final con.:1 μM).

The specific growth rate of target dinoflagellate, μ (d^{-1}), was calculated by averaging the growth rates obtained at each interval as follows:

$$\mu = [\mathrm{Ln}\ (G_t/G_0)] \quad (1)$$

Where G_0 is the initial concentration of the dinoflagellate at the beginning of each day and G_t is the final concentration at the end of the day. The first and last days in this calculation were Day 2 and Day 5, respectively.

Ingestion rate of dinoflagellates on *Vibrio*

Experiment 3 was designed to measure the ingestion rates of *Amphidinium carterae*, *Cochlodinium polykrikoides*, *Gymnodinium impudicum*, *Prorocentrum micans*, and *P. minimum* on *V. parahaemolyticus* as a function of the prey concentration.

One or two days before this experiment, *V. parahaemolyticus* cells collected from centrifugation were fluorescently labeled using the method of Sherr et al. (1987), and the fluorescently labeled bacteria (FLB) were added to triplicate 80 ml PC experimental bottles containing mixtures of *V. parahaemolyticus* (1×10^6-2×10^9 cells) and target dinoflagellate (80,000-1,600,000 cells). The abundance of the FLB was 30% of total bacteria. Triplicate control bottles containing only target dinoflagellate were also established. All bottles were filled to capacity with freshly filtered and autoclaved seawater, capped, placed on a shelf and incubated at 20 °C under continuous illumination of 30 μE $m^{-2}s^{-1}$ of cool white fluorescent light. After 1, 5, 10, 20, and 30 min incubation periods, 8 ml aliquots were removed from each bottle, transferred 20 ml vials, and then fixed with borate-buffered formalin (final concentration=3%). The fixed samples were stained using 4'6'-diamidino-2-phenylindole (DAPI. final con.: 1 μM), and then filtered onto 3 μm pore size PC white-membrane filters. The FLB inside a dinoflagellate cell were enumerated under an epifluorescence microscope with blue light excitation. Bacteria (both FLB and non FLB) outside dinoflagellates were also enumerated under an epifluorescence microscope with UV light excitation for non-FLB and blue light excitation for FLB. After subsampling, the bottles were capped, placed on a shelf, and incubated again, as described above. Each value of the ingestion rate (cells $alga^{-1}$ h^{-1}) was obtained. The relationship of ingestion rates to prey abundance was fitted to a Michaelis-Menten equation:

$$IR = I_{max}(x)/[K_{IR} + (x)] \quad (2)$$

Where I_{max} is the maximum ingestion rate (cells alga^{-1}h^{-1}); x the prey concentration (cells ml^{-1}), and K_{IR} the prey concentration sustaining 1/2 I_{max}. The prey concentration is the sum of living bacteria and FLBs.

A feeding experiment of *C. polikrikoides* on *V. parahaemolyticus* was also performed for 30 min. However, *C. polikrikoides* was killed within 30 min in high density of *V. parahaemolyticus* during this experiment. Thus, we could not measure the ingestion rate at the *V. parahaemolyticus* concentrations > 10^5 cells ml^{-1}.

Before these experiments were conducted, bacteria in the original dinoflagellate cultures were eliminated down to 1.4×10^4 cell ml^{-1} with a dilution method using filtered and autoclaved seawater.

3. Results

Effects of *V. parahaemolyticus* concentration and incubation time

The red tide dinoflagellates tested in the present study responded differently to a dense culture of *V. parahaemolyticus* or its filtrate. The body of *C. polikrikoides* was decomposed within 20 min after the addition of either dense *V. parahaemolyticus* culture (1×10^7 cells ml^{-1}) or filtrate of the culture (Fig. 1). However, the shape of *P. micans* did not changed by either *V. parahaemolyticus* nor the filtrate (Fig. 2). The shape of *P.minimum and A.carterae* did not change likewise. *Gymnoninium impudicum,* having a similar shape with *C. polykrikoides* was decomposed after 1 hour.

The abundance of all dinoflagellates tested in the present study was affected by *V. parahaemolyticus* (Fig. 3-7). However, the degree of effectiveness (i.e. growth rate) was species-dependent. In addition, the concentrations of *V. parahaemolyticus* in which each of the red tide dinoflagellates was killed were also different among the species.

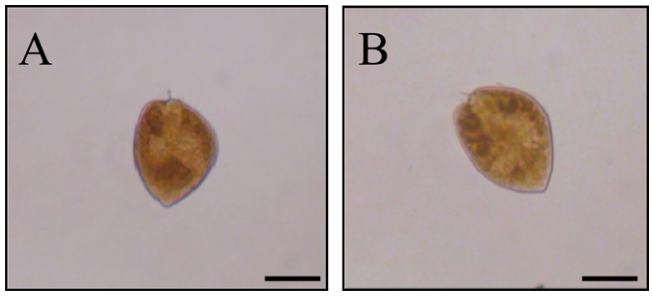

Fig. 2. The body shape of *Prorocentrum micans* cells at the beginning of the experiment (A) and 1 hour later after addition of 1×10^7 cells ml^{-1} *Vibrio parahaemolyticus*. Scale bar=10 μm

With increasing incubation time, the abundances of *A. carterae* in control and at all *V. parahaemolyticus* concentrations increased (Fig. 3A). However, the growth rate of *A. carterae* at all *V. parahaemolyticus* concentrations provided here were not significantly different from that in the control ($p>0.1$, one-tailed t test; Fig. 3B). This evidence suggests that the growth of *A. carterae* may be not significantly affected by *V. parahaemolyticus*.

With increasing incubation time, the abundances of *Prorocentrum minimum* increased at all *V. parahaemolyticus* concentrations, except the control and the highest concentration (Fig. 4A). The growth rate of *P. minimum* at *V. parahaemolyticus* concentration of 8.8×10^5 cells ml^{-1} was significantly higher than that in the control ($p<0.05$, one-tailed t test). However, the growth rates of *P. minimum* at the other *V. parahaemolyticus* concentrations were not significantly different from that in the control ($p>0.1$, one-tailed t test; Fig. 4B). This evidence suggests that the growth of *P. minimum* may only be stimulated by *V. parahaemolyticus* at concentrations of 8.8×10^5 cells ml^{-1}.

With increasing incubation time, the abundances of *Prorocentrum micans* increased in the control and at all *V.*

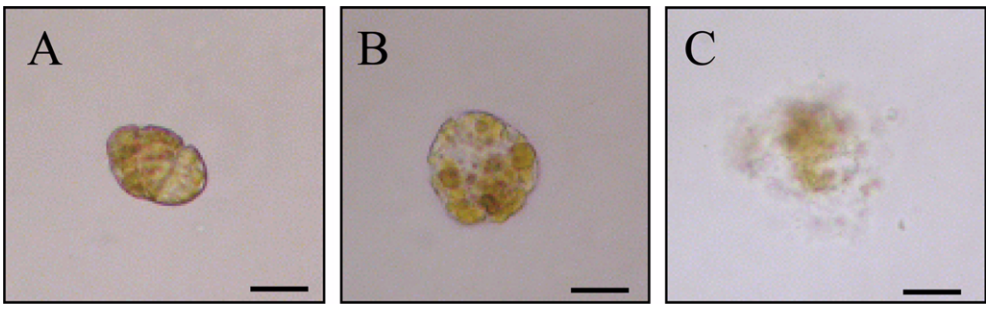

Fig. 1. The body shape of *Cochlodinium polykrikoides* cells at the beginning of the experiment (A) and 40 minute (B) and 1 hour later after addition of 1×10^7 cells ml^{-1} *Vibrio parahaemolyticus*. Scale bar=10 μm

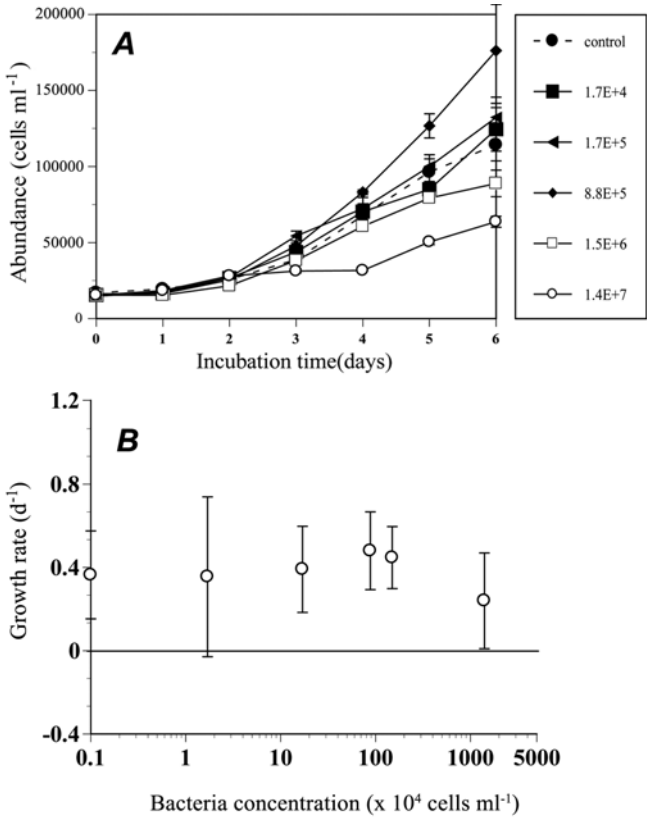

Fig. 3. The abundance (A) and growth rate (B) of *Amphidinium carterae* as functions of the abundance of *Vibrio parahaemolyticus* and elapsed incubation time. Legends represent the concentration of *V. parahaemolyticus*. Control: without *V. parahaemolyticus*. Symbols represent means ± standard errors. See text for calculation of the growth rate

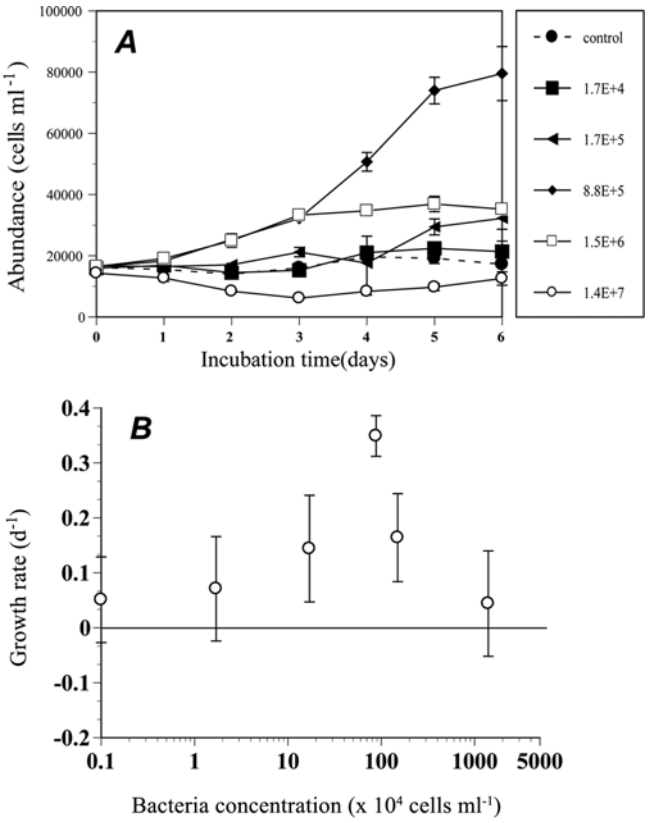

Fig. 4. The abundance (A) and growth rate (B) of *Prorocentrum minimum* as functions of the abundance of *Vibrio parahaemolyticus* and elapsed incubation time. Legends represent the concentration of *V. parahaemolyticus*. Control: without *V. parahaemolyticus*. Symbols represent means ± standard errors. See text for calculation of the growth rate

parahaemolyticus concentrations, except at the highest concentration, (Fig. 5A). The growth rates of *P. micans* at all *V. parahaemolyticus* concentrations were not significantly different from that in the control ($p > 0.1$, one-tailed t test; Fig. 5B). This evidence suggests that the growth of *P. micans* may be not affected by *V. parahaemolyticus*.

With increasing incubation time, the abundances of *G. impudicum* increased in the control and at the *V. parahaemolyticus* concentrations $\leq 1.5\times10^6$ cells ml^{-1}, but decreased at the *V. parahaemolyticus* concentration of 1.4×10^7 cells ml^{-1} (Fig. 6A). The growth rate of *G. impudicum* at the *V. parahaemolyticus* concentration of 1.4×10^7 was significantly lower than that in the control ($p<0.05$, one-tailed t test), while growth rates at the other *V. parahaemolyticus* concentrations were higher than in the control ($p<0.05$, one-tailed t test; Fig. 6B). This evidence suggests that the growth of *G. impudicum* may be negatively affected by *V. parahaemolyticus* at the concentration of 1.4×10^7 cells ml^{-1}, but it may be positively affected at lower bacterial concentrations.

With increasing incubation time, the abundances of *Cochlodinium polykrikoides* in control and at the *V. parahaemolyticus* concentration of 1.7×10^4-8.8×10^5 cells ml^{-1} did not markedly change, while those at the higher concentrations decreased (Fig. 7A). Most *C. polykrikoides* cells died after 1 day of incubation when the *V. parahaemolyticus* concentration was 1.4×10^7 cells ml^{-1}, while cells died within 2 days of incubation when the bacterial concentration was 1.5×10^6 cells ml^{-1}. The growth rates of *C. polykrikoides* at the *V. parahaemolyticus* concentration of 1.7×10^4-8.8×10^5 cells ml^{-1} was not significantly different from that in the control ($p>0.1$, one-tailed t test; Fig. 7B). However, the growth rates of *C. polykrikoides* at the *V. parahaemolyticus* concentrations of 1.5×10^6 and 1.4×10^7 cells ml^{-1} were -0.6

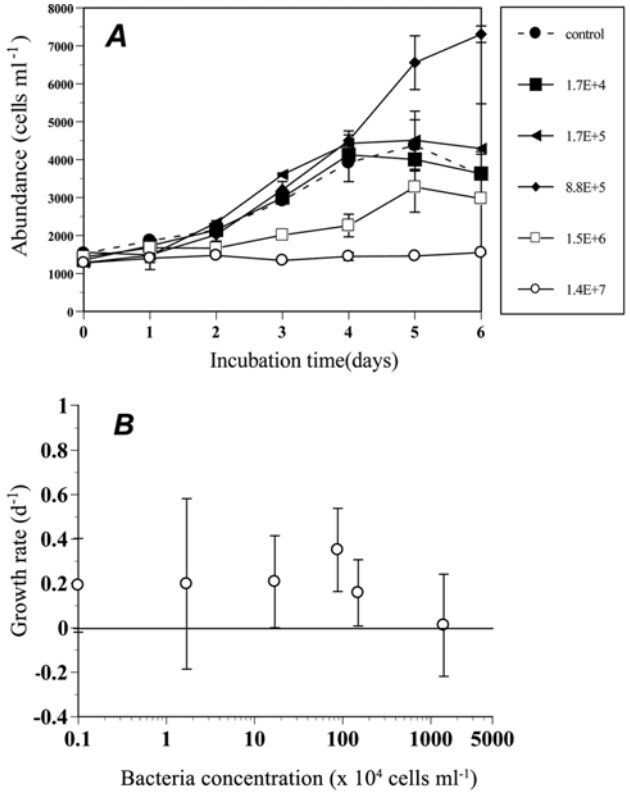

Fig. 5. The abundance (A) and growth rate (B) of *Prorocentrum micans* as functions of the abundance of *Vibrio parahaemolyticus* and elapsed incubation time. Legends represent the concentration of *V. parahaemolyticus*. Control: without *V. parahaemolyticus*. Symbols represent means ± standard errors. See text for calculation of the growth rate

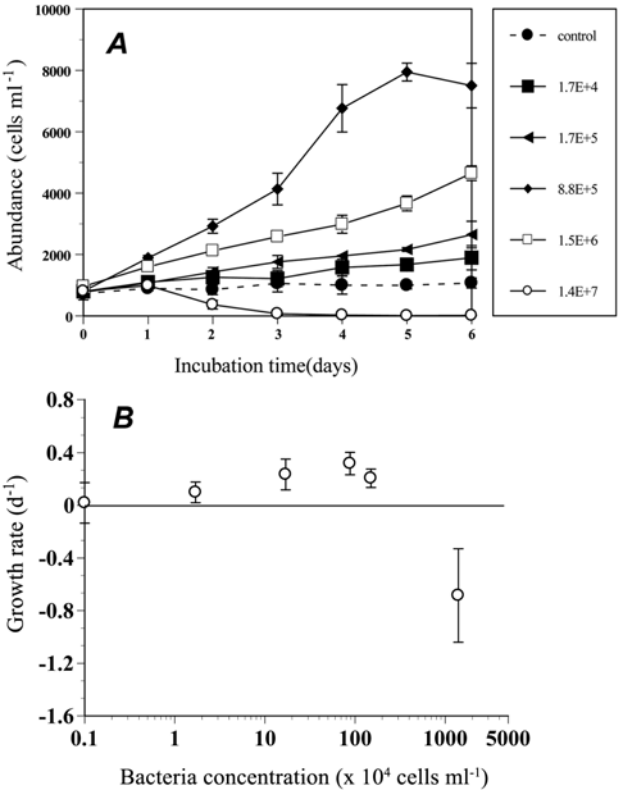

Fig. 6. The abundance (A) and growth rate (B) of *Gymnodinium impudicum* as functions of the abundance of *Vibrio parahaemolyticus* and elapsed incubation time. Legends represent the concentration of *V. parahaemolyticus*. Control: without *V. parahaemolyticus*. Symbols represent means ± standard errors. See text for calculation of the growth rate

and -1.0 d^{-1}, respectively. This evidence suggests that the growth of *C. polykrikoides* may be negatively affected by *V. parahaemolyticus* at the bacterial concentrations ≥1.5×10^6 cells ml^{-1}.

Ingestion rates of dinoflagellates on *V. parahaemolyticus*

The functional response of each of the 5 red tide dinoflagellates to *V. parahaemolyticus* concentration was different from that of the other dinoflagellates (Fig. 8). With increasing *V. parahaemolyticus* concentration, the ingestion rates of *A. carterae* and *P. minimum* increased rapidly at prey concentrations of < 1-3×10^6 cells ml^{-1} and slowly at higher prey concentrations (Fig. 8A,B). When the data were fitted to Eq. (2), the maximum ingestion rates of *A. carterae* and *P. minimum* on *V. parahaemolyticus* were 1.2 and 5.1 cells $alga^{-1}$ h^{-1}, respectively. The maximum clearance rates of *A. carterae* and *P. minimum* on *V. parahaemolyticus* were 0.1 and 0.3 nl $alga^{-1}h^{-1}$, respectively.

With prey concentrations of < 1.4×10^7 cells ml^{-1}, the ingestion rate of *P. micans* on *V. parahaemolyticus* increased linearly (Fig. 8C). The highest value among the ingestion rates was 55 cells $alga^{-1}h^{-1}$. The maximum clearance rate of *P. micans* on *V. parahaemolyticus* was 3.7 nl $algae^{-1}h^{-1}$.

The ingestion rates of *Gymnodinium impudicum* on *V. parahaemolyticus* were between 1.5-2.5 cells $alga^{-1}h^{-1}$ without any particular pattern (Fig. 8D).

With increasing prey concentrations, the ingestion rate of *C. polykrikoides* on *V. parahaemolyticus* decreased (Fig. 8E). The rate was not detected at prey concentrations of < 1.5×10^6 cells ml^{-1}.

4. Discussion

***Vibrio parahaemolyticus* as a killer**

The results of the present study show that the harmful pathogenic bacterium *V. parahaemolyticus* can be a killer and/or prey for red tide dinoflagellates. At *V. parahaemolyticus* concentrations of ≤1.5×10^6 cells ml^{-1}, *C. polykrikoides* is a

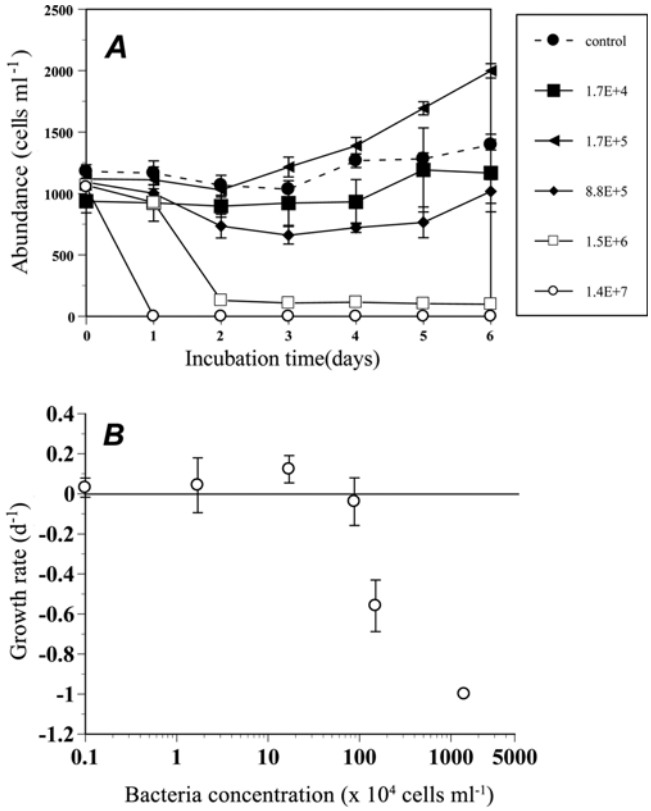

Fig. 7. The abundance (A) and growth rate (B) of *Cochlodinium polykrikoides* as functions of the abundance of *Vibrio parahaemolyticus* and elapsed incubation time. Legends represent the concentration of *V. parahaemolyticus*. Control: without *V. parahaemolyticus*. Symbols represent means ± standard errors. See text for calculation of the growth rate

victim of *V. parahaemolyticus*. Additionally, at the *V. parahaemolyticus* concentration of 1.4×10^7 cells ml^{-1}, *G.impudicum* was also a victim. However, *A.carterae*, *P. minimum*, and *P. micans* are mainly grazers on *V. parahaemolyticus* at *V. parahaemolyticus* concentrations of $\leq1.5\times10^6$ cells ml^{-1}, while they could be victims as well as grazers at the higher *V. parahaemolyticus* concentrations. Nayak et al. (2000) reported that the concentrations of *Vibrio harveyi, V. alginolyticus,* and *V. parahaemolyticus,* which was incubated with *A. carterae,* gradually decreased. *A. carterae* was likely to feed on these *Vibrios*, even though the authors did not mention this possibility.

Algicidal bacteria have been known to kill algae or inhibit their growth through direct contact with algal cells (Manage et al. 2000; Furusawa et al. 2003), or indirectly through release of toxic compounds into the ambient environment (Holmstrom and Kjelleberg, 1999; Nakashima et al. 2006). *Psedomonas* sp. *Flavobacteria* sp., *Alteromonas* spp., *Psedoalteromonas* spp., *Bacillus* sp., and *Hahella chejuensis* spp. are known to produce extracellular algicidal substances (Kim et al. 2008). Most of them are able to secrete metabolic compounds and might be used as biological control agent in natural seawater (Fukami et al. 1992; Wang et al. 2005). *C. polikrikoides* was decomposed within 20 min after the addition of either dense *V. parahaemolyticus* culture or filtrate of the culture. Thus, *C. polikrikoides* may be killed by direct physical contact and/or potential extracellular substances. *C. polykrikoides* has a thin surface membrane, while *A. carterae* and *G. impudicum* have relatively thick surface membrane, so called amphiesmal vesicles (Fraga et al. 1995). Furthermore, *P. minimum* and *P. micans* have theca (Roberts et al. 1995). Thus, *V. parahaemolyticus* or its excreting materials may easily penetrate and kill *C. polykrikoides* cells, while it has difficulty in penetrating and killing the cells of the other dinoflagellates. *V. parahaemolyticus* may deter the outbreak of red tides dominated by *C. polykrikoides* and/or accelerate the decline of red tides. Differential harmful effects by *V. parahaemolyticus* on *C. polykrikoides* compared to the other dinoflagellates may cause predominance by the other dinoflagellates over *C. polykrikoides*.

Red tides dominated by *C. polykrikoides* have caused great losses in many countries (Gárate-Lizárraga et al. 2004; Kim et al. 2004; Kim et al. 2007; Richlen et al. 2010). For example, its red tides have caused losses of up to USD $ 60 million per year in the Korean aquaculture industry (NFRDI 1998). Thus, diverse methods of controlling the outbreak and persistence of red tides dominated by *C. polykrikoides* and thereby reducing their economic impacts have been suggested (Jeong et al. 2002, 2008). Use of algicidal bacteria is one of the methods widely suggested (e.g. Imai et al. 1995). The bacterium *Micrococcus* sp. LG-5 and *Psedomonas* sp. LG-2 have also been reported to kill *C. polikrikoides* (Jeong et al. 2000; Lee et al. 2008). However, these bacteria also killed several other red tide dinoflagellates. Thus, *V. parahaemolyticus* can be the only effective algicidal bacterium against *C. polikrikoides*, and not the other red tide dinoflagellates (i.e. semi-species specific).

The bacterium *Psedoalteromonas haloplanktis* AFMB-008041 has been known to kill *P. minimum*, while *Micrococcus* sp. LG-5 and *Psedomonas* sp. LG-2 kill *P. micans* (Jeong et al. 2000; Kim et al. 2009; Table 1). However, the bacterium *Alteromonas* sp. which killed *Akashiwo sanguinea, C. polykrikoides, Gymnodinium catenatum, and Heterocapsa*

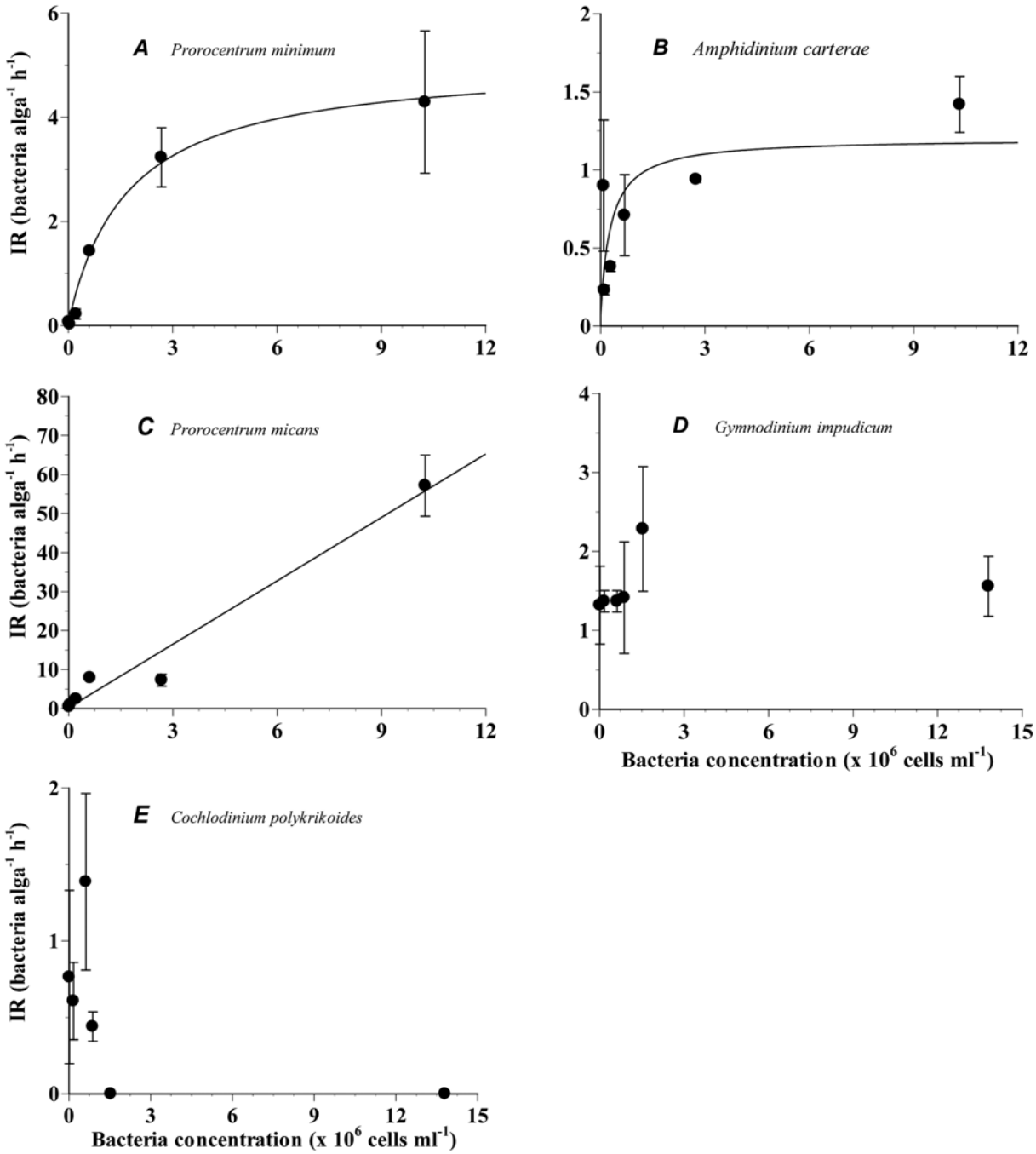

Fig. 8. Ingestion rate (cells alga h^{-1}) of *Prorocentrum minimum* (A), *Amphidinium carterae* (B), *P. micans* (C), *Gymnodinium impudicum* (D), and *C. polykrikoides*(E) on *Vibrio parahaemolyticus* a function of the initial bacterial concentration (x, cells ml^{-1}). Each ingestion rates was calculated by exploration from a linear regression curve on the number of prey cells inside a dinoflagellate predator cell over incubation time. Symbols represent treatment mean ± 1 SE. The curves were fitted by a Michaelis-Menten equation (Eq 2) in (A) and (B) and a linear equation in (C) using all treatments in the experiment. Ingestion rate (IR, cells dinoflagellate$^{-1}h^{-1}$)=5.1 [x/(1,700,000+x)], r^2=0.991 in (A), IR=1.2 [x/(270,000+x)], r^2=0.435 in (B), IR=5.378x+0.319, r^2=0.968 in (C)

triquetra did not kill *P. minimum* and *P. micans* (Lee et al. 2008). Therefore, the impact of algicidal activities on red tide algae including *P. minimum* and *P. micans* is *Psedoalteromonas haloplanktis* AFMB-008041, *Micrococcus* sp. LG-5, and *Psedomonas* sp. LG-2 > *Alteromonas* sp. > *V. parahaemolyticus*.

***Vibrio parahaemolyticus* as prey**

All red tide dinoflagellates tested in the present study were able to feed on *V. parahaemolyticus*. However, *C. polikrikoides* was not able to feed on this bacterium at bacterial concentrations of $\leq 1.5\times10^6$ cells ml^{-1} because the dinoflagellate was killed at this concentration.

The maximum ingestion rate of *P. minimum* on *V. parahaemolyticus* (5.1 cells $alga^{-1}h^{-1}$) were considerably lower than that on mixed bacteria, which originally lived in dinoflagellate culture (21.9 cells $alga^{-1}h^{-1}$; Seong et al. 2006). Furthermore, the maximum ingestion rate of *C. polikrikoides* on *V. parahaemolyticus* (1.3 cells $alga^{-1}h^{-1}$) was also much lower than that on mixed bacteria in Masan Bay (17.4 cells $alga^{-1}h^{-1}$; Seong et al. 2006). The size of *V. parahaemolyticus* used in the present study was similar to that of bacteria used in Seong et al. (2006). Thus, for the red tide dinoflagellate predators, *V. parahaemolyticus* may not be as good prey as mixed bacteria used in Seong et al. (2006).

The growth rate of *P. minimum* at the *V. parahaemolyticus* concentration of 8.8×10^5 was significantly higher than that in the control. The daily acquired bacterial carbon by *P. minimum* from *V. parahaemolyticus* [8.2 pg C ($5.1\times24\times0.067$ pgC)] was only 6.3% of the body carbon of *P. minimum*. Thus, *V. parahaemolyticus* cannot only support the positive growth of *P. minimum*. However, *V. parahaemolyticus* may stimulate or partially support the growth of *P. minimum*. Another bacterium, *Alteromonas* sp. strain A14, was known to stimulate the growth of *P. minimum* at the prey concentration of ~10^6 cell ml^{-1} (Lee et al. 2008).

In conclusion, *V. parahaemolyticus* can be killer and simultaneous prey for all red tide dinoflagellates tested in the present study; *V. parahaemolyticus* induces the most harmful effects on *C. polykrikoies*; Bacterial concentration and incubation time were important factors; With increasing *V. parahaemolyticus* concentration, ingestion rates of *P. minimum*, *P. micans*, and *A. carterae* on the prey increased, whereas ingestion rates on *C. polykrikoides* decreased.

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