

## 보 문

# Isolation of marine algicidal bacteria from surface seawater and sediment samples associated with harmful algal blooms in Korea

Sylvia Kristyanto and Jaisoo Kim\*

Department of Life Science, Kyonggi University, Suwon 16227, Republic of Korea

## 유해조류번성 주변의 해수와 침전물에서 살조균의 분리

실비아 그리스티안토 · 김재수\*

경기대학교 자연과학대학 생명과학과

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**ABSTRACT:** This study mainly focused on isolation of marine algicidal bacteria associated with phytoplankton blooms and characterization of algicidal activity against harmful algae. Harmful algal blooms (HABs) found naturally in surface waters have caused many environmental problems worldwide. In this study, forty bacterial strains that have capability of inhibiting harmful algal growth were isolated from Masan Bay, Jinhae Bay, Dol Island, Jangmok Bay, and the Tongyeong Sea, Republic of Korea. The bacteria were screened furthermore for the characteristics on algicidal activities against *Cochlodinium polykrikoides*, *Chattonella marina*, *Skeletonema costatum*, *Heterosigma akashiwo*, *Heterocapsa triquetra*, *Prorocentrum minimum*, and *Scrippsiella trochoidea*. As a result, the algicidal bacteria that were screened from double over layer agar and microscopic counts tests belonged to genera *Pseudomonas*, *Vibrio*, *Bacillus*, *Pseudoalteromonas*, *Ruegeria*, *Joostella*, *Marinomonas*, *Stakelama*, *Porphyrobacter*, and *Albirhodobacter*. One of the most important HAB species is *Co. polykrikoides* and the strongest algicidal activity against the dinoflagellate was 94.00% after 6 h treatment with 10% bacterial culture filtrate. In this study, *Marinomonas* sp. M Jin 1-8, *Stakelama* sp. ZB Yeonmyeong 1-11 & 1-13, *Porphyrobacter* sp. M Yeonmyeong 2-22, and *Albirhodobacter* sp. 6-R Jin 6-1 were found to be as new genera of bacteria having anti-algal activity. These results suggest that these bacteria might play an important role in controlling phytoplankton blooms.

**Key words:** *Albirhodobacter*, *Cochlodinium polykrikoides*, *Marinomonas*, *Porphyrobacter*, *Stakelama*, algicidal bacteria, harmful algal blooms

Harmful algal blooms (HABs), commonly termed as “red tides”, are massive proliferation of plankton algae that have occurred throughout the world in marine environment and have increased in frequency nowadays. The blooms have been caused by toxic and non-toxic harmful algae species that have affected negative impacts ecologically and have led to severe disruptions almost every year in marine ecosystems. The algal toxins have caused  $\pm$  2,000 human poisoning cases reported each year and the most toxic algal species are among dinoflagellates (Zingone and Enevoldsen, 2000; Kim *et al.*,

2009b). The first damage case of HAB was caused by *Karenia mikimotoi* in 1981 in Korea and resulted in losses 1.7 million dollars (Kim *et al.*, 2010). In the 1970s and 1980s, the dominant HABs species were diatom *Skeletonema costatum* and dinoflagellate *K. mikimotoi*. However, in the 1990s and 2000s, *Cochlodinium polykrikoides*, *Ceratium* spp., and *Chattonella* spp. were the dominant red tide species along Korean waters (Lee *et al.*, 2013; Park *et al.*, 2013). Particularly, *Co. polykrikoides* blooms have caused large economic losses in the Republic of Korea estimated at about 7.2 million dollars in 2003 (Kim *et al.*, 2008).

Causative HABs species can be classified into three different categories; species which cause discolorations of water, species

\*For correspondence. E-mail: jkimtamu@kgu.ac.kr;  
Tel.: +82-31-249-9648; Fax: +82-31-253-1165

which produce potent toxins that can be concentrated through food chains to humans causing gastrointestinal and neurological illnesses, and species which non-toxic to humans but harmful to fish and invertebrates by seriously damaging their gills through production of substances (Hallegraeff, 1993). HABs are usually caused by non-toxic microalgae (over 70%). However, the studies have concentrated to toxic microalgae rather than non-toxic microalgae that have resulted in lacking information about non-toxic organisms (Shi *et al.*, 2013).

HABs cause harm due to the toxins (harmful metabolites) or high biomass of phytoplankton that causes oxygen depletion in water environment, destruct habitat for water environment, and alter food web dynamics (Anderson *et al.*, 2002). There are several methods to mitigate the negative effects of HABs, including mechanical (application of yellow clay dispersion), biological (use of organisms as the biological agents), chemical (use of species-specific chemical control agents), genetic (genetic engineering of species), and environmental controls (environmental manipulation/physical or chemical modifications of the environment) (Anderson, 2009). Finding the effective and safe algicidal agent is urgently needed before applying to natural bloom areas. The classical approach for detecting HABs species is by direct observation (with microscopes) and flow cytometry for detection of HAB populations was developed as an alternative approach.

HABs have caused environmental problem worldwide and have increased in frequency nowadays. Although there are several efforts applied to mitigate the effect of HABs such as clay flocculation, no significant improvement has been achieved and might have some impacts on marine organisms (Sengco and Anderson, 2004). Many reports revealed that algicidal bacteria could be potential as effective killer of red tide organisms and many studies have focused on the relationships between algicidal bacteria and algae due to potential use of these bacteria for controlling HABs (Kim *et al.*, 1998; Mayali and Azam, 2004; Oh *et al.*, 2011; Yang *et al.*, 2012).

Biological control agents such as viruses, protozoa, zooplankton, protists, actinomycete, and macrophytes could be applied as potential killers of HABs species (Nagasaki *et al.*, 2004; Kang *et al.*, 2008; Zheng *et al.*, 2013; Yang *et al.*, 2014). However, the abundances of these organisms were low as compared to bacteria. Therefore, these organisms might not be

suitable as biological control agents. Many bacteria isolated from marine coastal waters have been known as potential killers of red tide organisms. These bacteria consist of various genera, including *Pseudomonas*, *Pseudoalteromonas*, *Vibrio*, *Bacillus*, *Micrococcus*, *Alteromonas*, *Cytophaga*, *Cellulophaga*, *Planomicrobium*, *Flavobacterium*, *Zobellia*, *Saprospira*, *Kordia*, *Hahella*, *Ruegeria*, and *Joostella* (Mayali and Azam, 2004; Amaro *et al.*, 2005; Seong and Jeong, 2013; Yang *et al.*, 2014). Recently, many scientists have focused on the use of algicidal bacteria as biocontrol to minimize the effects of HABs and correlations of marine bacteria-red tide species.

In this study, algicidal bacteria designated as effective strains were all indirect attack types and identified based on comparative 16S rRNA gene sequencing analysis. The effective strains that have the strongest algicidal activities and algicidal effect for each algal species will be selected for further study to investigate the basic knowledge about relationships between bacteria and microalgae.

## Materials and Methods

### Microalgal cultures

In this study, four dinoflagellates (*Co. polykrikoides*, *Hc. triquetra*, *P. minimum*, and *Sc. trochoidea*), two raphidophytes (*Ch. marina* and *Hs. akashiwo*), and one diatom (*Sk. costatum*) were used. *Co. polykrikoides* and *Ch. marina* cultures were provided by Korea Ocean Research & Development Institute (KORDI), and *Hc. triquetra*, *P. minimum*, *Sc. trochoidea*, *Hs. akashiwo*, and *Sk. costatum* cultures were supplied by the Korea Marine Microalgae Culture Center at Pukyong National University (KMMCC, Busan, Republic of Korea). All cultures except *Sk. costatum* were routinely maintained in f/2-Si medium (Guillard and Ryther, 1962) made of GF/F-filtered seawater at 20°C under white fluorescent light (38  $\mu\text{mol photons/m}^2/\text{sec}$ , 12: 12 h light: dark cycle). *Sk. costatum* was cultured in f/2 medium (Sigma Aldrich) made of GF/F-filtered seawater at 20°C under white fluorescent light (38  $\mu\text{mol photons/m}^2/\text{sec}$ , 12:12 h light:dark cycle). The cell numbers of axenic algal culture fixed with formaldehyde 1% (v/v) were counted with a Sedgewick-Rafter counting chamber at magnification  $\times 200$  using a light microscope (Korea Labtech; Olympus). Algal cell

densities were monitored routinely using microscopic counts.

### Bacterial culture

Marine bacterial strains used in this study were grown in eight different kinds of media. Marine broth (Difco) with/without seawater, R2A broth (MB Cell) with/without seawater, ZoBell broth (peptone 5 g, yeast extract 1 g,  $\text{FePO}_4 \cdot 4\text{H}_2\text{O}$  0.01 g, aged seawater 750 ml, distilled water 250 ml, pH 7.2), Super ZoBell broth (ZoBell containing 20 g of glucose), Diluted ZoBell broth (10% of ZoBell) (Park *et al.*, 2002), and pure seawater were used as bacterial media.

### Sampling

In this study, surface seawater and sediment samples were sampled on 31 March, 01 April, and 14 July 2014. Seawater samples were collected from a depth of 1 m using a Van Dorn sampler as well as sediment samples using a sediment sampler in Masan Bay (35°6'12.88"N and 128°36'14.31"E), Jinhae Bay (35°6'55.77"N and 128°41'7.16"E), Dol Island (35°11'0.44"N and 128°34'59.31"E), Jangmok Bay (34°59'38.19"N and 128°40'26.38"E), and the Tongyeong Sea (34°77'86.20"N and 128°39'62.73"E), Korea. The samples were placed into sterilized glass bottles, cooled into dark ice chest and transported to the laboratory.

### Isolation and screening of algicidal bacteria

Seawater samples were filtered through 0.22  $\mu\text{m}$  pore-size membrane filter (GF/F; Merck Milipore). A portion of the membrane filter paper was inoculated into the six-transwell plates containing bacterial media and the plates were incubated with shaking at 120 rpm in 1–2 weeks at 25°C. After serial 10-fold dilutions with bacterial media, 100- $\mu\text{l}$  aliquots of diluted samples were spread onto agar plates to isolate marine bacterial strains. The plates were incubated at 25°C for 1 week, and all colonies with different colour and morphology were transferred onto fresh bacterial media agar plates. Once purified, the isolates were then cryopreserved at -20°C containing 20% glycerol and subculture on Marine broth 2216 (Difco) before use as inoculums.

Initial screening for algicidal bacteria was conducted with double over layer agar method to isolate the putative algicidal

strains. The method used spreading of algal lawns (onto algal media agar plates (f/2 agar plates) as the bottom layer and spotting of bacterial cultures onto bacterial agar media as the upper layer. In this method, 100- $\mu\text{l}$  aliquots of algal cells were spread onto the bottom layer of agar plates. Then, the plates were incubated at 20°C using a 12 h:12 h light:dark cycle and after 3–4 days of incubation, the algal lawns were created onto f/2 media agar plates. For the upper layer, bacterial cells were spotted onto the surface of the plates and used to investigate the possibility of bacterial strains inhibiting harmful algal cells. Isolates were precultured first into Marine broth 2216 at 25°C for 3 days with shaking at 120 rpm. After cultivation, the cultures were spotted onto bacterial agar media and the plates were incubated at 25°C for 2–3 days. The appearance of inhibition zone around spotting area showed that the bacterial strains had algicidal activity against harmful algal species and were designated as effective strains that would be characterized furthermore for algicidal activities.

### Identification of algicidal bacteria

To identify the effective isolates, DNA isolation from bacteria was performed and for PCR amplification of 16S rRNA gene fragment, universal primers 27F (5'-AGAGTTTGATCMTGG CTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGAC TT-3') were used. Sequencing was done by Macrogen Korea. Identification of effective bacterial strains was carried out by comparing the almost full-length sequence of the bacteria with those of other 16S rRNA gene sequences from Eztaxon server database (Kim *et al.*, 2012).

### Determination of algicidal activity

To examine the algicidal activities of the effective strains, the strains were inoculated into Marine broth 2216 medium at 25°C for 3 days with shaking at 120 rpm (ca.  $10^6$ – $10^7$  cells/ml). Bacterial cells were centrifuged at  $5,000 \times g$  for 20 min (4°C) and then immediately filtered with 0.2  $\mu\text{m}$  pore-size membrane filter (Whatman, GE Healthcare). After filtration, bacterial culture filtrates were obtained and used for screening of algicidal activity furthermore. The algal cells were cultured in f/2-Si media and f/2 media (for *Sk. costatum*). 1,800  $\mu\text{l}$  algal cultures at mid-exponential growth phase (10–12 days after

incubation) were inoculated into each well of 24-well cell culture plate (SPL Life Sciences). 200  $\mu$ l of each bacterial culture filtrate (10% bacterial culture filtrates) was added to each plate containing algal cultures. The 24-well plates were then incubated for 6 hours and as control for this experiment, an equal volume of fresh Marine broth 2216 medium was added to 24-well plate containing algal cultures. After 6 h treatment, a portion of sample was fixed with a formaldehyde solution at final concentration 1% and the numbers of viable algal cells were counted directly on a Sedgewick-Rafter counting chamber at magnification of  $\times 200$  using a light microscope. The algicidal activity was calculated as follows: Algicidal activity (%) =  $\{1 - (\text{viable individuals of algal cells after the treatment} / \text{initial individuals of algal cells})\} \times 100$  (Byun *et al.*, 2002; Jung *et al.*, 2008; Kim *et al.*, 2008). The experiments were carried out in duplicate for control and treatment.

## Results and Discussion

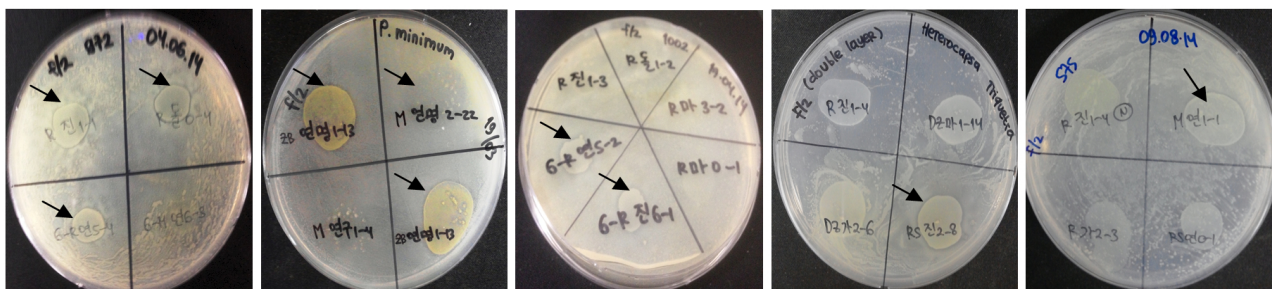
### Screening and identification of algicidal bacteria

In this study, we found forty effective strains from initial screening with double over layer agar method, and examined furthermore for determination of algicidal activities. The strains displayed inhibitory activity against harmful algal species tested and were designated as effective strains. The appearance of inhibition zone showed that the bacteria had anti-algal activity (Fig. 1). Double over layer agar method is culture-dependent method used during our study to isolate the possible bacterial strains that have capability to inhibit the growth of algal cells. This method was carried out as initial screening of algicidal bacteria because this method was more effective by

spending less time (about 2–3 days) than paper discs method to screen algicidal bacteria. Although paper discs method is better in displaying inhibition activity, but double over layer agar method could be potential method to screen the algicidal bacteria strains because this method could be fast screening method to give reliable screening results.

Algicidal strains designated as effective strains were identified with 16S rRNA gene sequence comparison through the bacterial sequence database, EzTaxon-e database (Table 1). Most of the sequences of effective strains showed the highest homology to sequences belonged to genera *Pseudomonas* (26 strains). Based on 16S rRNA gene sequence analysis, the effective strains belong to genera *Pseudomonas*, *Pseudoalteromonas*, *Vibrio*, *Ruegeria*, *Joostella*, *Bacillus*, *Marinomonas*, *Porphyrobacter*, and *Stakelama*.

Many reports revealed that alga-associating bacteria particularly belong to  $\alpha$ - and  $\gamma$ -*Proteobacteria* subclasses and Cytophaga-Flavobacterium-Bacteroides (CFB) group, and there is a correlation between decline of phytoplankton blooms and abundance of algicidal bacteria (Grossart *et al.*, 2005; Oh *et al.*, 2011; Yang *et al.*, 2013). Genera *Pseudomonas*, *Pseudoalteromonas*, *Vibrio*, *Ruegeria*, *Bacillus*, and *Joostella* are well known as algicidal bacteria found so far (Mayali and Azam, 2004; Amaro *et al.*, 2005; Yang *et al.*, 2014). However, in our study, uncommon algicidal bacteria identified as *Marinomonas*, *Porphyrobacter*, *Stakelama*, and *Albirhodobacter* that showed algicidal activity from initial screening with double over layer agar and microscopic counts. In marine environment, the  $\alpha$ - and  $\gamma$ -*Proteobacteria* subclasses are the majority of alga-associating bacteria (Oh *et al.*, 2011). It is consistent with our results during our study in which *Pseudomonas* sp., *Pseudoalteromonas* sp., *Vibrio* sp., *Marinomonas* sp. are included in  $\gamma$ -*Proteobacteria*



**Fig. 1.** Screening of algicidal bacteria by using double over layer agar method (left to right: algicidal activity against *Hs. akashiwo*, *P. minimum*, *Sk. costatum*, *Hc. triquetra*, and *Sc. trochoidea*).

**Table 1.** Summary of algicidal bacteria found in this study based on 16S rRNA gene sequences

Origin	Strain	Closest species	Identity (%)
Masan Bay	DZ Ma 1-19	<i>Pseudomonas cremoricolorata</i> NRIC 0181	99.73
	MS Ma 0-4	<i>Pseudomonas cremoricolorata</i> NRIC 0181	99.73
	R Ma 2-3	<i>Pseudomonas mosselii</i> CIP 105259	99.32
Jinhae Bay	M Jin 1-8	<i>Marinomonas alcarazii</i> IVIA-Po-14b	100
	M Jin 1-13	<i>Pseudomonas mosselii</i> CIP 105259	99.32
	MS Jin 1-1 (1)	<i>Vibrio crassostreae</i> CAIM 1405	99.93
	MS Jin 1-7	<i>Ruegeria mobilis</i> NBRC 101030	99.78
	R Jin 1-1	<i>Pseudomonas soli</i> F-279,208	99.51
	RS Jin 1-8	<i>Pseudomonas mosselii</i> CIP 105259	99.38
	RS Jin 2-8	<i>Pseudomonas taiwanensis</i> BCRC 17751	99.04
	ZB Jin 2-3	<i>Pseudomonas soli</i> F-279,208	99.23
	ZB Jin 2-9	<i>Joostella marina</i> DSM 19592	98.97
	6-R Jin 6-1	<i>Albirhodobacter marinus</i> N9	99.57
Dol Island	M Dol 1-8	<i>Pseudoalteromonas espejiana</i> NCIMB 2127	99.34
	R Dol 0-4	<i>Pseudomonas mosselii</i> CIP 105259	99.32
Jangmok Bay	DZ Ga 2-3	<i>Pseudomonas mosselii</i> CIP 105259	99.52
	M Ga 1-3	<i>Joostella marina</i> DSM 19592	99.86
	M Ga 1-6	<i>Pseudomonas taiwanensis</i> BCRC 17751	99.73
	MS Ga 1-5	<i>Ruegeria mobilis</i> NBRC 101030	99.35
Tongyeong Sea	M Yeon 1-1	<i>Pseudomonas taiwanensis</i> BCRC 17751	99.79
	M Yeon 3-2	<i>Pseudomonas cremoricolorata</i> NRIC 0181	99.66
	MS Yeon 1-4	<i>Pseudomonas cremoricolorata</i> NRIC 0181	98.98
	MS Yeon 1-1	<i>Pseudomonas cremoricolorata</i> NRIC 0181	99.86
	R Yeon 0-1	<i>Pseudomonas mosselii</i> CIP 105259	99.25
	R Yeon 0-2	<i>Pseudomonas cremoricolorata</i> NRIC 0181	99.59
	R Yeon 0-3	<i>Pseudomonas cremoricolorata</i> NRIC 0181	99.25
	R Yeon 0-4	<i>Pseudomonas taiwanensis</i> BCRC 17751	99.52
	R Yeon 0-6	<i>Pseudomonas taiwanensis</i> BCRC 17751	99.66
	R Yeon 0-9	<i>Pseudomonas mosselii</i> CIP 105259	99.52
	6-R Yeon 5-2	<i>Pseudomonas mosselii</i> CIP 105259	99.32
	6-R Yeon 5-4	<i>Pseudomonas taiwanensis</i> BCRC 17751	99.66
	DZ Yeongu 1-4	<i>Marinomonas alcarazii</i> IVIA-Po-14b	96.18
	M Yeongu 1-2	<i>Pseudomonas mosselii</i> CIP 105259	99.51
	S Yeongu 3	<i>Vibrio alginolyticus</i> NBRC 15630	99.67
	ZB Yeongu 2-15	<i>Pseudomonas taiwanensis</i> BCRC 17751	99.79
	DZ Yeonmyeong 1-2	<i>Bacillus thuringiensis</i> ATCC 10792	99.66
	M Yeonmyeong 2-18	<i>Pseudomonas parafulva</i> AJ 2129	99.8
	M Yeonmyeong 2-22	<i>Porphyrobacter dokdonensis</i> DSW-74	97.09
	ZB Yeonmyeong 1-13	<i>Stakelama pacifica</i> JLT832	98.36
	ZB Yeonmyeong 1-11	<i>Stakelama pacifica</i> JLT832	98.44

subclasses and *Ruegeria* sp., *Porphyrobacter* sp., *Stakelama* sp., and *Albirhodobacter* sp. are included in  $\alpha$ -Proteobacteria subclasses. *Marinomonas* sp. is classified into order Ocean-

ospirillales, the same order with algicidal bacteria *Hahella* sp. *Stakelama* sp. and *Porphyrobacter* sp. are included in  $\alpha$ -Proteobacteria subclasses and order *Sphingomonadales*. Lei et



**Table 2.** Summary of algicidal bacteria found in this study based on 16S rRNA gene sequences. The activities were examined after 6 h incubation in which 10% (v/v) bacterial culture filtrates were added to each algal cultures. For control, fresh Marine broth 2216E was added. Data are expressed as the mean  $\pm$  SD from duplicate assays

Harmful algal bloom-forming species	Effective strains	Algicidal activity (%)
<i>Cochlodinium polykrikoides</i>	<i>Pseudomonas</i> sp. R Jin 1-1	94.00 $\pm$ 0.00
<i>Heterosigma akashiwo</i>	<i>Pseudomonas</i> sp. ZB Jin 2-3	59.12 $\pm$ 3.91
<i>Heterocapsa triquetra</i>	<i>Marinomonas</i> sp. DZ Yeongu 1-4	73.26 $\pm$ 2.08
<i>Prorocentrum minimum</i>	<i>Pseudoalteromonas</i> sp. M Dol 1-8	60.37 $\pm$ 2.11
<i>Scrippsiella trochoidea</i>	<i>Pseudomonas</i> sp. MS Yeon 1-1	86.45 $\pm$ 2.74
<i>Skeletonema costatum</i>	<i>Pseudoalteromonas</i> sp. M Dol 1-8	68.07 $\pm$ 0.20
<i>Chattonella marina</i>	<i>Stakelama</i> sp. ZB Yeonmyeong 1-13	90.16 $\pm$ 1.80

*al.* (2014) reported that *Altererythrobacter* sp. LY02 (family *Erythrobacteraceae*) that was isolated from red tide seawater in China showed algicidal activity against *Alexandrium tamarense*. *Porphyrobacter* sp. also belonged to the family *Erythrobacteraceae* and showed algicidal activity against HABs species tested in this study. *Stakelama* sp. belonged to family *Sphingomonadaceae* and to our knowledge; this is the first report on family *Sphingomonadaceae* showing algicidal activity. *Albirhodobacter* sp. is classified into family *Rhodobacteraceae*. In family *Rhodobacteraceae*, *Sagittula* sp., and *Thalassobius* sp. showed algicidal activity against *Co. polykrikoides* and in previous report, these bacteria were isolated and identified from *Co. polykrikoides* mixed bacterial community (Oh *et al.*, 2011). *Ruegeria* sp. belonged to family *Rhodobacteraceae* also displayed anti-algal activity against the toxic dinoflagellate *A. catenella* (Amaro *et al.*, 2005). To our knowledge, this is the first record of genera *Marinomonas*, *Porphyrobacter*, *Stakelama*, and *Albirhodobacter* displaying algicidal activity to the HABs species. Further studies are needed to investigate furthermore about their algicidal mechanisms and their interactions with algae.

### Algicidal activities of effective strains against HABs species

Algicidal activities of forty effective strains were examined. During our study, bacterial culture filtrates were used for determination of algicidal activity. Among forty effective strains, the strongest algicidal activities were 94.00% for *Co. polykrikoides* (strain R Jin 1-1), 59.12% for *Hs. akashiwo* (strain ZB Jin 2-3), 73.26% for *Hc. triquetra* (strain DZ Yeongu 1-4), 60.37% for *P. minimum* (strain M Dol 1-8), 86.45% for *Sc.*

*trochoidea* (strain MS Yeon 1-1), 68.07% for *Sk. costatum* (strain M Dol 1-8), and 90.16% for *Ch. marina* (strain ZB Yeonmyeong 1-13) (Table 2). In this study, the algicidal activities were found in the culture filtrates (data not shown). The effective strains that displayed the strongest algicidal activities were selected for further study.

Algicidal bacteria can kill their algae prey by direct or indirect attack mode. In direct attack, direct physical contact between bacterial cells and algal cells has been occurred, but in indirect attack mode, algicidal bacteria release algicides (extracellular substances) and degrade most of algal cells. Approximately 70% of algicidal bacteria require indirect attack mode and 30% of algicidal bacteria require direct attack mode to lyse algal cells (Mayali and Azam, 2004; Roth *et al.*, 2008). In this study, algicidal activity was examined using the filtrates regard to the possibility of algicidal bacteria in releasing algicides (algicidal compounds) to degrade or lyse algal cells because all the effective strains were indirect attack types. Further studies were needed to identify the algicidal compounds and the characteristics of the secondary metabolites.

One of the most important red tide species is *Co. polykrikoides* and Kim *et al.* (2007) reported that *P. fluorescens* HAK-13 significantly inhibited the growth of *Co. polykrikoides* and caused cell lysis of *Co. polykrikoides*. In this study, the effective strain R Jin 1-1 (*Pseudomonas* sp.) also had significant algicidal activity against *Co. polykrikoides* and was selected for further study. Lee *et al.* (2000) reported that the algicidal bacterium *Pseudoalteromonas* sp. strain A28 displayed species-specific lysis of diatom *Sk. costatum* and produced an extracellular serine protease. Previous record reported by Kim *et al.* (2009a) considered that *Pseudoalteromonas haloplanktis* AFMB-008041

showed algicidal activity against red tide species *P. minimum*, but was not able to inhibit the growth of *Alexandrium tamarense*, *Akashiwo sanguinea*, *Co. polykrikoides*, *Hs. akashiwo*, and *Gymnodinium catenatum*. This strain had algicidal compounds that may have  $\beta$ -glucosidase activity. Based on our results, our isolated strains M Dol 1-8 (*Pseudoalteromonas* sp.) showed significant algicidal activity against *Sk. costatum* and *P. minimum*. The isolated strain also demonstrated as indirect attackers of red tide species *Sk. costatum* and *P. minimum*.

Four *Dinophyceae*, two *Raphidophyceae*, and one *Bacillariophyceae* were used in this study to examine the algicidal effect of effective strains against harmful algal species. The 10% bacterial culture filtrates concentration was used for determination of algicidal activity in this study. Kim *et al.* (2008) considered that culture filtrates inhibited the growth of HABs species in concentration-dependent manner. Previous studies reported by Kim *et al.* (2007) demonstrated that algicidal activity depends on different growth stages of bacteria (exponential>stationary>lag phase) and Seong and Jeong (2013) reported that algicidal activity depends on the bacterial concentration. The 10% (v/v) bacterial culture filtrates showed a relatively wide spectrum against HABs species tested. The effective strains demonstrate relatively wide algicidal effect against dinoflagellates, raphidophytes, and diatom. It is considered that these effective strains can prevent algal blooms caused by the 7 HAB species tested. The previous study performed by Kang *et al.* (2008) reported a broad host range with a good algicidal bacterium. The algicidal bacteria that display a wide host range have more advantages in possibility to control various host algal blooms.

## Conclusion

Forty effective algicidal strains were isolated from surface seawater and sediment samples of Masan Bay, Jinhae Bay, Dol Island, Jangmok Bay, and the Tongyeong Sea, Republic of Korea. The effective strains belonged to genera *Pseudomonas*, *Vibrio*, *Bacillus*, *Pseudoalteromonas*, *Ruegeria*, *Joostella*, *Marinomonas*, *Stakelama*, *Porphyrobacter*, and *Albirehodobacter*. The strains degrade algal cells indirectly and demonstrate a wide algicidal spectrum against *Co. polykrikoides*, *Hs. akashiwo*, *Hc. triquetra*, *P. minimum*, *Sc. trochoidea*, *Sk. costatum*, and *Ch.*

*marina*. From our knowledge, this is the first report of *Marinomonas* sp., *Stakelama* sp., *Porphyrobacter* sp., and *Albirehodobacter* sp. showing algicidal activity against HABs species. Our results suggest that these bacteria might play role in controlling HABs. Further studies are needed to investigate algicidal mechanisms and algicidal compounds associated with marine algicidal bacteria due to understanding the key role of algicidal bacteria in mitigating HABs.

## 적 요

본 연구는 식물성플랑크톤의 대량증식 조절과 관련된 해양성 살조능이 있는 박테리아의 분리와 유해조류에 대한 분리균주의 살조능 특성에 주로 초점을 맞추고 있다. 해수 표면에서 자연적으로 발생하는 유해조류번성(HAB)은 전세계적으로 많은 환경문제를 일으키고 있다. 본 연구에서는 유해조류 성장을 억제하는 능력을 가진 40개의 박테리아 균주를 마산만, 진해만, 돌섬, 거제도, 통영 앞바다에서 분리하였다. 분리된 균주들은 다양한 유해조류인 *Cochlodinium polykrikoides*, *Chattonella marina*, *Skeletonema costatum*, *Heterosigma akashiwo*, *Heterocapsa triquetra*, *Prorocentrum minimum*, *Scrippsiella trochoidea*에 대한 살조특성을 추가로 조사하였다. 살조균주의 선별은 이중층 아가배지와 현미경 계수법을 이용하여 진행하였고 *Pseudomonas*, *Vibrio*, *Bacillus*, *Pseudoalteromonas*, *Ruegeria*, *Joostella*, *Marinomonas*, *Stakelama*, *Porphyrobacter*, *Albirehodobacter*의 속들에 속하는 균주들이었다. 가장 중요한 유해조류인 *Co. polykrikoides*에 대한 가장 강력한 살조능은 10% 배양상등액으로 6시간 처리했을 때 94%를 보이는 균주였다. 이 연구를 통해 살조효과를 보이는 새로운 속으로 *Marinomonas* sp. M Jin 1-8, *Stakelama* sp. ZB Yeonmyeong 1-11 & 1-13, *Porphyrobacter* sp. M Yeonmyeong 2-22, *Albirehodobacter* sp. 6-R Jin 6-1를 새롭게 찾았다. 결론적으로 이들 해양박테리아를 이용하면 식물성플랑크톤 번성을 제어하는데 중요한 역할을 할 것으로 예상된다.

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