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Algicidal Activity of Strain MS-51 against a HAB Causing *Alexandrium tamarense*

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Abstract

The aim of this study was to isolate and identify algicidal bacterium against *Alexandrium tamarense*, and to determine the algicidal activity. During the declining period of *A. tamarense* blooms, three algicidal bacteria were isolated. The algicidal bacteria against *A. tamarense* were enumerated using the most probable number (MPN) method. The number of algicidal bacteria was high (3.3×10^3 /mL). The most algicidal bacterium was identified on the basis of biochemical and chemotaxonomic characteristics. The strongest bacterium, designated *Arthrobacer* sp. MS-51, is assumed to produce secondary metabolites. When 10% culture filtrate of this strain was applied to *A. tamarense* cultures, over 90% of *A. tamarense* cells were destroyed within 7 h. *Arthrobacer* sp. MS-51 showed significant algicidal activities against *A. tamarense*. Taken together, our results suggest that *Arthrobacer* sp. MS-51 could be a candidate for controlling HABs.

Keywords: *Alexandrium tamarense,* Algicidal bacterium, Algicidal activity, Dinoflagellate

INTRODUCTION

Most of microalgae are useful as primary producers in marine ecosystem. However, microalgal blooms can sometimes affect fish through oxygen deprivation and toxin. Microalgal toxins have harmful effect to marine organisms and human. *Alexandrium tamarense* is a marine toxic dinoflagellate which causes paralytic shellfish poisoning (PSP) of shellfish (Gracia et al., 2013). PSP is one of the most widely distributed and damaging HAB toxins (Lilly et al., 2007). Because of its huge impacts on marine fisheries and people's health, it has become a research hotspot (Murata et al., 2012).

Bacteria can role as control agents due to production of algicides and capability of decomposition of organic matters, and it will be very beneficial because they are abundant in marine ecosystems and replicate rapidly. Recent work has been focusing on algicidal bacteria as controllers of water blooms. Research into the relationship

between bacteria and algae has resulted in the isolation of several strains of bacteria capable of inhibiting or killing harmful algal blooms (HABs) species (Furusawa et al., 2003). Therefore, it was investigated algicidal bacterium to control of HABs in this study. The objectives of our study were to improve bacterial biomass and algicidal activity, characterize and identify algicidal bacterium.

MATERIALS AND METHODS

Cultures of *Alexandrium tamarense*

A. tamarense, the harmful dinoflagellate used in this study, was isolated from Masan Bay in Korea. Axenic clones were obtained by repeated washing with capillary pipettes (Droop, 1967) and repeated subcultures using enriched seawater medium containing antibiotic complex (Jeong et al., 2000). The concentration of antibiotic complex in the medium was: 100 μg/mL ampicillin, 10 μg/mL streptomycin, 10 μg/mL chloramphenicol, 10 μg/mL penicillin G, 50 μg/mL neomycin, 50 μg/mL gentamicin, 10 μg/mL kanamycin, and 1.5 μg/mL nystatin. All antibiotics and f/2-Si medium (Guillard and Ryther, 1962) were purchased from Sigma. *A. tamarense* cultures were routinely maintained in f/2-Si medium. The cultures were grown in disposable sterilized tissue culture flasks under an illumination of 100 μE m^{-2} s^{-1} under a 12 h light:12 h dark cycle at 20°C.

Isolations of algicidal bacteria against *A. tamarense* and identification of algicidal bacterium

The putative algicidal strains against *A. tamarense* were isolated from the positive wells in which the *A. tamarense* cells were completely destroyed. Wells with the highest possible dilution factor were selected to isolate the dominant species among bacteria populations, and subsamples were spread onto Zobell 2216E agar plates. The composition of Zobell 2216E agar was as follows: each liter of GF/F-filtered seawater contained 5 g of peptone, 1g of yeast extract, 0.1 g of $FePO_4$, and 1.5 g of Bacto agar. The viable bacterial number was determined by colony-forming unit (CFU) after 1 week of growth at 25°C on Zobell 2216E agar plates. All 12 colonies with different colony color and morphological shape were chosen for isolation. Each strain was cultured and was again inoculated into *A. tamarense* cultures in order to confirm its algicidal activities against *A. tamarense.* As a result, three algicidal strains were isolated in this study.

Three algicidal strains were grown at 25°C for 7 days on Zobell 2216E agar. Morphological characteristics were observed with a transmission electron microscope. Standard physiological and biochemical characteristics were examined according to the methods of MacFaddin (1984). Additional biochemical tests were performed using API kits.

Algicidal activities of algicidal strains against *A. tamarense*

To measure the algicidal activities of the three strains against *A. tamarense*, these strains were cultured in Zobell 2216E medium. Bacterial cultures were grown at 25°C for 7 days at 200 rev min^{-1} to reach stationary phases. Bacterial cells were then removed by centrifugation (6,000 *g* for 10 min) and filtration (0.2 μm pore-size membrane filter). The culture filtrates of three strains were investigated for algicidal activity. The *A. tamarense* cells were cultured in 300 mL tissue culture flasks containing 100 mL f/2-Si medium. Then, 180 μl of *A. tamarense* culture (1.0×10^4 cells/mL) at mid-exponential growth phase (10 days after incubation) was inoculated into each well of a 24-well tissue culture plate. 20 μL of each bacterial culture filtrate was added to a 24-well plate containing *A. tamarense*. To exclude the effect of bacterial culture medium, an equal volume of fresh Zobell 2216E medium was added as a control. The bioassay plates were then incubated at the above conditions. After 7 h, the numbers of surviving cells were directly counted on a Sedgewick-Rafter counting chamber at a magnification of ×200. The algicidal activity was calculated as follows (Byun et al., 2002): Algicidal activity (%) = {1 – (viable individuals of *A. tamarense* after the treatment / initial individuals of *A. tamarense*) × 100}. All experiments were repeated in triplicate, and results are given as the mean and standard deviation of raw data.

Algicidal effect of the culture filtrates of strain MS-51 against *A. tamarense*

The algicidal effect of the culture filtrates of strain MS-51 against *A. tamarense* was investigated at various concentrations of filtrates. The bacterial and *A. tamarense* cultures were prepared as above. The culture filtrates of strain MS-51 were added to *A. tamarense* cultures (1.0×10^4 cells/mL) at concentrations of 1, 5, and 10%, respectively. Zobell 2216E medium was added as the control, and the bioassay plates were incubated as above. After incubation for 1, 2, 5, and 7 h, the viable swimming cells in each well were counted with a Sedgewick-Rafter chamber using an inverted microscope.

RESULTS

Screening of algicidal bacteria

A total of 53 bacterial strains were isolated; three isolates showed algicidal activity against *A. tamarense*. The algicidal activity of three algicidal strains against *A. tamarense* was investigated. Strain MS-51 exhibited the strongest algicidal activity (95%) against *A. tamarense*.

Identification and culture conditions of strain MS-51

To identify the strains, morphological and biochemical analyses were performed. Strain MS-51 was gram-positive rods (Fig. 1A), and non-pigmented in a Zobell

2216E agar plate. Transmission electron microscope indicated that strain MS-51 displayed a coryneform morphology and was approximately 2.2 μm long and 0.7 μm in diameter (Fig. 1B). The morphological and biochemical characteristics of strain MS-51 are summarized in Table 1. This strain was grown on Zobell 2216E agar at 25°C for 7 days. By transmission electron microscope, we could observe that strain MS-51 was rods (Fig. 1B). This strain was catalase and oxidase positive. This strain contained L-arginine dehydrolase. Carbohydrate fermentation patterns were examined by API 50 CHL (BioMerieux, SA, France). Strains MS-51 assimilated produced acid from L-Rhamnose, Inositol, Sorbitol, Lactose, Mannitol, maltose, and sucrose (Table 1). The conventional tests showed close relationships between MS-51 and the genus *Arthrobacer*. Therefore, this strain was identified as *Arthrobacer* sp. via culture morphological and biochemical reactions.

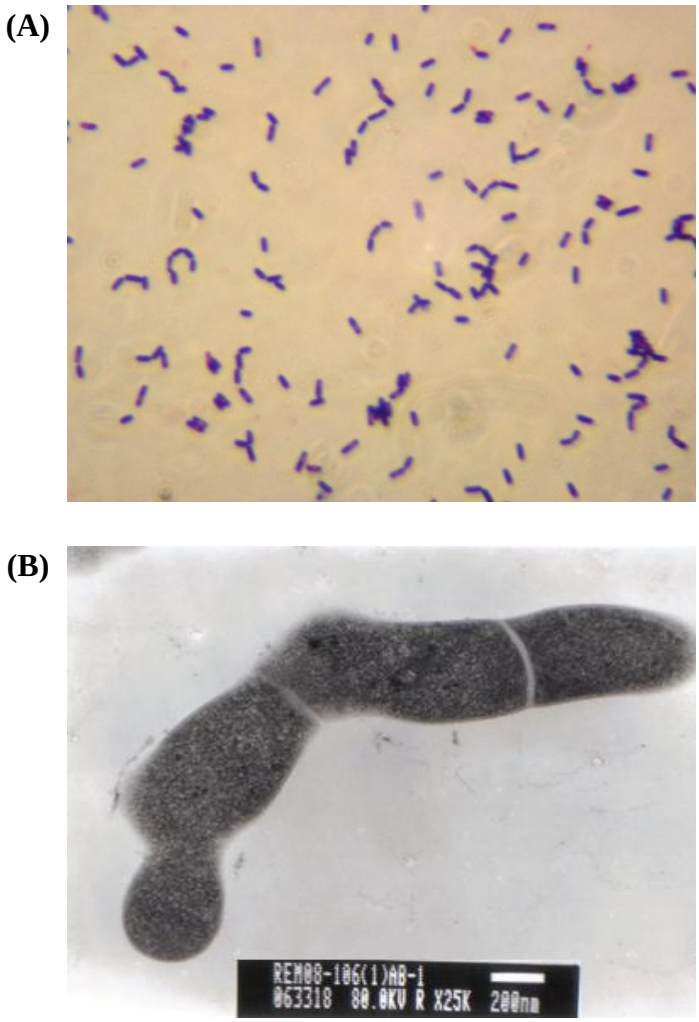

Fig. 1. Micrographs of strain MS-51. (A) optical micrograph of gram staining and (B) transmission electron micrograph (TEM). Scale bar represents 200 nm in size.

Table 1. Biochemical characteristics of strain MS-51

Characteristics	MS-51 strain	Characteristics	MS-51 strain
KIA (Kligler's iron agar) test	K/K	Utilization of:	
O/F (Oxidation/Fermentation) test	+/–	Glucose	–
Production of:		D-Xylose	–
Indole	–	D-Fructose	–
MR (Methyl red)	–	L-Rhamnose	+
VP (Voges-Proskauer)	–	D-Galactose	–
Citrate	–	Inositol	+
Catalase	+	D-Mannose	–
Oxidase	+	Sorbitol	+
L-lysine decarboxylase	+	Salicin	–
L-ornithine decarboxylase	+	Maltose	+
L-arginine dehydrolas	+	Adonitol	–
		L-Arabinose	–
		Lactose	+
		Sucrose	+
		Mannitol	+

+, positive result or growth; −, negative result or no growth

Relationship of algicidal activity and bacterial growth

The growth curve and algicidal activity of the strain MS-51 were inspected every 3 h, over a 36-h period (Fig. 2). It seemed that the algicidal activity of the isolated strain was bacterial growth-dependent, since the strongest algicidal activity occurred in early stationary-phase cultures, in treatments involving the addition of bacterial culture filtrates.

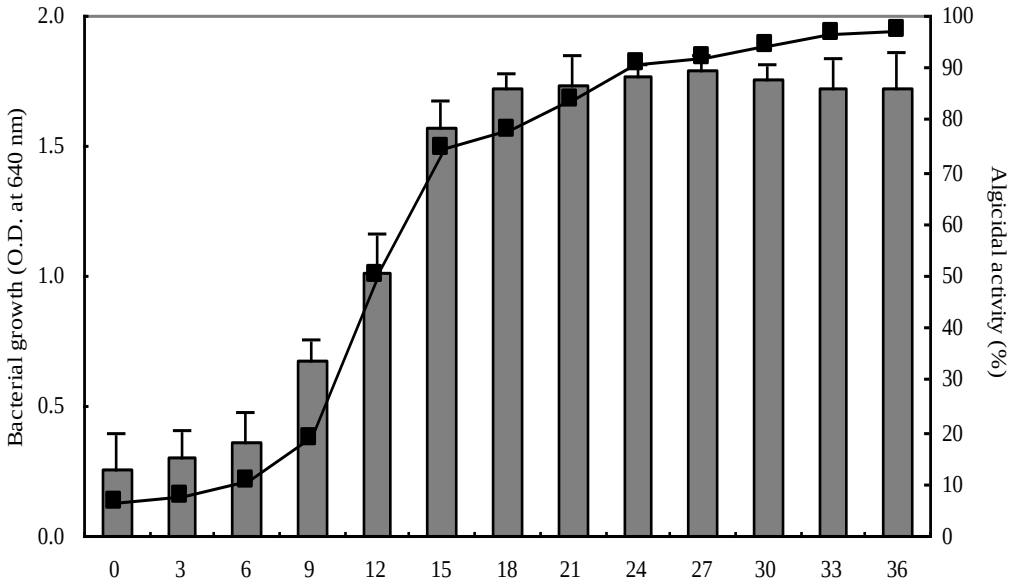

Fig. 2. Growth curve of *Arthrobacter* sp. MS-51 at optimal culture conditions (25°C, pH 7.0, 2.0% NaCl) and algicidal activity by the culture filtrate of each growth phase of *Arthrobacter* sp. MS-51 against *A. tamarense*. -■- = bacterial growth curve (O.D. at 640 nm). Bar graph= algicidal activity (control = algal cultures with Zobell 2216E broth added).

Algicidal activity of bacterial culture filtrates against *A. tamarense* cultures

To determine the effective algicidal threshold concentration of *Arthrobacter* sp. MS-51 against *A. tamarense*, the alga was inoculated with five different concentrations (10, 50, and 100 μL) of the culture supernatants. Algicidal effects were observed in the control wells, which involved 50 and 100% Zobell 2216E broth. Consequently, at a high concentration (> 50%), results were not significant; results with > 50% concentration are not shown (Fig. 3). Although all of the cells of *A. tamarense* were still motile, their speed of motility decreased markedly within 3 h at low concentration of culture supernatant, 50 μL added. The 10% (100 μL added) concentration showed strong algicidal activity and the culture filtrates of *Arthrobacter* sp. MS-51 inhibited the growth of *A. tamarense* in a concentration- and time-dependent manner.

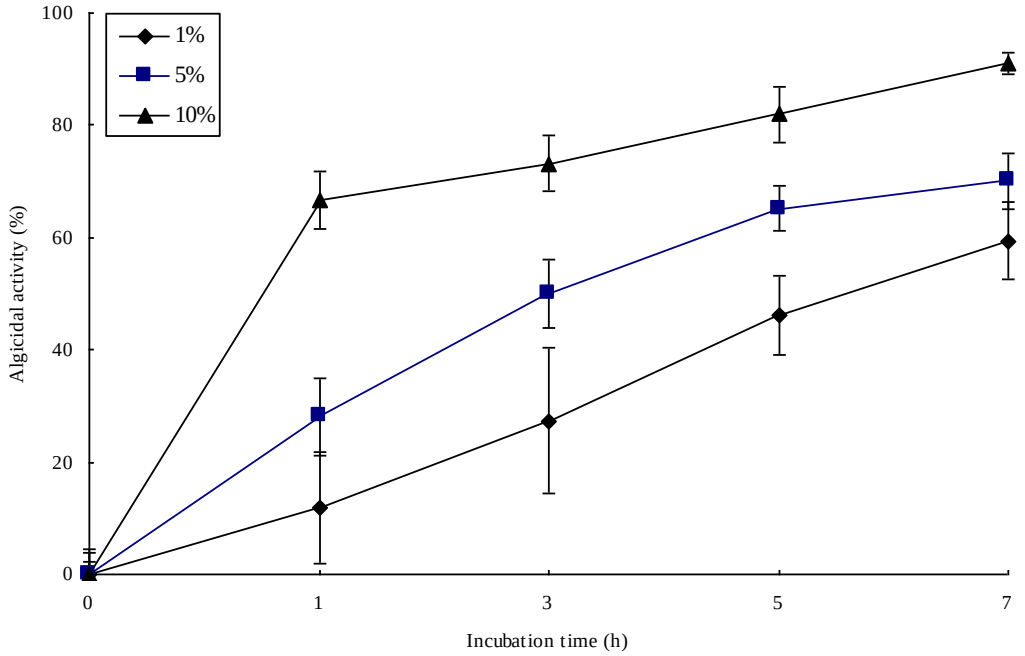

Fig. 2. Algicidal activity of the culture filtrates of *Arthrobacter* sp. MS-51 against *A. tamarense* at various concentrations (◆, 1% (10 μL); ■, 5% (50 μℓ); ▲, 10% (100 μℓ)). Control = algal cultures with Zobell 2216E broth added. Data are expressed as the mean ± standard deviation from ten-time assays.

DISCUSSION

Harmful algal blooms (HABs) are natural phenomena that occur across the world. HABs have occurred frequently and have drastically affected aquaculture industry in coastal waters. Over the past four decades, the occurrence of HABs has increased. In Korea, extreme blooms of dinoflagellates that have caused severe damage to fisheries have occurred annually. Many scientists have recently focused on the algicidal bacteria for the control of HABs (Lee et al., 2000; Jeong et al., 2003). Many algicidal bacteria have been isolated from various sources. These algicidal bacteria include species of various genera. For example, *Alteromonas*, *Bacillus*, *Cytophaga*, *Flavobacterium*, *Micrococcus*, *Pseudomonas*, *Pseudoalteromonas*, and *Vibrio* are included (Lovejoy et al., 1998; Jeong et al., 2000, 2003; Zheng et al., 2005). These findings have raised the possibility that HABs can be controlled by algicidal bacteria.

In the course of our screening procedure of algicidal bacteria, three marine bacteria against *A. tamarense* were isolated. Seawater samples were collected during the termination of *A. tamarense* blooms in Masan Bay in Korea. The number of algicidal bacteria against *A. tamarense* was high (3.3×10^3 /mL) in the field. Algicidal bacterium was identified on the basis of biochemical and chemotaxonomic

characteristics. In this study, we described the taxonomy of algicidal bacterium, and determine the algicidal activity.

In conclusion, Strain MS-51 showed significant algicidal activity against *A. tamarense*. In addition, algicidal activity against *A. tamarense* detected in the culture filtrates of strain MS-51, not in the cells. These results indicate that all of the seven algicidal strains are assumed to release certain algicidal compounds into the culture broth. Thus, *Arthrobacter* sp. MS-51 was thought to act as indirect attackers.

REFERENCES

[1] Byun HG, Jeong SY, Park YT, Lee WJ, Kim SK. 2002. Algicidal activity of substance purified from marine bacteria metabolites against *Cochlodinium polykrikoides*. J. Fish. Sci. Technol. 5: 150–155.

[2] Droop MR. 1967. A procedure for routine purification of algal cultures with antibiotics. Brit. Phycol. Bull. 3: 295–297.

[3] Furusawa G, Yoshikawa T, Yasuda A, Sakata T. 2003. Algicidal activity and gliding motility of *Saprospira* sp. SS98-5. Can. J. Microbiol. 49: 92–100.

[4] Gracia S, Roy S, Starr M. 2013. Spatial distribution and viability of *Alexandrium tamarense* resting cysts in surface sediments from the St. Lawrence Estuary, Eastern Canada. Estuarine Coastal Shelf Sci. 121–122: 20–32.

[5] Guillard RL, Ryther JH. 1962. Studies on marine planktonic diatoms. I. *Cyclotella nana* (Hustedt) and *Detonula confervacea* (Cleve) Gran. Can. J. Microbiol. 8: 229–239.

[6] Jeong SY, Park YT, Lee WJ. 2000. Isolation of marine bacteria killing red tide microalgae. III. Algicidal effects of marine bacterium, *Micrococcus* sp. LG-5 against the harmful dinoflagellate, *Cochlodinium polykrikoides*. J. Kor. Fish. Soc. 33: 331–338 (in Korean).

[7] Jeong SY, Ishida K, Ito Y, Okada S, Murakami M. 2003. Bacillamide, a novel algicide from the marine bacterium, *Bacillus* sp. SY-1, against the harmful dinoflagellate, *Cochlodinium polykrikoides*. Tet. Lett. 44: 8005–8007.

[8] Lee SJ, Kim Y, Kim HG, Seo GM, Jeong JH, Hong YK. 2000. Algalytic activity of α-mannosidase on harmful marine microalgae. J. Appl. Phycol. 12: 191–193.

[9] Lilly EL, Halanych KM, Anderson DM. 2007. Species boundaries and global biogeography of the *Alexandrium tamarense* complex (Dinophyceae). J. Phycol. 43: 1329–1338.

[10] Lovejoy C, Bowman JP, Hallegraeff GM. 1998. Algicidal effects of a novel marine *Pseudoalteromonas* isolate (Class Proteobacteria, Gamma Subdivision) on harmful algal bloom species of the genera *Chattonella*, *Gymnodinium*, and *Heterosigma*. Appl. Environ. Microbiol. 64: 2806.

[11] MacFaddin TF. 1984. Biochemical tests for identification of medical bacteria.

Williams & Wilkins. pp. 36–308.

[12] Murata A, Nagashima Y, Taguchi S. 2012. N. P ratios controlling the growth of the marine dinoflagellate *Alexandrium tamarense:* content and composition of paralytic shellfish poison. Harmful Algae 20: 11−18.

[13] Zheng TL, Su JQ, Maskaoui K, Yu ZM, Hu Z, Xu JS, Hong HS. 2005. Microbial modulation in the biomass and toxin production of a red-tide causing alga. Mar. Pollut. Bull. 51: 1018-1025.