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Algicidal activity of *Aeromonas hydrophila* sdPS-7 isolate against toxic marine dinoflagellate *Alexandrium minutum* KB- 5

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ABSTRACT

Aims: Several harmful dinoflagellate species are widely found in Malaysian marine waters. These microalgae are capable of producing a variety of toxins that can intoxicate humans through consumption of contaminated seafood. Therefore, the present work aimed to investigate the potential of a marine bacterium sdPS-7 isolate as a biological control agent against *Alexandrium minutum* KB-5.
Methodology and results: The isolate sdPS-7 was obtained from marine sediments in Malaysia and examined for its algicidal activity toward the toxic dinoflagellate *A. minutum* KB-5 in laboratory culture. Based on morphological and molecular characterization, this isolate was identified as *Aeromonas hydrophila* sdPS-7. *Alexandrium minutum* KB-5 cultures were exposed to cell free bacteria culture filtrate and the effect on dinoflagelate growth was evaluated based on direct cell counts in the treated samples compared to non-treated control cultures. The bacterial filtrate was treated at different temperatures, salinities and exposed to repeated freezing and thawing five times to study its algicidal activity stability thereafter. The results showed a potent inhibition of the growth of *A. minutum* KB-5. The strongest effect was observed when the bacterium culture filtrate was mixed with *A. minutum* KB-5 cultures that were in lag phase, resulting in 99% or higher mortality. The cell-free filtrate proved to be heat- stable when exposed to temperatures of 2 °C to 100 °C for one hour each. There was also no substantial salinity as well as (freezing – thawing) effect on the filtrate algicidal activity.
Conclusion, significance and impact of study: This study illustrated the potential use of the marine bacterial *Aeromonas hydrophila* sdPS-7 filtrates in controlling the growth of the toxic dinoflagellate *A. minutum* KB-5.

Keywords: Alexandrium minutum, Aeromonas hydrophila, algicidal, marine

INTRODUCTION

Harmful algal blooms (HABs) are masses of a particular algae species that can affect life forms including humans, fish, shellfish, birds and marine mammals which are correspond to the capability of these algae to produce highly potent toxins (Orellana-Cepeda *et al.*, 1998). The exact factors that cause HABs in a particular location vary. What is clear though is that over the last decade HABs have become more frequent and occur in more places worldwide (Usup *et al*., 2002; Kim *et al*., 2007).

In Malaysia, many seafood-poisoning cases are associated with dinoflagellate toxins. Paralytic shellfish poisoning (PSP) is the most significant form of these toxins. For example, *Pyrodiium bahamense* dinoflagellate is known as PSP in Malaysia water and considered as the most important PSP producing toxin in Malaysia and other countries (Philippines and America) (Rosales-Loessener *et al*., 1989; Orellana-Cepeda *et al*., 1998; Usup *et al*., 1998). *Alexandrium minutum* is another PSP producing toxic dinoflagellate that has been reported in Malaysian water. In 2001, a wide spread intoxication event was occurred following a huge marine dinoflagellate bloom. That dinoflagellate species was identified later as *A. minutum* based on wild specimens and cultures collected from the site.

In order to reduce the effect of HABs on fisheries and human health, several physical and ecological methods have been applied (Jeong *et al*., 2000; Kim *et al.*, 2009; Lee *et al*., 2013). Although these techniques exhibited some efficiency against HABs, pose risks to the existence of aquatic organisms are involved which limited the use of these strategies as alternative methods.

Several studies have investigated the algicidal activity of numerous marine bacteria and highlighted their role in controlling microalgae bloom in aquatic ecosystems

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(Furuki and Kobayashi 1991; Imai e*t al*., 1995; Yoshinaga *et al*., 1995; Imai *et al*., 1998; Iwata *et al*., 2004). Examples of reported algicidal bacteria are *Psudoalteromonas*, *Serratia* sp., *Vibrio ruber*, *Alteromonas*, *Flavobacterium*, *Micrococcus*, *Pseudomonas*, *Cytophaga*, *Bacillus*, *Bacteriolytica* (Lewis and Corpe 1964; Baker and Herson, 1978; Imai *et al.*, 1993, 1995; Lovejoy *et al.*, 1998; Park *et al.*, 1998; Sawabe *et al.*, 1998; Yoshinaga 1998; Adachi *et al.*, 2002; Jeong *et al.*, 2003; Shieh *et al.*, 2003; Kim *et al.*, 2009). However, these studies have not employed in Malaysia, which has a large area of the marine environment. Malaysia is of the most scenic states at the regional level due to the diverse marine environment, including the beaches. Thus, we aimed to obtain and characterize a powerful marine bacterium from Malaysian marine as a biological control of harmful algae.

In the current study, an indigenous isolated bacterium from marine sediments was screened for its algicidal activity against the toxic dinoflagellate *A. minutum* KB-5. This isolate was identified based on morphological and molecular characteristics. Bacterial filtrates of such organism were to test algicidal activity in all *A. minutum* KB-5 growth phases. The stability of algicides from the potent isolate at different temperatures, salinities, repeated freezing-thawing was tested.

MATERIALS AND METHODS

Cultures

The bacterial isolate used in this study was obtained from marine sediment collected from Pantai Senok, Kota Bharu, Kelantan, Malaysia. Bacteria were isolated on marine agar medium which composed of peptone 5.0 g/L, yeast extract 1.0 g/L, ferric citrate 0.1 g/L, sodium chloride 19.45 g/L, magnesium chloride 8.8 g/L, sodium sulfate 3.24 g/L, calcium chloride 1.8 g/L, potassium chloride 0.55 g/L, sodium bicarbonate 0.16 g/L, potassium bromide 0.08 g/L, strontium chloride 34.0 mg/L, boric acid 22.0 mg/L, sodium silicate 4.0 mg/L, sodium fluoride 2.4 mg/L, ammonium citrate 1.6 mg/L, disodium phosphate 8.0 mg/L and agar 15.0 g /L. The culture plates were incubated at 28 °C, 180 rpm for 24-48 h and stored in slant agar at 4 °C. For activation, stock cultures were grown in marine broth medium at 28 °C, 180 rpm for 24-48 h. Dinoflagellate cultures used in this study were collected from the marine microalgae culture collection at Universiti Kebangsaan Malaysia. The cultures were maintained in sterile ES-DK medium prepared with natural sea water taken directly from Port Dickson at around 27.91 ppt salinity and incubated at 26 °C under a 14 h : 10 h light-dark cycle. Dinoflagellate cultures were subcultured every two weeks.

Algicidal activity assay

The bacterium isolate sdPS-7 was screened in for its algicidal activity against the toxic *A. minutum KB-5*. The algicidal activity of sdPS-7 was studied during all growth phases of the algae. Bacteria were grown in marine broth medium and incubated at 28 °C/ 180 rpm agitation for 18-24 h. Bacterial culture was centrifuged (5,000 rpm /10 min at 4 °C) and the supernatant was separated from cells and filtered through 0.22 µm pore filters. Aliquots of the bacterial filtrates were inoculated into the *A. minutum* KB-5 culture. As negative control, the dinoflagellate cultures were incubated without bacterial filtrates. The assay cultures and controls were maintained at 26 °C under a 10 h: 14 h dark-light cycle. The growth of the dinoflagellates was examined under the microscope and samples from both treated and control cultures were taken every twenty minutes and counted using Sedgewick Rafter slide chambers. The algicidal activity was calculated using the following formula:

$$\text{Algicidal activity (\%)} = \left(1 - \frac{N_t}{N_0}\right) \times 100$$

N_t represents the number of algal cell after treatment while N_0 represents the number of living algal cells at the initial time.

Morphological changes in *A. minutum* KB-5 cells

In order to understand the probable mechanism of action of the algicidal compound, the morphology of the treated cells, movement, cell wall shape and cell content were examined under an inverted microscope and compared to the untreated cells.

Algicidal bacteria identification

The bacterium sdPS-7 isolate was cultured on marine agar and described morphologically and physiologically. Molecular identification of sdPS-7 was based on PCR amplification of the 16S rRNA gene. Two oligonucleotide primers were used: forward 5-'AGAGTTTGATCCTGGCTCAG-3' and reverse 5'-GGTTACCTTGTTACGACTT-3'. The PCR reaction was carried out using a thermal mini cycler (MJ Research, USA) and initial PCR cycle was as follows: 95 °C for 2 min (initial denaturation), 50 °C for 30 sec (annealing), and 72 °C for 45 sec (DNA synthesis). This was followed by 22 cycles of 95 °C for 30 sex, 50 °C and 72 °C for 2 min, with a further 10 min at 72 °C for final extension, using UniversAll™ tissue PCR kit. The amplified products were purified using the QIA quick PCR purification kit (Qiagen, Germany) based on the manufacturer's instructions and 20 µL of the purified DNA was sent for nucleotide sequencing (First Base Laboratory, Kuala Lampur). DNA sequences were first edited using Chromas and analysed using Bio Edit Sequence Alignment Editor program. The ribosomal gene sequence of isolate sdPS-7 was then compared with 16S rRNA gene sequences available in the NCBI databases. All sequences showed the highest similarity with the 16S rRNA gene from the genus *Aeromonas hydrophila*. A phylogenetic tree was generated using the Neighbour-

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joining algorithm provided in MEGA 4 program (Tamura *et al.*, 2011).

Stability of algicidal activity

The stability of algicidal activity of bacterial supernatant was investigated at different temperature values, salinities and at repeated freezing-thawing as well. The supernatant of sdPS-7 was separated from the cells and incubated at 2 °C, 4 °C, 25 °C, 30 °C, 35 °C, 40 °C, 50 °C, 100 °C (1 h each).

In order to test the effect of salinity on the algicidal activity, the sdPS-7 isolate was grown with different NaCl concentrations (0.5%, 1%, and 3 %). In the freezing treatment, the supernatant was subjected to repeated freeze and thaw for six times and then tested for its algicidal activity.

Aliquots of each treated supernatant were inoculated into *A. minutum* KB-5 cultures in exponential growth phase. The same volume of untreated supernatant was inoculated in to the algae culture as control. The growth of the algae was evaluated as described before in the algicidal assay section.

Statistical analysis

Experiments were performed in triplicate and results were recorded in the mean with standard deviation (SD) of triplicate measurements. Differences between means were evaluated mainly by one-way ANOVA (SPSS version 21.0) and were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Algicidal activity

The sdPS-7 isolate exhibited strong inhibition of *A. minutum* KB-5 growth phases after two hours of treatment. Algicidal activity was obtained from culture filtrate of sdPS- 7, indicating that this isolate can release potential antimicrobial agents capable of inhibiting dinoflagellate growth. More than 95% of the toxic algae cells in exponential growth phase were lysed after two hours of treatment with 10% (v/v) of culture- free cell filtrate.

The inhibition effect of sdPS-7 on *A. minutum* KB-5 was evident during all phases of the dinoflagellate growth cycle. Dinoflagellate cell numbers decreased by 92% after treatment at stationary phase, 95% at exponential phase and 99% at lag phase after two hours of treatment (Figure 1). Comparing our findings to other studies, Mayali and Doucette (2002) discovered that the harmful algae *K. brevis* was more affected by the algicidal bacterium *Cytophaga* strain 41-DBG2 at its stationary growth phase comparing to lag and log phases. In contrast Kang *et al.* (2008) found that lag and log growth phases of the tested dinoflagellate *Peridinium bipes* and the diatom *Stephanodiscus hantzchii* were more sensitive than stationary growth phase when treated by the algicidal bacteria *Stephanodiscus* spp. The differences in effectivity of sdPS-7 filtrate on *A. minutum* KB-5 growth phases suggesting that the algicidal effect is stage-specific.

Morphology changes of treated algae cells

Examination of treated *A. minutum* KB-5 cells under the microscope revealed rapid reduction of mobility during the first hour of the treatment by algicidal agents in the bacterial filtrate. The cell shape started losing its regularity

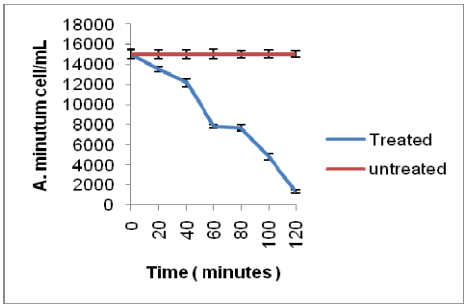

Figure 1: Algicidal activity of sdPS-7 filtrate on *A. minutum* phases (A) log phase, (B) lag phase, (C) stationary phase. Results were recorded in triplicates.

after 80 min. followed by the decomposing of the cell wall and releasing of the cell content (Figure 2). It has been demonstrated that the action mode of algicides is taken place via direct attack in which the activity requires direct contact with the algae cell or indirect attack where the effect is through extracellular components which showed inhibitory mechanism by decomposition of the algae cell, or stopping their movement. A study conducted on the algicidal activity of yellow pigmented bacteria strain Y and *P. carrageenovora* against the HABs *Gymnodinium catenatum,* described the morphological changes of the treated algae cells .The cells that treated by Y- strain got crumble and swelling at the initial phase of treatment followed by lysis where the cell got infiltration and cell content became destroyed. In contrast, *P. carrageenovora* didn't caused lysis *to G. catenatum* where the algae cells only got encystment. (Lovejoy *et al*., 1998). The results obtained in this study demonstrated that algicides extracted from sdpS-7 isolate have completely damaged *A. minutum* cell morphology, indicating the efficiency of this bacterium to be used as a biological control agent against toxic dinoflagellates.

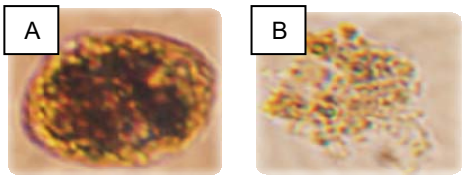

Figure 2: Lysis of *A. minutum* cells observed under a light microscope at 40× magnification. A, untreated cell; B, treated cell.

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Algicidal bacteria identification

On marine agar, the colonies of sdPS-7 obtained were round, intermediate in size with smooth surface, flat elevation and entire margins. The bacteria cell wall was Gram negative and rod-shaped. It was oxidase and catalase positive. Blast searches of the gene sequence showed that isolate sdPS-7 had 97% similarity to *Aeromonas hydrophila*. The phylogenetic tree is shown in Figure 3. The molecular data supported the identification of the isolate as *A. hydrophila* by conventional methods (Schubert, 1967) and Bergey's Manual of Determinative Bacteriology. *Aeromonas hydrophila* (Messi *et al.*, 2003) and many marine bacteria such as *Bacillus (*Motta and Brandelli 2008*)*, *Lactobacillus acidophilus* (Ivanova *et al.*, 2000) are known for their antimicrobial activities. Some species of *Aeromonas* like *A. hydrophia* and *A. media* are able to produce antimicrobial Bacteriocin like substance (BLS) which showed lytic activity against many indicator organisms (Messi *et al*., 2003). Recently, algicidal activity of the genus *Aeromonas* sp. against the cyanobacterium *Microcystis aerogonosa* has been reported (Liu *et al*., 2012, 2013; Yang *et al*., 2013). However, algicidal activity studies of this genus still limited.

To the best of our knowledge, this may be the first report of *A. hydrophila* obtained from marine sediments as an *A. minutum*-lysing bacterium. Our results about *A. hydrophila* and dinoflagellates increase our understanding of more rules of *Aeromonas* in marine environments.

Algicidal activity stability of sdPS-7 isolate

The bacterial culture filtrate was stable over a wide range of temperatures (Figure 4), representing that the algicidal components were thermostable. Previous studies have shown that some algicidal substances are thermolabile (Bujdakova *et al*., 1995) and some are thermostable (Skerratt *et al*., 2002). Growing the isolate with different NaCl concentrations as well as repeated freezing-thawing of the supernatant did not affect the filtrate activity (Figures 5 and 6).

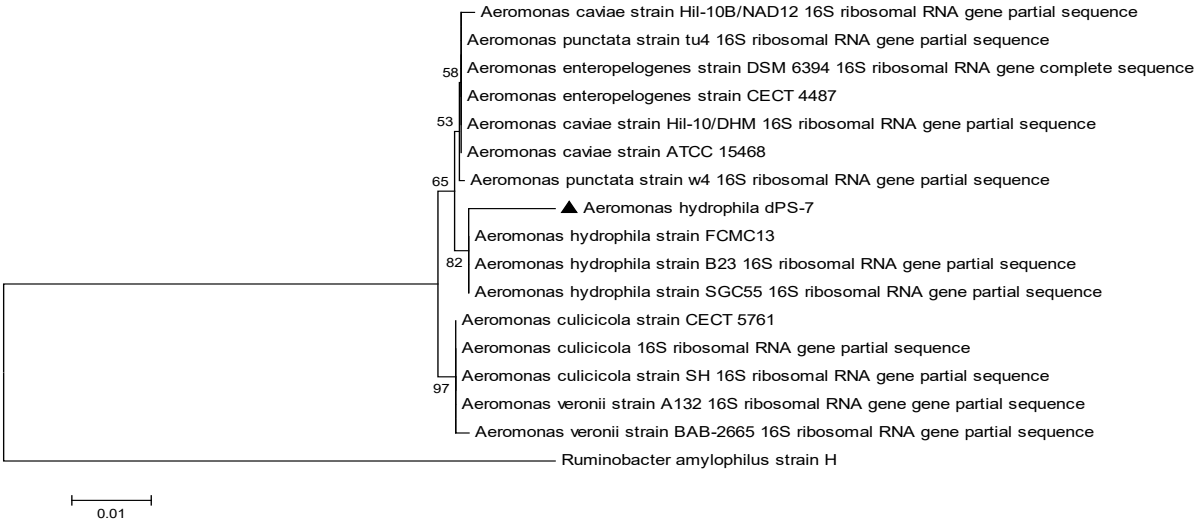

Figure 3: Phylogenetic position of sdPS-7 isolate based on the 16s ribosomal RNA gene sequence. The phylogenetic tree was performed using neighbour-joining method on the 16S rRNA.

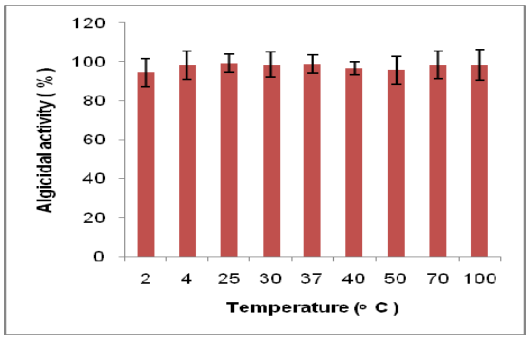

Figure 4: Heat stability of bacterial culture filtrate

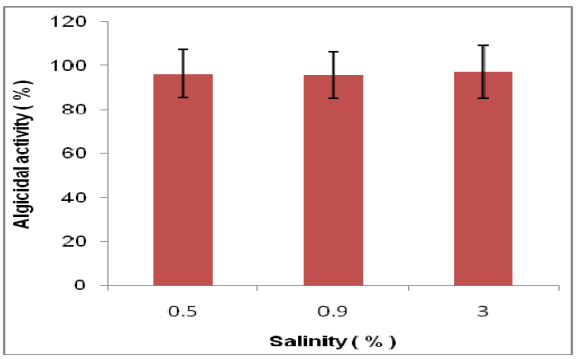

Figure 5: Freezing-thawing stability of bacterial culture filtrate.

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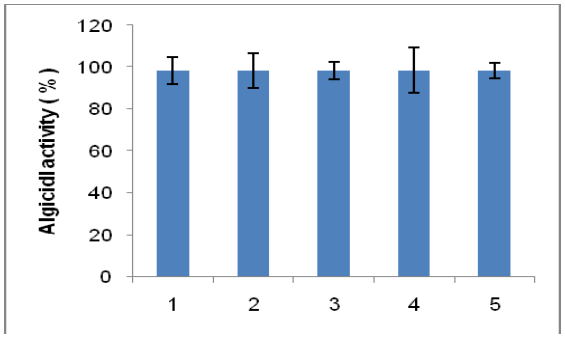

Figure 6: Salinity stability of bacteria culture filtrate.

CONCLUSION

In conclusion, the rapid inhibition activity of the marine *A. hydrophila* sdPS-7 culture filtrate indicated that the bacteria could release potent algicides against the toxic dinoflagellate *A. minutum* that may be used as potential component against HABs aquatic environment. Further work is needed to isolate and investigate the structural and molecular composition of the active compounds and to reveal the mechanism of activity.

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REFERENCES

Adachi, M., Fukami, K., Kondo, R. and Nishijima, T. (2002). Identification of marine algicidal *Flavobacterium* sp. 5 N-3 using multiple probes and whole-cell hybridization. *Fisheries Science* **68(4), 713-720.**

Baker, K. H. and Herson, D. S. (1978). Interactions between the diatom *Thallasiosira pseudonanna* and an associated pseudomonad in a mariculture system. *Applied and Environmental Microbiology* **35(4), 791-796.**

Bujdakova, H., Kral'ova, K. and Sidoova, E. (1995). Antifungal and antialgal activity of 3-(2-alkylthio-6-benzothiazolylaminomethyl)-2-benzoxazolinethi ones. *Pharmazie* **50(2), 156-156.**

Breed, R. S., Murray, E. G. D. and Smith, N. R. 1957. Bergyes Manual of Determinative Bacteriology. 7th edn. Williams & Wilkins Company, Baltimore.

Furuki, M. and Kobayashi, M. (1991). Interaction between *Chattonella* and bacteria and prevention of this red tide. *Marine Pollution Bulletin* **23, 189-193.**

Imai, I., Ishida, Y. and Hata, Y. (1993). Killing of marine phytoplankton by a gliding bacterium *Cytophaga* sp., isolated from the coastal sea of Japan. *Marine Biology* **116(4), 527-532.**

Imai, I., Ishida, Y., Sakaguchi, K. and Hata, Y. (1995). Algicidal marine bacteria isolated from northern Hiroshima Bay, Japan. *Fisheries Science: FS* **61(4), 628-636.**

Imai, I., Nakagiri, S., Nagai, K., Nagasaki, K., Itakura, S. and Yamaguchi, M. (1998). Fluctuations of algicidal microorganisms against the harmful dinoflagellate *Heterocapsa circularisquama* in Ago Bay, Mie prefecture, Japan. *Bulletin of the Nansei National Fisheries Research Institute* **31, 53-61.**

Ivanova, I., Kabadjova, P., Pantev, A., Danova, S. and Dousset, X. (2000). Detection, purification and partial characterization of a novel bacteriocin substance produced by *Lactococcus lactis* subsp. *lactis* B14 isolated from boza-Bulgarian traditional cereal beverage. *Biocatalysis* **41(6), 47-53.**

Iwata, Y., Sugahara, I., Kimura, T., Kowa, H., Matsumoto, A. and Noritake, K. (2004). Properties of an algicidal bacterium (*Flavobacterium* sp.) against Karenia mikimotoi isolated from Ise Bay, Japan. *Nippon Suisan Gakkaishi* **70(4), 537-541.**

Jeong, J.-H., Jin, H.-J., Sohn, C. H., Suh, K.-H. and Hong, Y.-K. 2000. Algicidal activity of the seaweed *Corallina pilulifera* against red tide microalgae**.** *Journal of Applied Phycology* **12(1), 37-43.**

Jeong, S.-Y., Ishida, K., Ito, Y., Okada, S. and Murakami, M. (2003). Bacillamide, a novel algicide from the marine bacterium, *Bacillus* sp. SY-1, against the harmful dinoflagellate, *Cochlodinium polykrikoides*. *Tetrahedron Letters* **44(43), 8005-8007.**

Kang, Y.-K., Cho, S.-Y., Kang, Y.-H., Katano, T., Jin, E.-S., Kong, D.-S. and Han, M.-S. (2008). Isolation, identification and characterization of algicidal bacteria against *Stephanodiscus hantzschii* and *Peridinium bipe*s for the control of freshwater winter algal blooms. *Journal of Applied Phycology* **20(4), 375-386.**

Kim, J.-D., Kim, B. and Lee, C.-G. (2007). Alga-lytic activity of *Pseudomonas fluorescens* against the red tide causing marine alga *Heterosigma akashiwo* (Raphidophyceae). *Biological Control* **41(3), 296-303.**

Kim, Y. S., Lee, D.-S., Jeong, S.-Y., Lee, W. J. and Lee, M.-S. (2009). Isolation and characterization of a marine algicidal bacterium against the harmful raphidophyceae *Chattonella* marina. *The Journal of Microbiology* **47(1), 9-18.**

Lee, Y.-C., Jin, E., Jung, S. W., Kim, Y.-M., Chang, K. S., Yang, J.-W., Kim, S.-W., Kim, Y.-O. and Shin, H.-J. (2013). Utilizing the algicidal activity of aminoclay as a practical treatment for toxic red tides. *Scientific Reports* **3. doi:10.1038/srep01292.**

Lewis, S. and Corpe, W. (1964). Prodigiosin-producing bacteria from marine sources. *Applied Microbiology* **12(1), 13-17.**

Liu, Y.-M., Chen, M.-J., Wang, M.-H., Jia, R.-B. and Li, L. (2013). Inhibition of *Microcystis aeruginosa* by the extracellular substances from an *Aeromonas* sp. *Journal of Microbiology and Biotechnology* **23(9), 1304-1307.**

Liu, Y.-M., Wang, M.-H., Jia, R.-B. and Li, L. (2012). Removal of cyanobacteria by an *Aeromonas* sp. *Desalination and Water Treatment* **47(1-3), 205-210.**

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Lovejoy, C., Bowman, J. P. and Hallegraeff, G. M. (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*. *Applied and Environmental Microbiology* **64(8), 2806-2813.**

Mayali, X. and Doucette, G. J. (2002). Microbial community interactions and population dynamics of an algicidal bacterium active against *Karenia brevis* (Dinophyceae). *Harmful Algae* **1(3), 277-293.**

Messi, P., Guerrieri, E. and Bondi, M. (2003). Bacteriocin-like substance (BLS) production in *Aeromonas hydrophila* water isolates. *FEMS Microbiology Letters* **220(1), 121-125.**

Motta, A. S. and Brandelli, A. (2008). Evaluation of environmental conditions for production of bacteriocin-like substance by *Bacillus* sp. strain P34. *World Journal of Microbiology and Biotechnology* **24 (5), 641-646.**

Orellana-Cepeda, E., Martínez-Romero, E., Munoz-Cabrera, L., Lopez-Ramirez, P., Cabrera-Mancilla, E. and Ramirez-Camarena, C. (1998). Toxicity associated with blooms of *Pyrodinium bahamense* var. compressum in southwestern Mexico, Harmful algae. Proc. VIII Int. Conf. On Harmful Algae. Xunta de Galicia and Intergovernmental Oceanographic Commission of UNESCO. Vigo, Spain. **pp. 60.**

Park, Y.-T., Park, J.-B., Chung, S.-Y., Song, B.-C., Lim, W.-A., Kim, C.-H. and Lee, W.-J. (1998). Isolation of marine bacteria killing red tide microalgae I. Isolation and algicidal properties of *Micrococcus* sp. LG-1 possessing killing activity for harmful dinoflagellate, Cochlodinium polykrikoides. *Journal-Korean Fisheries Society* **31, 767-773.**

Rosales-Loessener, F., Porras, E. and Dix, M. (1989). Toxic shellfish poisoning in Guatemala. *In:* Red Tides. Biology, Environmental Science, and Toxicology. Okaichi, T., Anderson, D. M. and Nemoto, T. (eds.) New York. **pp. 113-116.**

Sawabe, T., Makino, H., Tatsumi, M., Nakano, K., Tajima, K., Iqbal, M. M., Yumoto, I., Ezura, Y. and Christen, R. (1998). *Pseudoalteromonas bacteriolytica* sp. nov., a marine bacterium that is the causative agent of red spot disease of Laminaria japonica. *International Journal of Systematic Bacteriology* **48(3), 769-774.**

Schubert, R. H. (1967). The taxonomy and nomenclature of the genus *Aeromonas* Kluyver and van Niel 1936 Part I. Suggestions on the taxonomy and nomenclature of the aerogenic *Aeromonas* species. *International Journal of Systematic Bacteriology* **17(1), 23-37.**

Shieh, W. Y., Chen, Y.-W., Chaw, S.-M. and Chiu, H.-H. (2003). *Vibrio ruber* sp. nov., a red, facultatively anaerobic, marine bacterium isolated from sea water. *International Journal of Systematic And Evolutionary Microbiology* **53(2), 479-484.**

Skerratt, J., Bowman, J., Hallegraeff, G., James, S. and Nichols, P. (2002). Algicidal bacteria associated with blooms of a toxic dinoflagellate in a temperate Australian estuary. *Marine Ecology. Progress Series* **244, 1-15.**

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and Kumar, S. (2011). MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* **28(10), 2731-2739.**

Usup, G. and Azanza, R. V. (1998). Physiology and bloom dynamics of the tropical dinoflagellate *Pyrodinium bahamense*. *Nato Asi Series G Ecological Sciences* **41, 81-94.**

Usup, G., Pin, L. C., Ahmad, A. and Teen, L. P. (2002). *Alexandrium* (Dinophyceae) species in Malaysian waters. *Harmful Algae* **1(3), 265-275.**

Yang, F., Li, X., Li, Y., Wei, H., Yu, G., Yin, L., Liang, G. and Pu, Y. (2013). Lysing activity of an indigenous algicidal bacterium *Aeromonas* sp. against *Microcystis* spp. isolated from Lake Taihu. *Environmental Technology* **34(11), 1421-1427.**

Yoshinaga, I. (1998). Population structure of algicidal marine bacteria targeting the red tide forming alga *Heterosigma akasiwo* (Raphidophyceae), determined by restriction fragment length polymorphism analysis of the bacterial 16S ribosomal RNA genes. *Marine Ecology Progress Series* **170, 33-44.**

Yoshinaga, I., Kawai, T., Takeuchi, T. and Ishida, Y. (1995). Distribution and fluctuation of bacteria inhibiting the growth of a marine red tide phytoplankton *Gymnodinium mikimotoi* in Tanabe Bay (Wakayama Pref., Japan). *Fisheries Science: FS* **61(5), 780-786.**

 ISSN (print): 1823-8262, ISSN (online): 2231-7538