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Algicidal Characteristics of Marine Bacterium Strain OT-1 Isolated from Red-tide Area

Jae Hak Sohn, Jung-Hyun Lee, Tae-Young Ahn[1)] and Sang-Jin Kim
Microbiology Lab., Korea Ocean Research & Development Institute,
[1)] Dept. Microbiology, Dankook University

In the screening of algicidal bacteria from seawater of Masan Bay, Korea, an algicidal bacterium OT-1, which has killing activity for *Skeletonema costatum*, was isolated. The strain showed intensive algicidal activity on both lag and logarithmic phase cell of *S. costatum*. On the basis of phenotypic, chemotaxonomic, and phylogenetic comparison, strain OT-1 is classified as a novel genus designated *Kordimonas koreansis* gen. nov., sp. nov. in the family *Flavobacteriaceae* of *Cytophaga-Flavobacteria-Bacteroides* (CFB) group. Among 13 tested algal cultures, *Skeletonema costatum*, *Thalassiosira* sp., *Heterosigma akashiwo* and *Cochlodinium polykrikoides*, were selectively killed by the strain. Cell-free culture filtrates from monoculture of OT-1 and co-culture with *S. costatum* revealed algicidal activity against *S. costatum*. These facts indicate that strain OT-1 secretes algicidal extracelluar substance. The partially purified algicidal substances by DEAE-Sepharose and PAGE exhibited algicidal activity against *S. costatum* by agar diffusion method. Molecular mass of the proteinous substance was approximately 44 kDa, and it was composed of tetramer of 11 kDa subunit. N-terminal amino acid sequence of the protein was determined, but there is no match known protein.

Introduction

Bacteria are known to influence the dynamics of phytoplankton populations by stimulating or inhibiting phytoplankton growth through nutrient regeneration, endosymbiosis, and production of stimulatory or inhibitory compounds (7). In marine ecosystem, the rapid increase of algicidal bacteria, which kill and/or lyse algae, observed in the termination and decomposition phase of algal bloom (9, 12). Algicidal bacteria targeting specific phytoplankton may considered to be the one of the agents, which regulates the change of phytoplankton communitys structure (4, 6). Consequently, there is a possibility that algicidal bacteria could be useful tools in reducing the impact of noxious red tides.

Algicidal bacteria isolated from various coastal seawater during the period of algal blooming (7, 12) or that of non-algal bloom (3, 11) by using of different screening methods, such as a soft-agar overlay technique and an enrichment technique in liquid medium. The taxa of these algicidal bacteria are widely spread among genera; these genera are *Alteromonas*, *Flavobacterium*, *Pseudoalteromonas*, *Pseudomonas*, and *Vibrio* as well as gliding bacteria *Cytophaga* and *Saporospira*. These algicidal bacteria exhibit varying degrees of specificity for

their target alga, ranging from broad-spectrum effects across algal class (bacilliariophyceae, raphydophyceae and dinoflagellates) to narrow algicide effects against only one or more closely related species (5, 7). In the mode of algicidal action, some bacteria require for physical contact with algal cells (7) and others kill algae indirectly by excreting algicidal compound (s) into the surrounding water (3, 10). Evaluating the role of algicidal bacteria as natural regulators of algal dynamics, as well as critically assessing their possible use as a biological control strategy of algal blooming requires a more comprehensive understanding of how these microbes interact with algae at the molecular, cellular and population levels (3).

In this study, we carried out screening of algicidal bacteria against *Skeletonema costatum* from seawater samples of Masan Bay, South Korea, in which red tide occurred frequently. An algicidal bacterium showing strong algicidal activity was isolated. In this paper, we described the some characterizations of the algicidal bacterium and its algicide.

Isolation of Algicial Bacteria and Its Activity

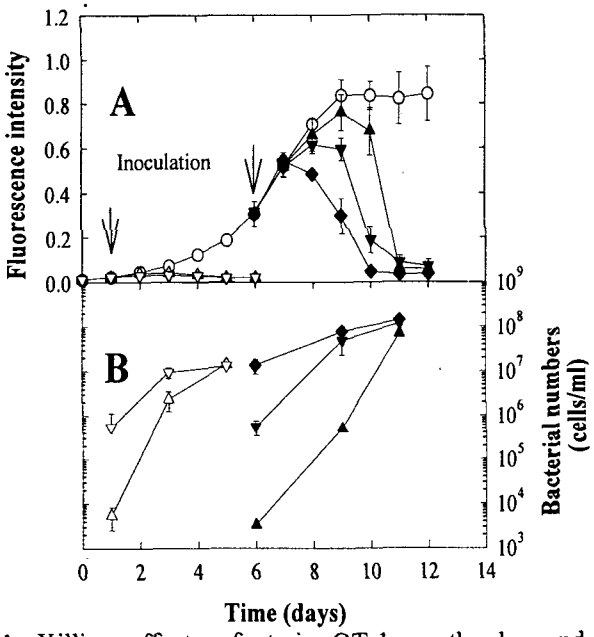

Fig. 1. Killing effects of strain OT-1 on the lag and logarithmic phase cells of *Skeletonema costatum*. A: Growth of *S. costatum* after addition of bacterial cell in log phase (1 day) (○; No inoculation as control, △; 10^3 cells/mℓ, ▽; 10^5 cells/mℓ) and logarithmic phase (▲; 10^3 cells/mℓ, ▼; 10^5 cells/mℓ, ◆; 10^7 cells/mℓ). B: Growth of strain OT-1. Arrows indicate bacterial inoculation.

Skeletonema costatum killing bacteria were detected to 5.6 MPN $m\ell^{-1}$ during the period of bloom caused by target algal species in Masan Bay on September 10, 1996. An algicidal bacterium designated as strain OT-1 was isolated from positive MPN tubes. The isolated strain showed algicidal activity in the lag and logarithmic phase culture of *S. costatum* (Fig. 1). When strain OT-1 was inoculated into the logarithmic phase of *S. costatum* culture ($7.0x10^5$ cells $m\ell^{-1}$), algal growth was dramatically inhibited after it reached at 10^7 cells $m\ell^{-1}$ in F/2 medium, which contained no organic nutrients (Fig. 1B). The bacterial cell number increased again with the progress of lysis and reached at *ca.* 10^8 cells $m\ell^{-1}$. Resultantly, the growth of strain OT-1 in F/2 medium limited in organic nutrients is considered to be due to the utilization of dead algal cells derived from algal cell lysis by unknown mechanism during co-cultivation.

Algicidal Ranges

In general, algicidal bacteria isolated from marine ecosystem have a wide prey range for diatom, raphidophycean flagellates and dinoflagellates (3, 5, 7-8, 10, 11). The algicidal ranges of strain OT-1 was determined by co-cultivation method with microalgal species listed in Table 1. Algicidal bacterium, strain OT-1 has a specific killing range for *Skeletonema costatum*, *Thalassiosira* sp., *Heterosigma akashiwo* and *Cochlodinium polykrikoides* (Table 1). The preyed algal species have been reported as mainly red-tide organisms in costal area of Korea. It is believed that strain OT-1 plays a significant controlling factor to terminate the red tide in coastal seawater and influences on the population dynamics of phytoplankton in nature. In the morphological observation by microscope, unialgal cells of *S. costatum* treated with OT-1 were swollen and rounded up like a protoplast and were lysed finally (Fig. 2A). *H. akashiwo* (initial density 3000 cells $m\ell^{-1}$) was decreased to the level of 10 cells $m\ell^{-1}$ after initial 2 days of incubation. After 4 days of incubation almost all of the cells of *H. akashiwo* was lysed. *Cochodinium polykrikoides* (initial density 600 cells $m\ell^{-1}$) was inhibited after 1 day and then decreased to 80 cells $m\ell^{-1}$ after 4 days. The lysis of *H. akashiwo* and *C. polykrikoides* occurred through swelling and rounding up of the cells (Fig. 2B and C).

Mode of Algicidal Action

Algicidal marine bacteria can be divided into 2 groups by mode of action; the first group directly attacks and lyses target algae after cell-to-cell attachment, and the second group produces and excretes algicidal substances to kill microalgae. When the filtrates (SC) of *S. costatum* lysed by strain OT-1 were added into fresh *S. costatum* culture, algal cell density was decreased and killed depending on the concentration (17-66%) of that (Fig. 3A). These results imply that the killing of *S. costatum* induced by unknown compound produced during cocultivation of bacteria and algae. The culture filtrates (MB) of strain OT-1 grown in Marine broth also showed higher killing effects than that of co-cultures with bacteria-algae (Fig. 3B). Consequently, strain OT-1 was demonstrated to have the mode of action by the algicidal compound, which excreted into culture medium during bacterial cultivation with or without algae.

Identification of Algicidal Strain

Phenotypic and chemotaxonomic data showed that the strain seemed to be closely related a member of *Cytophaga-Flavobacterium-Bacteroides* group (not shown data). On the 16S rDNA-based phylogenetic analysis the strain OT-1 showed closely relationships with the members of the *Cytophaga-Flavobacterium-Bacteroides* (CFB) phylum (Fig. 4) and belonged to the family *Flavobacteriaceae,* which was emended by Bernadet *et al.* (2). Strain OT-1 was the closest relative to algicidal bacterium *Cytophaga* sp. AA8-3, *Cytophaga* sp. J18/M01 with the very high percentage homology of 98~99%. This branch was constructed with only the algicidal bacteria, suggesting that this group of bacteria possessed algicidal activity. The 16S rDNA sequence similarities between these algicidal bacteria and *Cytophaga latercular* were

Table 1. Algicidal cultures used in this study and algicidal spectrum by strain OT-1

Class	Taxon	Strain No.	Algicidal Effects	Source and History
Bacillariophyceae	*Thalassiosira* sp.	KORDI-1	+	This study
	Skeletonema costatum	KORDI-4	+	This study
	Chaetoceros glycilis	KMCC-B23	-	J.H. Lee; Nacdong estuary
Raphidophyceae	*Heterosigma akashiwo* (Hada) Hada	NIES-10	+	M.M. Watanabe; Marima-Nada, Seto Inland sea, Japan
Dinophyceae	*Heterocapsa triestrum* Stein	NIES-235	-	S. Yoshimatsu; Marima-Nada, Seto Inland sea, Japan
	Prorocentrum micans	KORDI-14	-	This study
	Gymnodinium sp. *A8*	KORDI-12	-	This study
	Gymnodinium sp. *A9*	KORDI-13	-	This study
	Gym. mikimotoi	KORDI-10	-	This study
	Gym. sanguineum	KORDI-11	-	This study
	Cochlodinum polykrikoides	KORDI-8	+	This study
Cyanobacteria	*Microcystis aeruginosa*	KMCC-C13	-	Y.J. Kim; Chung-Mu Harbor
Chlorophyceae	*Chlorella vulgaris*	KMCC-FC1	-	UTEX 259

UTEX; University of Texas, USA, KMCC; Korea Marine Microalgae Culture Collection, NIES; National Institute for Environmental Studies, Japan.

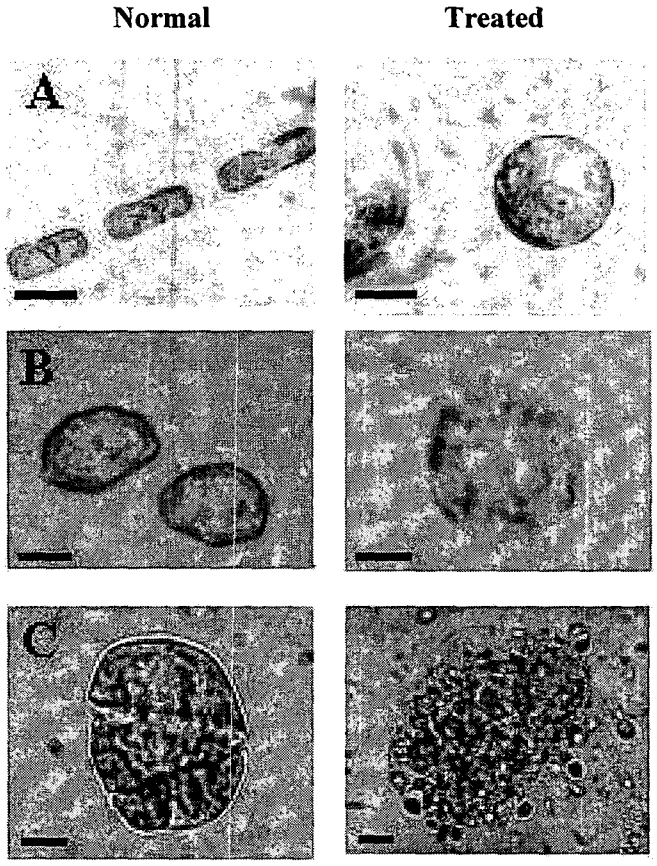

Fig. 2. Lytic effects of strain OT-1 on *Skeletonema costatum* (A), *Heterosigma akashiwo* (B) and *Cochlodinium polykrikoides* (C). Bar = 10 μm

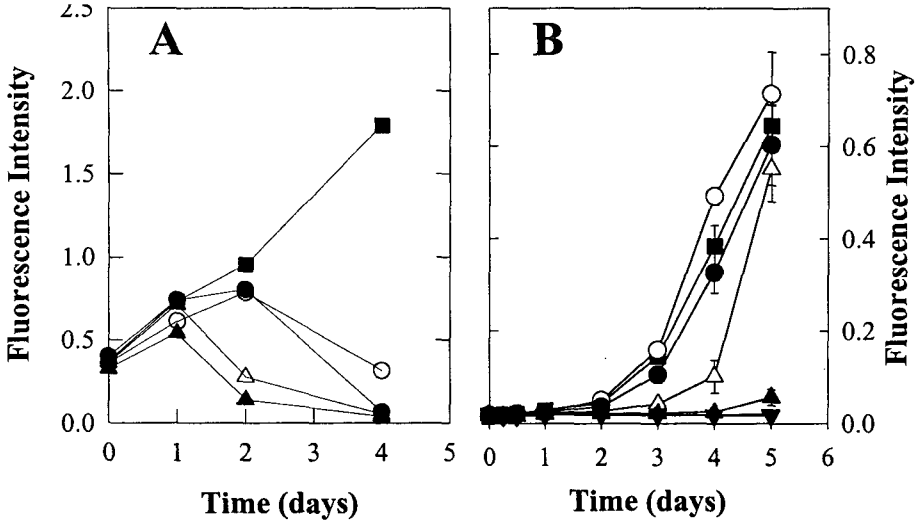

Fig. 3. Effects of culture filtrates from the co-cultivation of *Skeletonema costatum* and strain OT-1 (A) and pure culture of strain OT-1 (B) on the growth of *S. costatum* (■; Control, A: ○; 17%, ●; 33%, △; 50%, ▲; 67%. B : ○; 0.03%, ●; 0.06%, △; 0.125%, ▲; 0.25%, ▽; 0.5%, ▼; 1.0%).

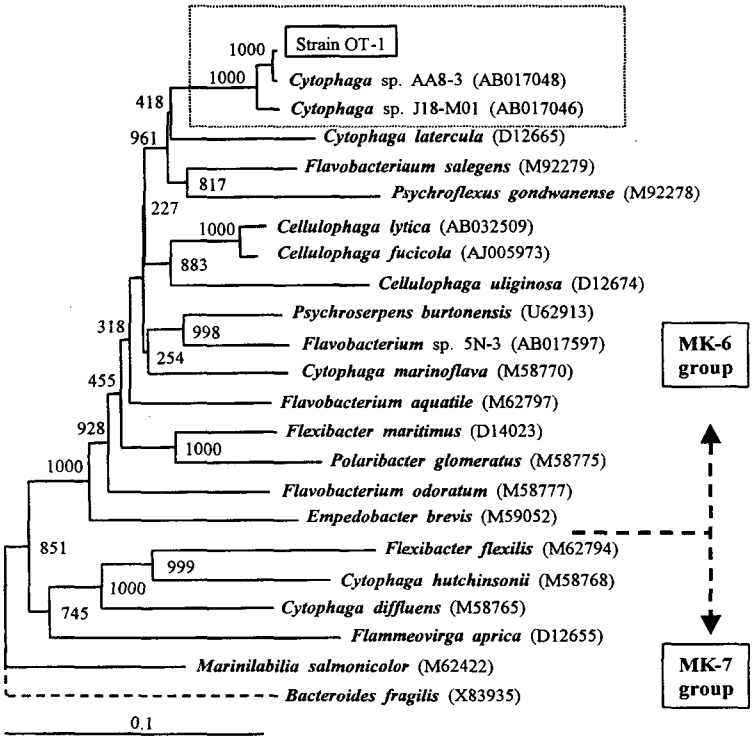

Fig. 4. Phylogenetic tree of strain OT-1. The scale bar corresponds to approximately 0.1 changes per nucleotide position

less than 92.5%. These results indicate that the algicidal bacteria including strain OT-1, *Cytophaga* sp. AA8-3 and *Cytophaga* sp. J18/M01 are a phylogenetically distinct group in the CFB group. Therefore, we propose that strain OT-1 is a novel bacterial species designated *Kordimonas koreansis* gen. nov., sp. nov. in the family *Flavobacteriaceae* of CFB group.

Purification of Algicidal Protein

The concentrated sample from bacterial culture filtrate was applied to anion exchange column and eluted with a non-linear NaCl gradient in Tris buffer (20 mM, pH 8.0). Main algicidal activity was detected at fraction eluted with 0.1M NaCl in Tris buffer. A protein band eluted from the gel after native-protein gel electrophoresis showed algicidal activity on the *S. costatum* lawns (Fig. 5). This protein was detected to single band on SDS-PAGE gels (Fig. 6). Molecular mass of algicidal protein was approximately 44 kDa in natural condition. Also it was determined to be approximately 11 kDa by SDS-PAGE (Fig. 5). The N-terminal amino acid sequence of purified algicide was Gln-Tyr-Val-Thr-Asn-Asn-Leu-Val-Ser-Pro-Gly-Val-Ile-Thr-Ile-Ile-Gly-Tyr-Thr-Phe. In comparison of the N-terminal sequence with known protein sequence in the NCBI and SWISSPROT databases, homologous protein was not identified.

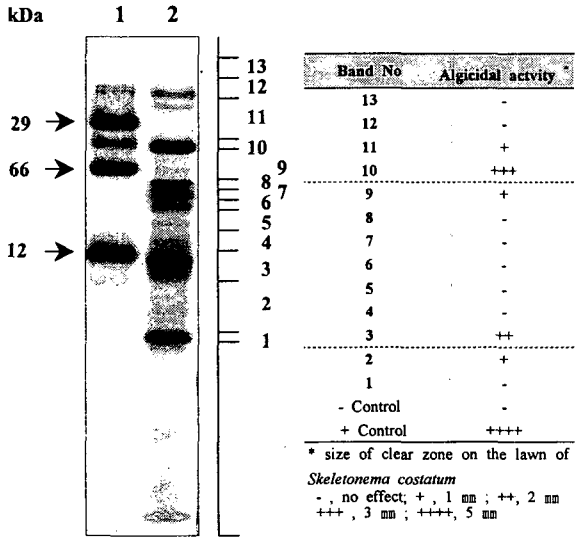

Fig. 5. Native-polyacrylamide gel eletrophoresis (12%) of partially purified sample (left) and results of algicidal asssay (right).
Lane 1; protein marker,
Lane 2; partially purified sample

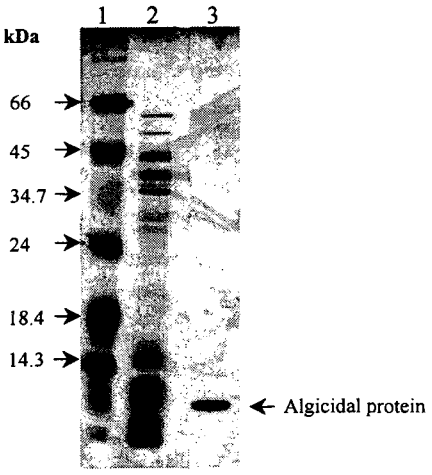

Fig. 6. SDS-polyacrylamide gel electrophoresis (12%) of active compound.
Lane 1; Protein marker
Lane 2; Partially purified protein
Lane 3; No. 10 band from the native gel

The present data showed that unidentified protein, secreted from our strain, from algicidal bacteria was responsible for the algicidal effect. Therefore, we are going to further research for solving a subject related to the identification and molecular properties of algicide, and understanding the killing mechanism of phytoplankton.

Acknowledgments

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