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Isolation and characterization of a marine algicidal bacterium against the toxic dinoflagellate *Alexandrium tamarense*

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Abstract

Interactions between bacteria and harmful algal bloom (HAB) species have been acknowledged as an important factor regulating both the population dynamics and toxin production of these algae. A marine bacterium SP48 with algicidal activity to the toxic dinoflagellate, *Alexandrium tamarense*, was isolated from the Donghai Sea area, China. Genetic identification was achieved by polymerase chain reaction amplification and sequence analysis of 16S rDNA. Sequence analysis showed that the most probable affiliation of SP48 was to the γ-proteobacteria subclass and the genus *Pseudoalteromonas*. Bacterial isolate SP48 showed algicidal activity through an indirect attack. Additional organic nutrients but not algal-derived DOM was necessary for the synthesis of unidentified algicidal compounds but β-glucosidase was not responsible for the algicidal activity. The algicidal compounds produced by bacterium SP48 were heat tolerant, unstable in acidic condition and could be easily synthesized regardless of variation in temperature, salinity or initial pH for bacterial growth. This is the first report of a bacterium algicidal to the toxic dinoflagellate *A. tamarense* and the findings increase our knowledge of bacterial–algal interactions and the role of bacteria during the population dynamics of HABs.

Keywords: *Alexandrium tamarense*; Algicidal bacteria; *Pseudoalteromonas*; Harmful algal blooms

1. Introduction

Harmful algal blooms (HABs), caused by rapid growth and accumulation of microalgae in the ocean, pose significant adverse impacts on public health, marine environments, aquaculture and natural resources. Recently, the apparently increasing occurrence of HABs throughout the world has led to extensive research to mitigate the blooms, and various management strategies, such as flocculation of microalgae with clay, have been developed (Sengco and Anderson, 2004).

The outbreak and termination of HABs in marine environments are affected by physical and chemical factors, as well as biological factors. Of the factors that regulate HABs dynamics, algal–bacterial interactions are increasingly cited as potential regulators both in the sense of decreasing and enhancing algal blooming (Doucette, 1995; Doucette et al., 1998). Thus, interactions range from beneficial trophic relationships to negative impacts on algal growth. Research into the relationship between bacteria and algae has resulted in

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the isolation of several strains of bacteria capable of inhibiting or killing HABs species. The algicidal bacteria isolated from marine environments have been assigned to the genera *Alteromonas*, *Bacillus*, *Cellulophaga*, *Cytophaga*, *Flavobacterium*, *Micrococcus*, *Planomicrobium*, *Pseudoalteromonas*, *Pseudomonas*, *Saprospira*, *Vibrio* and *Zobellia* (Mayali and Azam, 2004). In most cases, these algicidal bacteria are algal species-specific and their abundance increase during the decline of an algal bloom (Lovejoy et al., 1998; Yoshinaga et al., 1998; Doucette et al., 1999; Amaro et al., 2005). These facts have led to the suggestion that these algicidal bacteria may play a major role in controlling HAB dynamics, specifically near the end of the blooms.

Alexandrium tamarense is a notorious HABs species which is associated with the largest number of paralytic shellfish poison (PSP) poisoning cases (Anderson et al., 1996). There have been reports concerning the bacteria associated with laboratory maintained strains of *A. tamarense* (Kodama et al., 1996; Shimizu et al., 1996; Gallacher et al., 1997; Kopp et al., 1997; Babinchak et al., 1998; Hold et al., 2001a; Simon et al., 2002; Zheng et al., 2005) and also *A. tamarense* blooms (Wichels et al., 2004). These investigations mainly focused on the bacteria associated with laboratory cultures of *A. tamarense*, bacterial production of PSP and their effects on the PSP production of *A. tamarense*. No bacterial strain algicidal to *A. tamarense* has previously been isolated. However, in this study, a strain of bacterium algicidal to *A. tamarense* was isolated from the red-tide area in Donghai Sea area, China. Identification, characterization of the bacterium and of the mode of algicidal action were undertaken in this study.

2. Materials and methods

2.1. Algal cultures

A. tamarense ATGD98-006 cultures (supplied by the Algal Culture Collection, Institute of Hydrobiology, Jinan University, Guangzhou, China), were maintained in f/2 medium (without silicate) prepared with natural seawater (Guillard, 1975) at 20 ± 1 °C under a 12:12 h light–dark cycle with a light intensity of 50 μmol photons m^{-2} s^{-1}. After repeated washing, together with lysozyme/SDS and antibiotic (a mixture of gentamycin, streptomycin, cephalothin and rifampicin) treatments, the *A. tamarense* cultures were shown to be axenic using bacterial cultivation methods, epifluorescence microscopy examination and PCR amplification of 16S rDNA for both the eubacteria and archaea (Su et al., 2007).

2.2. Isolation and screening of algicidal bacteria

Bacteria to be screened for algicidal activity were isolated from a *Prorocentrum donghaiense* Lu bloom (cell concentration ~10^8 cells L^{-1}), in which *A. tamarense* was also detected (cell concentration ~10^4 cells L^{-1}). Water samples were collected during the National Science Foundation of China 973 project MC2003-2 cruise on 19 May 2003. Samples were serially diluted (10-fold dilution) and 0.1 mL aliquots of each dilution were spread onto Zobell 2216E agar plates followed by incubation for 7 days at 25 °C. Individual colonies of distinct morphology were streaked onto 2216E agar plates for purification and frozen at −70 °C in 10% (v/v) glycerol.

For screening of algicidal bacteria, bacterial isolates were grown in 3 mL 2216E broth (25 °C, 180 rpm) for 12–24 h. An aliquot (0.5 mL) of each bacterial culture was inoculated in triplicate into 50 mL axenic logarithmic-phase cultures of *A. tamarense* and 0.5 mL 2216E broth only was added into algal cultures instead of bacterial cultures as a control. The growth of *A. tamarense* was monitored by measuring the fluorescence density of the alga cultures at an excitation wavelength of 450 nm and an emission wavelength of 680 nm, and also by microscopic observation. A bacterial strain was considered to be algicidal when *A. tamarense* cultures were killed.

2.3. Determination of β-glucosidase activity (β-GlcA)

β-GlcA dynamics of the algal cultures was investigated to assessed bacterial activity during algicidal progress. β-GlcA was measured according to Hoppe's method, with a small modification (Hoppe, 1993). Samples were taken from the flask and MUF-β-D-glucopyranoside (Sigma Corp.) was added to a final concentration of 250 μM. The fluorescence of samples was measured after incubation for 0.5 h and 2.5 h in the dark at 20 °C using a SPECTRA max M2 (Molecular Devices, USA) at an excitation wavelength of 362 nm and an emission wavelength of 450 nm, respectively. β-GlcA was calculated using the following equation:

$$V = \frac{F_b - F_a}{\Delta T \times S}$$

where V (μmol L^{-1} h^{-1}) refers to the hydrolysis rate of β-glucosidase to substrate, F_a to the fluorescence inten-