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Comparative analysis of two algicidal bacteria active against the red tide dinoflagellate *Karenia brevis*

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Abstract

The red tide dinoflagellate *Karenia brevis* blooms annually along the eastern Gulf of Mexico, USA, and is often linked to significant economic losses through massive fish kills, shellfish harvest closures, and the potential threat to humans of neurotoxic shellfish poisonings as well as exposure to aerosolized toxin. As part of an effort to enhance the strategies employed to manage and mitigate these events and their adverse effects, several approaches are being investigated for controlling blooms. Previous studies have established the presence of algicidal bacteria lethal to *K. brevis* in these waters, and we aim to characterize bacterial–algal interactions, evaluate their role as natural regulators of *K. brevis* blooms, and ultimately assess possible management applications. Herein, the algicidal activity of a newly isolated *Cytophaga/Flavobacterium/Bacteroidetes* (CFB)-bacterium, strain S03, and a previously described CFB-bacterium, strain 41-DBG2, was evaluated against various harmful algal bloom (HAB) and non-HAB species (23 total), including multiple clones of *K. brevis*, to evaluate algal target specificity. Strains S03 and 41-DBG2, which employ direct and indirect modes of algicidal lysis, respectively, killed ~20% and ~40% of the bacteria-containing isolates tested. Interestingly, no bacteria-free algal cultures were resistant to algicidal attack, whereas susceptibility varied occasionally among bacteria-containing isolates of a single algal taxon originating from either the same or different geographic location. The dynamics of *K. brevis* culture death appeared to differ according to whether the algicidal bacterium did or did not require direct contact with algal cells, with the former most rapidly affecting *K. brevis* morphology and causing cell lysis. Both bacterial strains promoted the formation of a small number of cyst-like structures in the *K. brevis* cultures, possibly analogous to temporary cysts formed by other dinoflagellates exposed to certain types of stress. Results were also consistent with earlier work demonstrating that bacterial assemblages from certain cultures can confer resistance to attack by algicidal bacteria, again indicating the complexity and importance of microbial interactions, and the need to consider carefully the potential for using such bacteria in management activities.
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1. Introduction

The athecate dinoflagellate *Karenia brevis* (formerly *Gymnodinium breve*; Davis, 1948) G. Hansen and Moestrup is a harmful species often associated with massive fish and marine mammal mortality events in the Gulf of Mexico (Steidinger et al., 1998). As a result, upwards of millions of dollars are lost by the recreation and tourism industries during years with *K. brevis* red tide events (Anderson et al., 2001). In an effort to manage and mitigate the potentially devastating effects of these and other types of harmful algal blooms (HABs), several control strategies such as chemical algicides, flocculants and other physical manipulations, as well as biological agents, are currently under investigation (Hennes et al., 1995; Doucette et al., 1999; Anderson et al., 2001; Sengco et al., 2001; Kim, 2006). In marine and freshwater ecosystems, biological agents such as bacteria, viruses, protozoans, and fungi have all shown promise as potential algal bloom suppressors (Imai et al., 1998; Doucette et al., 1999; Castberg et al., 2001; Manage et al., 2001; Kang et al., 2005).

Bacteria have significant impacts on aquatic biogeochemical processes such as carbon flux and nutrient regeneration (Azam, 1998; Doucette et al., 1998; Copley, 2002). These microbes are known to be active in the decomposition of freshwater algal blooms (Kang et al., 2005) and may play a similar role in marine systems by influencing the initiation, growth, main-

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tenance, and/or termination of HAB populations (Imai et al., 1998; Kodama et al., 2006). Specifically, a bacterial assemblage can have inhibitory or stimulatory effects on algal growth during a bloom event (Riquelme et al., 1988; Fukami et al., 1997; Simon et al., 2002). Doucette et al. (1999) hypothesized that the relative and absolute abundances of algicidal bacteria, in particular, would increase as a bloom moves through its initiation, development, and maintenance phases. Ultimately, changes in the abundance and composition of the bacterial community may lead to bloom decline as these algicidal bacteria begin to negatively impact algal growth and/or render the alga more susceptible to other factors, such as grazing pressure and nutrient competition. The extent to which bloom termination is a reflection of bacterially mediated algal lysis remains unknown.

Algicidal bacteria are classified according to their mode of lysis, which can be either direct or indirect (see reviews by Mayali and Azam, 2004; Salomon and Imai, 2006). The former requires that a bacterium be in direct contact with the target algal species and lysis is thought to result from enzymatic digestion of the algal cell wall/membrane (e.g., Lee et al., 2000). Alternatively, algicidal bacteria exhibiting an indirect killing mechanism release a dissolved lytic agent(s) effective in the absence of physical contact with the target. Based on several recent reviews (Fukuyo et al., 2002; Mayali and Azam, 2004; Hare et al., 2005), at least 56 unique algicidal bacteria have been isolated and classified phylogenetically. Of these, 36 strains were characterized according to their lytic mechanism, with approximately 70% showing an indirect mode of attack and the remaining 30% requiring direct contact with the algal cells. Although the functional significance of cell lysis by algicidal bacteria remains to be determined, this activity clearly enhances the supply of algal-derived organic nutrients that may then provide a competitive advantage to this segment of the microbial community (Doucette, 1995; Mayali and Azam, 2004).

Most algicidal bacteria characterized thus far belong to the *Cytophaga/Flavobacterium/Bacteroidetes* group (Fandino et al., 2001; Yoshinaga et al., 1995) or to the γ-Proteobacteria group (Imai et al., 1995; Yoshinaga et al., 1995). Of the 56 algicidal strains noted above, about 50% belong to the CFB group while about 45% are members of the γ-Proteobacteria, with the remaining strains representing the gram-positive genera *Micrococcus*, *Bacillus*, and *Planomicrobium*. Although there is no conclusive link between phylogeny and the lytic mechanism, the available data suggest that gram-positive bacteria and γ-Proteobacteria employ primarily an indirect mode of attack, while the lytic activity of the CFB group can involve either direct or indirect interactions with target cells.

Considering the overall size of the CFB group, relatively few species are actually algicidal and the mode of attack does not appear to be consistent within or between the various genera. Doucette et al. (1999) isolated an algicidal *Cytophaga* sp., strain 41-DBG2, capable of killing *K. brevis* via the production of a soluble, heat-sensitive, algicidal compound (Twiner et al., 2004), yet a closely related *Cytophaga* sp., strain J18/M01, lyses the raphidophyte *Chattonella antiqua* by direct attack (Imai et al., 1993). Alternatively, *Aquimarina latercula* (formerly *Cytophaga latercula*; Nedashkovskaya et al., 2006), another marine bacterium within the CFB group, exhibits no algicidal activity and has been used as a negative control in many of our studies.

In this paper, we report the isolation of a new algicidal bacterium belonging to the family *Flavobacteriaceae* (strain S03; GenBank accession no. EU021292) from the Gulf of Mexico and compare its direct mode of algicidal attack with the indirect activity of *Cytophaga* sp. (strain 41-DBG2; GenBank accession no. AF427479) using *K. brevis* isolates determined previously to be susceptible or resistant to these algicidal bacteria. We also assess the effects of exposure to such bacteria on the morphology, growth characteristics, and bacterial succession patterns in the *K. brevis* cultures. The data obtained from this investigation will improve our understanding of the complex interactions between algicidal bacteria and their target algal species, as well as aid in evaluating the possible use of algicidal bacteria as part of a HAB management strategy.

2. Materials and methods

2.1. Field collections

Water samples were collected from the west Florida shelf in 2001 during the ECOHAB RV Suncoaster cruises (September 20–26; October 20–26). At most stations whole water samples were obtained from three depths, corresponding to surface, middle (mid-water column), and bottom (approximately 1 m from the bottom); occasionally, surface samples were also collected from a continuous flow-through system maintained on the ship's deck. All samples were pre-filtered through 80 μm nitex screen and three aliquots of the <80 μm filtrate containing both attached and free-living bacteria were amended with glycerol (10% final concentration) and stored in liquid nitrogen. These 'freeze-downs' were transported to the laboratory and used to screen for algicidal activity as outlined below.

2.2. Culture conditions and monitoring of growth

A non-axenic (i.e., bacteria-containing) *K. brevis* clonal isolate from Charlotte Harbor, FL, USA (isolate C2; provided by Dr. K. Steidinger, Fish and Wildlife Research Institute, St. Petersburg, FL, USA) was used for the initial screenings of algicidal activity and bacterial isolations. Additional studies employed the non-axenic *K. brevis* clonal isolate NOAA-1 and bacteria-free cultures of *Karenia mikimotoi* isolate G303ax-2 from Suo Nada, Japan (provided, respectively, by Dr. S. Morton, National Ocean Service/CCEHBR, Charleston, SC, USA and Dr. K. Fukami, Kochi University, Japan). The latter isolate was selected based on its phylogenetic similarity to *K. brevis* and its bacteria-free status as demonstrated by both direct microscopic observations and PCR-based methods (Mayali and Doucette, 2002). All algal cultures used in this study were grown in 25 mL of *f*/2 medium without silicate (Guillard, 1975) at 20 °C on a 16 h:8 h L:D regime with a photon flux rate of ~75 μmol m^{-2} s^{-1} (model QSL 100; Biospherical Instruments, San Diego, CA, USA).