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Growth-promoting effects of a bacterium on raphidophytes and other phytoplankton

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Abstract

On 29 April 2003, a *Heterosigma akashiwo* bloom (9.5×10^4 cells mL^{-1}) associated with a fish kill ($>10^4$ dead fishes estimated from aerial surveys) was observed offshore of Bulls Bay, McLellanville, South Carolina, USA. To assess a potential cause of this bloom event, we investigated the bacterial diversity and algal/bacterial interactions in the bloom microbial community. Thirty-five bacterial strains were isolated and screened for algicidal or algal growth-promoting activities. One strain (BBB25) had significant growth-promoting effects on all 7 algal species tested: three raphidophytes (*Heterosigma akashiwo*, *Chattonella subsalsa*, *Fibrocapsa japonica*), two diatoms (*Chaetoceros neogracile*, *Nitzschia* sp.), a cryptophyte (*Cryptomonas* sp.), and a chlorophyte, *Ankistrodesmus* sp. This strain (BBB25) is a Gram-positive, rod-shaped spore-forming bacterium. Partial 16S rDNA gene sequence and morphological characters indicated that BBB25 is related closely to the genus *Bacillus*. The general nature of the algal response indicates that the growth-promoting effects of BBB25 are not specific to *H. akashiwo*, and suggests potentially widespread effects. Since the presence or relative abundance of the other algal species was not assessed during the bloom initiation period, the selective stimulatory effect on *H. akashiwo* bloom formation in Bulls Bay is unknown. These results demonstrate, however, the potential for bacterial species to play a regulatory role in bloom formation.

Keywords: Algal growth-promoting bacteria; *Bacillus*; Harmful algal blooms; *Heterosigma akashiwo*; Marine bacteria; Raphidophytes; 16S rDNA

1. Introduction

Raphidophyte blooms have been noted frequently over the past decade, often in association with fish mortalities at mariculture facilities (Anderson, 1989, 1997; Honjo, 1993; Landsberg et al., 1995; Kim et al., 1998). Understanding the factors that regulate raphidophyte bloom dynamics is important to fishery managers, and may provide insight into methods for predicting and mitigating raphidophyte bloom occurrence. The interaction between bacteria and phytoplankton is increasingly recognized as an important factor in the physiology and dynamics of harmful algal blooms (HABs), including raphidophyte blooms (Doucette, 1995; Doucette et al., 1998). Different phylogenetic groups of bacteria have been observed to associate with different phases of algal blooms (Smith et al., 1995; Riemann et al., 2000) with impacts ranging from algal growth enhancement (Furuki and Kobayashi, 1991; Ogata et al., 1996; Suminto and Hirayama, 1997; Ferrier et al., 2002) to algicidal or algistatic effects

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(Imai et al., 1993; Doucette, 1995; Imai et al., 1995; Yoshinaga et al., 1995, 1997; Fukami et al., 1996; Lovejoy et al., 1998; Doucette et al., 1999; Skerratt et al., 2002; Liu et al., accepted).

The role of algicidal or algistatic bacteria in limiting raphidophyte bloom progression has received attention. Increasing evidence has revealed a close spatial and temporal association between the dynamics of algicidal bacterial populations and the termination of three raphidophycean flagellate (*Heterosigma*, *Chattonella*, and *Fibrocapsa*) blooms by use of the most probable number (MPN) method (Yoshinaga et al., 1995; Imai et al., 1998; Liu et al., accepted). Some bacteria algicidal to these marine microalgae have been isolated, and include a variety of genera ranging from *Pseudoalteromonas* and *Vibrio* of the γ-*Proteobacteria*, *Cellulophaga* and *Flavobacterium* of the *Cytophaga-Flavobacter/Flexibacter-Bacteroides* (CFB) group, and low G + C Gram-positive bacteria such as *Bacillus* (Yoshinaga et al., 1997; Lovejoy et al., 1998; Liu et al., accepted). The taxonomic specificities of these algicidal bacteria have been tested. Although some bacterial isolates demonstrated general algicidal effects to all three raphidophycean genera, such as a gliding bacterial *Cellulophaga* isolate J18/M01 (Imai et al., 1993), or a *Vibrio*-related isolate KMC7541 and a *Bacillus*-related isolate KMC0215 (Liu et al., accepted), other bacterial isolates showed high species-specific algicidal effects and their algicidal activity varied dramatically for bacterial isolates from different locations (Skerratt et al., 2002).

In comparison to algicidal bacteria, the role of bacteria as a stimulatory factor in raphidophyte growth and bloom formation has not been similarly explored. Bacteria have been shown to promote growth of other phytoplankton taxa by fixation of inorganic nitrogen (de-Bashan et al., 2002, 2004), remineralization and/or solubilization of inorganic nutrients (Doucette, 1995; Azam, 1998; Fandino et al., 2001; Vassilev et al., 2006), reduction of oxygen stress (Mouget et al., 1995), metabolic production of CO_2 (Chirac et al., 1985), and influencing pH and redox potential (Parker and Bold, 1961). However, most of these studies focused on either bacteria from culture collections known to have plant growth-promoting effects such as *Azosprillum* (Lebsky et al., 2001; de-Bashan et al., 2002, 2004) or on bacterial assemblages (Fukami et al., 1996; Ferrier et al., 2002). There is a paucity of information on the effects of individual bacterial strains and their *in vitro* and *in situ* effects on phytoplankton species and communities.

On 29 April 2003, a *Heterosigma akashiwo* bloom was observed offshore of Bulls Bay, McLellanville, SC during aerial surveys for stranded sea turtles (Keppler et al., 2005). This was the second major harmful bloom documented in SC offshore waters. It extended from inside the bay to about 8 km offshore and covered an area of over 207 km^2. *H. akashiwo* abundance was measured at 9.5×10^4 cells mL^{-1}. The bloom was associated with a fish kill ($>10^4$ dead fishes estimated from aerial surveys; Keppler et al., 2005). In an effort to assess the cause of this bloom event, we investigated bacterial diversity and algal/bacterial interactions in the bloom microbial community. We isolated 35 bacterial strains from the bloom samples. Preliminary experiments indicated that one of the strains (BBB25) had a strong algal growth-promoting effect on *H. akashiwo* strain CAAE 1663x, while other bacterial isolates showed no observable effect. Based on these preliminary results, the goal of this study was to determine the effect of bacterial strain BBB25 on the growth of raphidophyte and other phytoplankton isolates.

2. Materials and methods

2.1. Algal cultures

Three raphidophyte isolates from SC brackish lagoon ponds, two diatom species, one chlorophyte, and one cryptophyte were used in this study to test the bacterial effects (Table 1). All stock and experimental algal cultures were maintained on a 12:12 L:D photocycle under an irradiance of 70–80 μE m^{-2} s^{-1} at 25 °C. Medium f/2 (Guillard, 1975) made with 0.2 μm filtered Bulls Bay site water was used for each algal culture. The salinity was adjusted with ultrapure deionized water to 20 psu for the three raphidophytes, 30 psu for the two diatoms, and 16 psu for the chlorophyte and cryptophyte. For diatoms only, 107 μM silicate was also included in the f/2 medium (f/2 + Si). *H. akashiwo* CAAE 1663x and *Chattonella subsalsa* CAAE 1662x cultures were grown both axenically and non-axenically for this study and the other algal species were grown non-axenically.

2.2. Sample collection and bacterial isolation

Bacterial sampling was carried out in conjunction with the monitoring efforts in Bulls Bay by the South Carolina Algal Ecology Lab (SCAEL). The *H. akashiwo* Bulls Bay bloom samples were collected in 1-L acid-washed sample bottles. Samples were stored in the dark and processed in the laboratory within 4 h of collection. For total bacterial abundance, 10–20 mL aliquots of the bloom waters were fixed with filtered