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Allelopathic effects of the dinophyte *Prorocentrum minimum* on the growth of the bacillariophyte *Skeletonema costatum*

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ABSTRACT

We investigated growth interactions between the dinophyte *Prorocentrum minimum* and the bacillariophyte *Skeletonema costatum* using bi-algal cultures under axenic conditions. When low cell densities of *P. minimum* and high cell densities of *S. costatum* were inoculated into the same medium, growth of *P. minimum* was suppressed. Other inoculum combinations resulted in reduced *S. costatum* maximum cell densities. A mathematical model was used to simulate growth and interactions of *P. minimum* and *S. costatum* in bi-algal cultures. The model indicated that *P. minimum* always outcompeted *S. costatum* over time. Enriched filtrate from low-density *P. minimum* cultures significantly stimulated *S. costatum* growth, but enriched filtrate from high-density *P. minimum* cultures notably inhibited the growth of *S. costatum*. Growth of *P. minimum* was not affected by enriched filtrate from cultures of *P. minimum* at any density. Filtrates of *P. minimum* cultures were fractionated by ultrafiltration (molecular weight cutoff >3000 Da), and retentate that included polysaccharide(s) significantly inhibited the growth of *S. costatum*.

1. Introduction

Prorocentrum minimum (Pavillard) Schiller, a bloom-forming dinoflagellate which is potentially toxic, occurs in many coastal waters (Karentz and Smayda, 1984; Borkman et al., 1993; Kimor et al., 1985; Kondo et al., 1990a), and many physiological and ecological studies have been conducted to clarify the mechanism controlling blooms of this species (Tyler and Seliger, 1981; Granéli and Moreira, 1990; Grzebyk and Berland, 1996; Hajdu et al., 2000, 2005; Fan et al., 2003; Tango et al., 2005). Recently, Heil et al. (2005) reviewed this species, focusing on taxonomy and systematics, toxicology and harmful effects, direct effects of *P. minimum* exposure, biogeography including allelochemical interactions, and ecophysiology.

Smayda and Borkman (2003) hypothesized that *P. minimum* blooms in Narragansett Bay may related to blooms of not only *Skeletonema costatum* (Greville) Cleve, but also *Heterosigma akashiwo* (Hada) Hada ex Hara et Chihara. Blooms of *P. minimum* were observed following blooms of *S. costatum* in Obidos Lagoon, Portugal (Silva, 1985), and in the Romanian Black Sea (Bodeanu and Usurelu, 1979). In addition, Iwasaki (1979) showed that *P. minimum* inhibited growth of certain flagellates. Kondo et al. (1990b) reported *P. minimum* red tides at Lake Nakanoumi, Japan, in February and December 1975, April 1977, March 1979, and February 1980, and observed that in all cases *S. costatum* was abundant 1-month prior to the *P. minimum* blooms. They also observed that *P. minimum* and *S. costatum* bloomed concurrently in January 1976 and March 1979 and from November 1981 to April 1982 (Kondo et al., 1990b). Furthermore, when *P. minimum* was cultured with *S. costatum* at initial cell yields of 3.0×10^3 cells ml^{-1} and 3.3×10^3 cells ml^{-1}, respectively, *P. minimum* maximum cell yields were approximately three times the yield produced in mono-algal culture (Kondo et al., 1990c). They also reported that organic substances excreted by *S. costatum* and low molecular weight fractions collected from the interstitial water of bottom sediments stimulated *P. minimum* growth. However, details of growth interactions between *P. minimum* and *S. costatum* remain unresolved, particularly the effects of *P. minimum* on the growth of *S. costatum*. Thus, it is necessary to examine the growth

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interactions among many phytoplankton species to understand the mechanisms affecting succession in phytoplankton communities.

In this study, we conducted bi-algal culture experiments under axenic conditions using several combinations of initial cell densities of the two species. Second, we simulated the growth of *P. minimum* and *S. costatum* in bi-algal cultures using a mathematical model. Finally, we examined the chemical nature of the allelochemical(s) produced by *P. minimum*.

2. Materials and methods

2.1. Reagents

Hydrogen chloride methanol solution, pyridine, acetic anhydride, and silblender-HTP were purchased from Nacalai Tesque (Kyoto, Japan). Hexane was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

2.2. Algal species and culture conditions

An axenic strain of *S. costatum* (NIES-324) was obtained from the National Institute of Environmental Studies (NIES, Japan). *P. minimum* (Fig. 1) cells were isolated from the Hakozaki Fishing Port (lat 34°24′58″N, long 130°12′20″W), Fukuoka, Japan, in 1996 and were repeatedly washed using capillary pipettes. Tomas et al. (1997) described that *P. minimum*, small oval to triangular-shaped cell, had a short apical spine, evenly shaped broad-based spines that could appear as rounded papillae and small pores scattered. We identified this strain as *P. minimum* because these morphological features were observed by a scanning electron microscope (SEM, Fig. 1). Thereafter, the above strains were tested for bacterial contamination by the fluorochrome 4′, 6-diamidino-2-phenylindole (DAPI) staining method (Porter and Feig, 1980), and all were verified as axenic. Cultures were maintained in 100-ml flasks containing 50 ml of modified SWM-3 medium (Itoh and Imai, 1987) without calcium pantothenate, nicotinic acid, *p*-aminobenzonic acid, biotin, inositol, folic acid, and thymine addition (Yamasaki et al., 2007), at a salinity of 25 at 25 °C under 228 (±5) μmol m^{-2} s^{-1} of cool-white fluorescent illumination on a 12:12 h light:dark cycle. The modified SWM-3 medium was autoclaved (121 °C, 15 min) and contained a buffer (Tris (hydroxymethyl) aminomethane, Wako Pure Chemical Industries, Ltd.) to prevent change of pH during culture. Irradiance in the incubator was measured with a Quantum Scalar Laboratory irradiance sensor (Biospherical Instruments, San Diego, CA, USA).

2.3. Bi-algal culture experiments

Bi-algal culture experiments were conducted in 100-ml flasks containing 50 ml of medium. *S. costatum* cells in stationary phase (stock culture: 10–12 × 10^5 cells ml^{-1}) were inoculated to a final cell density of 10^2 or 10^4 cells ml^{-1} in all combinations into cultures of *P. minimum* (stock culture: 4–5 × 10^5 cells ml^{-1}) with cell densities of 10^2 or 10^4 cells ml^{-1}. The combinations of initial cell densities for the two species were: (1) *P. minimum*, 10^2 cells ml^{-1} and *S. costatum* 10^2 cells ml^{-1}; (2) *P. minimum*, 10^2 cells ml^{-1} and *S. costatum*, 10^4 cells ml^{-1}; (3) *P. minimum*, 10^4 cells ml^{-1} and *S. costatum*, 10^2 cells ml^{-1}; and (4) *P. minimum*, 10^4 cells ml^{-1} and *S. costatum*, 10^4 cells ml^{-1}. As controls, both *P. minimum* and *S. costatum* were cultured individually at cell densities of 10^2 and 10^4 cells ml^{-1}. Three replicate flasks were used for each treatment. All flasks were gently mixed by hand once a day and randomly rearranged to minimize the effects of light or temperature gradients in the incubator. *P. minimum* and *S. costatum* cells were counted under the microscope in 200- to 1000-μl subsamples collected at 2-d intervals (the total duration of the bi-algal culture experiments; 12 days). When cell densities exceeded 2 × 10^5 cells ml^{-1}, subsamples were diluted 10× to 20× with fresh modified SWM-3 medium before counting.

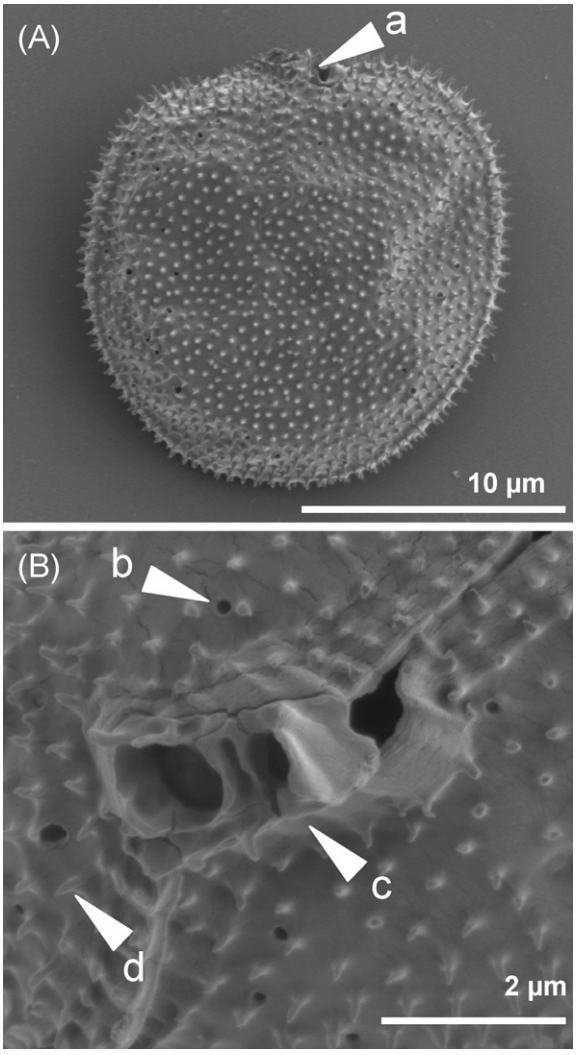

Fig. 1. Scanning electron microscope (SEM) photographs from the *Prorocentrum minimum* strain isolated from the Hakozaki Fishing Port, Fukuoka, Japan. The ventral view (A) and top view of the cell showing apical pore plates (B) of *P. minimum*. White arrowheads indicate the short apical spine (a), small pores (b) and the apical pore (c) evenly shaped broad-based spines (d).

2.4. Macronutrient analysis

At the beginning of the bi-algal experiments, 1 ml of each culture was passed through a 0.22-μm syringe filter (Millipore, Billerica, MA, USA) and the filtrate was frozen at −80 °C until analysis. At the end of the bi-algal experiments, 20 ml of each culture was gravity-filtered through a 5.0-μm pore size membrane filter (Millipore) on a 47-mm polysulfone holder (Advantec, Tokyo, Japan). Filtrates were then passed through 0.22-μm syringe filters and frozen at −80 °C until analysis of macronutrients. Nitrogen (NO_2^- + NO_3^-) and phosphorus (PO_4^{-3}) were measured with an autoanalyzer (TRACCS 800, Bran + Luebbe, Hamburg, Germany) after samples were diluted 40× to 90× with Milli-Q water (Millipore, Billerica, MA, USA).