Supplementary Information

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Supplementary Methods

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1. *Algal species and culture conditions.*

S. costatum (NIES-323 and NIES-324), *H. akashiwo* (NIES-10), *T. rotula* (CCMP 1647) and *P. minimum* strains were tested for bacterial contamination by the fluorochrome 4′,6-diamidino-2-phenylindole (DAPI) staining method (Porter & Feig, 1980), and were verified as axenic. *Prorocentrum triestinum*, *Heterocapsa circularisquama*, *Asterionellopsis gracialis*, *Thalassiosira* sp., *T. rotula* (HA) and *Chaetoceros didymus* were isolated from Hakata Bay, Fukuoka, Japan; these six strains were not axenic. Cultures were maintained in 100-ml flasks containing 50 ml modified SWM-3 medium (Yamasaki *et al*., 2007) without the addition of calcium pantothenate, nicotinic acid, p-aminobenzonic acid, biotin, inositol, folic acid and thymine, 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 prepared with seawater collected from the Tsushima warm current around Oki Island, Japan (latitude 34°24'58"N, longitude 130°12'20"E), and had been stored under laboratory conditions for >1 year. When the oceanic water was collected, few phytoplankter cells existed in 1 ml open seawater. In addition, the modified SWM-3 medium was autoclaved (at 121 °C for 15 min) and contained a Tris (hydroxymethyl) aminomethane buffer (Wako Pure Chemical Industries, Ltd.) to prevent pH change during culture. Irradiance in the incubator was measured with a quantum scalar laboratory irradiance sensor (Biospherical Instruments).

2. *Sampling and measurements of environmental factors.*

Surface seawater was sampled at Hakozaki Fishing Port from May to June 2007. The sampling was conducted every 3 or 4 days, or daily during the *H. akashiwo* bloom. The

water temperature, salinity, underwater light intensity (from the surface to the bottom layer at intervals of 0.5 m) and pH of the seawater were measured using a thermo-salinity meter (model 85, YSI/Nanotech Inc.), an underwater light photon meter (ALW-CMP; Alec Electronics Co. Ltd.) and a pH meter (F-51; Horiba), respectively. To evaluate whether the pH value in each sample during the dense *H. akashiwo* bloom was suitable for *S. costatum* growth, we referred to our previous report (Yamasaki *et al*., 2007). After sampling, the numbers of phytoplankters in each of the two 500 µl subsamples were counted microscopically without fixation. Then, a 50-ml portion of each 1,000 ml sample was passed through a 0.22-µm pore-size filter, and frozen at –80°C until the macronutrient analysis. The dissolved inorganic nitrogen (DIN), dissolved inorganic phosphorus (DIP) and silicate in these samples were measured with an autoanalyser (TRACCS 800; Bran+Luebbe) according to the previously described method (Strickland & Parsons, 1972). In addition, half-saturation constants (K_s) for the uptake of nitrate (Eppley *et al*., 1969), phosphate (Tarutani & Yamamoto, 1994) and silicate (Paasche, 1973) in *S. costatum* were used to examine whether the levels of these macronutrients in each sample during the dense *H. akashiwo* bloom were adequate for *S. costatum* growth.

3. *Effects of crude extract from* H. akashiwo *cultures on phytoplankton growth.*

Each strain (*S. costatum* (NIES 323 and NIES 324), *P. triestinum*, *H. circularisquama*, *A. gracialis*, *Thalassiosira* sp., *T. rotula* (HA), *C. didymus*, *H. akashiwo*, *T. rotula* (CCMP 1647) and *P. minimum*) was inoculated at a density of 10^2 cells ml^{-1} into 100-ml glass flasks (n = 10) containing 50 ml modified SWM-3 medium. A 100-µl sample of each cell suspension (initial cell density, 100 or 5,000 cells ml^{-1}) of each of the 11 test

phytoplankters was added to 900 µl of the crude extract solutions of *H. akashiwo* (80 µg protein ml^{-1}) in 48-well plates (Corning). As a control, each of these 11 strains was grown in the absence of the *H. akashiwo* extract. After incubation for 7 days (*H. akashiwo*), 6 days (*H. circularisquama*, *P. triestinum* and *P. minimum*) and 5 days (*A. gracialis*, *Thalassiosira* sp., *T. rotula*, *C. didymus* and *S. costatum*), the numbers of cells in each of five 10-µl subsamples from each well were counted under a microscope. This experiment had three replicates.

4. *Immunological studies.*

a) *Antibody production against APPCs in a rabbit.*

The crude extract obtained from filtrates of *H. akashiwo* cultures was purified as described above. The APPCs fractions were pooled, dialysed and lyophilized. An adult New Zealand white rabbit was used to produce polyclonal antibodies against APPCs of *H. akashiwo* according to the following procedure. APPCs dissolved in phosphate-buffered saline (PBS) were emulsified with an equal volume of Freund's complete adjuvant (first round of immunity) or Freund's incomplete adjuvant (second or subsequent rounds of immunity). This antigen mixture was injected subcutaneously into the back of a specific pathogen-free (SPF) rabbit at 10 different sites at weekly intervals over a period of 3 months. On days 25, 40 and 50, blood was collected from an ear vein, and the antisera were collected by centrifugation of the blood samples. The antibody levels of each antiserum collected on different days were measured by an enzyme-linked immunosorbent assay (ELISA). Each well of a 96-well plate was coated with 100 µl APPCs (5 µg ml^{-1} in 50 mM sodium carbonate buffer, pH 9.6), and incubated for 15 h at 4 °C. The plate was then washed three times with PBS containing 0.05% Tween-20

(T-PBS) and treated with 300 µl blocking solution (PBS containing 0.5% BSA, 0.2% gelatin and 0.1% thimerosal) for 2 h at room temperature (RT), then washed a further three times with T-PBS. The plate was reacted with 50 µl antiserum at six different dilutions (dilution ratio, 1:100 to 1:3200 in PBS) and was incubated for 15 h at 4 °C. The plate was washed a further three times with T-PBS, and 100 µl of the solution of peroxidase-labelled anti-rabbit immunoglobulin G (IgG; 2,000-fold dilution in PBS; Cappel) was added. After incubation for 1 h at 37 °C, the plate was washed a further three times, and 100 µl of the substrate solution mixed with 0.006% H_2O_2-0.2 M citrate buffer (pH 4.0) and 0.6 mg ml^{-1} 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt was added. After incubation for 30 min at 37 °C, the absorbance at 415 nm was measured. Finally, 60 ml antiserum was added to 0.02% sodium azide and frozen at –80 °C until use.

b) ***Immunological cross-reaction of polyclonal antibody against 11 species of phytoplankters using dot-blot analysis.***

Each strain of phytoplankton (*H. akashiwo*, *P. triestinum*, *P. minimum*, *P. dentatum*, *H. circularisquama*, *Chaetoceros* sp., *C. didymus*, *Thalassiosira* sp., *T. rotula*, *S. costatum* and *A. gracialis*) was inoculated at a density of 10^2 cells ml^{-1} into 100-ml glass flasks (n = 11) containing 50 ml modified SWM-3 medium. When each strain reached the stationary phase, each 50-ml sample from the 11 strains was passed through a 5.0-µm pore-size membrane filter on a 47-mm polysulphone holder under gravity filtration. Then, each filtrate was passed through a 0.22-µm pore-size filter (Millipore), and was frozen at –80 °C until use. Immunological cross-reactions of the polyclonal antibody against the 11 species of phytoplankters were detected by dot-blot analysis.

c) *Detection of APPCs from* H. akashiwo *culture at several growth phases.*

H. akashiwo was inoculated at a density of 100 cells ml^{-1} into three 200-ml glass flasks containing 100 ml modified SWM-3 medium. A 500 µl sample of the culture from each of the three flasks was combined to give a total of 1.5 ml, which was passed through a 0.22-µm pore-size membrane filter (Millipore) every other day during the experiment (days 0–22), and was frozen at –80 °C until use. To detect APPCs dissolved in the *H. akashiwo* cultures, filtrates from several growth phases were prepared for the dot-blot analysis using the method described above. APPCs produced by *H. akashiwo* at several growth phases were detected by dot-blot analysis.

Supplementary Notes

References

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Supplementary Figures

Supplementary Figure 1 Immunological cross-reactions of the polyclonal antibody against 11 species of phytoplankters using dot-blot analysis. When the 11 strains reached the stationary phase, each 50-ml sample was passed through a 5.0-μm pore-size membrane filter under gravity filtration. Then, each filtrate was passed through a 0.22-μm pore-size filter (Millipore), and was frozen at –80 °C until use. Dot-blot analysis was conducted as described in the text with two replicates. All samples were spotted at 5 μl per spot. Immunological cross-reaction of polyclonal antibody against other phytoplankters was not detected under experimental conditions because immunological cross-reaction of the polyclonal antibody against low cell density of *P. triestinum* (18×10^4 cells ml^{-1}) was not observed.

Supplementary Figure 2 Effects of combinations of allelochemical concentrations and inoculum densities on the growth of phytoplankters. Relative fluorescence when cultured alone (black circle), or together with 84 μg protein ml^{-1} (diamond), 59 μg protein ml^{-1} (square) or 37 μg protein ml^{-1} (triangle) from *H. akashiwo* filtrate. The numbers shown in the upper left of each graph indicate the inoculum cell density of each phytoplankton. Data are mean ± s.d. (relative values) of four replicate measurements.

Supplementary Figure 3 Detection of APPCs using dot-blot analysis. a, Detection of APPCs at several concentrations using dot-blot analysis. A detection limit of APPCs for the dot-blot assay under the experimental condition is between 2.5 to 1.25 μg protein

ml^{-1}. b, Detection of APPCs from *H. akashiwo* culture at several growth phases using dot-blot analysis. Each open circle indicates the cell density of *H. akashiwo*. Dot-blot analysis was conducted as described in the text with two replicates. All samples were spotted at 5 µl per spot. The dashed line indicates the first day of the detection of APPCs, and the cell density of *H. akashiwo* on that day.

Supplementary Figure 4 Macronutrient analysis of seawater samples collected from the surface water layer at Hakozaki Fishing Port from mid-May to June 2007. Fluctuations of concentrations of dissolved inorganic nitrogen (DIN; blue circles), silicate (green triangles) and dissolved inorganic phosphorus (DIP; pink squares, secondary *y*-axes) are shown. Dashed lines indicate half-saturation constants (K_s) for uptake of nitrate (blue), phosphate (pink) and silicate (green) in *S. costatum* according to previous studies (see Supplementary Methods).

Supplementary Figure 5 Fluctuations of several environmental factors in the field. Fluctuations of salinity (brown circles), water temperature (blue triangles) and pH value (purple squares, secondary *y*-axes) in the surface water layer at Hakozaki Fishing Port from mid-May to June 2007 are shown. The purple dashed line (pH value) indicates that *S. costatum* can grow at values lower than reported previously (see Supplementary Methods).

Supplementary Figure 6 Deactivation of inhibitory effect on the growth of *S. costatum* depending on storage time. This bioassay was conducted using 48-well. Samples (10 µl) of the *S. costatum* cell suspension (initial cell density, 100 cells ml^{-1}) were added to 990

µl filtrate of the *H. akashiwo* culture, prepared as described in the text, and stored from 0–10 days at 25 °C under 228 (±5) µmol m^{-2} s^{-1} of cool-white fluorescent illumination on a 12:12 h light:dark cycle. After incubation, the cell numbers in each of five 10-µl subsamples were counted under a microscope. Data are mean ± standard deviation (s.d.) of triplicate measurements.

Supplementary Figure 7 Effects of crude extract obtained from *H. akashiwo* culture on phytoplankton growth. a, b, A 100-µl sample of each cell suspension (initial cell density, 100 cells ml^{-1} (a) or 5,000 cells ml^{-1} (b)) of each of the 11 test phytoplankters was added to 900 µl of the crude extract solutions of *H. akashiwo* (80 µg protein ml^{-1}) in 48-well plates. After incubation, the cell numbers in each of five 10-µl subsamples were counted under a microscope. Data are mean ± s.d. of triplicate measurements.

Supplementary Figure 8 Variations of cell density of phytoplankton in the field. Cell density of *H. akashiwo* (orange circle), *S. costatum* (green triangle), *Prorocentrum* spp. (purple square), other diatoms (brown triangle) and other flagellates (light blue square). *S. costatum* and other diatoms were observed only at low concentrations when a dense bloom of *H. akashiwo* (45×10^4 cells ml^{-1}) was observed at the beginning of June. However, *Prorocentrum* spp. and other flagellates were observed at higher concentrations than on other sampling days during the same period.