Allelopathy of Baltic Sea cyanobacteria: no evidence for the role of nodularin

SANNA SUIKKANEN1*, JONNA ENGSTRÖM-ÖST1, JOUNI JOKELA2, KAARINA SIVONEN1 AND MARKKU VIITASALO1
1FINNISH INSTITUTE OF MARINE RESEARCH, PO BOX 2, FI-00561 HELSINKI, FINLAND AND 2DEPARTMENT OF APPLIED CHEMISTRY AND MICROBIOLOGY, DIVISION OF MICROBIOLOGY, PO BOX 36, FI-00014 UNIVERSITY OF HELSINKI, HELSINKI, FINLAND
*CORRESPONDING AUTHOR: sanna.suikkanen@fimr.fi

Received December 2, 2005; accepted in principle January 18, 2006; accepted for publication February 13, 2006; published online February 15, 2006

Communicating editor: K.J. Flynn

Extracts of Aphanizomenon flos-aquae and Nodularia spumigena, the two most common cyanobacteria forming recurrent blooms in the Baltic Sea, decrease the abundance of some phytoplankton species via the release of allelopathic substances. We investigated how cell-free filtrates of the two cyanobacteria, as well as purified hepatotoxin nodularin, produced by N. spumigena affected cell numbers, chlorophyll a content and 14CO2 uptake of the cryptophyte Rhodomonas sp. Both cyanobacterial filtrates significantly retarded the growth of Rhodomonas sp., A. flos-aquae filtrate up to 46%, whereas purified nodularin showed no significant effect on any of the growth parameters of the cryptophyte. These results suggest that the allelopathic effect of N. spumigena is most probably due to metabolite(s) other than nodularin, possibly acting via the damage of the target cells.

INTRODUCTION

The term allelopathy refers to inhibitory and stimulatory effects of plants and microorganisms on other plant species or microorganisms through the release of organic compounds (Rice, 1984). Such interactions occur in all aquatic habitats and can be caused by members from all groups of aquatic primary producers (Gross, 2003). The two nitrogen-fixing, brackish-water cyanobacteria, Aphanizomenon flos-aquae and Nodularia spumigena, are the most common species that form recurrent cyanobacterial blooms in the Baltic Sea. Recently, both species were found to decrease the abundance of the cryptophyte Rhodomonas sp. and the diatom Thalassiosira weissflogii grown in monocultures (Suikkanen et al., 2004). Also when applied to a natural Baltic Sea plankton community, cyanobacterial cell-free filtrates decreased the amounts of cryptophytes, whereas they increased the abundance of other cyanobacteria, the chlorophyte Oocystis sp., the dinoflagellate Amphidinium sp. and nanoflagellates (Suikkanen et al., 2005). However, the mechanisms of the observed allelopathic actions, underlying the decrease in cell numbers, or the chemical compound(s) causing them, are still unknown.

Most of the cyanobacterial inhibitory allelochemicals are directed against oxygenic photosynthetic processes of other cyanobacteria or algae; more specifically, they inhibit the electron transport in the vicinity of photosystem II (Smith and Doan, 1999). Thus, through the inhibition of photosynthesis, the allelochemicals decrease the production and cell numbers of the target species. The effects are probably first expressed at the level of the photosynthetic pigments and primary production capacity and, finally, in cell numbers.

To date, some cyanobacterial allelochemicals have been isolated and characterized (Smith and Doan, 1999 and references therein), and, e.g., the antialgal compounds produced by the freshwater Microcystis aeruginosa and Anabaena spores have been identified as peptides (Ishida and Murakami, 2000; Kaya et al., 2002). The cyanobacterial peptide hepatotoxin, microcystin, has been found to cause allelopathic effects both on higher plants (MacKintosh et al., 1990; Pfugmacher, 2002) and phytoplankton (Kearns and Hunter, 2000, 2001; Singh et al., 2001). In a comparison between the allelopathic effects of exponential and stationary N. spumigena cultures on cell numbers of a diatom and a cryptophyte species, allelopathic activity was only expressed by the exponential culture, with a lower nodularin concentration (Suikkanen et al., 2004). Thus it was concluded that nodularin was unlikely the cause of the observed
allelopathic activity. Nodularin, however, being structurally closely related to microcystin, may also be capable of acting as an allelochemical, and therefore its potential allelopathic effects warrant more specific attention.

The aims of the present study were (i) to investigate and compare the allelopathic effects of the selected cyanobacteria on different growth parameters, i.e., cell numbers, chlorophyll a (Chl a) concentration and $^{14}$CO$_2$ uptake, of the target species and (ii) to test if purified nodularin had an effect on the target species, comparable to that of $N$. spumigena filtrate with a corresponding nodularin concentration. We hypothesized that both cyanobacteria would inhibit photosynthetic processes of $Rhodomonas$ sp. (demonstrated by inhibition of $^{13}$CO$_2$ uptake and Chl a concentration), that the effects on Chl a and $^{14}$CO$_2$ uptake would not necessarily be reflected in cell numbers and that purified nodularin would not have as strong allelopathic effects as the crude $N$. spumigena filtrate.

**METHOD**

**Culture conditions**

The cyanobacterial strains, $N$. spumigena AV1 and $A$. flos-aquae Tr183, were isolated at the Division of Microbiology, University of Helsinki, Finland. The $N$. spumigena strain produces nodularin, and the $A$. flos-aquae strain is nontoxic (Lehtimäki et al., 1997; Lyra et al., 2001). Based on the enzyme-linked immunosorbent assay (ELISA; procedure described below), conducted shortly before the experiments, the $N$. spumigena culture filtrate contained 27.7 ng nodularin equivalents ($\mu$g Chl a$^{-1}$), whereas the $A$. flos-aquae filtrate was non-toxic. As target algae we used the cryptophyte $Rhodomonas$ sp. (cf. salina) KAC 30, obtained from the Kalmar Algal Collection, University of Kalmar, Sweden. All strains were originally isolated from the Baltic Sea, where $Rhodomonas$ spp. typically occur during summer (Hill, 1992), approximately simultaneously with the cyanobacterial summer maximum.

All strains were grown as batch cultures in f/10 medium (Guillard, 1975) at 18°C, in ~30 $\mu$mol photons m$^{-2}$s$^{-1}$, in a 16:8 h light:dark regime, and with continuous air supply. The culture medium was prepared from filtered seawater (Whatman GF/F glass microfibre filter) with a salinity of 3.7, and with pH adjusted to 8.2. The culture biomass was monitored by Chl a measurements to confirm exponential growth of the strains in the beginning of the experiment.

**Preparation of purified nodularin**

Nodularin-R [hereafter nodularin; Rinehart et al., 1988] is the dominant nodularin variant both in natural $N$. spumigena blooms in the Baltic Sea and in strains isolated from the same source, including the AV1 strain that was used in the present experiment (Sivonen and Jones, 1999). For nodularin extraction, 1.6 g of freeze-dried cyanobacterial bloom sample from sampling station SR5 (Kononen et al., 1993) was loaded into a 11-mL stainless steel extraction cell and extracted with Dionex ASE 200 Accelerated Solvent Extractor (Dionex Corp., Sunny Vale, CA, USA) by using one extraction cycle with methanol and the following method settings: preheat time 2 min, heat-up time 5 min, static extraction time 13 min and pressure 10.3 MPa, extraction temperature 40°C, flush% 60 and purge time 20 s with nitrogen gas.

The methanol extract (40 mL) was prepurified with an SPE cartridge (10 g/60 mL Mega Bond Elut C$_{18}$, Varian, Palo Alto, CA, USA). The methanol from the cartridge was evaporated with a Heto Maxi dry plus vacuum centrifuge (Heto-Holten A/S, Allerød, Denmark). The residue was dissolved into 1 mL 30% (v/v) acetonitrile in water and filtered over a 0.2 $\mu$m Gelman Acrodisc filter (13 mm).

Nodularin was purified from the extract with an Agilent 1100 high-performance liquid chromatography (HPLC), equipped with a diode array detector and a Phenomenex Luna C$_{18}$ (150 x 4.6 mm, 5 $\mu$m particle size) at 40°C. The extract was injected into the column in 100 $\mu$L portions, and isocratically eluted (1 mL min$^{-1}$) with 24% (v/v) acetonitrile and 76% of 0.1% trifluoracetic acid (TFA) in water (pH adjusted to 7.1 with 24% ammonium hydroxide solution).

Nodularin was detected at 238 nm, and the effluent was collected from 19.6 to 24.5 min. The fractions were pooled, diluted with an equal volume of water and desalted with an SPE cartridge (0.2 g/6 mL Oasis HLB, Waters, Milford, MA, USA). After washing with 10 mL of water, purified nodularin was eluted from the cartridge with 4 mL of acetonitrile. The solution contained 395 $\mu$g of nodularin, calculated as microcystin-LR equivalents at 238 nm. The purity of nodularin in the solution was 99% (238 nm), when analysed with the HPLC method employed in the purification. For storage, the solvent was removed by evaporation using a Heto Maxi dry plus vacuum centrifuge.

For the experiments, dry toxin was dissolved in 99.8% methanol and subsequently diluted to 20% with MilliQ water. The final methanol concentration did not exceed 5 ppm (v/v) in the experimental units.

**Experimental design**

We investigated the effects of (i) cell-free filtrates of $N$. spumigena and $A$. flos-aquae and that of (ii) purified...
nodularin, on the cell numbers, Chl a content and 14CO2 uptake of Rhodomonas sp.

To test the effects of cyanobacterial cell-free filtrates, we gently filtered aliquots of the cyanobacterial cultures through Whatman GF/F filters, with a pressure difference lower than –2 kPa. Freshly prepared filtrates were added to triplicate 1-L erlenmeyer flasks containing the target algae (final volume 400 mL). Based on the Chl a contents of the original cultures, the ratio of cyanobacteria to target species in the flasks was adjusted to 2:1 (256.1 µg Chl a L–1 cyanobacteria and 128.0 µg Chl a L–1 Rhodomonas). This ratio represents an underestimate of those in cyanobacterial bloom conditions, where the amounts of cyanobacteria, in relation to other phytoplankton species, may be much higher (Kononen, 1992). Concentrations of up to 400 µg Chl a L–1 have been reported during blooms of N. spumigena (Potter et al., 1983). Triplicate controls were made by adding a volume of f/10 medium, equal to the volume of cyanobacterial filtrate added to the treatment flasks. Prior to the experiment, the nitrate and phosphate concentrations were adjusted to the same level as in the f/10 growth medium. Therefore both the treatments and the controls contained the same amount of nutrients. The experiment lasted 6 days, and samples for cell counts, Chl a and 14CO2 uptake were taken and pH was measured (from one bottle per treatment) at least every second day. The volume removed during every sampling occasion (50 mL) was replaced with an equal volume of fresh cyanobacterial filtrate or control medium.

In another experiment, we investigated the effects of purified nodularin on Rhodomonas sp. We used a nodularin concentration of 10 µg L–1, which corresponded to the nodularin concentration in the experiment using cell-free filtrate of N. spumigena (7 µg L–1). In the field, the concentration of free nodularin in the water may vary from 0.01 to 18.7 µg L–1 (Kononen et al., 1993). The initial Chl a concentration of the target algae was ~125 µg L–1, corresponding to ca. 161 x 106 Rhodomonas sp. cells L–1. The total volume in the 1-L experimental flasks was 400 mL. At the beginning of the experiment, 85 mL 570 µM nodularin solution (corresponding to 10 µg nodularin L–1) was added to triplicate experimental units, and 85 µL distilled water, with a methanol concentration corresponding to that of the nodularin solution (2 µL MeOH mL–1), was added to triplicate control units. Every second day of the 6-day experiment, we took samples for determination of Chl a, 14CO2 uptake and cell numbers, measured pH and replaced the sampling volume (50 mL) with an equal amount of f/10 medium, containing 11 µL of either 570 µM nodularin or the control solution. Nodularin uptake of the target algal cells or adherence to their surface was monitored by taking samples for ELISA on the first and the last experimental day.

Analyses

For Chl a analysis, 5 ml samples of the cultures were filtered over GF/F filters (Whatman, Middlesex, UK), which were sonicated for 10 min (Branson GBW 2200) and extracted in 96% ethanol [Jespersen and Christoffersen, 1987]. The Chl a fluorescence was measured with a Shimadzu spectrofluorophotometer RF 5000 (Japan).

Cell numbers of Rhodomonas sp. were determined from live samples with an electronic particle analyser (Elzone 282 PC, Particle Data, Elmhurst, IL, USA) directly after sampling. The particle analyser was calibrated with a standard solution of latex particles of two different sizes.

Apparent net production (14CO2 uptake) was measured according to Niemi et al. (1983). From each experimental unit, duplicate 10 mL light samples and a dark sample were incubated with 0.5 µCi (18.5 kBq) of NaH14CO3 (14C Agency, Denmark) for 4 h in experimental conditions. Thereafter, 4 mL of water were transferred to a glass scintillation vial and acidified with 100 µL of 1 N HCl. The open vials were allowed to stand in a fume hood for 24 h, in order to release inorganic 14CO2, after which 7 mL of OptiPhase ‘HiSafe’ 3 scintillation cocktail (Wallac) was added. Radioactivity was assayed with a Wallac WinSpectral 1414 liquid scintillation counter, using the external standard channel ratio method for standardization.

The hepatotoxin concentration in the cyanobacterial filtrates was analysed from 10-mL samples taken from GF/F filtered N. spumigena and A. flos-aquae cultures. The transfer of purified nodularin from the medium to the target algal cells, and/or its adsorption on the cell surfaces, was quantified by taking duplicate 20-mL samples from both the nodularin treatment and the control and filtration through Whatman GF/C filters. All samples were stored in –20°C until analysis. Before the measurement, the filters were freeze-dried (GBW Edwards Super Modulyo, West Sussex, UK), dissolved in 5 mL 100% methanol, sonicated (MSE Soniprep 150 Ultrasonic Disintegrator, Sanyo Gallenkamp, UK) and extracted for 12 h. Subsequently the samples were filtered through GF/F filters and dried with N2 gas. Then 40 µL 50% methanol (v/v) and 280 µL MilliQ water were added to the samples, starting with the methanol. During the following 4 days, 70 µL MilliQ was added each day, until a final concentration of 6.3% methanol was
reached. On the fifth day, the samples were analysed by means of ELISA (Chu et al., 1990), using a microcystin plate kit (EnviroLogix, Portland, ME, USA), according to the kit instructions. The nodularin concentration was determined from absorbance at 450 nm, measured with an EMS Reader MF (Labsystems, Vantaa, Finland) photometer. The nodularin concentration values obtained for the controls, without nodularin additions, were probably false positive results of the ELISA. They were thus considered as a background level and subtracted from the concentrations obtained for the respective nodularin treatments of the target species.

**Statistical analyses**

Repeated measures analysis of variance (ANOVA) (Zar, 1999) was used to test for differences in the growth parameters between the target algae cultures treated with cyanobacterial filtrates or purified nodularin and the control, over the entire experimental period. A post hoc test (Tukey’s HSD) was used to show which treatments significantly differed from the control and from each other. The data were tested for normality and homogeneity of variances. All tests are two-tailed, with a significance level of \( P = 0.05 \), and data are reported as means ± SD. The statistical analyses were performed using the software SPSS 10.0.7 for Windows.

**RESULTS**

**Effects of cyanobacterial filtrates**

The growth and production of *Rhodomonas* sp. were retarded by both cyanobacterial filtrates (Fig. 1A–C). Repeated measures ANOVA revealed significant differences in the cell numbers, as well as cellular Chl a content and CO\(_2\) uptake of *Rhodomonas* sp. between the cyanobacterial filtrate treatments and the control (Table I). Both cyanobacterial filtrates significantly decreased the cell number of *Rhodomonas* sp., compared to the control (Fig. 1A). This inhibition was already observed after the first experimental day, but the effects of the two cyanobacteria only started to diverge after 3 days, after which *A. flos-aquae* extract inhibited the cell number development significantly more than that from *N. spumigena*. By day 6, *A. flos-aquae* and *N. spumigena* filtrates had decreased the cell numbers of *Rhodomonas* sp. by 29 and 14%, respectively.

A similar pattern was observed for the cellular Chl a content of *Rhodomonas* sp. (Table I, Fig. 1B), but in this case, the effects of the cyanobacterial filtrates only differed significantly from the control after two experimental days. By the end of the experiment, the cyanobacterial filtrates had decreased the cellular Chl a content by 34% (*A. flos-aquae*) and 12% (*N. spumigena*).

There was also a significant difference in the cellular 14CO\(_2\) uptake between the filtrate treatments and the control (Table I, Fig. 1C), although the effects of the two cyanobacteria did not differ. At first, the *A. flos-aquae* filtrate increased the 14CO\(_2\) uptake of *Rhodomonas* sp., but from day 4 onwards, the 14CO\(_2\) uptake was lower in both filtrate treatments, compared to the control. By day 6, the 14CO\(_2\) uptake was 46 or 43% lower in the cultures treated with *A. flos-aquae* and *N. spumigena* filtrates, respectively.

The average pH was 8.3 ± 0.2 (mean ± SD, \( n = 4 \)) both in the control *Rhodomonas* sp. cultures and in those treated with *N. spumigena* filtrate, and 8.1 ± 0.2 in the cultures treated with *A. flos-aquae* filtrate.

**Effects of purified nodularin**

The addition of purified nodularin did not significantly affect any of the growth parameters of *Rhodomonas* sp. (Table I, Fig. 1D–F).

According to the ELISA, the cells of *Rhodomonas* sp. contained more nodularin \(73.5 \times 10^{-6} \) pg nodularin equivalents cell\(^{-1}\); mean, \( n = 2 \)) shortly after the first nodularin addition at the beginning of the experiment, than at the last experimental day \(2.2 \times 10^{-6} \) pg nodularin equivalents cell\(^{-1}\), after three subsequent nodularin additions.

The average pH of both the control and nodularin-treated target cultures was 9.4 ± 0.6 (mean ± SD, \( n = 4 \)).

**DISCUSSION**

The results indicate that nodularin is not the cause of allelopathic effects of *N. spumigena*. Nodularin did not significantly affect *Rhodomonas* sp. at an environmentally relevant concentration, corresponding to the nodularin concentration in *N. spumigena* culture filtrate, which significantly decreased the growth and production of *Rhodomonas* sp. Moreover, the non-toxic *A. flos-aquae* filtrate caused even stronger inhibition of *Rhodomonas* sp. growth than the toxic filtrate of *N. spumigena*. The observed allelopathic effects of both cyanobacterial filtrates were thus most likely caused by other biologically active substances than the known toxins. This conclusion agrees with other studies that have not found any correlation between toxicity and antibiotic or allelopathic effects in cyanobacteria (Campbell et al., 1994; Lahti et al., 1995; Østensvik et al., 1998; Suikkanen et al., 2004) or other phytoplankton species (Arzul et al., 1999; Fistarol et al., 2004; Sugg and VanDolah, 1999; Tillmann
and John, 2002) and studies where stronger effects on aquatic organisms have been detected by crude cyanobacterial extracts or a co-exposure of hepatotoxins and endotoxins, e.g. lipopolysaccharides, than by equivalent concentrations of purified cyanotoxins (Best et al., 2002; Bury et al., 1996; Pietsch et al., 2001). Nodularin may have some effect on phytoplankton under field conditions with much smaller phytoplankton concentrations than those used in the present study, but metabolites other than nodularin are likely to be mainly responsible for the allelopathic effects of N. spumigena. These metabolites appear to be extracellular, because the effects are caused by the cell-free culture medium.

Suggestions for the ecological role of cyanobacterial toxins have ranged from cell–cell signalling to defence against grazers and competitors, but a consensus is still lacking (Kaebernik and Neilan, 2001). The mechanism of action is similar in nodularin and closely related microcystins: they inhibit protein phosphatases in eukaryotic cells (Yoshizawa et al., 1990). In addition to various animal poisonings, microcystins have also been observed to inhibit microalgal growth (Kearns and Hunter, 2000, 2001; Singh et al., 2001). On one hand, it would be logical to assume that nodularin has similar effects on the aquatic primary producers, especially as it is capable of direct action on cell membranes (Spassova et al., 1995), although in the present study, no

Fig. 1. Cell numbers (A, D), chlorophyll a (B, E) and 14CO2 uptake (C, F) of Rhodomonas sp. treated with (A–C) cell-free filtrates of Aphanizomenon flos-aquae (Aph filtrate) and Nodularia spumigena (Nod filtrate) as well as the control medium (Control) and (D–F) treated with purified nodularin (Nodularin) and the control medium (Control; n = 3, mean ± SD).
such effects were found. On the other hand, the allelopathic effects of microcystins and nodularin have been studied using different target algae. The effects of microcystins have been tested on chlorophytes and cyanobacteria (Kearns and Hunter, 2000, 2001; Singh et al., 2001), whereas effects of nodularin have only been studied using cryptophytes. Although it is possible that nodularin has negative effects on target organisms other than cryptophytes, cell-free extracts of *N. spumigena*, the principal nodularin producer, added to a whole phytoplankton community were only found to negatively affect cryptophytes and diatoms (Suikkanen et al., 2005).

Cryptophytes generally seem to be sensitive to chemicals released by other phytoplankton groups, including cyanobacteria (Granéli and Johansson, 2003; Infante and Abella, 1985; Rengefors and Legrand, 2001). In the present study, cell-free filtrates of both cyanobacteria, but especially *A. flos-aquae*, significantly inhibited the growth and production of *Rhodomonas* sp. In another monoculture experiment, *A. flos-aquae* was also observed to inhibit *Rhodomonas* sp. more strongly than *N. spumigena* (Suikkanen et al., 2004). In an experiment employing a natural phytoplankton community, only *N. spumigena* inhibited cryptophytes (Suikkanen et al., 2005), but the cryptophytes present in the community were other species than *Rhodomonas* sp.

Cyanobacteria produce bioactive substances affecting a range of biochemical processes, but most of their allelochemicals characterized to date are directed against photosynthesis (Gross et al., 1991; Smith and Doan, 1999; Sukenik et al., 2002). Light-dependent processes of prokaryotic cyanobacteria and eukaryotic algae would be the logical targets for competing organisms capable of producing bioactive metabolites. In the present study, however, the pattern of inhibition by the cyanobacterial extracts proceeded from the effects on cell numbers of *Rhodomonas* sp. after the first day, to inhibition of $^{14}$CO$_2$ uptake and Chl *a* concentration only after two or three days. Also there was first an increase in primary production simultaneously with the decrease in cell numbers. Therefore the results do not support the hypothesis that the growth inhibition caused by the filtrates would be associated directly with the inhibition of photosynthetic processes. The observed decrease in cell numbers may have been caused by, e.g. breakage of the cell membranes, as has been demonstrated in several studies of phytoplankton allelopathy (reviewed by Legrand et al., 2003).

At least part of the purified nodularin added to the target algal cultures was incorporated into the algal cells by either transport into the cells or adsorption on the cell surface. To our knowledge, nodularin has not previously been reported to attach or be transported into phytoplankton cells. The decrease of the toxin concentration in the cells of *Rhodomonas* sp. over the course of the experiment may have been due to either a dilution effect during cell division, or to the utilization of nodularin by *Rhodomonas* sp., as, e.g., a nutrient source. Also, detoxifying enzyme systems have been found in many plant species, including marine macroalgae (Pflugmacher and Steinberg, 1997). It is unlikely that the observed decrease in the cellular nodularin concentration was due to bacterial or physical degradation of the toxin, because nodularin was added to the cultures every second day.

To conclude, our results support the hypothesis that the hepatotoxin, nodularin is not the main allelopathic compound produced by *N. spumigena*, although nodularin is incorporated, at least to some extent, into phytoplankton cells. The growth inhibition of *Rhodomonas* sp. caused by *N. spumigena* and *A. flos-aquae* filtrates may be associated with the action on target cell surfaces, causing damage to the cell membranes rather than affecting photosynthesis after entry to the target cells. In the field, allelopathy is probably one of the competitive strategies of cyanobacteria, mediated via specific chemicals that decrease cell numbers of certain phytoplankton species.

### Table I: Repeated measures analysis of variance (ANOVA) and Tukey’s HSD post hoc test were applied to reveal differences in the three growth parameters between the target algae cultures in the control and treatments with cyanobacterial filtrates or purified nodularin

| Parameter       | Filtrate experiment |                 |                 |                 |                       |                 |                       |                       |                       |
|-----------------|---------------------|-----------------|-----------------|-----------------|-----------------------|-----------------|-----------------------|-----------------------|
|                 | ANOVA               | Repeated measures | Tukey’s HSD post hoc test | Repeated measures | ANOVA |                       |                       |                       |
|                 | $F_{(2,4)}$ | $P$-value | $n$ | $P$-value (Aph-Nod) | $P$-value (Aph-ctrl) | $P$-value (Nod-ctrl) | $F_{(1,4)}$ | $P$-value | $n$ |
| Cell number L$^{-1}$ | 57.2 | 0.000 | 3 | 0.009 | 0.000 | 0.002 | 0.11 | 0.76 | 3 |
| Chl *a* cell$^{-1}$ | 55.5 | 0.000 | 3 | 0.002 | 0.000 | 0.01 | 1.3 | 0.32 | 3 |
| $^{14}$CO$_2$ uptake cell$^{-1}$ | 21.3 | 0.002 | 3 | 0.43 | 0.007 | 0.002 | 5.1 | 0.09 | 3 |
REFERENCES


S. SUUKANEN ET AL. | ALLELOPATHY OF BALTIC SEA CYANOBACTERIA

ACKNOWLEDGEMENTS

Prof E. Granéli kindly provided the Rhodomonas sp. strain KAC 30. We are also grateful to A.-M. Åström and U. Sjölund for nutrient analyses and to M. Wahlsten for help in nodularin purification. R. Autio and M. Karjalainen are acknowledged for assistance with 14CO2 uptake and nodularin analyses, respectively. This study was financed by the Maj and Tor Nestling Foundation and the Walter and Andree de Nottebeck Foundation.