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Interactions between marine bacteria and axenic diatoms (*Cylindrotheca fusiformis*, *Nitzschia laevis*, and *Thalassiosira weissflogii*) incubated under various conditions in the lab

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ABSTRACT: Bacteria-phytoplankton interactions in aquatic systems range from symbiosis to parasitism and are highly variable in space and time. Three marine diatoms (*Cylindrotheca fusiformis*, *Nitzschia laevis*, and *Thalassiosira weissflogii*) were grown in axenic culture and incubated under controlled lab conditions with single bacterial isolates, mixed bacterial populations, and seawater off Scripps Pier. Growth of both bacteria and algae was significantly higher when incubated together in *f*/2 medium or artificial seawater which were rich in inorganic nutrients, vitamins, and trace metals. In contrast, growth of diatoms in a vitamin and trace metal free medium was reduced or even negative when incubated together with bacterial isolates or seawater bacteria. In addition, the amount and quality of exopolymeric matter in mixed cultures were different from those of pure cultures. In general, aminopeptidase and β-glucosidase activities of bacteria in mixed cultures strongly increased after 70 h whereas those of a pure culture (Fl 7) were slightly higher until 50 to 70 h of incubation but strongly decreased thereafter. High growth and enhanced hydrolytic ectoenzyme activities of bacteria in the presence of algae and polymer particles led to high bacterial remineralization of organic nutrients increasing phytoplankton growth. However, bacteria compete with phytoplankton for nutrients and can inhibit algal growth under certain environmental conditions. Thus, changes in eutrophication and pollution can alter bacteria-phytoplankton interactions, which influence the flux and cycling of nutrients and carbon at both micro- and global scale.

KEY WORDS: Bacteria · Diatoms · Exopolymers · Bacterial colonization · Carbon cycle

INTRODUCTION

Bacteria can be loosely or tightly associated with phytoplankton (ZoBell 1941, Sieburth 1968, Caldwell 1977), leading to a multitude of possible interactions between these organisms in aquatic environments (see review Cole 1982). Algal blooms create 'phycospheres' in which microbial activity is altered from that of the surrounding milieu (Bell & Mitchell 1972). Bacteria in phycospheres can be free-living, directly attached to phytoplankton surfaces, or live inside phytoplankton cells (Kochert & Olson 1970, Kodama et al. 1990, Rausch de Traubenberg & Lassus 1991).

The following bacteria-phytoplankton interactions, predominatly studied in laboratory experiments, were identified: (1) Bacteria and algae form symbioses in which bacteria benefit from phytoplankton products such as exudates (Bell et al. 1974, Cole 1982) whereas phytoplankton profits of bacterial products such as remineralized nutrients (Golterman 1972), vitamins (Pringsheim 1912, Haines & Guillard 1974), and other growth factors (Paerl & Pinckney 1996). (2) Bacteria are parasites of phytoplankton which penetrate and become lodged in the periplasmatic space of the host cell

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and may lead to cell lysis and death (see review Cole 1982, Imai et al. 1993). To prevent bacterial parasitism many algae have the capability to produce antibiotics (Steeman-Nielsen 1955, Sieburth 1968, Sastry & Rao 1994). (3) Commensalic bacteria benefit from phytoplankton without having any negative effect on it (Barbeyron & Berger 1989). However, the distinction between commensalism and parasitism is transient and bacterial commensalism may result in parasitism when the phytoplankton becomes stressed. (4) Bacteria are loosely associated with phytoplankton and can compete for limited nutrients such as phosphate (Rhee 1972, Rothhaupt & Güde 1992). In natural environments including heterogeneous communities of bacteria and phytoplankton multiple interactions and relations exist simultaneously which can be highly variable in time and space.

Specific bacteria-phytoplankton interactions such as the clustering of chemotactic bacteria in the vicinity of phytoplankton cells lead to patchy distributed bacterial communities and activities (Blackburn et al. 1997, Grossart in press). Most pelagic bacteria have a repertoire of hydrolytic enzymes which allows them to efficiently utilize polymeric organic material such as phytoplankton exudates (Hoppe 1993, Martinez et al. 1996). Thus, small-scale variations in bacterial numbers and species composition can significantly alter the distribution of hydrolytic enzyme activities. Bacterial action on components of the organic matter field in the vicinity of phytoplankton influences carbon and nutrient fluxes in various pathways: microbial loop, sinking, grazing food chain, storage, and fixation (Azam 1998). For example, high hydrolytic activities of bacteria colonizing phytoplankton cells of macroscopic organic aggregates led to 'uncoupled solubilization' of exopolymers which reduced the sinking flux (Smith et al. 1992, Grossart & Simon 1998). On the other hand, bacteria clustered around or attached to phytoplankton cells significantly enhanced (Bell & Mitchell 1972) or reduced (Brussaard & Riegman 1998) primary production by release or uptake of phytoplankton nutrients, respectively.

To study bacteria-phytoplankton interactions under more controlled conditions we have incubated 3 axenic marine diatoms (*Cylindrotheca fusiformis*, *Nitzschia laevis*, and *Thalassiosira weissflogii*) with specific bacterial isolates, mixed bacterial populations, and coastal seawater from Scripps Pier, La Jolla, USA. Bacterio- and phytoplankton growth as well as exopolymer production were followed during incubation of various cultures to characterize bacteria-phytoplankton interactions. In addition, potential hydrolytic ectoenzyme activities were measured to test the hypothesis that bacteria become highly metabolically active in the presence of living algae, dead algae, and polymer particles. Furthermore, we incubated phytoplankton and bacteria in a vitamin and trace metal free medium to test if various environmental conditions may alter bacteria-phytoplankton interactions.

MATERIAL AND METHODS

Study site and sampling. From June 1996 to March 1997 water was collected off Scripps Pier (32° 53'N, 117° 15'W) by lowering a 1 l polycarbonate flask into the upper metres of the sea.

Bacterial isolates. Three bacterial strains of marine bacteria (Fl 2, Fl 3, and S 3) were used as test organisms isolated in fall and winter 1993 off Scripps Pier (Martinez et al. 1996). The frozen stocks were spread on agar plates (1.5% wt/vol. agar, Difco) enriched with ZoBell 2216E medium (5 g peptone + 1 g yeast extract in 1 l of 0.45 µm pre-filtered seawater, autoclaved at 121°C for 30 min) and grown overnight at 21°C. To gain a sufficient number of bacteria all isolates were grown at 21°C in fluid ZoBell 2216E medium without agar overnight. Harvested bacteria were washed 3× by dialysis (12 h) in dialysis bags (~0.3 to 0.35 nm, neoLab) filled with sterile artificial seawater (Darley & Volcani 1969) or sterile Guillard seawater medium (*f*/2) to minimize input of bacteria-derived matter into the phytoplankton cultures.

In July 1996, 4 mixed populations of bacteria were obtained by incubating seawater off Scripps Pier in fluid ZoBell 2216E medium without agar (B 1) and in axenic cultures *of Cylindrotheca fusiformis* (B 2), *Nitzschia laevis* (B 3), and *N. angularis* (B 4). Bacteria and algae were separated by gravity filtration through 5.0 µm polycarbonate filters (Nuclepore). To remove dissolved organic matter from the filtrate bacteria were rinsed with sterile seawater medium and concentrated by tangential flow filtration (0.2 µm).

Axenic algal cultures. Axenic cultures of 3 marine diatoms (*Cylindrotheca fusiformis*, *Nitzschia laevis*, and *Thalassiosira weissflogii*) were obtained from Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP, Maine, USA). The algae were grown at 16°C in sterile artificial seawater (Darley & Volcani 1969) or sterile Guillard seawater medium (*f*/2) in a 12:12 h dark:light cycle at approximately 85 µE m^{-2} s^{-1}.

Growth of bacteria-phytoplankton cultures. Mixed cultures were obtained by adding 1 ml of washed bacteria to diatom cultures immediately after transferring them to 20 ml of fresh medium. The density of added diatoms was 2000 to 4000 cells ml^{-1} and that of bacteria 1 to 3×10^6 cells ml^{-1}. Bacterial growth in monospecific and mixed cultures was followed by cell counting (see

Table 1. Histochemical dyes and procedures used to stain free and surface-bound exopolymers in pure and mixed cultures of bacteria and diatoms

Dye	Protocol	Specificity	Source
Toluidine Blue (TB)	TB (final conc. 0.1%) in na-acetate buffer at pH 4.2, 5.0, and 5.6	Proteoglycanes Acid polysaccharides Highly polymeric Molecules, e.g. DNA	Chayen et al. (1973)
Alcian Blue (AB)	AB (8GX, final conc. 0.05%) in na-acetate buffer at pH 5.8	Acid polysaccharides	Logan et al. (1994)
Comassie Blue (CB)	CB (G-250, final conc. 0.04%) in filtered seawater at pH 7.0	Proteins	Long & Azam (1996)
FITC labeled lectins	Lectins (final conc. 0.001%) in 50 mM TRIS buffer plus 2 M NaCl and $CaCl_2$, $MnCl_2$, $MgCl_2$ (ea. 1 mM)	Carbohydrates	Liener et al. (1986)
(a) Concanavalin A		a-man, a-glc	
(b) Bandeiraea s. II		glnNAc galNAc	
(c) Glycine max			
(d) Wheat germ		glcNAc, neuNAc	

below) whereas that of diatoms was monitored in a fluorometer (Beckman, USA), which measured the autofluorescence of chlorophyll *a*. Phytoplankton growth is given as relative fluorescence since it was linearly correlated to *Thalassiosira weissflogii* cell numbers for more than 300 h of incubation ($r^2 = 0.98$, n = 12). This holds also true for *Cylindrotheca fusiformis* but chlorophyll *a* content per cell and cell growth were much higher as compared to *T. weissflogii* and *Nitzschia laevis* (data not shown). Cultures were continously shaken at low speed to prevent sedimentation and accumulation of cells at the bottom of the tube. To test whether bacteria-phytoplankton interactions change with stress we incubated *C. fusiformis* and *N. laevis* in *f*/2 medium missing vitamins and trace metals. All cultures were kept at 16°C in a 12:12 h dark:light cycle at approximatey 85 µE m^{-2} s^{-1}.

Numbers of bacteria and algae. Two-hundred µl samples were diluted 1:10 in sterile filtered seawater, fixed with 0.2 µm pre-filtered, borate-buffered formalin (2% final), and stained with 4', 6-diamidino-2-phenylindole (DAPI; 1 µg ml^{-1} final conc.) for 3 min. Thereafter, samples were filtered onto 0.2 µm pore-sized black polycarbonate filters (Nuclepore) and bacterial numbers were determined by epifluorescence microscopy at 1250× (Porter & Feig 1980). For counting algae 2 ml of sample were fixed, stained, and filtered onto 5.0 µm pore-sized polycarbonate filters (Nucleopore). Algal numbers were counted at 500× magnification using the DAPI and blue light filter set to detect the autofluorescence of chlorophyll *a*. All samples were usually counted within a few hours after sampling.

Staining of algal and bacterial exopolymers. To detect free and surface-bound exopolymers during incubation of pure and mixed cultures of bacteria and diatoms various histochemical staining procedures were used (Table 1). For staining with Toluidine Blue, Alcian Blue, and Comassie Blue, aliquots of 1 to 5 ml were filtered onto 0.2 µm pore-sized polycarbonate filters (Nuclepore). Filters were mounted on frosted slides (Cytoclear™, Poretics Corp., CA, USA) and examined with light microscopy. In contrast, staining with lectins directly took place in 100 µl subsamples. Further details of all protocols used for staining are given in references of Table 1.

Hydrolytic ectoenzyme activities. Leucine aminopeptidase (Leu-AMP) and β-glucosidase (β-GlcAse) activities were measured by using L-leucine-methyl coumarinylamide (MCA, aminopeptidase) and methyl umbelliferyl β-D-glucoside (MUF, β-glucosidase) as fluorogenic substrate analogs (Hoppe 1993). Hydrolytic ectoenzyme activities were measured by incubation of 5 ml subsamples with analogs at saturating concentration (250 µM final conc.) for 1 h at 16°C in the dark. A heat-killed sample (80°C for 20 min) served as control. Fluorescence was measured at 380/365 nm excitation and 440/455 nm emission for MUF/MCA substrates, respectively, in a Hoefer TKO-100 fluorometer. Cell specific activities were calculated by dividing the volume specific ectoenzyme activities by corresponding bacterial numbers.

RESULTS

Bacterial and algal growth in *f*/2 medium

Cell numbers of *Thalassiosira weissflogii* almost exponentially increased until more than 300 h of incubation in *f*/2 seawater medium (Fig. 1). Phytoplankton growth was significantly higher whenever bacteria were present. Addition of the bacterial isolate S 3 led to the most pronounced increase in phytoplankton growth and resulted in almost twice the algal cell number as compared to axenic cultures.

In parallel, bacterial numbers of single isolates were significantly higher when incubated together with the diatom as compared to *f*/2 seawater medium without any organic material (Fig. 1). In all bacteria-phytoplankton cultures, increase in bacterial numbers was strongest during the first 100 h of incubation. Thereafter, cell numbers of the bacterial isolate Fl 2 remained almost the same whereas those of Fl 7 and S 3 further increased until the end of the experiment. Abundances of Fl 7 and Fl 2 in *f*/2 medium only slightly increased during the first 50 h of incubation

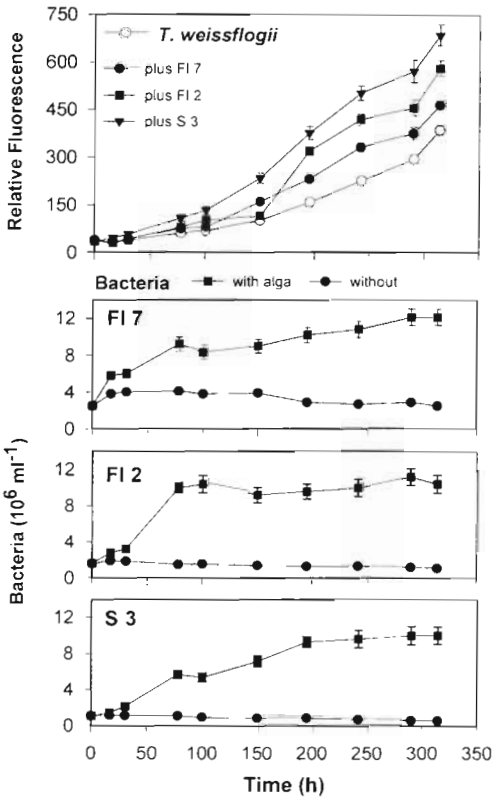

Fig. 1. Relative fluorescence of *Thalassiosira weissflogii* incubated without and with bacterial isolates (Fl 7, Fl 2, and S 3) and bacterial numbers of the 3 isolates incubated without and with alga

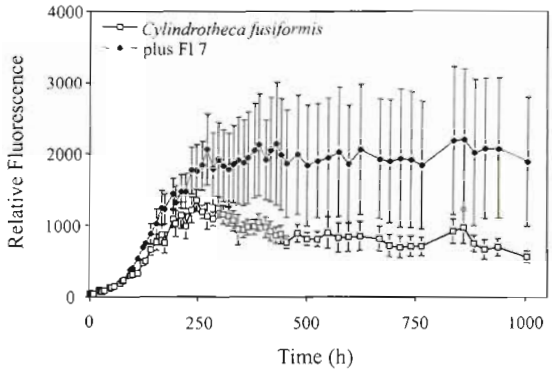

Fig. 2. Relative fluorescence of *Cylindrotheca fusiformis* incubated in *f*/2 medium with and without a bacterial isolate (Fl 7)

whereas that of S 3 did not show any increase. In all diatom-free cultures bacterial numbers continuously decreased after 100 h of incubation.

Cell numbers of *Cylindrotheca fusiformis* were continuously higher when incubated together with Fl 7 for 1000 h (Fig. 2) even though of all tested bacterial isolates Fl 7 had the lowest stimulation on growth of *Thalassiosira weissflogii*. Cell densities *of C. fusiformis* in axenic culture were maximal after 250 h and decreased thereafter. In contrast, in the presence of Fl 7 algal numbers were highest after more than 270 h and remained at almost the same level thereafter suggesting an equilibrium between phytoplankton growth and bacterial remineralization of nutrients. The high standard deviation in relative fluorescence of *C. fusiformis* in the presence of Fl 7 was due to significantly lower algal numbers in one of 3 parallels.

Maximum growth yield of *Cylindrotheca fusiformis* in *f*/2 medium was increased by 43 to 92% when the alga was incubated with mixed populations of bacteria isolated from seawater off Scripps Pier. Interestingly, seawater bacteria grown and isolated on axenic marine diatoms had a more pronounced effect on algal growth than those isolated on ZoBell 2216E medium (73 to 92% and 43% higher algal growth yield, respectively). In contrast to the previous experiment, maximum algal density in axenic culture was reached after 480 h and persisted until 700 h of incubation, indicating different growth conditions in both experiments. However, maximum algal density in mixed populations occurred even later (>550 h).

Nitzschia laevis grown in *f*/2 medium together with Fl 7 also showed significantly higher algal numbers as compared to axenic cultures (Fig. 3). When adding Fl 7 to an axenic culture in the stationary growth phase (after 400 h) a further increase in cell number up to the level of an initially mixed culture occurred. Again, maxima in algal number were persistent in the presence of Fl 7.

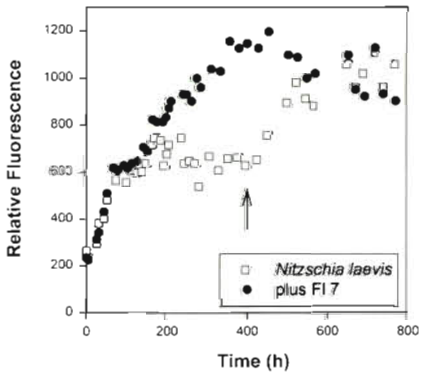

Fig. 3. Relative fluorescence of *Nitzschia laevis* incubated in *f*/2 medium without and with a seawater bacterium (Fl 7). (↑) Time point when Fl 7 was added to the axenic control

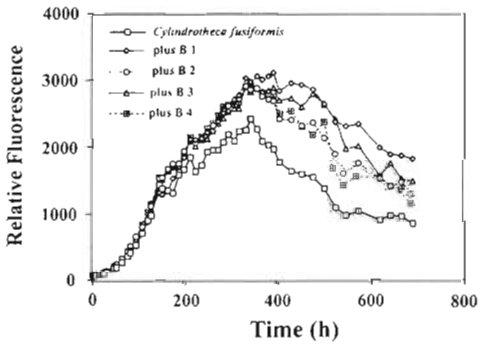

Fig. 4. Relative fluorescence of *Cylindrotheca fusiformis* incubated in artificial seawater rich in organics without and with 4 bacterial populations from Scripps Pier (B 1, B 2, B 3, and B 4, see 'Results')

Bacterial and algal growth in artificial seawater

The growth pattern of *Cylindrotheca fusiformis* in artificial seawater (Fig. 4) was dramatically changed as compared to that in *f*/2 medium. In axenic culture maximum algal numbers occurred after >350 h and rapidly decreased thereafter. In mixed cultures numbers of *C. fusiformis* peaked between 350 and 390 h but also strongly decreased thereafter. In contrast to incubations in *f*/2 medium, bacterial populations grown and isolated on ZoBell 2216E medium (B 4) led to strongest stimulation of algal growth. Artificial seawater contains 5 mM glycylglycine and, thus, offers good conditions for growth of heterotrophic bacteria such as those selected on nutrient-rich ZoBell 2216E medium. Bacterial numbers in all mixed populations were extremely high and reached up to 5.2×10^9 cells ml^{-1}, approximately 2 orders of magnitude higher than in *f*/2 medium.

Algal growth in vitamin and trace metal free *f*/2 medium

In contrast, incubation of *Cylindrotheca fusiformis* in vitamin and trace metal free *f*/2 medium resulted in no visible algal growth (Fig. 5). The addition of the bacterial isolate Fl 7 led to a slight increase and consecutive slight decrease in algal cell number. In contrast, addition of the marine isolate Fl 2 and of natural assemblages of seawater bacteria off Scripps Pier resulted in a steady decline of relative fluorescence, suggesting reduced nutrient availability or even a parasitic relationship between bacteria and algae.

Incubation of axenic *Nitzschia laevis* under the same limiting conditions led to cell growth (Fig. 5) which was much reduced as compared to non-limiting conditions (Fig. 3). The addition of bacterial isolates and fresh seawater did not result in a complete stop but in reduced algal growth. Again, the presence of bacteria had a negative effect on algal growth under the given limiting conditions.

Exopolymer production

Lectine staining in all algal cultures revealed enhanced concentrations of exopolysaccharids in the presence of bacteria on the surface of cells with reduced autofluorescence or in cell aggregates (Fig. 6) in mixed cultures. Cells with bright autofluorescence

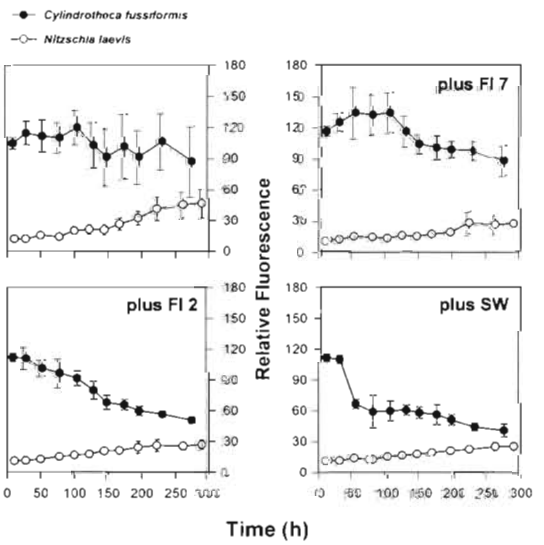

Fig. 5. Relative fluorescence of *Cylindrotheca fusiformis* and *Nitzschia laevis* incubated in *f*/2 medium free of vitamins and trace metals without and with bacterial isolates (Fl 7, Fl 2, and SW)

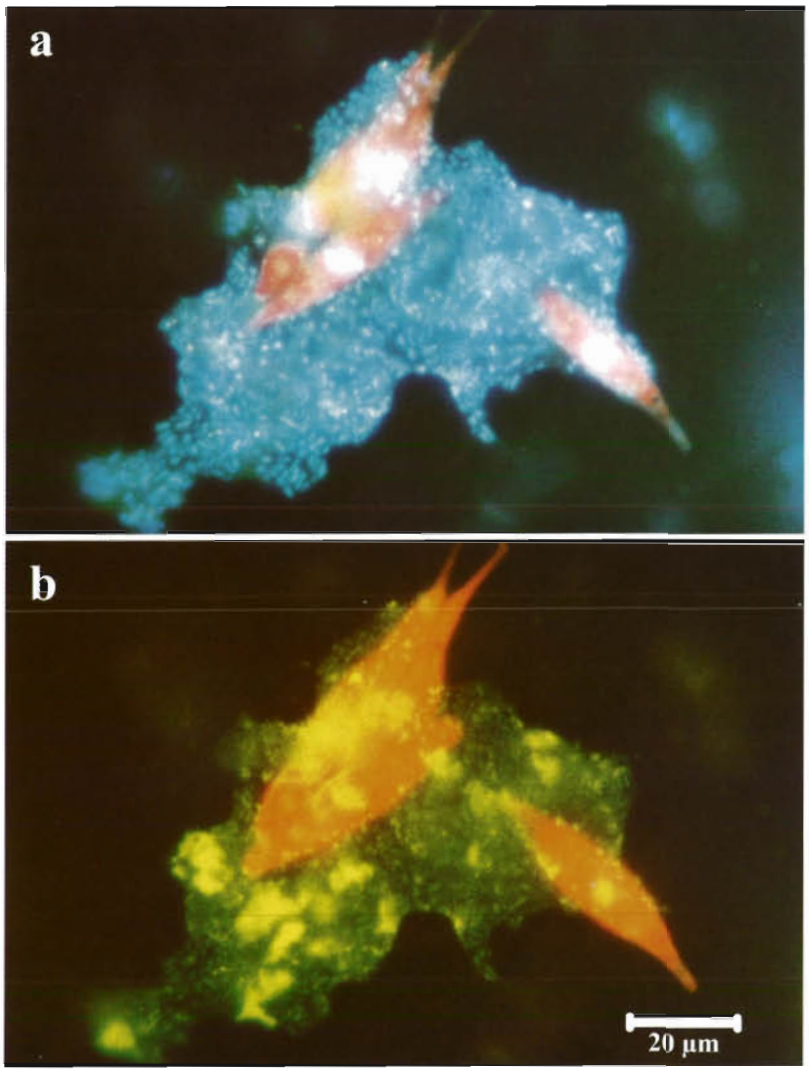

Fig. 6. Exopolymer production after 12 h of incubation of *Cylindrotheca fusiformis* and the seawater bacterium Fl 7. (a) DAPI staining. (b) Concanavalin A staining. Epifluorescence micrograph (1000× magnification)

only showed a thin layer of Concanavalin A stainable material in the absence and presence of bacteria whereas all other lectines (Bandeiraea s. II, Glycine max., and Wheat germ) did not stain at all. In contrast, small amounts of material stainable with Bandeiraea s. II, Glycine max, and Wheat germ were found on algal cells and algal aggregates both with dense bacterial colonization. Changes in the quality of exopolymer material according to the degree of bacterial colonization may also reflect production and degradation of exopolymers by bacteria. However, mucus and cells of the bacterial isolate Fl 7 in ZoBell 2216E medium were only stainable with Concanavalin A.

Staining with Toluidine Blue also revealed high amounts of exopolymers on the surface of algal cells with reduced autofluorescence and in cell aggregations. In all axenic cultures a continuous increase in the amount of free and cell-bound exopolymer material was observed whereas in mixed cultures no uniform trend was found.

Staining with Alcian Blue and Coomassie Blue yielded a complex pattern during different incubations: In axenic cultures of *Cylindrotheca fusiformis* free particles of both acid polysaccharides (TEP: transparent exopolymer particles) and proteins (CSP: Comassie Blue stainable particles) accumulated during phytoplankton growth (Fig. 7a). Initial concentrations of TEP and CSP were relatively high and may be introduced with the inoculated algae. In pure cultures of Fl 7 numbers of TEP and CSP increased during bacterial growth whereby the increase in TEP was much higher than in CSP (Fig. 7b). TEP and CSP almost completely disappeared in parallel to a strong decrease in bacterial numbers. Some TEP and CSP might be also intro-

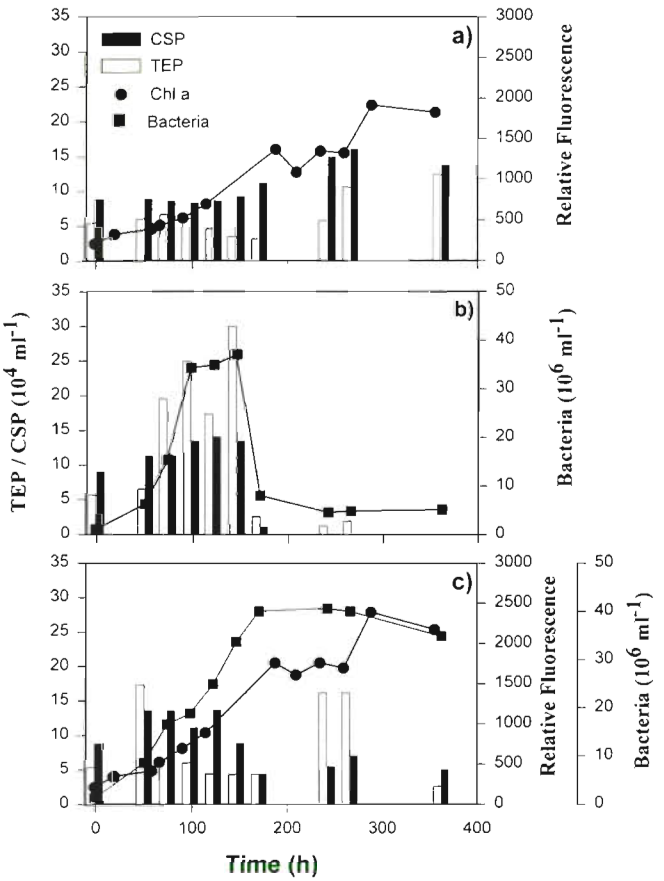

Fig. 7. Abundance of transparent exopolymer particles (TEP) and of Comassie Blue stainable particles (CSP), relative fluorescence, and bacterial numbers in axenic and mixed cultures of *Cylindrotheca fusiformis* and the seawater bacterium Fl 7. All parameters were measured in triplicate and mean values varied within 5 to 15%. (a) Axenic culture of *C. fusiformis*. (b) Pure culture of Fl 7. (c) Mixed culture of *C. fusiformis* and Fl 7

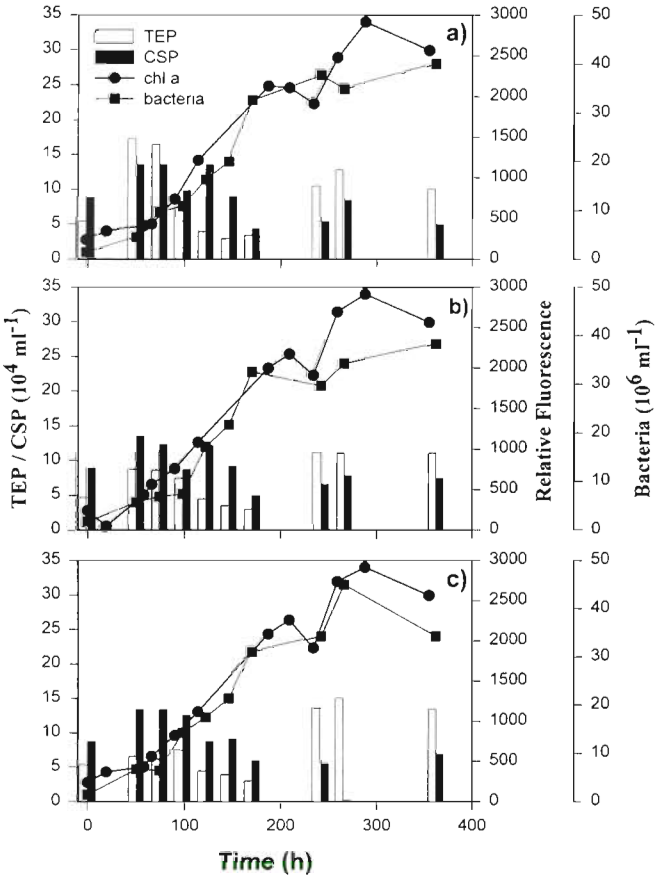

Fig. 8. Abundance of transparent exopolymer particles (TEP) and of Comassie Blue stainable particles (CSP), relative fluorescence, and bacterial numbers in mixed cultures of *Cylindrotheca fusiformis* and seawater populations: (a) B 2, (b) B 3, and (c) B 4 (see 'Results'). All parameters were measured in triplicate and average values varied within 5 to 15%

duced with the inocculum since particles >0.3 nm could not be separated by dialysis. In mixed cultures of *C. fusiformis* and Fl 7, numbers of TEP peaked at 50 h and 250 to 270 h whereas those of CSP were highest at 50 to 120 h and were only slightly enhanced at 250 to 370 h of incubation (Fig. 7c). Bacterial numbers remained constant or slightly decreased after 170 h whereas those of the algae increased until 290 h.

In mixed cultures of *Cylindrotheca fusiformis* and B 2 numbers of TEP peaked at 50 to 70 h and at 250 to 370 h whereas those of CSP remained highest at 50 to 120 h and were only slightly enhanced at 250 to 370 h (Fig. 8a). Both algae and bacteria continuously increased until 290 h of incubation. Mixed cultures of *C. fusiformis* and B 3 (Fig. 8b) or B 4 (Fig. 8c) had comparable low numbers of TEP at 50 to 70 h. In addition, in cultures with B 4 higher numbers of TEP occurred at 250 to 370 h. In contrast, numbers of CSP in all cultures were almost the same. The comparison of axenic (Fig. 7a) and mixed cultures (Figs. 7b,c & 8a–c) shows that abundance of CSP in mixed cultures increased between 50 and 120 h and was much lower after a steep decline at 120 to 170 h. TEP were almost always more abundant in mixed than in axenic cultures. In contrast to axenic cultures, abundance of TEP in bacterial and mixed cultures showed a first peak at 50 to 100 h, which was highly variable in the various cultures. In all mixed cultures a further peak of TEP appeared at 250 to 270 h.

Hydrolytic ectoenzyme activity

Aminopeptidase activity in cultures of *Cylindrotheca fusiformis* and bacteria increased dramatically during incubation (Fig. 9). The most pronounced increase in

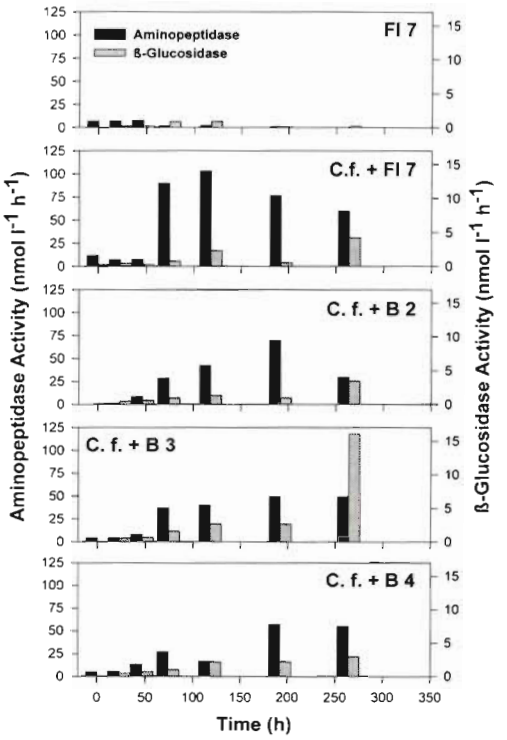

Fig. 9. Activities of aminopeptidase and β-glucosidase in a pure culture of Fl 7 and in mixed cultures of bacterial isolates (Fl 7, B 2, B 3, and B 4) and *Cylindrotheca fusiformis* in *f*/2 seawater medium

total as well as cell specific proteolytic activity occurred after 70 h but was highly variable in different cultures. Total and cell specific activities were highest in cultures with Fl 7. In contrast, Fl 7 in *f*/2 medium without algae showed an increase in cell specific aminopeptidase activity until 50 h when numbers of CSP were high. Later it decreased to almost zero at the end of the experiment when no CSP were detected. High proteolytic activities in mixed cultures, especially with Fl 7, may explain a reduced accumulation of protein particles during incubations.

Activity of β-GlcAse was significantly lower and more variable in different cultures (Fig. 9) as compared to that of aminopeptidase. Increased total and cell specific activity of β-GlcAse in pure cultures of Fl 7 were measured at 50 h when concentrations of free TEP were high. After 50 h total and cell specific β-GlcAse activities in mixed cultures were higher than in pure cultures and may explain the consecutive decrease in TEP number. In contrast, total as well as cell specific β-GlcAse activities in mixed cultures with B 2 and B 4 were relatively low but slightly increased during incubation. Mixed cultures with B 3 showed an extreme increase in total and cell specific β-GlcAse activity after 270 h. The lower activity of β-GlcAse as compared to that of aminopeptidase may explain the higher abundance of TEP than that of CSP at 250 to 270 h.

DISCUSSION

Our study shows that interactions between specific bacteria (Fl 7, Fl 2, or natural populations of seawater bacteria) and a marine diatom (*Cylindrotheca fusiformis*, *Thalassiosira weissflogii*, or *Nitzschia laevis*) strongly depend on the varying environmental conditions. Media rich in inorganic but without organic nutrients led to enhanced growth of both bacteria and algae in mixed as compared to single bacterial or algal cultures (Fig. 1). Media rich in organic nutrients increased bacterial abundance by 2 orders of magnitude as compared to media without organic nutrients. Algal growth in media rich in organic nutrients was also enhanced in the presence of bacteria but bacterial and algal numbers never reached a steady state as observed for media without any organic nutrients (Fig. 2). Stressing phytoplankton by incubation in media free of vitamins and trace metals led to lower algal growth or even reduction in living cells when bacteria were present (Fig. 5).

Stimulation of microbial activity by phytoplanktonic release of extracellular products has been shown by Bell et al. (1974), supporting the 'phycosphere' as a useful concept in discussing bacteria-phytoplankton interactions in aquatic environments. In the 'phycosphere' bacterial growth is directly stimulated by extracellular products of the alga (Wang & Priscu 1994a). Following continuous production and excretion of dissolved organic matter by phytoplankton, utilizable for bacteria, Azam & Ammerman (1984) formulated the 'cluster' hypothesis. Bacterial mineralization of the relatively N- and P-rich lysed algal cellular components (Garber 1984, Caron et al. 1988, Jürgens & Güde 1990, Goldman & Dennett 1991) is supposed to be an important process for supplying algae with ammonium or phosphate (Brussaard & Riegman 1998). Increasing ectoenzyme activities, especially of aminopeptidase, throughout our study (Fig. 9) indicate enhanced bacterial mineralization of algal detritus and particles which are rich in carbon and nitrogen. Since there were no protozoa present in any of our incubations stimulation of nutrient-limited phytoplankton by bacterivorous flagellate grazing (Rothhaupt 1992) can be excluded. Stimulation of algal growth observed in our relatively simple laboratory systems is, thus, only the result of bacterial action.

Furthermore, phytoplankton growth can be also enhanced by stimulatory substances which are not of direct bacterial origin but can be released during microbial colonization and degradation of senescent algae. In all mixed cultures, and in cell aggregations,

cells with reduced autofluorescence were densely colonized by bacteria which possibly released stimulatory substances such as cytokins. On the other hand, high microbial activities in aggregates and detritus lead to release of nitrate and ammonium (Paerl & Pinckney 1996), phosphorus (see above), and CO_2, which increase and sustain algal growth in mixed cultures. In the presence of the bacterial isolate Fl 7 the relative fluorescence of *Cylindrotheca fusiformis* remained at almost maximum value for >1000 h of incubation whereas that of axenic cultures strongly decreased (Fig. 2). This indicates that microbial action even of a single isolate (Fl 7) is sufficient to sustain enhanced growth of the diatom for a prolonged period of time.

Under *in situ* conditions, however, competition between algae and aquatic bacteria for limiting nutrients such as phosphate (Rhee 1972, Currie & Kalff 1984, Thingstad et al. 1993, Guerrini et al. 1998) frequently occurs. Bacterioplankton growth can be directly limited by inorganic P and N when these elements are in short supply (Wang & Priscu 1994b). In media without organic nutrients, increase in organic matter and simultaneous reduction in inorganic nutrients due to high algal and bacterial production led to a symbiotic relationship between bacteria and algae. However, in artificial seawater enriched with glycylglycine no steady state between algal and bacterial production could be observed. Extremely high concentrations of bacteria (5.2×10^9 cells ml^{-1}) in artificial seawater enhanced turbidity, bacterial colonization of algal cells, and presumably competition for inorganic nutrients. Eutrophication increases input of organic matter, which may result in high bacterial biomass and subsequent reduction of phytoplankton health and biomass. In addition, a tremendous number of viruses were found in estuarine and other eutrophic environments (Cochran et al. 1998), indicating that prophage induction can further reduce health and biomass of phytoplankton in these environments.

Phytoplankton growth in vitamin and trace metal free *f*/2 media was much lower (*Nitzschia laevis*) or even zero (*Cylindrotheca fusiformis*) as compared to media replenished with vitamins and trace metals. Bacteria which enhanced and sustained phytoplankton growth under nutrient-rich conditions reduced algal growth and biomass when vitamins and trace metals were absent. Microscopical observations revealed very dense colonization of the cell surface of the already stressed phytoplankton whereas the cell surface of healthy phytoplankton is hardly colonized by bacteria (Cole 1982). This demonstrates that bacteria-phytoplankton interactions of the same species are not constant and alter with changes of environmental conditions. In addition, under axenic culture conditions algal cell lysis is directly affected by the degree of nutrient deficiency (Brussaard et al. 1997) and the presence of bacteria can further increase phytoplankton stress by competition for limiting nutrients and increased phytoplankton lysis.

Various marine algae prevent bacterial colonization of their cell surfaces by the production of antibacterial substances (Sieburth 1964, Kellam & Walker 1989, Sastry & Rao 1994). The extent of antibiotics production depends on phytoplankton activity and, thus, is reduced in stressed cells. As a result bacterial colonization will increase on algae growing under stress conditions such as vitamin and trace metal depletion. In natural bacterial communities there are certain species known which directly attack and lyse marine phytoplankton including diatoms (Imai et al. 1993). The shape of bacteria-phytoplankton interactions, thus, controls primary production to a varying degree, depending on the environmental conditions present.

Depending on bacteria-phytoplankton interactions the extent and quality of phytoplanktonic carbon release may dramatically change, resulting in temporarily enhanced or decreased carbon transfer to higher trophic levels. Algal carbon exudation in the form of TEP and CSP was increased in the presence of bacteria. Polysaccharide production of *Cylindrotheca fusiformis* was significantly increased in the presence of bacteria even though diatom cell growth was not negatively affected. On the other hand, bacteria can produce significant amounts of exopolymers such as polysaccharides (Grobben et al. 1998) and a large fraction of bacterial glucose uptake is incorporated into capsules when seawater bacteria are not C-limited (Stoderegger & Herndl 1998). Production of TEP and CSP in pure cultures of Fl 7 were extremely high between 50 and 150 h of incubation, but with increasing starvation numbers of TEP and CSP dramatically decreased. This indicates that depending on environmental conditions bacteria are able to produce and also to degrade various amounts of exopolymeric matter.

In general, hydrolytic ectoenzyme activities were significantly enhanced in mixed cultures as compared to pure cultures of Fl 7 which only showed low aminopeptidase and β-GlcAse activities. However, in the presence of artificial substrate analoga Fl 7 is characterized by high aminopeptidase and relatively low β-GlcAse activity (Martinez et al. 1996). Preferential hydrolysis of proteins by bacteria in contrast to polysaccharides has been shown for different environments, e.g. macroscopic organic aggregates (Grossart & Simon 1998). The preferential hydrolysis of CSP rich in N may explain the usually lower accumulation of CSP compared to TEP at 250 to 270 h of incubation. This indicates that high bacterial mineralization of organic nitrogen occurred in these experiments, which presumably increased and sustained phytoplankton growth.

The high variability in production and in quality of exopolymers was also detected by lectine staining. Enhanced concentrations of exopolysaccharides on the surface of algae with reduced autofluorescence and in aggregates in the presence of dense bacterial colonization suggest changes in phytoplankton polysaccharide production due to the presence of bacteria. P limitation increases polysaccharide production not only of algae but also of heterotrophic bacteria (Mohamed et al. 1998). Polysaccharides stainable with Bandeiraea s. II, Glycine max, and Wheat germ were only found on algal cells and aggregates with dense bacterial colonization, indicating modifications of exopolysaccharides by bacterial production and ectoenzyme activity.

Modification of exopolymeric material by bacteria could result in slow-to-degrade polymeric but also dissolved organic matter (Azam 1998) and, thus, enhance carbon storage. Bacterial action on exopolymeric matter in microzones such as the 'phycosphere' will change ocean basin-scale carbon fluxes: microbial loop, sinking, grazing, storage, and fixation. Changes in bacteria-phytoplankton interactions due to environmental conditions such as eutrophication and pollution may have huge impacts on global carbon cycling but are largely neglected in ecosystem management and modelling (Azam et al. 1998). Our very simple laboratory system shows that bacteria-phytoplankton interactions are highly variable with environmental conditions and may be even more complex in natural systems.

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