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Interactions of planktonic algae and bacteria: effects on algal growth and organic matter dynamics

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ABSTRACT: Interactions between phytoplankton and heterotrophic bacteria have been intensively studied, but many aspects of these interactions are still unknown because most of the studies were performed under non-axenic conditions. Therefore, we investigated the growth and release of dissolved organic matter of the marine diatom *Thalassiosira rotula* in axenic culture in comparison to *T. rotula* cultures inoculated with either a natural marine bacterial community (German Wadden Sea) or 3 different bacterial isolates. The isolates affiliated to the *Roseobacter* group (HP50), *Hyphomonas* (HP48) and *Flexibacteriaceae* (HP49) and were previously obtained from a *T. rotula* culture inoculated with a natural marine bacterial community (German Wadden Sea). To test whether the availability of inorganic nutrients, vitamins and trace metals affects algal growth, organic matter release and interactions with heterotrophic bacteria, we performed experiments with either Guillard's *f*/2 or *f*/10 medium, which differ in their inorganic nutrient, vitamin and trace metal concentration by a factor of 5. The bacterial community promoted growth of *T. rotula* in both media, as shown by the higher algal numbers relative to the axenic cultures, but also led to a rapid decline after the growth phase. Isolate HP50 promoted algal growth in the *f*/2 medium, but inhibited growth in the *f*/10 medium, whereas isolate HP48 showed the opposite patterns. Isolate HP49 prevented algal growth in both media. *T. rotula* exhibited distinct release patterns of dissolved organic carbon (DOC), dissolved amino acids, dissolved neutral carbohydrates, transparent exopolymer particles (TEP) and protein-containing particles (Coumassie Blue-stainable particles, CSP), which were modulated by the bacteria added. TEP and CSP were produced by the growing alga only in the presence of bacteria, indicating that the bacterial modification of algal exudates and/or the bacterial decomposition of the alga are prerequisites for formation of these microparticles. Our results show that the presence of the bacterial community and of specific populations have distinct effects on the growth and organic matter release of *T. rotula* and presumably also on other algae.

KEY WORDS: Marine diatoms · Bacteria · DOC · Amino acids · Carbohydrates · TEP · CSP · Organic matter cycling

INTRODUCTION

Algal–bacterial interactions have received increasing attention in the recent past (Ferrier et al. 2002, Schäfer et al. 2002, Green et al. 2004, Pinhassi et al. 2004, Grossart et al. 2005, 2006a,b, Rooney-Varga et al. 2005). These interactions comprise the following 4 major modes: (1) bacteria and algae form symbioses, in which bacteria benefit from phytoplankton exudates, and algal growth is favoured by bacterial products such as re-mineralised nutrients, vitamins and other growth factors; (2) bacteria act as parasites on phytoplankton and, thus, can lead to lysis and death of their hosts, while algae can also inhibit bacterial growth by releasing antibiotic compounds; (3) commensalistic bacteria have no actual negative effect on phytoplankton, but the transition between commensalism and parasitism is highly variable in time; and (4) bacteria

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are only loosely associated with phytoplankton and, thus, can efficiently compete for limiting nutrients such as phosphate. For more details of these interactions see Grossart (1999) and references therein. These interactions have often been neglected, but may be as important as inorganic nutrient supply, grazing and viral lysis in controlling the development of diatom blooms.

It has been shown that the same bacteria can either stimulate or even inhibit algal growth, depending on the physiological status of the algae (Grossart 1999). Further, algal exudation affects the presence and activity of distinct bacterial communities, which, in turn, control the amount and composition of the dissolved organic matter (DOM) released (Grossart et al. 2005, 2006a).

The composition of exuded organic matter is often characteristic of the algae releasing it (Myklestad 1974, 1995). *Skeletonema costatum*, for example, releases large amounts of DOM rich in carbohydrates, while *Emiliania huxleyi* exudes small quantities of DOM with lower proportions of carbohydrates (Biersmith & Benner 1998). There are many reports on massive production of extracellular polymers in various aquatic environments, especially in times of nutrient depletion, as the synthesis and exudation of polymers often increase under these conditions (e.g. Obernosterer & Herndl 1995, Biddanda & Benner 1997, Søndergaard et al. 2000). Roughly 10% of the photosynthetically fixed carbon is released by phytoplankton (Baines & Pace 1991, Carlson et al. 1998), but this fraction is highly variable in the field, ranging between 1 and 70% (Baines & Pace 1991). The high variability points to great fluctuations in algal DOM release and also in bacterial DOM consumption. Today, we still lack a detailed understanding of the bacterial decomposition of specific phytoplankton-derived DOM compounds and how changes in algal–bacterial interactions affect the amount and quality of DOM in aquatic systems.

The role of heterotrophic bacteria in algal–bacterial interactions seems to be ambiguous. The presence of bacteria affects the net-release of DOM and the formation of transparent exopolymer particles (TEP), proteinaceous Coomassie Blue-stainable particles (CSP) and aggregates (Grossart et al. 2006a). TEP have been identified as an important agent for aggregation (Passow 2002a), and various studies have shown that TEP is produced by phytoplankton, but also by bacteria and from dissolved precursor material (Zhou et al. 1998, Passow 2002b, Engel et al. 2004). These processes greatly depend on the physiological state of the algae (Grossart et al. 2006a,b), and there are distinct differences in the quality of the organic matter released by different diatoms, its bacterial consumption and growth. Algal–bacterial interactions can also increase phytoplankton aggregation and sedimentation by modifying phytoplankton-derived DOM and POM, but, on the other hand, may reduce sedimentation of algal-derived organic matter due to bacterial decomposition (Grossart et al. 2006a).

We hypothesise that algal growth and release of organic matter greatly depend on the presence of distinct bacterial communities and that algal–bacterial interactions vary with changing concentrations of inorganic nutrients, vitamins and trace metals. Therefore, we studied whether changes in algal–bacterial interactions result in (1) changing concentrations of DOM, TEP and CSP, (2) variable decomposition patterns of phytoplankton-derived organic material by specific bacteria, and (3) differences in algal nutrition and growth, as well as organic matter dynamics. We measured algal growth, release of DOM and concentrations of TEP and CSP in axenic cultures of *Thalassiosira rotula* incubated in *f*/2 or *f*/10 medium and compared these values with those from the same cultures that were either incubated with a natural community of marine bacteria or with single isolates. Our results show that phytoplankton growth, dynamics of DOM release and numbers of TEP, as well as CSP vary with the bacteria added and/or algal nutrition.

MATERIALS AND METHODS

Experimental design and sampling. An axenic culture of *Thalassiosira rotula* (CCMP 1647) was obtained from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP, Maine, USA). The alga was tested for axenic conditions by (1) epifluorescence microscopy, (2) cultivation on agar plates, and (3) PCR-based methods (Grossart et al. 2005). The alga was incubated in batch cultures in Guillard's *f*/2 or *f*/10 medium to generate different availabilities of inorganic nutrients. To test for the effect of specific algal–bacterial interactions, the diatom culture (ca. 1×10^3 cells ml^{-1}) was either inoculated with 10 ml of seawater (containing the natural bacterial community, ca. 1×10^6 cells ml^{-1}) or with 10 ml of bacterial isolates (ca. 1×10^6 cells ml^{-1}) previously isolated from the same diatom culture (see next paragraph). All cultures were run in triplicate in 1.2 l rolling tanks (5 rpm) at 15°C and in a 12 h light:12 h dark cycle for 30 d.

The seawater used for inoculation was collected in June 2000 from the German Wadden Sea (53° 42′ N, 7° 50′ E) and filtered through 5.0 µm Nuclepore membranes to reduce contamination by the natural algal community and protozoan growth. The bacterial strains (HP48, HP49 and HP50) were isolated from *Thalassiosira rotula* and grown on MB agar plates

(Difco). Before inoculation all bacterial strains were rinsed 3 times in sterile seawater and diluted to ca. 1×10^6 cells ml^{-1}. HP48 is closely related to the alphaproteobacterium HP40 of the *Hyphomonas* group, HP49 to the Sphingobacterium HP34 (*Flexibacter aggregans*) (Grossart et al. 2004) and HP50 to the alphaproteobacterium HP44w of the *Roseobacter* group. All isolates were sequenced as described in Grossart et al. (2004) and have been deposited in GenBank under the Accession Numbers DQ148413 (HP48), DQ150528 (HP49) and AY841778 (HP50).

Samples for all measured parameters were collected periodically under sterile conditions (clean bench) and immediately processed for further analyses. The water withdrawn was replaced by sterile seawater to avoid air bubbles inside the rolling tanks.

Algal and bacterial abundance. Free-living and attached bacteria from 1 to 5 ml sub-samples were counted on 0.2 and 5.0 µm Nuclepore membranes, respectively, after staining with DAPI (4′6′diamidino-2-phenolindole) by epifluorescence microscopy (Axioplan) at 1000× magnification (Porter & Feig 1980). Bacteria retained on the 5.0 µm membranes were considered attached to either algal cells or organic particles, which formed during incubation. We are aware of the fact that bacterial colonies and cells >5 µm may have also been retained on the filters; however, microscopy revealed that this was seldom the case. The algae were counted by simultaneously using light and epifluorescence microscopy at 400× and 1000× magnification. A minimum of 10 replicates was counted for each bacterial and algal sample. Algal cells without any visible chlorophyll *a* autofluorescence were defined as dead algal cells.

Dissolved organic carbon (DOC). Samples (10 ml) were collected in glass ampoules after filtration through 0.2 µm polycarbonate membranes (Nuclepore). Samples were acidified with 100 µl of 85% H_3PO_4, flame sealed and stored until analysis at 4°C in the dark. DOC was analysed by high-temperature combustion (Shimadzu TOC-5000) as described in Grossart et al. (2006a).

Amino acids. Samples (10 ml) were filtered through 0.22 µm pore size low-protein-binding filters (Acrodisc, Pall Corporation) and stored frozen at –20°C until analysis. Concentrations of dissolved free amino acids (DFAA) were analysed by HPLC after ortho-phthaldialdehyde derivatisation according to Lindroth & Mopper (1979) using 2 injections per sample. Dissolved combined amino acids (DCAA) were hydrolysed with 6 N HCl at 155°C for 1 h and analysed as DFAA.

Neutral carbohydrates. Samples (10 ml) were filtered using 0.2 µm pore size polycarbonate filters (Nuclepore) and stored frozen at –20°C until analysis. Concentrations of dissolved free neutral monosaccharides (DFCHO) were analysed by HPLC using a Carbopac PA 10 column (Dionex) and pulsed amperometric detection according to Mopper et al. (1992). In general, 2 injections per sample were analysed. We used 20 mM NaOH as eluent. Prior to analysis, samples were desalted by ion-exchange chromatography according to Borch & Kirchman (1997). Dissolved combined neutral monosaccharides (DCCHO) were analysed by HPLC as DFCHO after 20 h of hydrolysis with 0.09 N HCl at 100°C.

TEP and CSP. Subsamples (2 ml) were filtered in duplicates onto polycarbonate membranes (0.2 µm pore size) under low vacuum (<10 mbar) to enumerate TEP and CSP. TEP samples were stained with 0.22 µm pre-filtered 0.02% Alcian Blue prepared in 0.06% glacial acetic acid (pH 2.5) (Alldredge et al. 1993). CSP samples, which were available only until Day 11, were stained with 1 ml of 0.04% Coomassie Brilliant Blue (G-250) according to Long & Azam (1996). Samples were filtered dry, placed over a drop of oil on a frosted slide (Cytoclear TM, Poretics) and enumerated using a Zeiss Axioplan microscope under bright field illumination (100× to 200× magnification). Image analysis (analySIS V 3.0, Soft Imaging System) was used to assess the equivalent spherical area of TEP and CSP.

Statistical analyses. Statistical analyses were done by 'post hoc' standard least square contrast analyses after ANCOVA, with time as the covariate and culture type as the nominal predictor. All statistical analyses were performed with the software JMP 4.02 using average values. Significance was given at p-values <0.05.

RESULTS

Phytoplankton and bacterial abundance

Abundances of living and dead *Thalassiosira rotula* greatly differed between axenic and non-axenic cultures and between cultures incubated in *f*/2 and *f*/10 media (Fig. 1A, B, Table 1). The onset of growth in the axenic *f*/2 *T. rotula* culture was delayed by 10 d relative to that in the *f*/10 medium, even though maximum numbers were similar in both treatments. In contrast, in the presence of the natural marine bacterial community, the onset of algal growth occurred much faster in the *f*/2 than in the *f*/10 medium. During the whole experiment we did not observe any small phytoplankton, cyanobacteria, or protozoa that may have slipped through the 5 µm Nuclepore membranes. The presence of HP50 led to persistently high algal numbers in the *f*/2 medium, whereas no algal growth was observed in the respective *f*/10 culture. The isolate HP48 inhibited growth of *T. rotula* in the

Table 1. *Thalassiosira rotula.* To test for significant differences between axenic and non-axenic cultures of the diatom in *f*/2 and *f*/10 media, statistical analyses using 'post hoc' analyses after ANCOVA, with time as the covariate and culture as the nominal predictor, were performed with the software JMP 4.02. DOC: dissolved organic carbon; DFAA: dissolved free amino acids; DCAA: dissolved combined amino acids; DFCHO: dissolved free neutral monosaccharides; DCCHO: dissolved combined neutral monosaccharides; TEP: transparent exopolymer particles; NS: not significant

	Significance value	
	f/2	*f*/10
Living algae	<0.0001	<0.0001
Dead algae	0.0224	<0.0001
Free bacteria	<0.0001	<0.0001
Attached bacteria	<0.0001	<0.0001
DOC	NS	NS
DFAA	0.0472	0.0309
DCAA	NS	0.0111
DFCHO	NS	NS
DCCHO	NS	0.0079
TEP	0.0019	<0.0001

f/2 medium completely, but led to persistently high algal numbers in the *f*/10 medium. The isolate HP49 prevented growth of *T. rotula* completely in both media.

Numbers of free and attached bacteria also varied significantly among the different treatments and media (Fig. 1A,B, Table 1). The abundance of free-living cells in the natural bacterial community increased in both media together with algal cell numbers. Numbers declined after the algal peak in the *f*/2 medium, but persisted at an enhanced level in the *f*/10 medium (Fig. 1A,B). The abundance of attached bacteria in this treatment peaked during the decline of algal cell numbers in both media and decreased thereafter. Numbers of free and attached HP50 in the algal *f*/2 culture continuously increased during the incubation period and reached the highest values of all treatments, thus responding to the high algal cell numbers in this treatment. In the respective *f*/10 culture with no algal growth, the cell numbers of HP50 remained low. Free and attached bacteria of the isolate HP49, preventing algal growth in both media, also remained low throughout the whole incubation. This was also true for the isolate HP48 in the *f*/2 culture; however, in the *f*/10 culture, the numbers of free and attached HP48 constantly increased, thus responding to the algal growth in this treatment.

In both media — that initially axenic and that inoculated with axenic algae — free and attached bacteria were detected on Days 25 and 30, indicating that the axenic control tanks became contaminated by bacteria on the last 2 sampling dates.

Dissolved organic carbon

Concentrations of DOC did not significantly differ between the various treatments, but they did vary with medium (Fig. 2A,B, Table 1). Initial concentrations of DOC in the *f*/2 medium, however, were substantially higher than in the *f*/10 medium (Fig. 2A). DOC measurements of the compounds added (such as vitamins and EDTA) to the medium revealed that the 5-fold higher concentrations of these compounds in the *f*/2 medium accounted for an additional input of ca. 150 µM DOC in comparison to the *f*/10 medium. In the *f*/2 culture, with the natural bacterial community, initial DOC concentrations were reduced and remained low during the main growth phase of the alga and bacteria until Day 11 and increased thereafter, covarying with the axenic treatment (Fig. 2A). In the *f*/10 medium of this treatment, with low bacterial growth, DOC concentrations increased and peaked together with algal cell numbers. In the cultures to which bacterial isolates were added, DOC concentrations increased systematically only in those treatments in which algal growth was promoted, i.e. in the treatment with HP50 in *f*/2 medium and that with HP48 in *f*/10 medium. In the other cultures, DOC concentrations fluctuated independently of the growth of the alga and the bacterial isolate.

Dissolved amino acids

In the *Thalassiosira rotula* cultures, in which algal growth was detected, the dynamics of DFAA concentrations co-varied with those of algal numbers, i.e. in the axenic treatments and in those inoculated with natural bacteria, in *f*/2 culture with the isolate HP50 and in *f*/10 culture with HP48 (Fig. 3A,B,E,F). The dynamics of DFAA concentrations in the axenic *f*/10 culture and in the culture inoculated with the natural bacterial community lagged a few days behind the dynamics of algal cell numbers. DFAA concentrations in the axenic *f*/10 culture were substantially higher than in the culture with the natural bacterial community towards the end of the incubation. Interestingly, highest concentrations occurred in the *f*/10 culture inoculated with HP48, showing persistently high algal numbers with 2 distinct peaks at Days 11 and 25 (Fig. 3F). In the other cultures inoculated with bacterial isolates and no or low algal growth, DFAA concentrations always remained low.

Average compositions (mol%) of DFAA in axenic *f*/2 and *f*/10 *Thalassiosira rotula* cultures were almost the same, with aspartate, glutamate and serine representing 15 to 18%, 17 to 20% and 20 to 21%, respectively. The presence of bacteria in *T. rotula* cultures with high

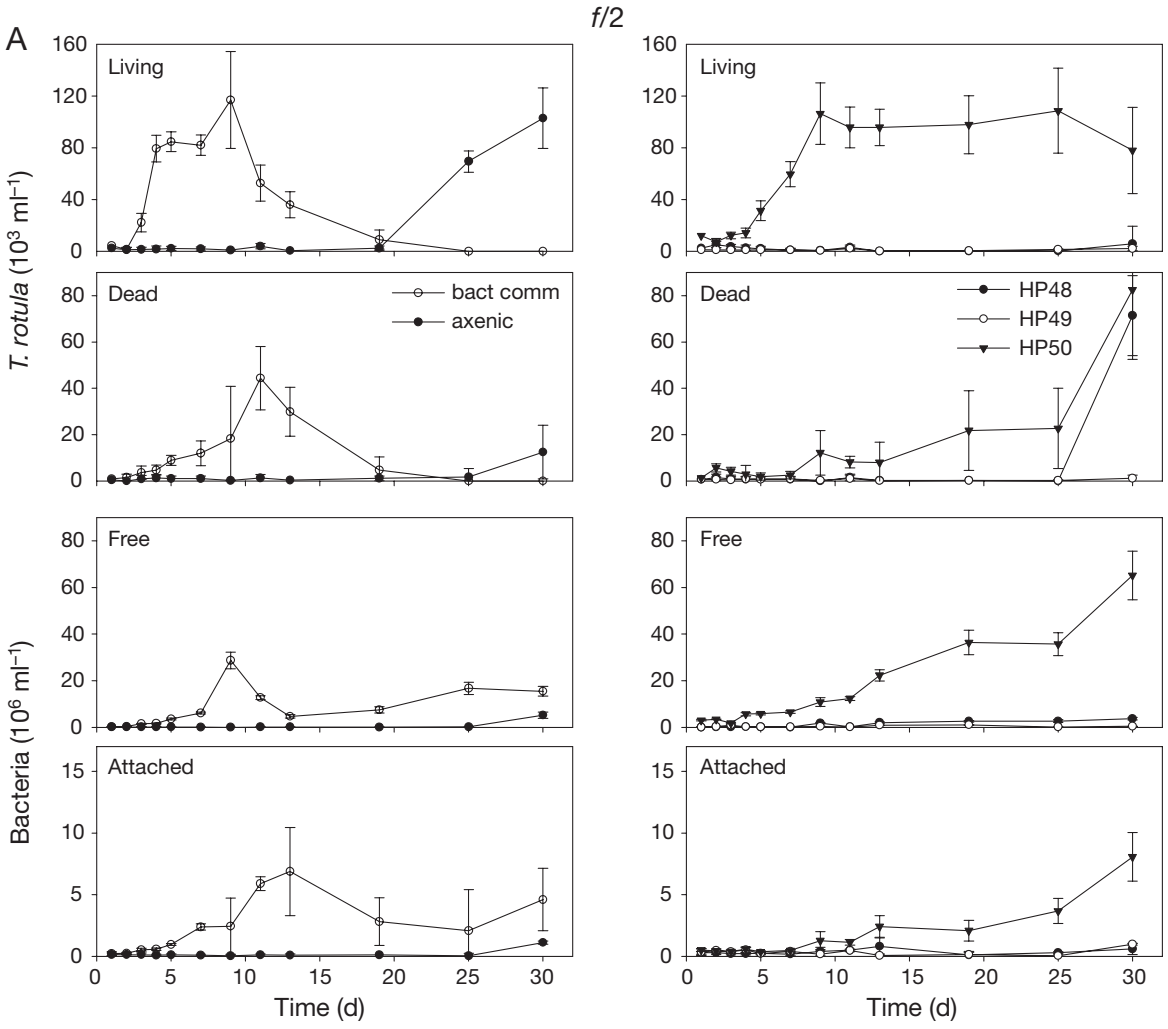

Fig. 1 (and overleaf). *Thalassiosira rotula.* Numbers of living and dead diatoms as well as free and attached bacteria grown in (A) *f*/2 and (B) *f*/10 media without (axenic) and inoculated with a natural bacterial community (bact comm) or bacterial isolates HP48, HP49 and HP50. Error bars: SD of 3 independent measurements for each given parameter

algal growth (*f*/2 and *f*/10 cultures with natural bacteria, *f*/2 culture with the isolate HP50 and *f*/10 culture with HP48) resulted in increased mol% of aspartate and glutamate (up to 45%) and in reduced mol% of serine (≤15%), compared to the axenic cultures. In non-axenic cultures with low or no algal growth (*f*/2 cultures with HP48 or HP49 and *f*/10 cultures with HP49 or HP50) the mol% of aspartate and glutamate was reduced (<40%) and that of serine was increased (≥25%).

The concentration dynamics of DCAA in the axenic treatments and in those inoculated with natural bacteria in both media as well as in those inoculated with HP50 in *f*/2 medium and HP48 in *f*/10 medium were, in general, similar to the dynamics of DFAA (Fig. 3C,D,G,H). In the treatment with the natural bacterial community in *f*/2 medium, however, highest DCAA concentrations occurred in the second half of the experiment. At this time, algal and bacterial numbers decreased. In the *Thalassiosira rotula* cultures growing in *f*/2 medium and inoculated with the isolates HP48 and HP49, DCAA concentrations also increased in the second half of the experiment, whereas they did not increase in the respective *f*/10 cultures (Fig. 3D,F).

In contrast to DFAA, DCAA were characterised by a higher mol% of glycine/threonine, ß-alanine and alanine (10 to 15% each). In all non-axenic algal cultures with high algal growth (*f*/2 and *f*/10 cultures with natural bacteria, *f*/2 culture with the isolate HP50 and *f*/10 culture with HP48) the mol% of aspartate and glutamate was increased (>25%), whereas the values for glycine/threonine and ß-alanine were reduced (10 to 15%), compared to the axenic cultures. In non-axenic cultures with low or no algal growth (*f*/2 cultures with HP48 or HP49 and *f*/10 cul-

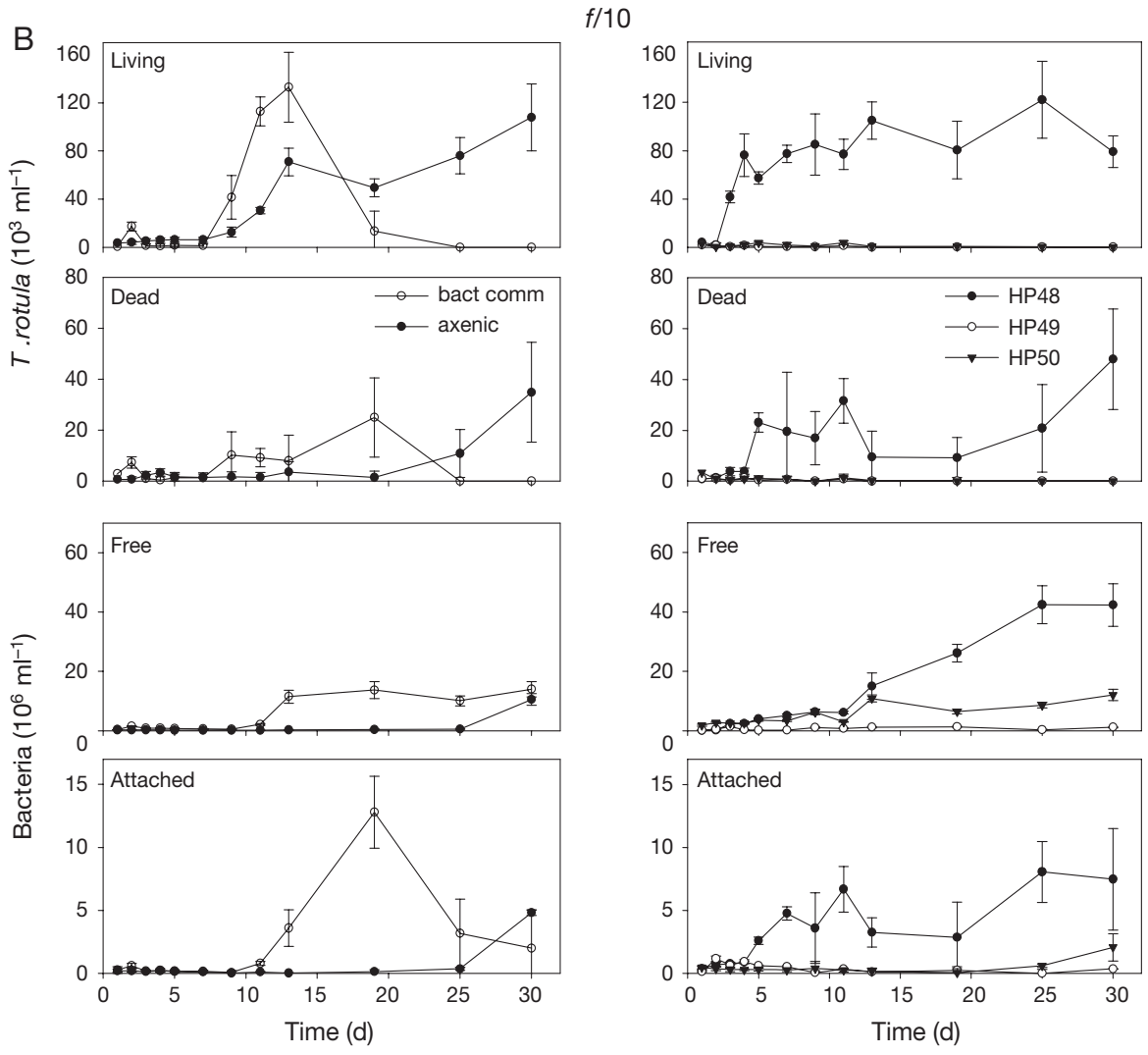

Fig. 1 (continued)

tures with HP49 or HP50) the mol% of aspartate and glutamate (<10%) was reduced, whereas the values for serine, glycine/threonine and ß-alanine were increased (≥50%). These results indicate that algal exudation and bacterial hydrolysis may affect DCAA mol% in different ways.

Dissolved carbohydrates

Concentrations of DFCHO remained low and without fluctuations in the axenic *f*/2 *Thalassiosira rotula* culture and in those inoculated with natural bacteria (Fig. 4A,E). In the axenic *f*/10 culture, DFCHO concentrations increased between Days 16 and 25 after the alga started growing (Fig. 4E) and before the culture became non-axenic. High concentrations of DFCHO also occurred in the *f*/10 culture of isolate HP48 over most of the incubation period, especially when algal numbers were high. In all other cultures with bacterial isolates, DFCHO concentrations remained low, except on Day 25, and independent of the growth dynamics of the alga (Fig. 4B,F). DFCHO in all cultures consisted of >80% of glucose and showed only little variation with time and among the different treatments.

Concentrations of DCCHO in axenic cultures remained lower than concentrations in cultures inoculated with the natural bacterial community (Fig. 4C,G). In the *f*/2 culture with natural bacteria, DCCHO concentrations increased when algal numbers declined. In the axenic culture, as well as in that with natural bacteria in *f*/10 medium, however, DCCHO concentrations already increased during the growth phase and remained higher thereafter. In the *f*/2 culture with the isolate HP50, promoting algal growth, DCCHO concentrations were higher than in the respective cultures

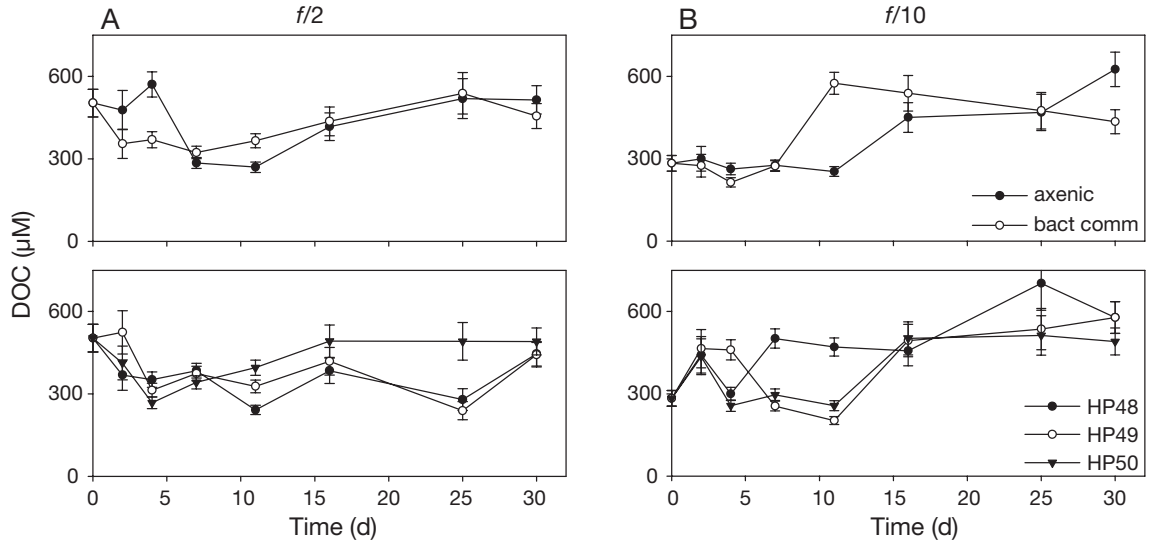

Fig. 2. *Thalassiosira rotula.* Concentrations of dissolved organic carbon (DOC) in cultures of the diatom grown in (A) *f*/2 and (B) *f*/10 media without (axenic) and inoculated with a natural bacterial community (bact comm) or bacterial isolates HP48, HP49 and HP50. Error bars: SD (as in Fig. 1)

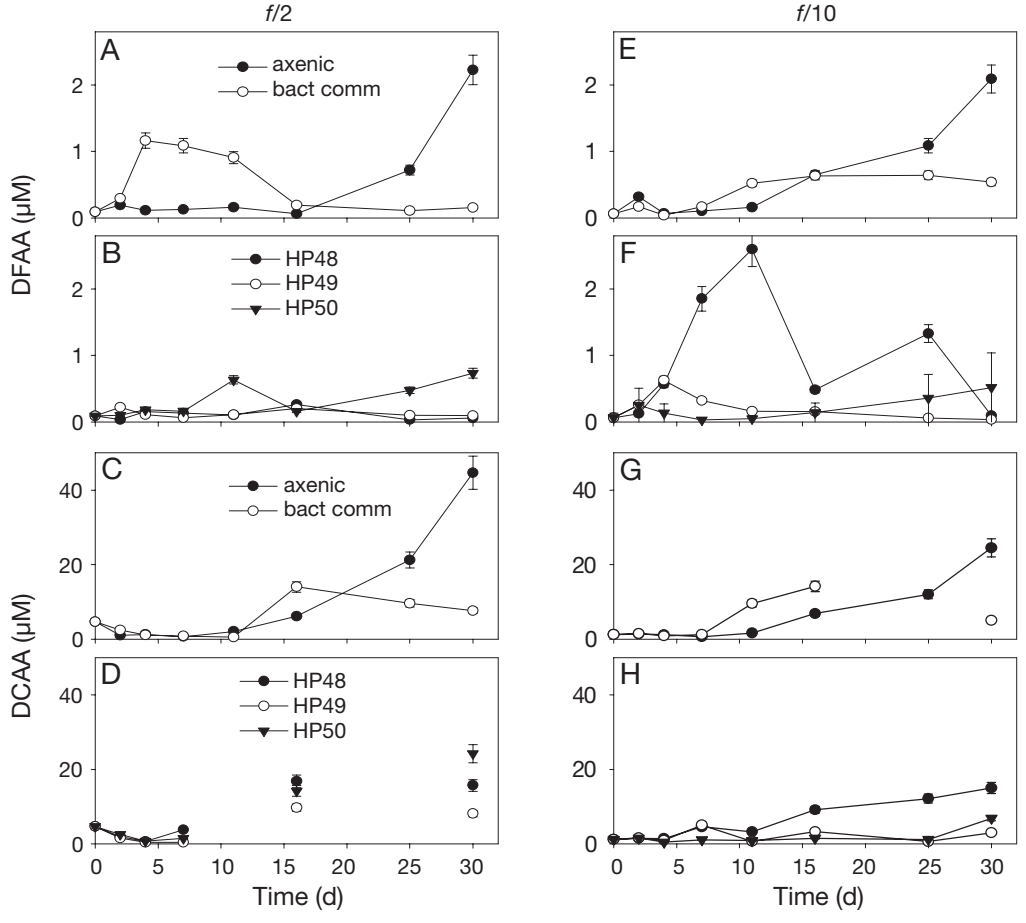

Fig. 3. *Thalassiosira rotula.* Concentrations of dissolved free amino acids (DFAA) and dissolved combined amino acids (DCAA) in cultures of the diatom grown in *f*/2 and *f*/10 media. (A,E) DFAA in axenic and bacterial community-inoculated (bact comm) cultures; (B,F) DFAA in cultures inoculated with the bacterial isolates HP48, HP49 and HP50; (C,G) DCAA in axenic and bacterial community-inoculated cultures; and (D,H) DCAA in cultures inoculated with the bacterial isolates HP48, HP49 and HP50. Error bars: SD (as in Fig. 1)

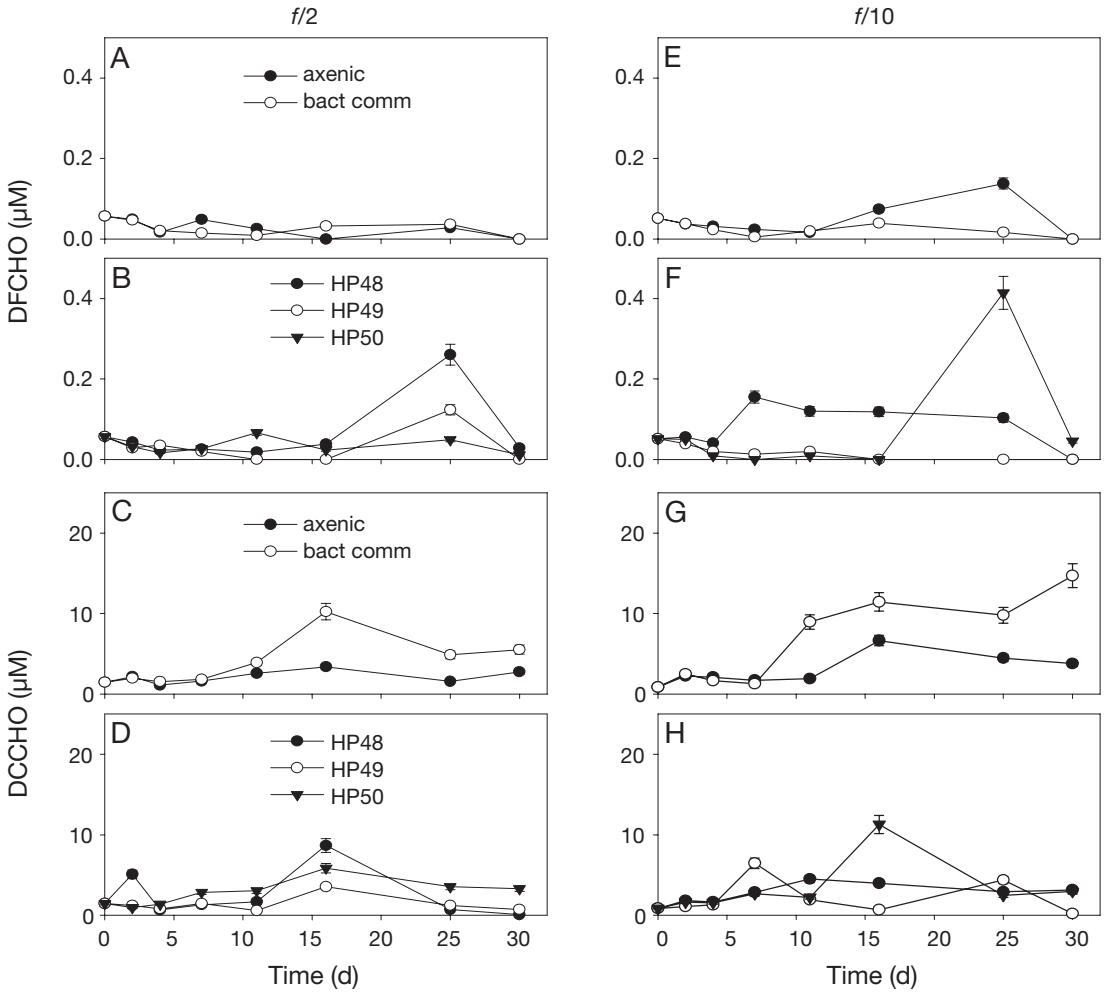

Fig. 4. *Thalassiosira rotula.* Concentrations of dissolved free neutral monosaccharides (DFCHO) and dissolved combined neutral monosaccharides (DCCHO) in cultures of the diatom grown in *f*/2 and *f*/10 media. (A,E) DFCHO in axenic and bacterial community-inoculated (bact comm) cultures; (B,F) DFCHO in cultures inoculated with the bacterial isolates HP48, HP49 and HP50; (C,G) DCCHO in axenic and bacterial community-inoculated cultures; and (D,H) DCCHO in cultures inoculated with the bacterial isolates HP48, HP49 and HP50. Error bars: SD (as in Fig. 1)

inoculated with the other isolates, except on Day 16, when a single value with HP48 exceeded the other values (Fig. 4D). Generally, DCCHO concentrations remained low in the treatments with *f*/2 medium and bacterial isolates in which algal growth was inhibited (Fig. 4D). In *f*/10 cultures inoculated with bacterial isolates, DCCHO concentrations fluctuated without any relationship to the growth of the alga or bacteria (Fig. 4H).

Average mol% compositions of DCCHO in axenic *f*/2 and *f*/10 *Thalassiosira rotula* cultures were very similar, with glucose and mannose contributing 65 to 70% and 15 to 18%, respectively. In non-axenic algal cultures with high algal growth (*f*/2 and *f*/10 cultures with natural bacteria, *f*/2 culture with the isolate HP50 and *f*/10 culture with HP48) the mol% of glucose was decreased (<50%), whereas the values of fucose and mannose were increased (>10 and 20%, respectively). In non-axenic cultures with low or no algal growth (*f*/2 cultures with the isolate HP48 or HP49 and *f*/10 cultures with HP49 or HP50) the mol% of glucose increased (>70%), whereas that of mannose decreased (<10%).

Transparent exopolymer particles

All *Thalassiosira rotula* cultures that exhibited significant growth produced TEP, even though pronounced differences were recorded (Fig. 5, Table 1). Highest abundances of TEP occurred in *f*/2 cultures inoculated with natural bacteria and with the isolate HP50 and in the *f*/10 culture inoculated with the isolate HP48. In all these cultures, but also in the *f*/10 culture

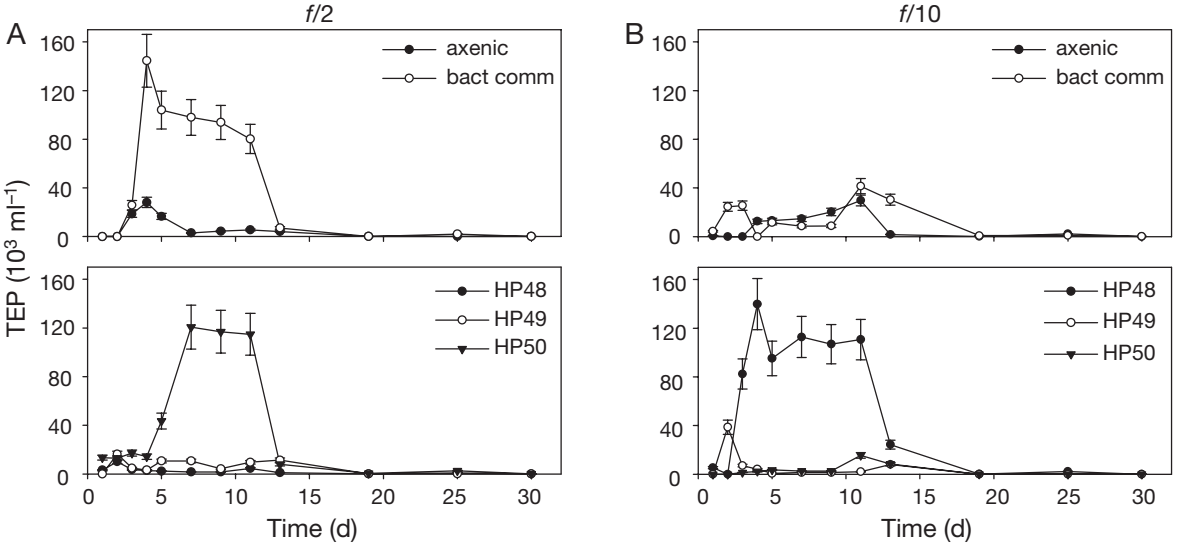

Fig. 5. *Thalassiosira rotula.* Abundance of transparent exopolymer particles (TEP) in cultures of the diatom grown in (A) *f*/2 and (B) *f*/10 media without (axenic) and inoculated with a natural bacterial community (bact comm) or the bacterial isolates HP48, HP49 and HP50. Error bars: SD (as in Fig. 1)

with the natural bacterial community, TEP abundance strongly decreased when the bacterial growth phase began. This decrease occurred irrespective of whether algal numbers declined, as in the *f*/2 and *f*/10 cultures with the natural bacterial community, or remained high, like in the 2 cultures with the bacterial isolates. Axenic cultures produced substantially less TEP and only in the early growth phase (Fig. 5A,B). In the cultures inoculated with bacterial isolates that inhibited algal growth, TEP abundance always remained low (Fig. 5A,B).

Coomassie Brilliant Blue-stainable particles

CSP were only monitored until Day 11 of the experiment, and no significant differences were found between *f*/2 and *f*/10 cultures (Table 1). Axenic *f*/2 cultures as well as *f*/10 cultures of *Thalassiosira rotula* did not show any production of CSP until Day 11. In contrast, in the *f*/2 cultures inoculated with the natural bacterial community, numbers of CSP increased from Day 5 to 11. This increase paralleled that of dead algal cells, which reached a maximum on Day 11. In the respective *f*/10 culture, the number of CSP remained very low until Day 11, but highest algal numbers only occurred on Day 13 (Fig. 1B). In the *f*/10 culture inoculated with the isolate HP48, leading to high algal numbers (Fig. 1B), CSP abundance remained low at the beginning but greatly increased on Day 11. CSP numbers remained very low in all cultures inoculated with bacterial isolates that inhibited algal growth.

DISCUSSION

Interactions of bacteria and *Thalassiosira rotula*

Our results show that different bacteria affected the growth of *Thalassiosira rotula* very differently, and that the strain-specific effects were also dependent on environmental conditions such as concentrations of nutrients, vitamins and trace metals. The presence of a natural bacterial community collected from a coastal marine site, the Wadden Sea, promoted growth of this alga in *f*/2 as well as in *f*/10 medium. In *f*/2 medium, promotion was such that algal growth started much earlier than in the axenic culture, whereas in *f*/10 medium promotion resulted in higher cell numbers than in the axenic culture without any time shift in the onset of algal growth. Inoculation with natural bacteria always prevented accumulation of algal cells after the peak of the algal bloom, presumably by ectoenzymatic lysis of the alga (Grossart et al. 2006a). This change in function of the mixed bacterial community may have been linked to changes in bacterial community composition. In fact, in a comparable study, we have shown that a succession of the bacterial community took place during the growth of a *T. rotula* culture, leading to the dominance of *Sphingobacteria/Flavobacteria* and enhanced proportions of *alpha-* and *gammaproteobacteria* in the particle-associated fraction during the late exponential and stationary phases (Grossart et al. 2005). This succession has important implications for the modes of interactions between the alga and the bacteria present (Grossart et al. 2005, 2006a). We

hypothesise that the breakdown of the algal culture was mainly a result of nutrient stress and subsequent bacterial decomposition. Similarly, declines in the abundance of free-living and attached bacteria may have been a result of the reduced availability of organic substrates. However, phage infection and protozoan grazing may have contributed to this decline and succession in community composition. Our microscopic observations (even with Sybr Gold staining, H. P. Grossart unpubl. data), however, do not give any hint that viral lysis or grazing have been important in our experimental setup.

The addition of single bacterial isolates, also obtained from *Thalassiosira rotula* cultures inoculated with bacteria from the Wadden Sea, resulted either in almost complete growth inhibition of the alga (HP49) or in growth promotion in *f*/2 (HP50) or *f*/10 medium (HP48). These different growth patterns of the alga as a function of the presence of single bacterial isolates support our observation that modes of interactions between algae and bacteria vary with changes in bacterial community structure and environmental conditions. It has been previously shown that (1) environment and source community of bacteria control availability of nutrients, vitamins and trace metals (Grossart 1999), (2) complex bacterial communities tend to vary over time and with algal growth (Lebaron et al. 1999, Schäfer et al. 2002, Pinhassi et al. 2004, Grossart et al. 2005), and (3) the presence of different bacteria effects algal exudation and organic matter dynamics in different ways (Grossart et al. 2006a).

We have no direct indications of which controlling factors changed under the varying growth conditions, except that concentrations of inorganic nutrients, vitamins and trace elements differed by a factor of 5. This apparently rather small difference obviously induced major changes in the physiology of the alga and/or bacteria, leading to such strikingly different interactions. In addition to these differences, bacteria may have changed the availability of inorganic carbon and affected pH in the microenvironment of the alga. Both factors are well known to affect algal growth (e.g. Gavis & Ferguson 1975). We did not assess inorganic carbon and pH in the microenvironment of the alga, and assume that these factors were of minor importance in controlling the growth of *Thalassiosira rotula*. Maximum algal numbers were rather similar in both assays and had not yet reached the stationary phase in the axenic treatments, indicating that the availability of inorganic carbon was not limiting.

The given conditions may have been favourable for some isolates to produce growth-promoting substances such as vitamins (Pringsheim 1912, Haines & Guillard 1974). Growth inhibition of the alga could have been caused by (1) production of antibiotic substances by the bacteria (Cole 1982, Imai et al. 1993), (2) synthesis of very effective ectoenzymes hydrolysing the algae (Martinez et al. 1996, Bidle & Azam 2001), and (3) competition for inorganic nutrients (Rhee 1972, Thingstad et al. 1993, Guerrini et al. 1998) and/or lack of vitamins and trace elements (Grossart 1999). These properties and different modes of interactions have been reported for various bacteria, but not for single bacterial isolates growing at different growth conditions.

Our experiments were performed with a single *Thalassiosira rotula* culture. Hence, the algal bacterial interactions we observed may be typical for this alga, but not necessarily for other algae. In fact, we have shown that modes of interactions and composition of mixed bacterial communities, mainly attached bacterial subcommunities, differ throughout growth of *T. rotula* and *Skeletonema costatum* batch cultures (Grossart et al. 2005, 2006a). Hence, and as outlined above, specific interactions of algae and bacteria appear to be general phenomena, and further studies may reveal other modes or modifications of interactions between algae and heterotrophic bacteria.

Potential role of specific isolates

Isolates HP48 and HP50 are both members of the family *Rhodobacteriaceae*, but exhibit completely different interactions with *Thalassiosira rotula* and indicate that specific physiological properties mediate these interactions. The isolate HP48 affiliates to the marine prosthecate and budding *Hyphomonas* group and is closely related to the marine alphaproteobacterium HP40 (Grossart et al. 2004). So far, only little is known about the physiology of this marine bacterium, which often exhibits a surface-associated life style (Weiner et al. 2000). Members of this group have also been detected during phytoplankton blooms in the Southern California Bight and the North Sea (Fandino et al. 2001, Pinhassi et al. 2003).

The isolate HP50 belongs to the *Roseobacter* clade, which is often affiliated with phytoplankton blooms (Zubkov et al. 2001, Pinhassi et al. 2004, Grossart et al. 2005). There are good indications that 1 factor for close interactions between this bacterial group and specific algal communities is the release of dimethylsulfoniumpropionate (Zubkov et al. 2001). Our results suggest that this bacterial group also favours phytoplankton growth.

The isolate HP49 affiliates to the *Sphingobacteria* and is closely related to the marine Flexibacterium HP34 (Grossart et al. 2004). *Bacteroidetes* can greatly dominate the attached fraction of bacteria on marine phytoplankton (Fandino et al. 2001, Schäfer et al. 2002, Pinhassi et al. 2004, Grossart et al. 2005), and are capa-

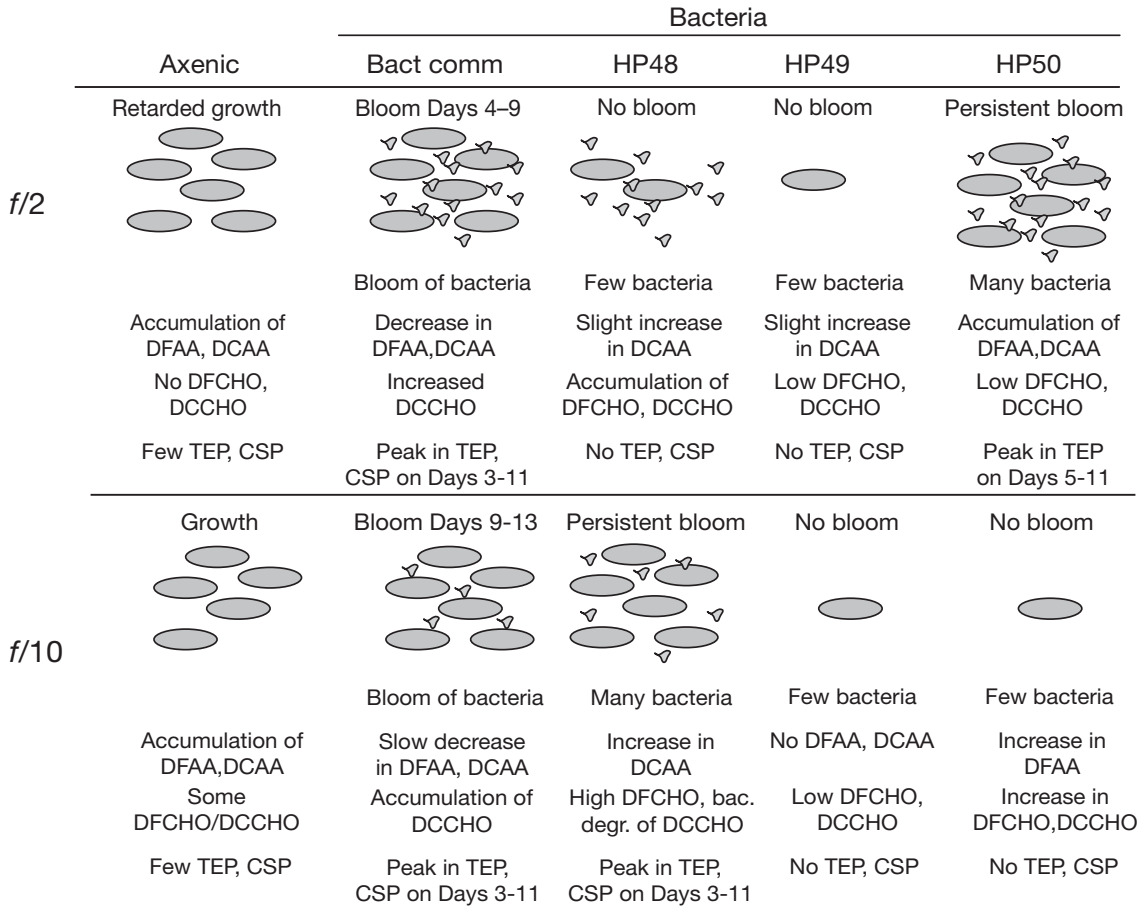

Fig. 6. Schematic summary of the major differences between the various approaches used. (⬭) diatoms; (ᵠ) bacteria; bact Comm: natural bacterial community; HP48, HP49 and HP50: bacterial isolates; bac. degr.: bacterial degradation; CSP: Coumassie Blue-stainable particles; for other abbreviations, see Table 1

ble of degrading a variety of polymeric substrates (Kirchman 2002). In addition, a high algicidal activity has been reported for this bacterial group (Mayali & Azam 2004) and may explain growth inhibition of *Thalassiosira rotula* by this isolate.

Dynamics of DOM

Bacteria affected the dynamics of DOC, amino acids and the carbohydrates of *Thalassiosira rotula*, in different and highly specific ways as can be seen by comparing axenic versus non-axenic cultures (Fig. 6). The observed variable patterns of DOM dynamics may be due to changes in release as well as selective decomposition and removal by bacteria. For example, the much higher DOC concentration on Day 11 in the *f*/10 culture with mixed natural bacteria compared to that without bacteria indicates stimulation of DOC release in the presence of these bacteria. After Day 11, DOC concentrations continuously decreased in the presence of natural bacteria, but continuously increased in the absence of bacteria, indicating bacterial decomposition and removal of DOC. Since DOC release and removal may take place at the same time in our non-axenic cultures, we were not able to clearly distinguish between these 2 opposing processes. However, concentrations of DOC always decreased when bacterial numbers and presumably bacterial decomposition was high, e.g. in the early breakdown phase of the alga in *f*/2 cultures inoculated with natural bacteria.

Cultures with single bacterial isolates showed pronounced differences in DOC dynamics depending both on the presence of specific bacteria and environmental conditions. For example, the presence of HP50 in *f*/2 cultures led to removal of DOC, whereas in *f*/10 cultures, where bacterial numbers remained low, release of DOC increased throughout the incubation even though algal numbers were low. This evidence indicates that bacterial DOC utilisation requires sufficiently high levels of inorganic nutrients (Stoderegger & Herndl 1999) and presumably vitamins and trace metals. In addition, bacteria may actively transform

DOC into particulate organic carbon, e.g. generate TEP (Passow 2002a), especially when concentrations of inorganic nutrients and trace metals are low.

DFAA accumulated in the axenic cultures over time, and concentrations co-varied with algal and bacterial dynamics. Highest concentrations occurred in periods when high numbers of bacteria were recorded (*f*/2 cultures with natural bacteria and with the isolate HP50, and *f*/10 culture with the isolate HP48). Because axenic *Thalassiosira rotula* do not release substantial amounts of DFAA (Grossart et al. 2006a), the high concentrations were most likely a result of intense DCAA hydrolysis. This notion is supported by the fact that non-axenic cultures with high algal growth (*f*/2 and *f*/10 cultures with natural bacteria, *f*/2 culture with HP50 and *f*/10 culture with HP48) showed a different DFAA mol% composition than those with low or no algal growth. These findings indicate that algal exudation, bacterial hydrolysis and DFAA uptake not only affect the quantity (Grossart et al. 2006a), but also the composition of DFAA.

DCAA accumulated over time in the axenic cultures, but also in the other cultures in which the growth of *Thalassiosira rotula* was promoted by bacteria. This accumulation was a result of the breakdown of algal biomass, i.e. lysis and bacterial decomposition. In both algal cultures inoculated with natural bacteria, DCAA accumulation was less pronounced, reflecting more intense decomposition and consumption by the mixed bacterial community. As for DFAA, the mol% composition of DCAA in all non-axenic algal cultures with high algal growth was different from that in non-axenic cultures with low or no algal growth. Because phytoplankton blooms have a reduced long-term impact on the composition of amino acids in the sea (Meon & Kirchman 2001), degradation pathways by bacteria seem to be more important than production processes in determining the molecular composition of amino acids in marine pelagic systems.

Whereas DFCHO concentrations remained low in most cultures, DCCHO accumulated in cultures inoculated with bacteria, but also in the axenic *f*/10 culture. DCCHO concentrations were higher in cultures with natural bacteria compared to those in which algal growth was promoted by the isolates HP48 and HP50. These results indicate that the alga growing in *f*/10 medium released some DCCHO without any bacterial impact and that the bacterially promoted growth resulted in enhanced DCCHO release under these conditions. Furthermore, the isolates consumed some DCCHO, mainly towards the end of the experiment. On the other hand, the mixed bacterial community even released DCCHO, mainly in the *f*/10 medium. Similarly to amino acid dynamics, algal exudation and bacterial removal led to changes in the quantity and quality of DCCHO.

Dynamics of microparticles

Formation of TEP predominantly occurred in the growing algal cultures, irrespective of the type of bacteria present, but disappeared when the bacteria started growing. This result suggests that TEP was mainly of algal origin and that the presence of bacteria favoured its formation but also its degradation. It has been shown that natural marine bacterioplankton is capable of producing significant amounts of exopolymeric substances (Stoderegger & Herndl 1999), which may increase the concentration of TEP (Passow 2002b). On the other hand, marine bacteria are known to hydrolyse exopolymers such as TEP (Passow 2002a). Another possibility for the disappearance of TEP in our experiments would be aggregate formation. However, from our macroscopic and microscopic observations we do not have any indications of massive aggregation. Therefore, we assume that bacterial decomposition was the major sink for TEP, suggesting that the TEP we detected were not recalcitrant, as postulated for the TEP-mediating aggregation (Passow 2002b).

CSP were consistently detected only in those cultures in which dead algae occurred, which suggests that algal death due to bacterial hydrolysis led to the appearance of CSP. It has previously been shown that CSP are decomposed by bacteria in various pelagic ecosystems (Long & Azam 1996, Berman & Viner-Mozzini 2001), but our observation adds to published reports that CSP are also formed from decaying algae. In more complex marine food webs other decaying organisms releasing protein-containing particles, such as zooplankton and bacteria, may also contribute to the formation of CSP.

CONCLUSIONS

We have shown that algal bacterial interactions are very specific, strongly affecting algal growth, and that they depend on environmental conditions such as concentrations of inorganic nutrients, vitamins and trace metals (Fig. 6). The mode of interaction of individual bacteria can change at varying growth conditions at least for *Thalassiosira rotula*, but presumably also for other algae. The presence of specific bacterial communities and environmental conditions led to pronounced changes in the quantity and quality of phytoplankton-derived DOM and microparticles. Natural bacterial communities promote algal growth in the early, i.e. exponential, growth phase, but transitionally turn into a competitive and parasitic life style, leading to the breakdown of the algal bloom. Our experiments with single isolates indicate that various modes of interac-

tion within a given bacterial community occur, even with a single alga. In the field these interactions are much more complex, as they also include reactions among various bacteria (Long & Azam 2001, Grossart et al. 2004), as well as temporal changes in the composition and activities of bacterial and phytoplankton communities.

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