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Virus-induced transfer of organic carbon between marine bacteria in a model community

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ABSTRACT: Viral lysis results in the transformation of living cells into dissolved and colloidal organic matter referred to as lysate. When viruses are included in food web models it is generally assumed that lysates are readily metabolized by bacteria in the community. We hypothesized that the production of lysate by viruses could also influence microbial community composition by mediating the diversification of carbon sources. To test this hypothesis, we established simple model communities containing various combinations of 2 marine bacteria (*Cellulophaga* sp. and *Photobacterium* sp.) and 2 viruses (one specific to each bacterial type) grown in a seawater-based medium with lactose as the sole carbon source. This medium supported vigorous growth of *Cellulophaga* sp., but not of *Photobacterium* sp. In control experiments, where *Photobacterium* sp. was cultured with either *Cellulophaga* sp. or *Cellulophaga*-specific virus, the biomass of *Photobacterium* sp. increased by 50% or less. In contrast, the *Photobacterium* sp. biomass significantly increased by 8-fold ($p < 0.001$, $n = 3$) in co-cultures with the *Cellulophaga* sp. virus-host pair. These data indicate that the substrate supporting growth by *Photobacterium* sp. was primarily *Cellulophaga* lysate and not material introduced with the host and virus inocula nor material secreted by *Cellulophaga* during normal growth. Estimates of the trophic transfer suggested that 28% of the *Cellulophaga* sp. lysate was converted into new bacterial biomass, which indicated that at least 62% of the lysate was metabolized by *Photobacterium* sp. Our results from this simple marine model community illustrate that the activity of a virus-host system can effect the transfer of organic material from one bacterial type to another whose growth would otherwise be limited by a lack of suitable substrates.

KEY WORDS: Virus · Lysate · Bacterial uptake · Carbon cycling · Diversity

INTRODUCTION

Viral lysis is a significant source of bacterial mortality in pelagic marine systems (Wommack & Colwell 2000). In the process of killing infected host cells, viral lysis causes the release of new viruses and host cell contents, including cytoplasmic and structural material, to the environment. Experimental studies performed during the last decade suggest that regeneration of dissolved organic carbon and nutrients by viral lysis can affect bacterial community structure (Van Hannen et al. 1999) and have a large impact on bacterial carbon cycling (Middelboe et al. 1996, Gobler et al. 1997, Noble et al. 1999, Middelboe & Lyck 2002, Riemann & Middelboe 2002). Inclusion of viruses in theoretical food-web models suggests that, if viral lysis is significant and lysates are consumed by bacteria, then bacterial growth and respiration will be enhanced at the expense of biomass at higher trophic levels (Fuhrman 1992, Wilhelm & Suttle 1999).

Our understanding of how viral lysates influence pelagic carbon cycling as well as bacterial diversity is

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limited by lack of data on the bioavailability of viral lysates and, therefore, the degree to which they fuel the productivity and respiration of bacterial populations. It is generally accepted that lysates of bacteria and algae are recycled within the microbial community (e.g. Middelboe et al. 1996, Gobler et al. 1997, Fuhrman 1999, Wommack & Colwell 2000), but there have been few studies that directly test this assumption. Experimental studies by Middelboe et al. (1996) suggested that viral lysis of a specific bacterial-host population stimulated organic carbon uptake by non-host bacteria in complex communities. The results also indicated that bacterial growth efficiency was reduced for bacteria utilizing the viral lysates, presumably due to increased energy requirements associated with the degradation of the lysates prior to assimilation (Middelboe et al. 1996). In a subsequent study, Noble & Fuhrman (1999) added radiolabeled lysates to natural bacterial communities and followed the fate of the material in terms of its breakdown and assimilation. The results indicated that lysates were relatively labile, but that little of the bulk (^{3}H-labeled) material was taken up by bacteria in 30 h incubations. In contrast, experiments with ^{33}P-labeled lysates indicated that P was rapidly assimilated (>50% within 7 h) in P-limited seawater. As discussed by the authors, the filtration and purification of the labeled lysates was likely to have affected their quality (Noble & Fuhrman 1999). In addition, the experiments were performed with natural communities. This has the advantage of including the complexity of a real community, but can obscure interpretation of the specific underlying processes. The study is, however, probably the most direct attempt to assess the utilizability of lysates reported so far.

The mechanisms by which viruses influence bacterial diversity are even less well established experimentally. It is typically assumed that viruses help maintain bacterial diversity by a density-dependent mechanism sometimes referred to as 'kill the winner' (Thingstad & Lignell 1997, Thingstad 2000). The basis of this model is the assumption that dominant bacterial strains are more susceptible to lysis as a result of increased contact rates with their respective viruses, and that viruses therefore counteract dominance of specific bacterial populations. We hypothesize that viruses may also influence bacterial diversity by altering organic matter quality through the production of bioavailable substrates by viral lysis.

The purpose of this study was to directly test under simplified, controlled conditions, whether, and with what efficiency, the unmanipulated viral lysate of one bacterial type can be used for growth by another bacterial type. Due to the experimental design, we were able to control the growth of individual bacterial isolates and to identify the effects of viral lysis of one species on the dynamics of another. Using this approach, we demonstrated that the lysate derived from growth of a the phage-host system was used with high efficiency by an unrelated bacterial isolate, and supported significant growth of that isolate in an environment in which it was otherwise unable to grow. Transmission electron microscopy (TEM) also illustrated that lysing bacterial cells may serve as 'hot spots', which appear to attract other bacteria.

MATERIALS AND METHODS

Characterization of the virus-host systems. Two virus-host systems were isolated from the surface waters of southern Kattegat, Denmark at a coastal estuarine location with a salinity of 15 to 20. Water samples were spread on Zobell agar plates (5 g peptone, 1 g yeast extract, 15 g agar, 800 ml filtered seawater [GF/F, Whatman], 200 ml distilled water, autoclaved at 121°C, 20 min) and bacterial colonies were purified and stored in 50% glycerol at –80°C and later screened for susceptibility to viruses in the water samples. Viruses in 0.2 µm filtered water samples were concentrated approximately 200 times, after multiple spins in 15 ml Amicon Ultra centrifugal filter devices with a 30 kD regenerated cellulose membrane (Millipore) by centrifugation at less than 200 × *g* to a final volume of 1 to 2 ml. For identification of specific virus-host systems, plaque assays were performed with bacterial isolates and virus concentrates from the same water sample. In case of plaque formation, the viruses were isolated and purified. Two phage-host systems showing no cross-infectivity were further characterized for use in this study.

The phylogenetic affiliations of the bacterial isolates were determined from small-subunit ribsomal RNA gene sequences. The 16S rRNA genes were amplified using the bacteria-specific 27F and the universal 1492R primers and cloned into pGEM-T easy® vector (Promega). Cycle sequencing reactions were performed using the BigDye® terminator kit (ABI) with a suite of 10 primers (27F, 357F, 533F, 805F, 1080F, 1492 R, 1389R, 906R, 515R, and 338R, where numbers represent position of the 3′ end of the primer relative to *Escherichia coli* numbering and letters indicate forward or reverse orientation). Reactions were run on an ABI 310 capillary sequencer and the resulting sequences assembled in Autoassembler (Applied Biosystems). Assembled sequences were analyzed using online tools at the Ribosomal Database Project II web site (http://rdp.cme.msu.edu) and were submitted to GenBank (Accession numbers: AF497997, *Cellulophaga* sp.; and AF497998, *Photobacterium* sp.).

The ability of the 2 bacterial isolates to metabolize a variety of sole carbon sources was tested using the BIOLOG GN MicroPlate method (BIOLOG). Based on these assays, 2 different substrates were identified, which could be used to select for one or the other bacterial type. According to the assay, α-D-lactose supported growth of *Cellulophaga* sp., but not *Photobacterium* sp., while N-acetyl-D-glucosamine (NAG) supported growth of *Photobacterium* sp., but not *Cellulophaga* sp. To verify the BIOLOG results, the 2 bacterial strains were grown separately in batch cultures with autoclaved seawater enriched with either α-D-lactose (Merck 1.07660) or N-acetyl-D-glucosamine (Calbiochem 1079) each at a final concentration of 2 mM C equivalents (control experiments). The cells were inoculated from overnight cultures after 2 sequential washes in sterile seawater at a final density of approximately 5×10^5 cells ml^{-1}. Growth of the strains was measured as increased cell abundance determined by epifluorescence microscopy after staining with 4′,6-diamidino-2-phenylindole (DAPI) according to Porter & Feig (1980).

Experimental design. To test whether lysate could be used for growth, *Photobacterium* sp. was used as the indicator species in a series of mixed-culture experiments. *Photobacterium* sp. was inoculated into a lactose-enriched seawater medium along with different combinations of the *Cellulophaga* sp. and the *Cellulophaga*- and *Photobacterium*-specific viruses (Table 1). Since the *Photobacterium* sp. isolate was unable to use lactose as a sole carbon source, we used net growth of this bacterial type as an indicator that other substrates had become available. A large batch of medium was prepared from 70% 0.2 µm filtered seawater and 30% distilled water. The medium was enriched with 0.52 mM NH_4Cl and 0.14 mM Na_2PO_4, and pasteurized at 80°C for 40 min. Lactose (2 mM C, final concentration) was added and the medium was sterile filtered (0.2 µm capsule filter, Millipore) directly into acid-rinsed, autoclaved 2.5 l polycarbonate bottles (Nalgene) in a laminar flow hood using autoclaved silicone tubing. Triplicate cultures with 5 different combinations of *Photobacterium* sp., *Cellulophaga* sp. and the 2 viruses (Table 1) were incubated for 120 to 160 h at 18°C on a rotating wheel at ~5 rpm. Subsamples for determination of the abundance of individual bacterial and viral populations were taken every 8 to 12 h. After ca. 112 h, 2 mM C (final concentration) of the *Photobacterium*-selective substrate NAG was added to the cultures in selected experiments, to assess the nutritional status of the *Photobacterium* sp. population.

Table 1. Experimental design. All experiments were performed in triplicate cultures with α-D-lactose medium (2 mM C). After 112 h incubation N-acetyl-D-glucosamine (2 mM C) was added to all cultures. Only the data on *Photobacterium* sp. biomass are shown for Expt 1 (Fig. 7)

Expt	*Cellulophaga* sp.	*Photobacterium* sp.	*Cellulophaga*-specific virus	*Photobacterium*-specific virus
1		X	X	
2	X	X		
3	X	X	X	
4	X	X		X
5	X	X	X	X

Bacterial abundance and morphology. Polyclonal antibodies directed against *Cellulophaga* sp. or *Photobacterium* sp. were prepared by the State Serum Institute (Copenhagen, Denmark). In brief, rabbits (strain Ssc:CPH) were immunized with a Proteinase K-digested whole cell lysate containing lipopolysaccharides as described by Chart & Rowe (1992).

Cellulophaga sp. and *Photobacterium* sp. cells were detected by immunofluorescence microscopy (Nybroe et al. 1992, Middelboe et al. 1996). At each sampling, 2×0.5 ml samples were fixed (2% formaldehyde, final concentration) and filtered onto 0.2 µm polycarbonate filters (MFS). For immunofluorescence staining, filters were incubated for 2 h at room temperature on drops of 1:500 dilutions of the *Cellulophaga* sp. or the *Photobacterium* sp. antibody, respectively. Filters were then transferred back to the filtration manifold, washed 3 times with buffer (50 mM Tris-HCl, 150 mM NaCl, 0.05% Tween 20, 0.05% bovine serum albumin, pH: 7.6), and incubated for 2 h on drops of Alexafluor 568-conjugated swine-anti-rabbit immunoglobulins (0.02 mg ml^{-1}, Molecular Probes). After 3 more washes as above, the filters were stained with DAPI according to Hoff (1988) and subsequently mounted on glass slides.

Two samples were taken from each of the triplicate cultures at every time point. The abundance of *Cellulophaga* sp. and *Photobacterium* sp. as well as total bacterial abundance were determined by epifluorescence microscopy. For each set of samples, 200 to 500 *Cellulophaga* sp. and *Photobacterium* sp. cells were counted. Comparing the sum of the *Cellulophaga* sp. and *Photobacterium* sp. cell counts with total bacterial counts assured that the cultures were not contaminated with other bacterial strains.

Cellulophaga-specific viruses and *Photobacterium*-specific viruses were enumerated by plaque assay using the top agar overlay method (Sambrook et al. 1989) with ZoBell medium. Samples for plaque assay were chloroform-treated (3% final concentration) to kill bacteria after verification that the treatment did not affect infectivity of the viruses (data not shown). Mid-log-phase cultures of *Cellulophaga* sp. and *Photobacterium* sp. in Zobell medium were used as host bacteria. A total of 100 to 500 plaques were counted on duplicate plates for each subsampling.

Epifluorescence microscopy of the bacterial strains showed indications of morphological changes and aggregation of bacteria during the incubations, and for a closer examination of these changes, we prepared samples at selected time points for analyses by TEM. Formaldehyde-fixed samples (1 ml) were centrifuged (100 000 × *g*, 3 h) onto 200 mesh formvar-coated Cu grids (Ted Pella), stained for 20 s with 5 % uranyl acetate, and examined by TEM (Zeiss EM 900). At selected time points, cell volumes of *Cellulophaga* sp. and *Photobacterium* sp. were derived from measurements of cell dimensions on TEM photographs for estimation of cell carbon content. This allowed us to estimate the transfer of organic carbon between the 2 bacterial populations from changes in abundance and cell carbon content of the individual bacterial populations from inoculation to the steady state densities prior to NAG addition. The volumes of 25 to 50 cells of each of the 2 populations were calculated from their length and width (rod-shaped cells) or their diameter (round cells) at each selected time point. We assume that any shrinking of the cells due to preparation for TEM would be similar for all the cells, thus allowing examination of relative changes in cell size within the experiment. Bacterial biomass was estimated using a carbon:volume conversion factor of 310 fg C μm^{-3} (Fry 1990).

RESULTS

Characterization of bacterial isolates

Analysis of the 16S rRNA genes of the bacteria revealed that one of the isolates shared 99% nucleotide sequence identity with *Photobacterium angustum* (D25307). The other isolate shared 99% nucleotide sequence identity with *Cellulophaga baltica* (AJ005972), a Baltic Sea isolate described by Johansen et al. (1999). Members of this *Cellulophaga* group were recently re-classified from the genus *Cytophaga*. Our isolates will be referred to here as *Photobacterium* sp. and *Cellulophaga* sp., respectively.

The ability of each isolate to grow on lactose or NAG was tested in batch cultures (Fig. 1). Consistent with results from initial BIOLOG assays, *Cellulophaga* sp. grew well on lactose as the sole carbon source (exponential increase from 5.4×10^5 to 5.2×10^7 cells ml^{-1}), but grew poorly on NAG (reaching only 2.6×10^6 cells ml^{-1}, Fig. 1A).

The opposite pattern was seen for *Photobacterium* sp., which grew well on NAG (increasing exponentially from 4.8×10^5 to 4.8×10^7 cells ml^{-1}) but poorly on lactose (increasing from 4.3×10^5 to 1.3×10^6 cells ml^{-1}, Fig. 1B). Growth of *Photobacterium* sp. in lactose medium was unaffected by the presence of *Cellulophaga*-specific virus (Expt 1, data not shown).

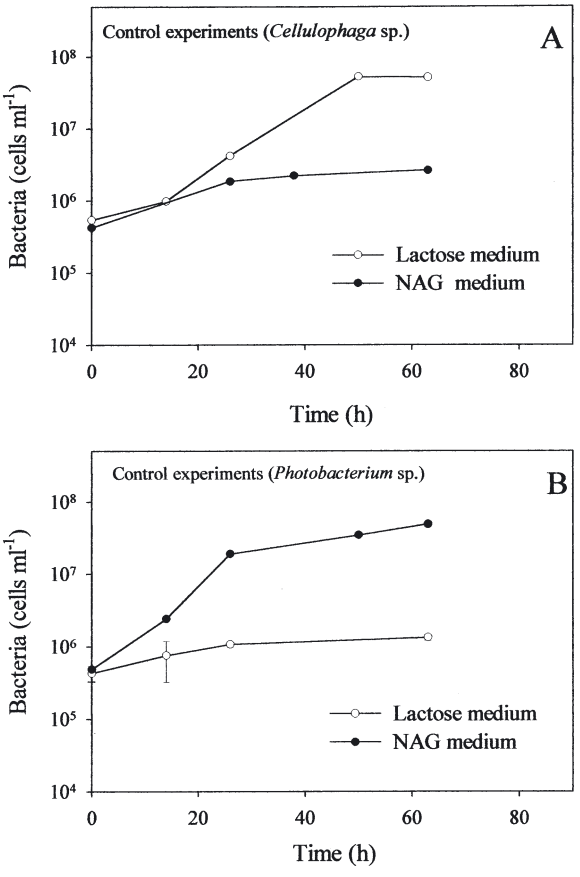

Fig. 1. Control experiments. Abundance of (A) *Cellulophaga* sp. and (B) *Photobacterium* sp. cultured alone in α-D-lactose and N-acetyl-D-glucosamine medium (NAG)

By culturing *Cellulophaga* sp. and *Photobacterium* sp. in lactose medium that only supported growth of *Cellulophaga* sp., we ensured that significant growth of the *Photobacterium* sp. population would depend on a supply of available substrate derived from the *Cellulophaga* sp. population. Accordingly, all of the following experiments were performed in lactose-based seawater medium.

Population dynamics in mixed cultures

Co-culture of *Photobacterium* sp. and *Cellulophaga* sp. (Expt 2). When *Photobacterium* sp. was co-cultivated with *Cellulophaga* sp., the population dynamics of each bacterial type were similar to those observed for individual cultures of the 2 strains. From an initial density of 5.3×10^5 cells ml^{-1}, the *Cellulophaga* sp. population increased exponentially to a maximum of ca. 9.5×10^8 cells ml^{-1}, while the *Photobacterium* sp. reached ca. 1.5×10^6 cells ml^{-1} (Fig. 2). After addition of NAG (at 112 h), there was a significant ($p < 0.01$)

increase in *Photobacterium* sp. abundance to 3.3×10^6 cells ml^{-1} by the end of the experiment (Fig. 2).

Co-culture of *Photobacterium* sp. and *Cellulophaga* sp. in the presence of *Cellulophaga*-specific viruses (Expt 3). When *Photobacterium* sp. was cultivated with both *Cellulophaga* sp. and the *Cellulophaga*-specific virus, the dynamics of both bacterial species (Fig. 3A) were altered relative to when they were grown alone. After an initial exponential increase to 1.2×10^7 cells ml^{-1}, the abundance of *Cellulophaga* sp. decreased to 1.8×10^5 cells ml^{-1} by 88 h. The abundance of *Cellulophaga*-specific viruses increased by almost 8 orders of magnitude from 40 pfu (plaque-forming units) ml^{-1} to a maximum abundance of 1.3×10^9 pfu ml^{-1} at 88 h and remained relatively constant throughout the rest of the incubation (Fig. 3B). As the *Cellulophaga* sp. population crashed, the abundance of *Photobacterium* sp. increased to 4.8×10^6 cells ml^{-1} at 112 h (Fig. 3A), which was significantly higher than the maximum abundance obtained in lactose medium in Expt 2 (*t*-test; $p < 0.01$, Fig. 2). Following the crash in the *Cellulophaga* sp. population, there was a secondary exponential growth of *Cellulophaga* sp. cells (presumably virus-resistant), which reached a density of 4.1×10^6 cells ml^{-1}. After the addition of NAG at 112 h, the virus-resistant *Cellulophaga* sp. entered stationary phase while *Photobacterium* sp. increased further to a density of 2.4×10^7 cells ml^{-1} (Fig. 3A).

Co-culture of *Photobacterium* sp. and *Cellulophaga* sp. in the presence of *Photobacterium*-specific viruses (Expt 4). When the *Photobacterium*-specific virus was added to a co-culture of *Photobacterium* sp. and *Cellulophaga* sp., there was an increase in the virus from 350 pfu ml^{-1} to 1.8×10^6 pfu ml^{-1} (Fig. 4B), but there was no detectable effect on the population dynamics of either

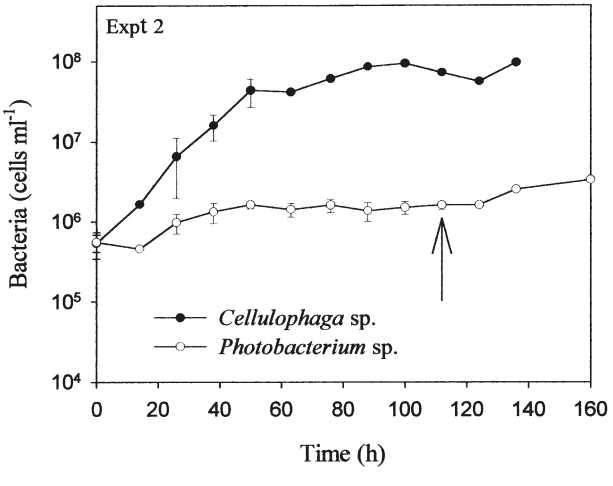

Fig. 2. Abundance of *Photobacterium* sp. and *Cellulophaga* sp. in mixed cultures with α-D-lactose medium (Expt 2). Arrow indicates addition of N-acetyl-D-glucosamine

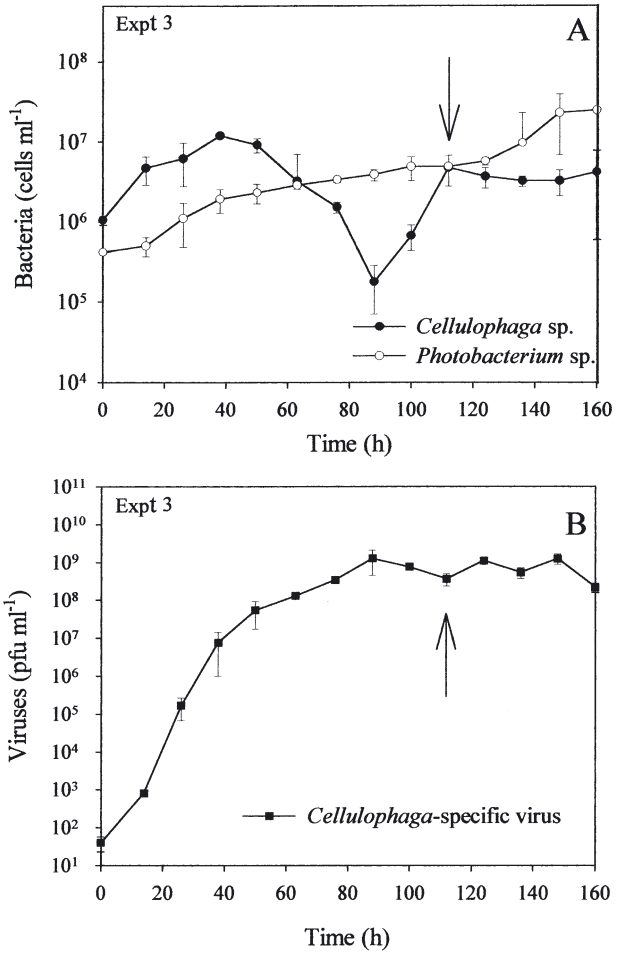

Fig. 3. Abundance of (A) *Photobacterium* sp. and *Cellulophaga* sp. and (B) *Cellulophaga*-specific viruses in mixed cultures with α-D-lactose medium (Expt 3). Arrows indicate addition of N-acetyl-D-glucosamine

bacterial type relative to when they were grown alone (Fig. 4A). Stationary-phase abundance of *Cellulophaga* sp. and *Photobacterium* sp. was approximately 1.1×10^8 cells ml^{-1} and 2.0×10^6 cells ml^{-1}, respectively. Addition of NAG at 112 h incubation was followed by a second increase in viral abundance to 2.0×10^8 pfu ml^{-1} and a reduction in the *Photobacterium* sp. to 5.3×10^5 cells ml^{-1} by the end of the experiment (Fig. 4A). *Cellulophaga* sp. abundance remained unchanged.

Co-culture of *Photobacterium* sp. and *Cellulophaga* sp. in the presence of their specific viruses (Expt 5). Results from the co-cultivation of both virus-host systems are presented in Fig. 5. The abundance of *Cellulophaga* sp. followed the pattern observed in Expt 3 (Fig. 3A) with an increase to 1.4×10^7 cells ml^{-1} followed by a decrease to 2.3×10^5 cells ml^{-1} (Fig. 5A), as the *Cellulophaga*-specific viruses increased from 250 pfu ml^{-1} to approximately 1×10^9 pfu ml^{-1} (Fig. 5B). As

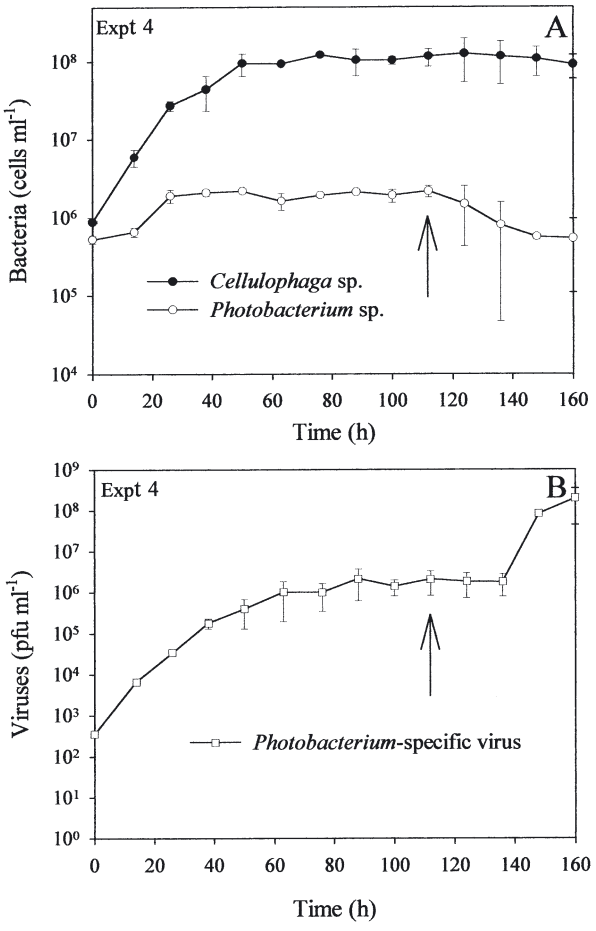

Fig. 4. Abundance of (A) *Photobacterium* sp. and *Cellulophaga* sp. and (B) *Photobacterium*-specific viruses in mixed cultures with α-D-lactose medium (Expt 4). Arrows indicate addition of N-acetyl-D-glucosamine

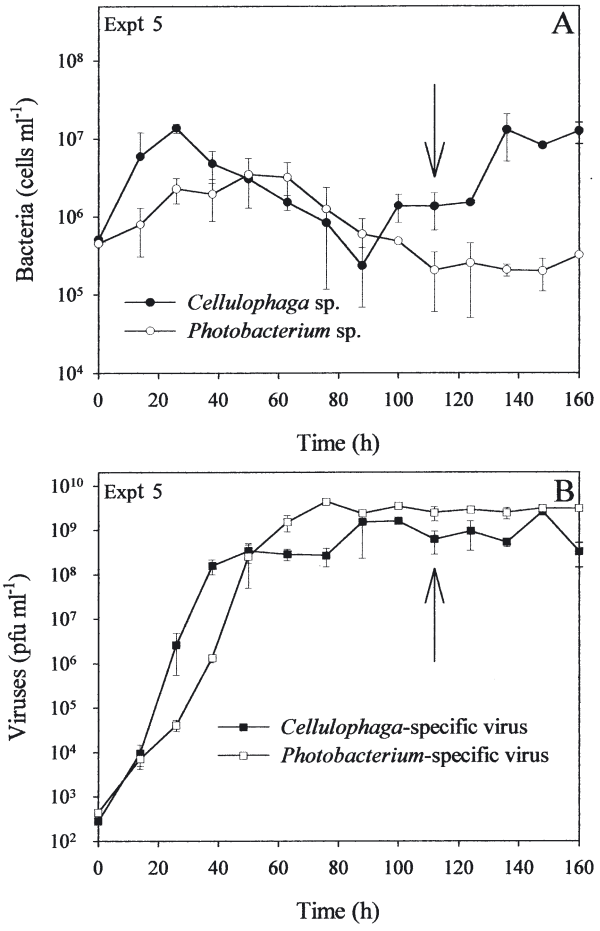

Fig. 5. Abundance of (A) *Photobacterium* sp. and *Cellulophaga* sp. and (B) *Photobacterium*-specific viruses and *Cellulophaga*-specific viruses in mixed cultures with α-D-lactose medium (Expt 5). Arrows indicate addition of N-acetyl-D-glucosamine

in Expt 3, there was a positive response of the *Photobacterium* sp. to the crash of the *Cellulophaga* sp. population; however, in the presence of *Photobacterium*-specific viruses there was a subsequent breakdown of the *Photobacterium* sp. population (Fig. 5A). Accordingly, there was a large production of *Photobacterium*-specific viruses, which peaked at a density of 4.4×10^9 pfu ml^{-1} (Fig. 5B). Following the addition of NAG at 112 h, there was no discernible change in the abundance of *Photobacterium* sp, but *Cellulophaga* sp. increased by an order of magnitude.

Morphological observations

The individual bacterial populations could be identified in the transmission electron microscope due to differences in morphology, *Cellulophaga* sp. cells being relatively long and thin, while *Photobacterium* sp. cells were shorter and thicker (Fig. 6A,B). Viral infection of *Photobacterium* sp. (Expt 5) resulted in a dramatic change in morphology (Fig. 6D,E,F). The infected cells became significantly enlarged and rounded with a volume of 5.1 ± 0.6 µm^3 compared to an inoculated cell size of 0.55 ± 0.21 µm^3 ($p < 0.001$). An aggregation of *Cellulophaga* sp. was observed around these large infected *Photobacterium* sp. cells (Fig. 6E,F), while healthy *Photobacterium* sp. cells were not colonized. The *Cellulophaga* sp. cells did not show a similar volume increase associated with virus infection (Fig. 6C), and there was no aggregation of *Photobacterium* sp. cells around infected *Cellulophaga* sp. cells.

Effect of experimental treatments on *Photobacterium* sp. biomass

To statistically compare the influence of viral lysis of *Cellulophaga* sp. on *Photobacterium* sp. growth, the response of *Photobacterium* sp. in the different experi-

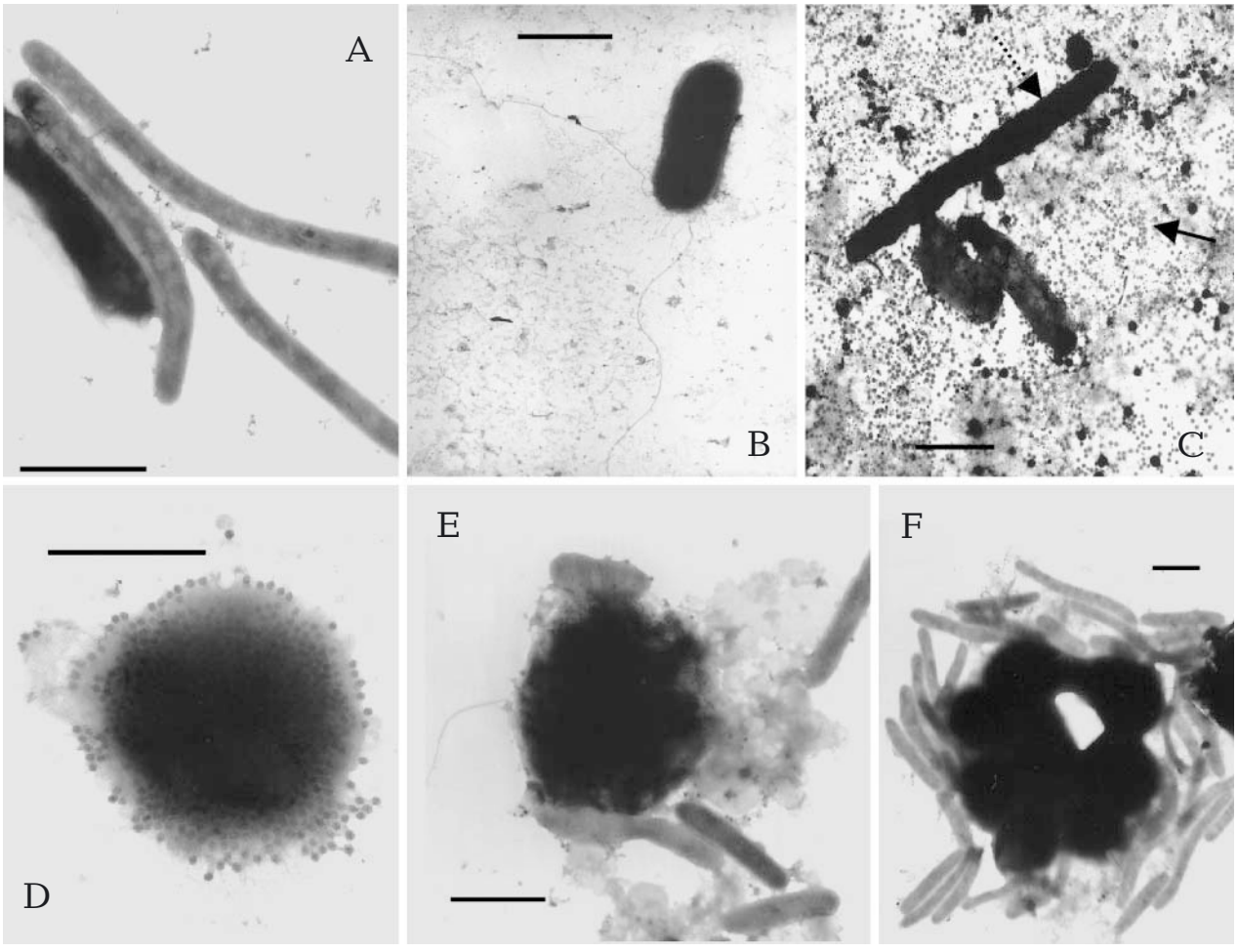

Fig. 6. TEM micrographs of *Cellulophaga* sp. and *Photobacterium* sp. at different stages during the incubations. (A) *Cellulophaga* sp. cells in Expt 1. (B) *Photobacterium* sp. cell at beginning of Expt 1. (C) Virus-infected *Cellulophaga* sp. cell (dotted arrow) surrounded by 2 *Photobacterium* sp. cells and numerous *Cellulophaga*-specific viruses (arrow) in Expt 2. (D) Virus-infected *Photobacterium* sp. cell. (E) and (F) Infected *Photobacterium* sp. cells (round) colonized by *Cellulophaga* sp. cells (elongated; Expt 4). Scale bars = 1 µm

mental treatments was quantified in terms of increase in total biomass per ml from time zero to the steady-state condition prior to addition of NAG (Fig. 7). The significance of the observed differences in biomass increases was tested using a *t*-test. The observed increase in *Photobacterium* sp. abundance in lactose medium (Fig. 1) was accompanied by a significant decrease in cell size (from 0.55 ± 0.21 to 0.19 ± 0.07 μm^3, $p < 0.001$). Hence, we did not observe any significant increase in *Photobacterium* sp. biomass from time zero and until stationary phase conditions when grown alone or in the presence of only the *Cellulophaga*-specific virus ($p = 0.38$ and 0.35, respectively) (Fig. 7).

When co-cultivated with the *Cellulophaga* sp. host alone, *Photobacterium* sp. biomass increased significantly ($p < 0.01$) relative to the control experiment. However, the population was able to undergo less than a doubling in biomass (Fig. 7). In contrast, when cultivated with the *Cellulophaga* sp. host and virus together (Expt 3), there was an 8-fold increase in *Photobacterium* sp. biomass, which was significantly higher than when co-cultured with the *Cellulophaga* sp. host or virus alone ($p = 0.0004$ and 0.0002, respectively).

DISCUSSION

The experimental setup was designed to elucidate the transfer of cell lysates generated by viral lysis of one bacterial type to another. The growth of *Photobac-*

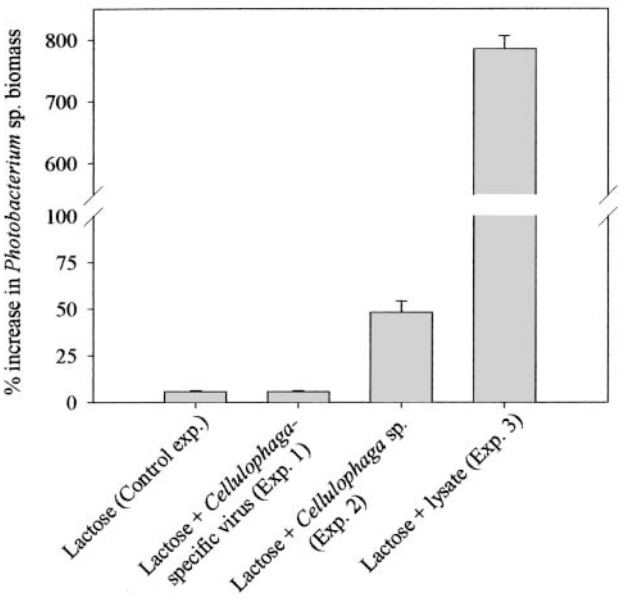

Fig. 7. *Photobacterium* sp. Percent increase in biomass from time zero to the steady-state condition prior to addition of N-acetyl-D-glucosamine (NAG) in the control experiment and Expts 1, 2, and 3

terium sp. was used as a bioassay for the availability of viral lysates, and by this approach we were able to demonstrate and quantify the recycling of viral lysates within a simple bacterial model community.

Bacterial recycling of lysate

The data on biomass increase (summarized in Fig. 7) show that *Photobacterium* sp. was not growing on material introduced along with the inoculum of the *Cellulophaga*-specific virus. In addition, the small increase in *Photobacterium* sp. biomass in the presence of only *Cellulophaga* sp. (~0.5 doublings, Fig. 7), suggest that, in the absence of virus, there was little material released by the *Cellulophaga* sp. to support the growth of *Photobacterium* sp. The stimulation of *Photobacterium* sp. growth following addition of NAG at 112 h in Expt 2 (Fig. 2) indicates that the failure of cells to grow in this case was due primarily to carbon limitation and could not be explained strictly by an antagonistic relationship between *Cellulophaga* sp. and *Photobacterium* sp. These data strongly support the conclusion that growth of *Photobacterium* sp. in the presence of *Cellulophaga* sp. and *Cellulophaga*-specific virus was directly supported by lysate from the *Cellulophaga* sp.

The utilizability of *Cellulophaga* sp. lysate was further evaluated using data from Expt 3 (Fig. 3). Abundance and volume data were used to estimate the loss of *Cellulophaga* sp. biomass due to viral lysis as well as the concurrent increase in *Photobacterium* sp. biomass. The ratio between the loss of *Cellulophaga* sp. biomass and the subsequent production of *Photobacterium* sp. indicates that at least 28% of the *Cellulophaga* lysate was converted to *Photobacterium* sp. biomass. Assuming a maximum growth efficiency of 45%, which is typical for bacteria growing at high rates (>1 d^{-1}; Middelboe et al. 1992), the result implies that at least 62% of the lysed *Cellulophaga* sp. biomass was used by *Photobacterium* sp. for growth and respiration. Expressed in another way, the estimated transformation of *Cellulophaga* sp. lysates into new *Photobacterium* sp. production indicate a minimum *Photobacterium* sp. growth efficiency of 28%, if it is assumed that *Photobacterium* sp. consumed 100% of the lysate. These minimum estimates suggest that the lysate was readily utilizable.

The recycling of lysate is relevant to our understanding of carbon flow in the marine food web. Bacterial net production is considered to be roughly 20% of primary production on a volumetric basis (Cole et al. 1988), indicating that ca. 50% of the primary production is channeled through the bacteria. However, the estimated input of organic carbon from the primary producers cannot always account for the measured bacterial carbon demand (e.g. Strayer 1988), suggesting that bacteria themselves may contribute to bacterial carbon demand either by direct predation by other bacteria or via bacterial exudates or lysis products (Cole & Caraco 1993). Based on measurements of bacterial and viral production, Wilhelm & Suttle (2000) calculated that viral lysates potentially supplied 4 to 30% of bacterial carbon demand in the Gulf of Mexico and up to 80 to 95% of the carbon demand in stratified locations in the Strait of Georgia, USA. The importance of viral lysis as a contributor to bacterial carbon demand has already been suggested in a number of studies (e.g. Fuhrman 1992, Middelboe et al. 1996, Wilhelm & Suttle 1999, 2000, Middelboe & Lyck 2002). However, our data are the first to directly demonstrate, under carefully controlled experimental conditions, an efficient transfer of biomass from one bacterial type to another mediated by viral lysis. The results support the idea that viral lysis may be a key mechanism supplying substrate to the bacterioplankton and contribute to the high rates of heterotrophic bacterial production relative to primary production often observed in marine systems (i.e. Strayer 1988).

Nutrient limitation of viral replication

The carbon limitation of *Photobacterium* sp. in lactose medium affected not only growth of the bacterial type, but also replication of its virus as was illustrated

in Expt 4 (Fig. 4). Assuming a burst size of 100, the initial increase in viruses implied that only ca. 1% of the population was lysed prior to reaching steady state. Consequently, the increase in *Photobacterium* sp. biomass as a result of exudates from growing *Cellulophaga* sp. (Fig. 7) could support a production of 1.8×10^6 viruses ml^{-1}, corresponding to <0.05% of the number of viruses produced in the presence of *Cellulophaga* sp. lysates (Fig. 5). Addition of NAG resulted in a substantial production of viruses with no preceding increase in host abundance, which implies that the virus production was restricted by nutrient limitation of the host rather than host density. The increase in *Photobacterium*-specific viruses following the release of *Cellulophaga* sp. lysates (Fig. 5) supported the conclusion that lysates stimulated growth of *Photobacterium* sp. and emphasized the potential impact of this nutrient recycling on the dynamics of *Photobacterium* sp. and its specific virus (Fig. 5).

Implications for bacterial diversity

The density-dependence of virus-host interactions and observed successions in bacterial communities has led to the proposal that viruses help to maintain bacterial diversity by 'killing the winner' (Thingstad & Lignell 1997, Thingstad 2000). In this model, viruses are agents that prevent numerical dominance of any given bacterial species via density-dependent control of the population. The implication is that viruses primarily function as a handicap to the most successful bacteria and other bacteria benefit by a reduction in competition as well as some return of resources. Our results supplement this scenario. Due to the nature of our experimental design we empirically demonstrated that (1) viral lysis of one bacterial type did not simply recycle dissolved organic material, but transformed it in the process and (2) this transformation provided utilizable substrate for a bacterial type that was otherwise unable to grow. This illustrates that a virus-host interaction can benefit a competing bacterial population specifically via the conversion of non-utilizable substrate (in this case lactose) into utilizable substrates (the lysate). The results from such simple experiments with 2 different bacterial types can obviously not be directly applied to a natural environment with complex community structure and substrate composition. However, we speculate that substrate transformation, as demonstrated in this experimental approach, is a mechanism that also occurs in natural environments and by which viruses may promote coexistence of diverse bacteria in natural communities. This process need not involve boom-and-bust cycles as implied by 'kill the winner', but could also function in steady state.

The TEM observations of *Cellulophaga* sp. colonizing infected *Photobacterium* sp. cells further indicate that viral lysis of bacteria may not only transform the chemical composition of the available substrates, but also how they are distributed at the microscale (Fig. 6). The aggregation of non-infected bacteria onto the enlarged infected bacteria suggested that the infected cells functioned as nutrient point sources, which attracted other bacteria. Lysing protozoan cells have been shown to act as microscale nutrient patches, which are consumed by bacteria clustering around the source (Blackburn et al. 1998). This, in combination with our observations, suggests that lysing cells may function as 'hot spots' of bioavailable substrate contributing to the patchiness of the pelagic environment and, thus, supporting higher bacterial diversity (Azam 1998, Long & Azam 2001). *Cellulophaga*-like bacteria have previously been shown to attack and lyse cyanobacteria during the decline of a cyanobacterial bloom (Rashidan & Bird 2001), hence *Cellulophaga* sp. cells colonizing weak or stressed bacterial cells may be a common phenomenon and not only related to infection.

In conclusion, by using marine virus-host systems in simple model communities under controlled conditions, we have provided direct empirical evidence that the lysate of one bacterial species can be used with high efficiency by another. We speculate that substrate transformation due to viral lysis is a potential mechanism by which viruses could influence bacterial community composition. While the present study illustrates some fundamental phenomena based on a simple model community, a verification of the quantitative importance of lysate recycling and substrate transformation in complex natural communities will require further iterations of empirical investigation and theoretical modelling.

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