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Direct and Indirect Effects of Protist Predation on Population Size Structure of a Bacterial Strain with High Phenotypic Plasticity

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We studied the impact of grazing and substrate supply on the size structure of a freshwater bacterial strain (*Flectobacillus* sp.) which showed pronounced morphological plasticity. The cell length varied from 2 to >40 μm and encompassed rods, curved cells, and long filaments. Without grazers and with a sufficient substrate supply, bacteria grew mainly in the form of medium-sized rods (4 to 7 μm), with a smaller proportion (<10%) of filamentous forms. Grazing experiments with the bacterivorous flagellate *Ochromonas* sp. showed that freely suspended cells of <7 μm were highly vulnerable to grazers, whereas filamentous cells were resistant to grazing and became enriched during predation. A comparison of long-term growth in carbon-limited chemostats with and without grazers revealed that strikingly different bacterial populations developed: treatments with flagellates were composed of >80% filamentous cells. These attained a biomass comparable to that of populations in chemostats without grazers, which were composed of medium-sized rods and c-shaped cells. Carbon starvation resulted in a fast decrease in cell length and a shift towards small rods, which were highly vulnerable to grazing. Dialysis bag experiments in combination with continuous cultivation revealed that filament formation was significantly enhanced even without direct contact of bacteria with bacterivores and was thus probably stimulated by grazer excretory products.

Grazing by small protists is considered a major mortality factor, and therefore an important selective force, for aquatic bacteria (42). Studies from recent years have demonstrated that different bacterial strains are not equally vulnerable towards grazers and have evolved different mechanisms to resist capture, ingestion, or digestion by bacterivores (reviewed in references 13 and 18). Phenotypic bacterial properties which have been identified to influence grazing mortality are size and morphology (8, 44), swimming speed (31), toxic pigments (29), and the physicochemical surface structure (32, 34).

In planktonic environments, size-structured predator-prey interactions are of particular importance (22), and bacterial cell size must also be considered a major feature which impacts vulnerability towards different grazers (10). Size-selective grazing by bacterivorous nanoflagellates exerts the strongest grazing pressure on medium-sized cells, whereas bacteria obtain a predation refuge at the lower end of the prey size spectrum as well as at the upper end (10). For the smallest bacteria, this is partly due to a reduced number of encounters and partly due to the inability of certain grazers to capture small cells (17, 18). Elongated or filamentous cells and complex morphologies might be grazing resistant, as they are too large to be ingested by the smallest bacterivores (9). The interplay between size-selective grazing and differences in the cell size distribution of different bacterial strains, which finally results in an altered bacterial community composition, has also been demonstrated in chemostat experiments with mixed bacterial assemblages and bacterivorous nanoflagellates (14, 36, 47).

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Grazing-resistant, filamentous bacteria are frequently observed in freshwater lakes, where they can temporarily attain significant concentrations (20, 37, 40, 48). The appearance of filamentous, suspended bacteria has also been observed, but less frequently, in brackish (7) and marine (5) systems. The enhanced development of filamentous morphotypes has often been correlated with an increased abundance of small bacterivores (heterotrophic nanoflagellates), and this link could also be demonstrated in food web manipulation experiments (16, 19, 45, 46). It is assumed that the appearance of grazing-resistant bacteria has important ecological implications such as the stabilization of bacterial biomass and a decreased trophic transfer efficiency (17).

Although these observations point towards grazing as a causative factor for the development of morphologically predation-resistant cells in natural bacterial assemblages, little is known regarding the underlying regulatory mechanisms. Many bacterial taxa possess a considerable degree of phenotypic plasticity with respect to cell size and morphology (21). In studies with pure bacterial cultures and single predator species, it has been shown that selective grazing and morphological plasticity of bacteria result in a shift towards resistant morphotypes such as filamentous forms (14, 43) or bacteria growing in microcolonies (15, 30). It has been demonstrated that filament formation can be stimulated by an increase in the bacterial growth rate (14), whereas the existence of predator-mediated chemical signals has not been proven so far. The high diversity of bacterial resistance mechanisms, which act at different stages of predator-prey interactions (18) and which vary between different bacterial grazer species (2), suggests that not just one general mechanism is responsible.

Similar to previous studies (12, 28), we used a bacterium-flagellate chemostat system to select and enrich for grazing-

resistant bacterial strains. Interestingly, by this approach we isolated a facultative filamentous bacterium belonging to the same genus (*Flectobacillus*) which has been used in previous studies of bacterium-protist interactions (14). We examined how the interaction of grazing pressure and substrate supply determined the size structure and vulnerability of this highly phenotypically plastic bacterial strain. In order to test for the chemical induction of phenotypic changes, we performed different types of dialysis experiments in batch and continuous culture. In several aspects, our study confirmed the results of Hahn et al. (14), but with respect to the underlying mechanism of filament formation, our isolate did not have the suggested growth rate dependency. Additionally, we present for the first time evidence that excretory products of bacterial grazers seem to be involved in the observed morphological shift towards resistant filaments.

MATERIALS AND METHODS

Isolation, cultivation, and identification of microorganisms. Bacterial and flagellate strains were isolated from the euphotic zone of a mesotrophic lake in North Germany (Lake Schöhsee). A culture of the mixotrophic flagellate *Ochromonas* sp. was obtained from a 10-μm-filtered water sample and inoculated on artificial mineral medium (WC) (11) supplemented with heat-killed bacteria (*Pseudomonas putida* strain MM1) (6) but without organic carbon substrates. The chrysophyte genus *Ochromonas* is characterized by mixotrophic nutrition (i.e., photosynthesis and bacterivory). Our isolate (4 to 7 μm in diameter) was also able to grow purely heterotrophically in the dark. Stock cultures of *Ochromonas* sp. were kept in WC mineral medium supplemented with a wheat grain to promote bacterial growth.

Axenic cultures of *Ochromonas* were obtained by antibiotic treatments and dilution (12). From a dense flagellate culture growing on a suspension of heat-killed bacteria, aliquots were inoculated into 24-well cell culture plates containing WC mineral medium, heat-killed bacteria (10^7 ml^{-1}), and a mixture of bacterial antibiotics (streptomycin, chloramphenicol, and gentamicin; weight ratio, 1:1:0.5). To account for flagellate sensitivity to antibiotics, a gradient of different concentrations of the antibiotic mixture (range, 20 to 100 mg per liter) was used. After 12 h, the culture plates were inspected by light microscopy, and samples from wells which supported the growth of flagellates were inoculated into new cell culture plates enriched with heat-killed bacteria but without antibiotics. After 4 days, the presence of bacteria was examined microscopically and by plating subsamples on nutrient broth (NB) agar plates. After repeating this procedure twice, we obtained subcultures of *Ochromonas* sp. which did not contain live bacteria. These axenic cultures were maintained in Erlenmeyer flasks in the light and supplemented with heat-killed bacteria. For detection of bacterial contamination, subsamples were regularly checked microscopically and by plating subsamples on NB agar.

For enrichment and isolation of potentially grazing-resistant bacteria, a one-stage chemostat was operated which was inoculated with a natural mixture of planktonic bacteria from Lake Schöhsee and axenic *Ochromonas* sp. The culture in the chemostat reactor was fed WC medium supplemented with glucose as an organic substrate (10 mg per liter). The dilution rate was held at 0.5 day^{-1}. The system was installed in a climate room at 16°C with a 12-h–12-h light-dark cycle. The microbial populations were quantified by epifluorescence microscopy after staining of formalin-fixed samples with DAPI (4′,6′-diamidino-2-phenylindole) (38). After 4 weeks of continuous cultivation, the bacterial community was dominated by filamentous bacteria which were obviously too large to be ingested by *Ochromonas* sp. For the isolation of bacterial strains, the culture suspension was plated on different agar plates (either NB or WC medium plus 1 g glucose per liter). Isolated bacterial strains were kept in glycerol medium at −80°C until further examination.

From the isolated bacteria, one strain was chosen for further experiments. This strain grew on agar plates in small, well-defined red colonies (diameter, 0.5 to 2 mm) and developed elongated and variable cell morphologies (a "c" shape, long rods, cell chains, etc.) when grown in liquid media. None of the examined morphotypes showed any motility. For an examination of taxonomic affiliation, the 16S rRNA gene was amplified by PCR with the primers 27f and 1492r (24). PCR products were sequenced by *Taq* cycle sequencing with universal 16S rRNA-specific primers, using an ABI377 (Applied Biosystems, Inc.) sequencer. Sequence data (1,358 bp) were aligned against sequences in GenBank by using BLAST. There was a 98% similarity with *Flectobacillus mayor*, the type species of the genus *Flectobacillus* (26). Therefore, we use the name *Flectobacillus* sp. strain GC-5 for this isolate.

Grazing experiments with *Flectobacillus* sp. strain GC-5 in batch and continuous cultures. For a first analysis of the morphological and size structure in response to flagellate grazing and to obtain information about the vulnerability of the strain, bacteria were grown with and without *Ochromonas* sp. (starting concentration, 130 cells ml^{-1}) in short-term (3 to 4 days) batch cultures on WC medium supplemented with either glucose (10 mg per liter) or, to test for higher growth rates and cell densities, yeast extract (20 mg per liter). Subsamples were taken regularly, fixed with formalin (2% final concentration), and stored at 4°C until DAPI staining.

In order to compare the morphology and size structures of *Flectobacillus* sp. strain GC-5 growing under prolonged protist grazing pressure versus carbon-limited growth without predators, we performed a chemostat experiment. It consisted of eight 250-ml cylindrical chemostat reactors (filling volume) which were continuously aerated and mixed by fine bubbling with sterile air from below. WC mineral medium supplemented with glucose (10 mg per liter) served as the growth medium and was pumped by peristaltic pumps into the reactors to achieve a dilution rate of 1.0 day^{-1}. The chemostat system was assembled in a climate chamber at 16 ± 1°C under dim light.

Flectobacillus sp. strain GC-5 cells from a preculture were inoculated (100 μl, or about 7×10^5 bacterial cells) into all eight reactors. After 3 days, when bacteria reached relatively stable numbers, axenic *Ochromonas* sp. cells were inoculated into four of the reactors to achieve an initial density of approximately 250 cells ml^{-1}. Subsamples were taken daily from all reactors and fixed with formalin (2% final concentration) for counts of bacteria and protists and for bacterial size and volume measurements. The total chemostat run time was 4 weeks. The purity of the chemostat cultures was checked by immunofluorescence microscopy, using strain-specific polyclonal antibodies which were produced from rabbits immunized with *Flectobacillus* sp. strain GC-5 (Eurogentec, Herstal, Belgium). Staining with primary and secondary antibodies and assessment by epifluorescence microscopy were done with a simplified version of a previously described procedure (6) (i.e., permeabilization and use of the blocking buffer were skipped).

Transfer of bacteria in chemostats to batch cultures. In order to obtain information about the conservation of bacterial population size structures and for a comparison of the growth dynamics of the differently preadapted subpopulations, bacteria from the different chemostat treatments were transferred to batch growth cultures at the end of the chemostat experiment. In order to eliminate predation, the flagellates were killed by the addition of a eukaryotic inhibitor (cycloheximide; final concentration, 40 mg per liter; exposition time, 6 h). Bacteria (500 μl) from the reactors were inoculated into batch cultures (100-ml flasks filled with 50 ml WC medium) which contained different concentrations of glucose (10 and 20 mg per liter, each in triplicate). The flasks were incubated for 60 h in the same climate chamber as the chemostat systems. In order to examine the morphological alterations of *Flectobacillus* sp. strain GC-5 when transferred from continuous cultivation to starvation conditions, 50-ml samples collected from reactors with and without flagellates were transferred to batch cultures (100-ml flasks) without any substrate addition and incubated for 60 h in the dark at 16 ± 1°C. For all batch incubations, subsamples for determinations of cell numbers of bacteria and flagellates and for morphological analyses of bacteria were taken every 4 to 12 h.

Dialysis bag experiments. In order to examine the bacterial response to the excretory products of grazers without any direct predation impact, we performed dialysis bag experiments with continuous cultivation, using the same type of chemostat system as that described above. The sterilized continuous-flow system was comprised of 12 cylindrical vessels which were continuously aerated, mixed by bubbling, and filled with 500 ml WC medium (plus 20 mg glucose per liter) and was set up under the same conditions as those in the first chemostat experiment. A dialysis bag (regenerated cellulose with a 15,000-molecular-weight cutoff; ZelluTrans/Roth, Karlsruhe, Germany) with a volume of 100 ml was attached inside each reactor. Schemes of inoculations with bacteria and flagellates inside and outside of the dialysis bags are shown in Fig. 1. *Flectobacillus* sp. strain GC-5 cells from a single preculture clone were inoculated (100 μl, or about 7×10^5 bacterial cells) into all 12 reactors outside the bags. Either *Flectobacillus* sp. strain GC-5 (nine chemostats) or *P. putida* (three chemostats) was inoculated into the bags as prey bacteria.

After 3 days, when bacterial growth in the reactors approached a steady state, *Ochromonas* sp. was inoculated inside three of the dialysis bags with *Flectobacillus* sp. (Fig. 1C) and in the three bags with *P. putida* (Fig. 1D) (final concentration, approximately 150 cells ml^{-1}). For these treatments, prey bacteria were

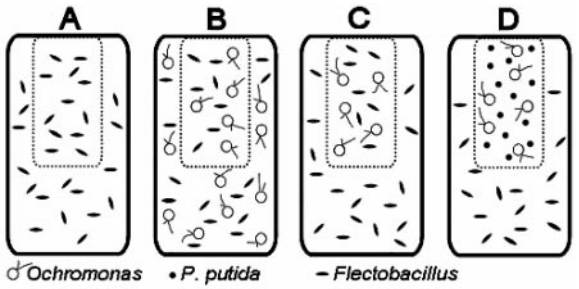

FIG. 1. Scheme of the experimental setup in the dialysis bag chemostat experiment, showing microbial populations inside and outside the dialysis bag (dashed line). (A) Control treatment with *Flectobacillus* GC-5 only; (B) control treatment with *Ochromonas* sp. and *Flectobacillus* GC-5 inside and outside the dialysis bag; (C) grazing treatment with *Ochromonas* sp., with *Flectobacillus* GC-5 as the prey bacterium; (D) grazing treatment with *Ochromonas* sp., with *Pseudomonas putida* MM1 as the prey bacterium.

restocked three (MM1) or four (GC-5) times during the chemostat run in order to maintain sufficient substrates for flagellate growth. Three replicates of the remaining chemostats with *Flectobacillus* sp. strain GC-5 inside the dialysis bags served as flagellate-free controls to account for possible leaching effects of the dialysis bags (Fig. 1A). In three other replicates, *Ochromonas* sp. was inoculated inside and outside the dialysis bags (Fig. 1B). These served as controls to compare for direct predation effects on *Flectobacillus*. The dilution rates of all reactors were adjusted to 1 day^{-1} after inoculation of the flagellates. Subsamples for determinations of bacterial and protozoan abundance and bacterial size, shape, and biovolume were taken from all reactors every day for a period of 20 days.

Cell numbers and bacterial cell size. Bacterial and flagellate cell numbers were determined from formalin (2% final concentration)-fixed and DAPI-stained samples, which were filtered onto 0.2-μm-pore-size polycarbonate filters and counted by epifluorescence microscopy. At least 400 bacteria and 100 flagellates were counted per sample. We did not enumerate the abundance of bacterial cells but rather that of bacterial "morphological units," which comprised, besides free single cells, filaments which consisted of several connected cells. However, for simplicity, we include all morphological units in the terms "bacterial cells" and "bacterial abundance."

Bacterial cell size measurements were taken with DAPI-stained samples. For some samples with mainly small morphotypes, an automated image analysis system (SIS GmbH, Münster, Germany) was used which measures the area and perimeter of 300 to 500 cells, which are used to calculate cell dimensions (length, width, and volume) according to previously given algorithms (27). Since morphological changes (e.g., cell elongation) were mainly reflected in changes in the cell length, whereas the cell width stayed relatively constant, for most samples only cell length was assessed. For this, the lengths of at least 100 randomly selected cells (morphological units) per filter were estimated with the help of an ocular grid.

Statistical analysis. Statistical analyses were carried out using the program package Statistica (version 5.1; StatSoft Inc.). Differences in the development of bacterial numbers, biomass, and cell length were tested for all chemostat experiments, using analysis of variance with repeated measures (rm ANOVA). A multivariate analysis of variance (MANOVA) was used to test for differences in cell length distribution in the chemostat dialysis experiments. Differences in the daily values of the bacterial parameters were tested with one-way ANOVA, with comparison of the means by Tukey's post hoc test. When necessary, bacterial numbers, biomasses, cell lengths, and uptake rates were log transformed to obtain a normal distribution of the data.

Nucleotide sequence accession number. The accession number of the 16S rRNA gene sequence obtained for this study is DQ145723.

RESULTS

Growth of *Flectobacillus* sp. strain GC-5 in batch culture. The polymorphic phenotypic appearance of *Flectobacillus* sp. strain GC-5 and the grazing-mediated shift in population size

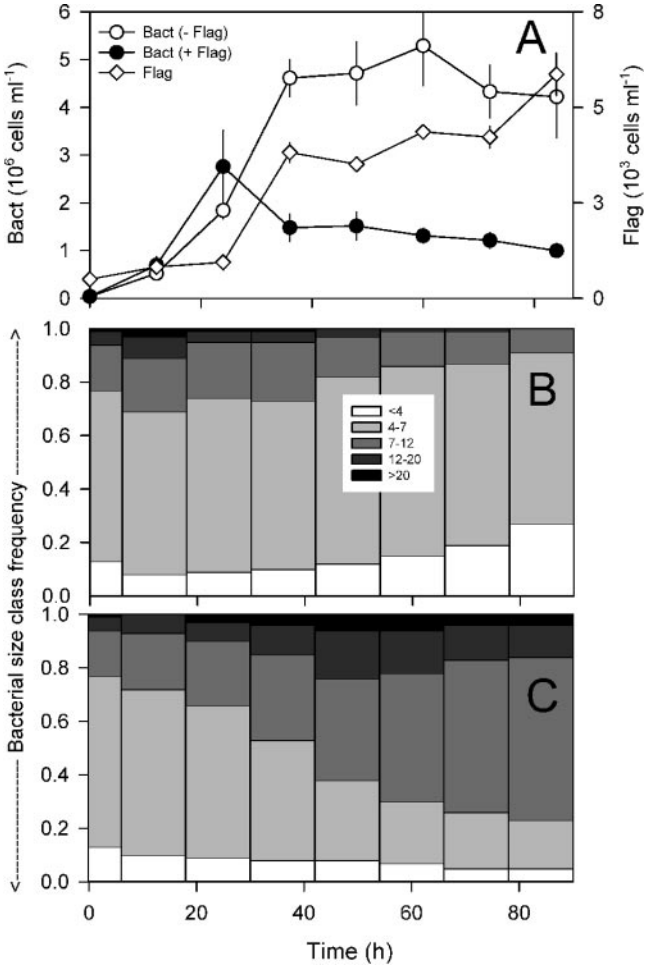

FIG. 2. Growth of *Flectobacillus* GC-5 in batch culture on WC medium (plus 10 mg glucose per liter) in the presence and absence of the bacterivorous flagellate *Ochromonas* sp. (A) Development of bacteria and flagellates. Values are expressed as means of three replicates ± standard deviations. (B and C) Development of different cell length classes (μm) of *Flectobacillus* GC-5 in the absence (B) or presence (C) of *Ochromonas* sp. (% of cell numbers).

structure became apparent during short-term growth in batch cultures with either glucose or yeast extract as a carbon source. In the absence of flagellates and supplemented with 10 mg glucose per liter, bacteria grew exponentially (doubling time, about 3.5 h) to about 5×10^6 cells ml^{-1} and afterwards remained in the stationary phase at more or less constant cell numbers for 3 to 4 days (Fig. 2A). When flagellates were present, grazing reduced the bacterial numbers, but not below approximately 1×10^6 cells ml^{-1} (Fig. 2A).

There was always a mixture of differently sized and shaped cells present, with a range of cell length from 2 μm (single straight or c-shaped rods) to 40 μm (filaments or chains of 3 to 10 elongated rods). Without predation, the mean bacterial cell length at the end of the exponential phase was 5.0 ± 0.7 μm, and the population was composed of about 80% single cells shorter than 7 μm, which increased to >90% in stationary phase (Fig. 2B). Strong shifts in population size structure became apparent when *Flectobacillus* sp. strain GC-5 was grown

in batch culture together with *Ochromonas* (Fig. 2C). Freely suspended cells of <7 μm appeared to be completely edible for the flagellates, as judged by the continuous decline of this size class parallel to the growth of *Ochromonas* sp. In contrast, bacterial cells of >7 μm (mainly chains of two to five elongated cells) increased in relative proportion in the grazing treatments, constituting 75 to 80% of the bacteria at the end of the batch experiment shown in Fig. 2. Based on these results, we therefore used an operational size limit of 7 μm to classify *Flectobacillus* sp. strain GC-5 cells into edible and inedible (resistant) morphotypes for *Ochromonas* sp. The cell widths of the bacteria remained fairly constant (0.5 to 0.7 μm), independent of the impact of grazing and the realized cell length. Estimates of total bacterial biovolume were therefore based on cell length measurements and an assumed constant cell width. Increasing the substrate concentration in additional batch experiments to 20 mg glucose per liter approximately doubled the bacterial concentration in grazer-free trials but did not change the morphological composition (data not shown). Using yeast extract instead of glucose as the carbon source, the average cell length was slightly larger (6.3 ± 0.9 μm), but still about 80% of the population consisted of single cells of <7 μm at the end of the exponential phase (data not shown).

Long-term bacterial growth in continuous cultivation. *Flectobacillus* sp. strain GC-5 was grown for 4 weeks in carbon-limited chemostats, with and without grazers, in order to examine long-term adaptations during continuous grazing pressure. Immunofluorescence microscopy revealed that both populations contained no contamination by other bacteria and were composed until the end of the experiment with 100% target strain. Bacterial concentrations in the flagellate-free chemostats fluctuated in the range of 3.0×10^6 to 4.4×10^6 cells ml^{-1}, and those in the chemostats with *Ochromonas* ranged between 1.7×10^6 and 2.5×10^6 cells ml^{-1} (Table 1). In the latter, flagellates were present until the end of the experiment (range, 3.0×10^3 to 8.2×10^3 ml^{-1}).

The significant reduction of bacterial numbers in the grazer chemostats ($P < 0.01$; rm ANOVA) was not reflected in differences in total bacterial biovolume between chemostats with and without grazers ($P > 0.1$; rm ANOVA) (Table 1). The mean bacterial biovolume values for the period from day 10 to day 28 were identical for the two treatments ($3.6 \times 10^6 \pm 0.6 \times 10^6$ μm^3 ml^{-1}). The reason for the different cell numbers but approximately similar total biovolumes could easily be deduced when looking at the bacterial morphologies in the treatments with and without grazers (Fig. 3). After 1 month, we obtained two *Flectobacillus* populations in the different reactors with completely different morphologies and size structures. Chemostats without grazers were dominated by curved-rod and c-shaped morphologies consisting of one or two cells, mostly <7 μm in length, whereas only a few filamentous cells occurred (Fig. 3A). In contrast, in the chemostats with *Ochromonas*, single cells of <7 μm were nearly eliminated, and the population of *Flectobacillus* sp. strain GC-5 cells was entirely dominated by filamentous cells, mostly 10 to 25 μm in length, and chains of 3 to 10 cells reaching up to 40 μm in length (Fig. 3B). The detailed morphometric analysis of the two bacterial populations revealed that the mean cell width had not significantly changed and that only shifts in the mean cell length affected the total biovolume (Table 1). The mean cell length and the length-to-width ratio were about three times higher in the chemostats with *Ochromonas* sp. (Table 1). Considering our size limit of 7 μm for edibility of the bacteria, on average 76% of the bacterial population was grazing resistant in the chemostats with flagellates, in contrast to 17% in the treatments without predators.

TABLE 1. Comparison of cell size characteristics of *Flectobacillus* GC-5 during growth in chemostat culture in the presence and absence of the bacterivorous flagellate *Ochromonas* sp.[a]

Parameter	Type of data	Value	
		With flagellates	Without flagellates
Cell volume (μm^3)	Mean ± SD	2.55 ± 1.88	1.06 ± 0.38
	Min.	0.28	0.33
	Max.	7.77	3.00
Cell width (μm)	Mean ± SD	0.57 ± 0.07	0.51 ± 0.05
	Min.	0.36	0.40
	Max.	0.59	0.62
Cell length (μm)	Mean ± SD	14.5 ± 9.7	4.3 ± 1.7
	Min.	2.2	2.2
	Max.	48.6	12.5
Ratio of length to width	Mean ± SD	30.4 ± 21.2	10.5 ± 3.8
	Min.	5.3	4.7
	Max.	84.1	23.3
% Inedible morphotypes	Mean ± SD	76 ± 11	17 ± 4
	Min.	69	15
	Max.	86	21
Cell number (10^6 bacteria ml^{-1})	Mean ± SD	1.9 ± 0.3	3.5 ± 0.3
	Min.	1.7	3.0
	Max.	2.5	4.4
Biovolume (10^6 μm^3 ml^{-1})	Mean ± SD	3.6 ± 0.6	3.6 ± 0.6
	Min.	2.1	2.3
	Max.	3.7	3.7
Flagellates (cells ml^{-1})	Mean ± SD	6,807 ± 3,122	
	Min.	2,996	
	Max.	8,247	

[a] The period between day 10 and day 28 of the chemostat run was analyzed.

Regrowth of chemostat bacteria in batch cultures. Bacterial inocula were derived from the two chemostat populations with contrasting size structures and morphological compositions (as described above) but similar bacterial biovolumes. The inoculum from the grazer-free chemostat consisted of >80% single straight or c-shaped rods, whereas the inoculum from the grazer chemostat was dominated by >80% long filaments and chains. Despite these differences, the bacterial growth dynamics, maximal cell numbers, and final mean cell lengths attained in batch cultures were comparable (Fig. 4). Thirty to 40 h after inoculation, bacteria reached stationary phase, in which cell numbers stayed constant and only the proportions of edible and inedible morphotypes changed. At both substrate concentrations, 10 and 20 mg glucose per liter, batch cultures inoculated from grazer-free and grazer-containing chemostats had similar growth rates (doubling times during logarithmic growth were in the range of 3.4 to 4.0 h) and achieved approximately similar maximal cell numbers (5.0×10^6 to 5.2×10^6 cells ml^{-1}

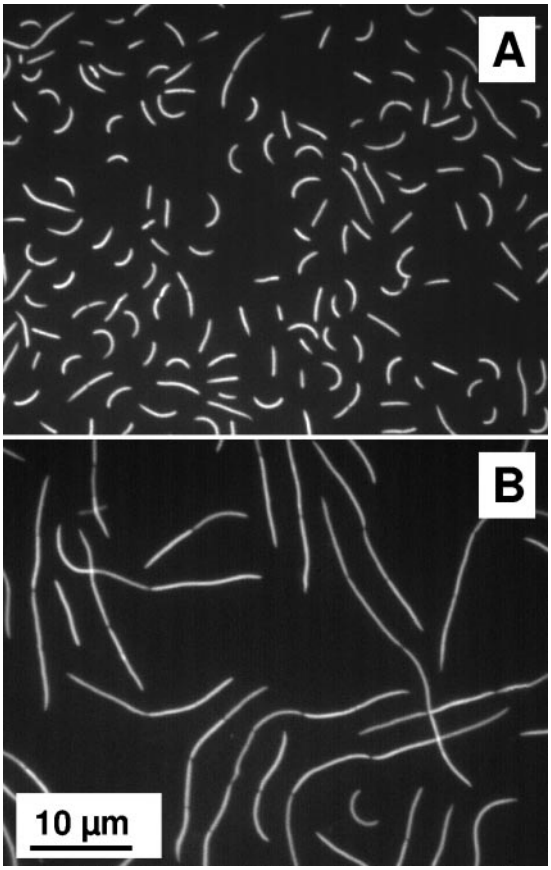

FIG. 3. Photomicrographs of DAPI-stained cells of *Flectobacillus* GC-5 grown in chemostat cultures in the absence (A) or presence (B) of *Ochromonas* sp. Samples were taken on day 28 of the chemostat run. The scale bar in panel B refers to both photographs.

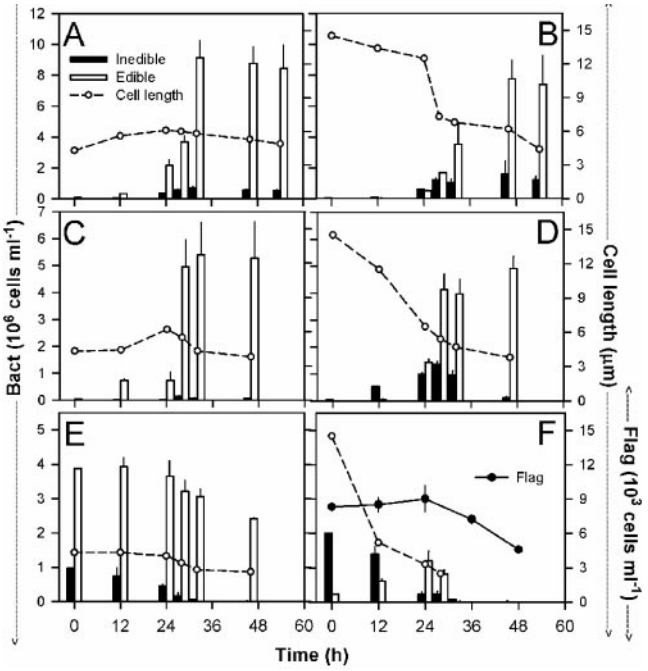

FIG. 4. Regrowth of *Flectobacillus* GC-5, derived from grazer-free (A, C) and grazer-containing (B, D) chemostats, in batch cultures. Growth media were supplemented with 20 (A, B) or 10 (C, D) mg glucose per liter. Chemostat bacteria derived from grazer-free (E) and grazer-containing (F) chemostats were also transferred to starvation conditions in batch culture. The development of flagellates and bacteria, differentiated into edible (<7 μm) and inedible (>7 μm) bacterial cell length classes, and the mean bacterial cell length after transfer into batch culture are shown. Values are means ± standard deviations of three replicates.

with 10 mg glucose and 9.9×10^6 to 10.3×10^6 cells ml^{-1} with 20 mg glucose per liter).

Although in all treatments mainly edible (<7 μm) morphotypes developed, some differences between the two populations became apparent. The populations derived from grazer-free inocula were dominated by characteristic curved (c-shaped) morphologies, often consisting of two connected cells. This "double c shape," presumably recently divided but not separated cells, was permanently present during the batch experiment. Populations derived from grazer-adapted inocula developed only a few c-shaped cells (<5%) and instead developed straight rods that were roughly similar in length and volume to the c-shaped cells. The abundance of filamentous cells, which were dominant in the inoculum, decreased, and newly developed cells seemed to consist mainly of short rods. Bacteria from both inocula achieved similar mean cell lengths of about 6 μm during the exponential growth phase. For cells derived from the grazer chemostats, this implied a reduction in cell length from an average of 14.5-μm-long filaments, and for cells derived from grazer-free chemostats, it involved an increase from an initial mean length of 4.3 μm.

A shift towards smaller cell sizes became apparent when chemostat bacteria were transferred to batch cultures without a substrate supply (Fig. 4E and F). Bacteria from the grazer-free chemostats declined about 30% in numbers within 48 h (Fig. 4E). The mean cell length was reduced to 2.6 ± 0.3 μm, and inedible size classes (>7 μm) nearly disappeared under these starvation conditions. More drastic changes occurred in the populations taken from the grazer chemostats (Fig. 4F): the mean cell length shifted in less than 24 h from grazing-resistant long filaments to straight rods in the edible size range (<7 μm). Since flagellates were still present in this starvation culture, the size reduction and grazing pressure finally resulted in a severe decline in bacterial abundance to $<0.1 \times 10^6$ ml^{-1} after 48 h.

Dialysis bag experiments with chemostat cultures. The growth and morphological changes of *Flectobacillus* sp. strain GC-5 in the presence of flagellates, but without a direct predation impact, were examined in chemostat experiments which contained dialysis bags attached inside the chemostat vessels. Whereas *Flectobacillus* cells were continuously cultivated in the chemostat reactors, inside the dialysis bags *Ochromonas* sp. was grazing on different prey bacteria (either *Flectobacillus* sp. or *P. putida*). Flagellates were still present at the end of the experiment, meaning that grazing and resulting excretory products could permanently influence the *Flectobacillus* sp. population growing outside the bags.

Control treatments achieved steady-state-like conditions after approximately 1 week and had fairly stable biomass levels of *Flectobacillus* sp. strain GC-5 until the end of the experiment

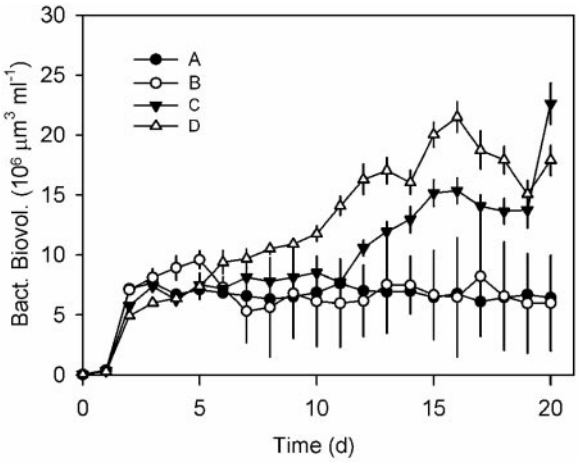

FIG. 5. Growth of *Flectobacillus* sp. strain GC-5 growing outside dialysis bags in the chemostat dialysis experiment. (A) Control with bacteria only; (B) control with bacteria and flagellates inside and outside the dialysis bags; (C) grazing treatments with flagellates and *Flectobacillus* sp. strain GC-5 inside the dialysis bags; (D) grazing treatments with flagellates and *P. putida* MM1 inside the dialysis bags. Cells numbers are given as means of three replicates for each treatment ± standard deviations.

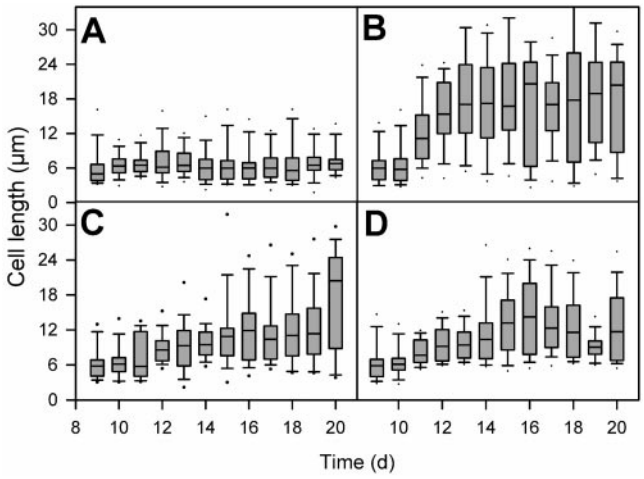

FIG. 6. Development of cell length of *Flectobacillus* sp. strain GC-5 growing outside dialysis bags during the last 12 days of the chemostat dialysis experiment. Treatments A to D refer to the same treatments as in Fig. 1 and 5. (A) Only *Flectobacillus* GC-5 (inside and outside); (B) *Flectobacillus* GC-5 and *Ochromonas* sp. (inside and outside); (C) inside, *Flectobacillus* GC-5 and *Ochromonas* sp.; outside, *Flectobacillus* GC-5; (D) inside, *P. putida* MM1 and *Ochromonas* sp.; outside, *Flectobacillus* GC-5. Vertical boxes with error bars in the graphs represent the median and 10th, 25th, 75th, and 90th percentiles of bacterial cell lengths (means of two replicates) of *Flectobacillus* GC-5.

(Fig. 5). The results for the two types of control treatments, i.e., reactors with *Ochromonas* sp. inside and outside the dialysis bags and reactors without any predators (only bacteria inside the bags), resembled those for the chemostat experiment shown before. Although the average concentrations of bacteria (morphological units) which were achieved under steady-state-like conditions were $8.0 \times 10^6 \pm 0.5 \times 10^6$ cells ml^{-1} without and $3.1 \times 10^6 \pm 2.1 \times 10^6$ cells ml^{-1} with *Ochromonas* sp., the bacterial biovolumes were not significantly different between the treatments. The bacterial biovolumes in the reactors in which flagellates grazing inside the dialysis bags occurred started to increase after 1 week and achieved much higher values than the controls towards the end of the experiment (Fig. 5).

The size structure of *Flectobacillus* sp. strain GC-5 outside the dialysis bags was followed for the last 12 days of the chemostats run in the different reactors (Fig. 6). For statistical comparison of the different treatments, data for the last 6 days of the experiments were pooled because prolonged direct and indirect effects of flagellate bacterivory should have become evident during this time. In the controls with and without flagellates, the same contrasting size distributions as in the first chemostat experiment could be observed. Without flagellates (Fig. 6A), about 75% of the population was composed of cells of <7 µm (edible size range for *Ochromonas* sp.), and the median cell length was 6.2 µm. With a direct grazing impact of flagellates (Fig. 6B), on average 86% of the cells were >7 µm long (median, 18.6 µm) and thus inedible for *Ochromonas* sp. The most interesting result of this experiment was the bacterial size distribution of the populations which were not directly grazed but potentially influenced by flagellate excretory products from the dialysis bags (Fig. 6C and D). Here the mean cell lengths of *Flectobacillus* sp. strain GC-5 cells started to increase after about 1 week of cultivation. With *Flectobacillus* as the prey bacterium inside the dialysis bag (Fig. 6C), the external bacterial population achieved a median cell length of 11.4 µm (50% between 7 and 15 µm and 80% between 5 and 24 µm), which was significantly higher than that in the flagellate-free control (MANOVA; $P < 0.001$). A similar effect was observed when grazers in the dialysis bags had *P. putida* as a prey bacterium (Fig. 6D): 11.6 µm was the median cell length, with 50% of the cells being between 7 and 20 µm and 80% being between 5 and 24 µm (significant difference between panels A and D; $P < 0.0001$). For both groups of reactors (panels C and D) with *Ochromonas* in the dialysis bags, <30% of the cells were in the edible size range (<7 µm) for flagellates.

DISCUSSION

Similar experimental model systems to the one used here have been successfully used in previous studies of bacterium-protist interactions, providing insights into mechanisms of bacterial adaptations to predation (12, 14, 28). Chemostat and batch culture experiments with bacterial monocultures and axenic (or monoxenic) bacterivores allow one to follow phenotypic shifts in the prey bacteria and resulting alterations in their vulnerability and grazing mortality. Such simplified model systems are not designed to mimic natural conditions but to examine potential underlying mechanisms in predation-mediated changes in bacterial phenotypic properties. Along this line, our experiments revealed insights into general mechanisms for how direct protist grazing and size selection as well as chemical induction of defended morphotypes can determine the population size structure of a bacterial strain with high phenotypic plasticity.

Effect of substrate supply and flagellate grazing on the population size structure of *Flectobacillus* sp. strain GC-5. The

Flectobacillus strain investigated in our study is obviously a highly polymorphic bacterium, covering a range of cell lengths from small rods and curved cells of <2 μm to filamentous cell chains of >40 μm. The whole range of morphotypes can be found within a clonal culture, but the distribution and frequencies of the different morphologies are determined by the substrate concentration (and resulting growth rate) as well as the actual grazing pressure. The reported size limits for separating grazing-vulnerable from grazing-resistant bacterial cells range from 2.4 μm to 10 μm in different study systems and depend on the bacterial and protist species under consideration (13). Recently, it was shown that even filamentous bacteria are not absolutely resistant to flagellate grazing but have a selective advantage due to a strongly reduced ingestion efficiency by bacterivorous flagellates (53). For our model system, a bacterial cell length of approximately 7 μm defined the size above which grazing by *Ochromonas* seemed to become too inefficient. This absolute or relative resistance of *Flectobacillus* cells of >7 μm can explain the grazing-mediated shift in the population size structure (Fig. 2).

Prolonged grazing pressure in the chemostat experiment shifted the *Flectobacillus* population size structure nearly completely towards resistant filaments with a mean length of 14 to 15 μm but encompassing a wide range of cell lengths (Table 1). A minor portion (about 20%) of edible bacterial cells sustained a relatively small flagellate population which was able to maintain the bacterial size structure under grazing control. This shift in population size structure resulted in a compensation of bacterial grazing mortality and nearly identical bacterial biomasses in grazed and ungrazed systems (Fig. 5). A similar degree of compensation has been found in chemostats with mixed bacterial assemblages and bacterivorous flagellates, but this was due to the occurrence of different resistance mechanisms (33). A continuous size distribution and co-occurrence of vulnerable and resistant morphotypes have also been observed for several filament-forming bacterial strains (14).

The *Flectobacillus* cells from chemostats with flagellates, which consisted mainly of resistant filamentous cells, returned quickly to the smaller, vulnerable phenotype when transferred to a predation-free growth medium (Fig. 4). Thus, when only competition for the organic substrate was the main selective force, cells with a 4- to 5-μm length seemed to be best adapted. Cells in the edible size range (<7 μm) constituted a fairly constant proportion of about 80%, including during exponential growth on higher concentrations of organic substrates (glucose and yeast extract). This transient nature of phenotypic alterations in response to the environment is characteristic of phenotypic plasticity and indicates that it is not an adaptive mutational event. Phenotypic variation by mutation and selection (39), but also by other underlying genetic mechanisms, is a well-known mechanism which can result in rapid speciation of bacteria. In the case of bacterial resistance against *Bdellovibrio*, both processes have been demonstrated, with the appearance of resistant mutants (51) and a plastic phenotypic response (41).

With the starvation experiment, it became obvious that a sufficient substrate supply is required for the development of elongated and filamentous cell forms. When *Flectobacillus* cultures were transferred to a starvation medium, a fast decline in cell length (to <3 μm) occurred, thereby shifting the population into the edible size range for *Ochromonas* sp. Thus, when grazers were present in these nongrowing cultures, the *Flectobacillus* population became almost completely eliminated (Fig. 4F). This also indicates that no other resistance mechanisms were expressed.

The observation that a fraction of a genetically homogenous bacterial population can survive strong grazing pressure is somewhat comparable to the well-known fact that there is always a persisting fraction when a bacterial population is exposed to some kind of lethal stress such as antibiotics (4). This is due to inherent phenotypic heterogeneity, a recognized property of bacterial cultures, including morphological (21, 53) and metabolic (50) properties. It is assumed that heterogeneity arises at different levels of cell regulation (e.g., transcription or translation) and involves some stochasticity, for example, in the synthesis of different proteins (4). Switching between different growth phases has also been shown to generate population heterogeneity (1). Even in cases of strong phenotypic heterogeneity, cells surviving exposure to stress possess the same genetic potential as the original population and can generate a similar mixed population profile when returned to the previous conditions (4). This polymorphism within populations should enhance their adaptability and potential to survive environmental changes caused by abiotic or biotic factors.

Underlying mechanisms of bacterial filament formation. The most detailed laboratory studies on grazing-mediated development of resistant morphologies (filaments and aggregates) have been performed by Hahn and coworkers (12, 14). They presented an intriguingly simple theory for the underlying mechanism of how flagellate grazers shift bacterial populations towards the dominance of resistant filaments. An increase in mean cell size of filament-forming strains was also observed in the absence of grazers when the growth rate was increased, either during logarithmic growth in batch culture or at increased dilution rates in chemostat cultures. This was particularly pronounced for a *Flectobacillus* strain grown with continuous cultivation and much less so for *Comamonas acidovorans* (14). Therefore, the suggested underlying mechanism of filament formation was a growth-rate-controlled increase in cell length. For predation treatments, the increase in specific growth rate should be due to the elimination of nonresistant competitors and the excretion of dissolved substrates by grazers.

The pattern of filament formation observed in our experiments with *Flectobacillus* sp. strain GC-5 resembles in several aspects the phenomenon observed by Hahn et al. (14) for other filament-forming bacteria. With all these strains, a high morphological variability occurred, with cell lengths in the range of <1 μm to >10 μm and thus with co-occurring resistant and vulnerable size classes even under strong grazing pressure. We also observed some dependence of cell size on growth conditions, e.g., no elongated cell forms were present under starvation conditions. However, we could not observe a strong increase in the proportion of filamentous cells, such as that reported for *Flectobacillus* sp. strain MWH38 (14), in any of the treatments without predators. If filament formation were controlled by the growth rate, this should have been visible, particularly in the batch experiments where nearly maximal growth rates were achieved. Even on a relatively rich medium (20 mg yeast extract per liter), the *Flectobacillus* strain used in

our experiments did not develop a larger proportion of filamentous cells when predators were absent. In our case, a simple negative selection mechanism of filamentous cells would be sufficient to explain the observed shift in the polymorphic *Flectobacillus* population as long as there are substrates available that allow further growth of resistant morphotypes.

Our chemostat dialysis experiments imply, however, that this selection process becomes enhanced due to a chemically stimulated transformation of cells into filamentous morphotypes. The grazing activity of bacterivorous protists involves the release of dissolved and colloidal organic carbon (35). Whereas mineral nutrients were not limited in our system, dissolved organic carbon excretion was presumably important and significantly increased the bacterial concentrations in the chemostat dialysis experiments (Fig. 5). The input of extra organic carbon from the dialysis bags in the chemostat reactors is conceptually similar to a higher substrate inflow concentration and produced a larger bacterial biomass but not a higher growth rate, which was similarly controlled in all reactors by the dilution rate.

The chemostat dialysis experiment showed a clear chemical effect of the flagellate grazers on bacterial morphologies (Fig. 6). The *Flectobacillus* cell length in the treatments where bacteria were exposed to flagellate excretory products (Fig. 6C and D) continuously increased and reached a median cell length of >10 μm after about 1 week of continuous cultivation. The proportion of resistant morphotypes (defined as cells of >7 μm) was not as high as that for the treatment with direct grazing impact (Fig. 6B) but still exceeded 50% of the population. The fact that treatments with different prey bacteria inside the dialysis bags, either *Flectobacillus* GC-5 or *P. putida* MM1, produced similar results makes it less probable (but does not exclude) that "infochemicals" were released by the bacteria themselves. Therefore, this experiment can be seen as the first strong evidence that grazing-resistant morphologies in bacteria might be induced by excretory products of bacterivores.

It has long been speculated that grazer-mediated chemicals might trigger phenotypic responses in bacteria and the development of grazing resistance (17, 36), similar to that known for other predator-prey interactions in planktonic systems (23, 49). However, attempts to demonstrate inducible phenotypic shifts in bacteria, e.g., by adding grazer-conditioned water to bacterial cultures, have failed until now (Jürgens, unpublished results). One reason might be that the development of resistant bacteria depends on the right balance between bacterial growth and grazing losses in such experimental systems. The chemostat results further indicate that sufficiently long exposure to a grazer-mediated environment is required for the shift towards the development of filaments. This fact is in line with other published examples showing that it may take several days of protist predation impact before filamentous bacteria develop, e.g., in chemostat experiments (12) and in freshwater plankton (37). We could not find a larger proportion of filamentous *Flectobacillus* cells in dialysis experiments performed in batch growth cultures (data not shown). We assume that the faster shift towards filament dominance in batch experiments containing grazers is mainly due to the rapid elimination of nonresistant morphotypes and not necessarily to strong growth of the filaments. It remains to be seen whether a predator-released "infochemical" is really involved or whether the flagellate excretions change the bacterial growth medium in a way which favors filamentous morphotypes. Until the active chemical compound has been determined, it will not be possible to answer this question.

How widespread this mechanism is and whether it also plays a role under natural conditions, e.g., during short-lasting blooms of filamentous bacteria in planktonic systems (37), also remain to be elucidated. Situations in which protist grazing is the only selective predation pressure are probably only temporary events, and vulnerabilities towards metazoan filter feeders (25), bacteriophages (52), and predatory bacteria (41) must also be considered and imply multiple tradeoffs in natural bacteria (3).

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REFERENCES

1. **Balaban, N. Q., J. Merrin, R. Chait, L. Kowalik, and S. Leibler.** 2004. Bacterial persistence as a phenotypic switch. Science **305:**1622–1625.
2. **Boenigk, J., and H. Arndt.** 2002. Bacterivory by heterotrophic flagellates: community structure and feeding strategies. Antonie Leeuwenhoek **81:**465–480.
3. **Bohannan, B. J. M., B. Kerr, C. M. Jessup, J. B. Hughes, and G. Sandvik.** 2002. Trade-offs and coexistence in microbial microcosms. Antonie Leeuwenhoek **81:**107–115.
4. **Booth, I. R.** 2002. Stress and the single cell: intrapopulation diversity is a mechanism to ensure survival upon exposure to stress. Int. J. Food Microbiol. **78:**19–30.
5. **Caron, D. A., J. C. Goldman, and M. R. Dennett.** 1988. Experimental demonstration of the roles of bacteria and bacterivorous protozoa in plankton nutrient cycles. Hydrobiologia **159:**27–40.
6. **Christoffersen, K., O. Nybroe, K. Jürgens, and M. Hansen.** 1997. Measurement of bacterivory by heterotrophic nanoflagellates using immunofluorescence labelling of ingested cells. Aquat. Microb. Ecol. **13:**127–134.
7. **Engström-Öst, J., M. Koski, K. Schmidt, M. Viitasalo, S. H. Jonasdottir, M. Kokkonen, S. Repka, and K. Sivonen.** 2002. Effects of toxic cyanobacteria on plankton assemblage: community development during decay of *Nodularia spumigena*. Mar. Ecol. Prog. Ser. **232:**1–14.
8. **González, J. M., E. B. Sherr, and B. F. Sherr.** 1990. Size-selective grazing on bacteria by natural assemblages of estuarine flagellates and ciliates. Appl. Environ. Microbiol. **56:**583–589.
9. **Güde, H.** 1979. Grazing by protozoa as selection factor for activated sludge bacteria. Microb. Ecol. **5:**225–237.
10. **Güde, H.** 1989. The role of grazing on bacteria in plankton succession, p. 337–364. *In* U. Sommer (ed.), Plankton ecology. Succession in plankton communities. Springer Verlag, Berlin, Germany.
11. **Guillard, R., and C. Lorenzen.** 1972. Yellow-green algae with chlorophyllide c. J. Phycol. **8:**10–14.
12. **Hahn, M. W., and M. G. Höfle.** 1999. Flagellate predation on a bacterial model community: interplay of size-selective grazing, specific bacterial cell size, and bacterial community composition. Appl. Environ. Microbiol. **65:** 4863–4872.
13. **Hahn, M. W., and M. G. Höfle.** 2001. Grazing of protozoa and its effect on populations of aquatic bacteria. FEMS Microbiol. Ecol. **35:**113–121.
14. **Hahn, M. W., E. R. B. Moore, and M. G. Höfle.** 1999. Bacterial filament formation, a defense mechanism against flagellate grazing, is growth rate controlled in bacteria of different phyla. Appl. Environ. Microbiol. **65:**25–35.
15. **Hahn, M. W., E. R. B. Moore, and M. G. Höfle.** 2000. Role of microcolony formation in the protistan grazing defense of the aquatic bacterium *Pseudomonas* sp. MWH1. Microb. Ecol. **39:**175–185.
16. **Jürgens, K., H. Arndt, and K. O. Rothhaupt.** 1994. Zooplankton-mediated changes of bacterial community structure. Microb. Ecol. **27:**27–42.
17. **Jürgens, K., and H. Güde.** 1994. The potential importance of grazing-resistant bacteria in planktonic systems. Mar. Ecol. Prog. Ser. **112:**169–188.
18. **Jürgens, K., and C. Matz.** 2002. Predation as a shaping force for the phenotypic and genotypic composition of planktonic bacteria. Antonie Leeuwenhoek **81:**413–434.
19. **Jürgens, K., J. Pernthaler, S. Schalla, and R. Amann.** 1999. Morphological

and compositional changes in a planktonic bacterial community in response to enhanced protozoan grazing. Appl. Environ. Microbiol. **65:**1241–1250.
20. **Jürgens, K., and G. Stolpe.** 1995. Seasonal dynamics of crustacean zooplankton, heterotrophic nanoflagellates and bacteria in a shallow, eutrophic lake. Freshwater Biol. **33:**27–38.
21. **Koch, A.** 1996. What size should a bacterium be? Annu. Rev. Microbiol. **50:**317–348.
22. **Lampert, W.** 1987. Predictability in lake ecosystems: the role of biotic interactions, p. 333–346. *In* E. D. Schulze and H. Zwölfer (ed.), Potential and limitations of ecosystem analysis. Ecological studies, vol. 61. Springer-Verlag, Berlin, Germany.
23. **Lampert, W., K. O. Rothhaupt, and E. Von Elert.** 1994. Chemical induction of colony formation in a green alga (*Scenedesmus acutus*) by grazers (*Daphnia*). Limnol. Oceanogr. **39:**1543–1550.
24. **Lane, D.** 1991. 16S-23S rRNA sequencing, p. 115–175. *In* E. Stackebrandt and M. Goodfellow (ed.), Nucleic acid techniques in bacterial systematics. Wiley, Chichester, United Kingdom.
25. **Langenheder, S., and K. Jürgens.** 2001. Regulation of bacterial biomass and community structure by metazoan and protozoan predation. Limnol. Oceanogr. **46:**121–134.
26. **Larkin, J., and R. Borrall.** 1984. Family I. *Spirosomacea*, p. 125–132. *In* N. R. Krieg and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 1. Williams & Wilkins Co., Baltimore, Md.
27. **Massana, R., J. M. Gasol, P. K. Björnsen, N. Blackburn, A. Hagström, S. Hietanen, B. H. Hygum, J. Kuparinen, and C. Pedrós-Alió.** 1997. Measurement of bacterial size via image analysis of epifluorescence preparations—description of an inexpensive system and solutions to some of the most common problems. Scientia Marina **61:**397–407.
28. **Matz, C., J. Boenigk, H. Arndt, and K. Jürgens.** 2002. Role of bacterial phenotypic traits in selective feeding of the heterotrophic nanoflagellate *Spumella* sp. Aquat. Microb. Ecol. **27:**137–148.
29. **Matz, C., P. Deines, J. Boenigk, H. Arndt, L. Eberl, S. Kjelleberg, and K. Jürgens.** 2004. Impact of violacein-producing bacteria on survival and feeding of bacterivorous nanoflagellates. Appl. Environ. Microbiol. **70:**1593–1599.
30. **Matz, C., P. Deines, and K. Jürgens.** 2002. Phenotypic variation in *Pseudomonas* sp. CM10 determines microcolony formation and survival under protozoan grazing. FEMS Microbiol. Ecol. **39:**57–65.
31. **Matz, C., and K. Jürgens.** 2005. High motility reduces grazing mortality of planktonic bacteria. Appl. Environ. Microbiol. **71:**921–929.
32. **Matz, C., and K. Jürgens.** 2001. Effects of hydrophobic and electrostatic cell surface properties of bacteria on feeding rates of heterotrophic nanoflagellates. Appl. Environ. Microbiol. **67:**814–820.
33. **Matz, C., and K. Jürgens.** 2003. Interaction of nutrient limitation and protozoan grazing determines the phenotypic structure of a bacterial community. Microb. Ecol. **45:**384–398.
34. **Monger, B. C., M. R. Landry, and S. L. Brown.** 1999. Feeding selection of heterotrophic marine nanoflagellates based on the surface hydrophobicity of their picoplankton prey. Limnol. Oceanogr. **44:**1917–1927.
35. **Nagata, T.** 2000. Production mechanisms of dissolved organic matter, p. 121–152. *In* D. Kirchman (ed.), Microbial ecology of the oceans. Wiley-Liss, New York, N.Y.
36. **Pernthaler, J., T. Posch, K. Šimek, J. Vrba, R. Amann, and R. Psenner.** 1997. Contrasting bacterial strategies to coexist with a flagellate predator in an experimental microbial assemblage. Appl. Environ. Microbiol. **63:**596–601.
37. **Pernthaler, J., E. Zöllner, F. Warnecke, and K. Jürgens.** 2004. Bloom of filamentous bacteria in a mesotrophic lake: identity and potential controlling mechanism. Appl. Environ. Microbiol. **70:**6272–6281.
38. **Porter, K. G., and Y. S. Feig.** 1980. The use of DAPI for identifying and counting aquatic microflora. Limnol. Oceanogr. **25:**943–947.
39. **Rainey, P.** 2000. An organism is more than its genotype. Environ. Microbiol. **2:**8–9.
40. **Schauer, M., and M. W. Hahn.** 2005. Diversity and phylogenetic affiliations of morphologically conspicuous large filamentous bacteria occurring in the pelagic zones of a broad spectrum of freshwater habitats. Appl. Environ. Microbiol. **71:**1931–1940.
41. **Shemesh, Y., and E. Jurkevitch.** 2004. Plastic phenotypic resistance to predation by *Bdellovibrio* and like organisms in bacterial prey. Environ. Microbiol. **6:**12–18.
42. **Sherr, E. B., and B. F. Sherr.** 2002. Significance of predation by protists in aquatic microbial food webs. Antonie Leeuwenhoek **81:**293–308.
43. **Shikano, S., L. S. Luckinbill, and Y. Kurihara.** 1990. Changes of traits in a bacterial population associated with protozoan predation. Microb. Ecol. **20:**75–84.
44. **Šimek, K., and T. H. Chrzanowski.** 1992. Direct and indirect evidence of size-selective grazing on pelagic bacteria by freshwater nanoflagellates. Appl. Environ. Microbiol. **58:**3715–3720.
45. **Šimek, K., P. Kojecka, J. Nedoma, P. Hartman, J. Vrba, and J. R. Dolan.** 1999. Shifts in bacterial community composition associated with different microzooplankton size fractions in a eutrophic reservoir. Limnol. Oceanogr. **44:**1634–1644.
46. **Šimek, K., J. Pernthaler, M. G. Weinbauer, K. Hornak, J. R. Dolan, J. Nedoma, M. Masin, and R. Amann.** 2001. Changes in bacterial community composition and dynamics and viral mortality rates associated with enhanced flagellate grazing in a mesoeutrophic reservoir. Appl. Environ. Microbiol. **67:**2723–2733.
47. **Šimek, K., J. Vrba, J. Pernthaler, T. Posch, P. Hartman, J. Nedoma, and R. Psenner.** 1997. Morphological and compositional shifts in an experimental bacterial community influenced by protists with contrasting feeding modes. Appl. Environ. Microbiol. **63:**587–595.
48. **Sommaruga, R., and R. Psenner.** 1995. Permanent presence of grazing-resistant bacteria in a hypertrophic lake. Appl. Environ. Microbiol. **61:**3457–3459.
49. **Tollrian, R., and C. Harvell.** 1999. The ecology and evolution of inducible defenses. Princeton University Press, Princeton, N.J.
50. **Varma, A., and B. Palsson.** 1994. Metabolic flux balancing: basic concepts, scientific and practical use. Biotechnology **12:**994–998.
51. **Varon, M.** 1979. Selection of predation-resistant bacteria in continuous culture. Nature **277:**386–388.
52. **Weinbauer, M. G., and M. G. Höfle.** 1998. Significance of viral lysis and flagellate grazing as factors controlling bacterioplankton production in a eutrophic lake. Appl. Environ. Microbiol. **64:**431–438.
53. **Wu, Q. L., J. Boenigk, and M. W. Hahn.** 2004. Successful predation of filamentous bacteria by a nanoflagellate challenges current models of flagellate bacterivory. Appl. Environ. Microbiol. **70:**332–339.