Applied and Environmental Microbiology, Feb. 1997, p. 596–601 Vol. 63, No. 2
0099-2240/97/$04.00+0
Copyright © 1997, American Society for Microbiology

Contrasting Bacterial Strategies To Coexist with a Flagellate Predator in an Experimental Microbial Assemblage

JAKOB PERNTHALER,[1]* THOMAS POSCH,[1] KAREL ŠIMEK,[2] JAROSLAV VRBA,[2] RUDOLF AMANN,[3] AND ROLAND PSENNER[1]

Institute of Zoology and Limnology, University of Innsbruck, A-6020 Innsbruck, Austria[1]; Hydrobiological Institute of the Czech Academy of Sciences, Ceské Budejovice 37005, Czech Republic[2]; and Lehrstuhl für Mikobiologie, Technische Universität München, D-80260 Munich, Germany[3]

Received 20 June 1996/Accepted 11 October 1996

We studied predator-induced changes within a slowly growing mixed microbial assemblage that was sustained by algal exudates in a continuous cultivation system. In situ hybridization with fluorescent monolabeled oligonucleotide probes was used for a tentative community analysis. This method also allowed us to quantify the proportions of predators with ingested bacteria of different taxonomic groups. In addition, we determined grazing rates on bacteria with fluorescently labelled prey. Bacteria belonging to the alpha and beta subdivisions of the phylum *Proteobacteria* (α- and β-Proteobacteria, respectively) showed very different responses to the addition of a bacterivorous flagellate, *Bodo saltans*. Within one day, filamentous protist-inedible bacteria developed; these belonged to the β-Proteobacteria and constituted between 8.7 and 34% of bacteria from this subgroup. Total abundance of β-Proteobacteria decreased from 3.05×10^6 to 0.23×10^6 cells ml^{-1}, and estimated cell division rates were low. Other morphologically inconspicuous protist-edible bacteria belonging to the α-Proteobacteria were found to respond to predation by an increase in growth rate. Although these bacteria were heavily grazed upon, as on average >85% of flagellate cells had ingested α-Proteobacteria, they numerically dominated after the addition of *B. saltans* (mean, 1.35×10^6 cells ml^{-1}). It was thus mainly those fast-dividing strains of α-Proteobacteria that supported the growth of the flagellate population. We conclude that bacteria in mixed assemblages can adopt at least two distinct strategies as a reaction to intense flagellate predation: to outgrow predation pressure or to develop inedible, inactive filaments. Since these strategies occurred within 24 h after the addition of the flagellate, we hypothesize that chemical stimuli released by the predator may have triggered bacterial responses.

Top-down and bottom-up control have become widely known concepts in aquatic microbial ecology (e.g., see reference 22). There is still disagreement as to whether the abundance and productivity of pelagic bacteria are determined mainly by predators (28, 30) or nutrients (22), and the two control modes may interchange rapidly (27). A key issue within this topic is the relationship between protist predation and bacterial metabolic state. Does protist grazing release nutrients that stimulate bacterial productivity (22, 29), or do protists selectively eliminate growing or dividing cells (15, 30) and thus shift microbial assemblages toward slowly growing, small bacteria? As both of these somehow contradictory propositions have been verified, there must be unknown differences in the studied natural bacterial communities that determine whether so-called top-down or bottom-up forces explain the contrasting system responses. Aquatic microbial ecologists tend to focus on parameters that describe microbial assemblages as a whole, such as secondary production (11), enzyme activity (40), or total mortality rates (26, 32). Thus the controversy may partly reflect the lack of information about, e.g., the fraction of bacteria that is responsible for secondary production (7, 10), the neglected size distributions of bacterial biomass (24), and most importantly, the unknown taxonomic variability of communities that add up to total predator and bacterial abundance (1, 8, 34).

* Corresponding author. Mailing address: Institute of Zoology and Limnology, University of Innsbruck, Technikerstr. 25, A-6020 Innsbruck, Austria. Phone: 43 512 507 6126. Fax: 43 512 507 2930. E-mail address: Jakob.Pernthaler@uibk.ac.at.

Recently, there have been calls for more information on bacterial community structure (23), and much effort has been put into detailed descriptions of the genetic diversity of aquatic microbial assemblages (8, 12). A new protocol allows us to quantify different taxonomic groups of planktonic bacteria by single-cell in situ hybridization with rRNA targeted fluorescent probes on membrane filters even in oligotrophic systems (1, 13). The method is still limited by the lack of taxonomic knowledge about freshwater procaryotes, as it can be used only to study those bacterial species or higher taxonomic groups that have already been set into the phylogenetic framework of rRNA sequences. Furthermore, fluorescent oligonucleotide probes can easily penetrate the cell membranes of protists, and they have been successfully applied to detection of bacterial symbionts inside the macronucleus of a ciliate (4). Thus it is possible to hybridize intact or even partly digested bacteria inside protistan food vacuoles and determine the proportion of the predator population ingesting bacteria of a particular taxonomic group. Grazing pressure on different bacterial taxa could be estimated from their relative abundances in the community and total grazing on fluorescently labeled prey (32). But only the simultaneous inspection of protistan food vacuoles will verify or dismiss the underlying assumption of nonselective, density-dependent protistan feeding, as we may, e.g., find that bacteria from some taxonomic groups are ingested only by a small fraction of the predator population, although their abundance in the environment is high.

In this study we investigated contrasting features of a mixed microbial assemblage in a predation-free and a simple predator-prey system. We used in situ hybridization with fluorescent

oligonucleotide probes for a simple description of the microbial community. This method was also employed to detect bacteria of different taxonomic positions inside protistan food vacuoles. In particular, we combined taxonomic analysis with the measurement of protist feeding rates in order to estimate the growth and mortality of bacteria belonging to different subdivisions of the phylum *Proteobacteria* in the presence and absence of a bacterivorous nanoflagellate. By doing this we attempted to distinguish between contrasting bacterial survival strategies under heavy grazing pressure.

MATERIALS AND METHODS

Continuous-flow system. The two-stage continuous culture we used as a model system is described in detail by Šimek et al. (36). In the first stage we cultivated a phosphorus-limited population of a *Rhodomonas* sp. on a modified WC medium containing exclusively inorganic nutrients (36) at a dilution rate of 0.46 day^{-1} and densities of around 5×10^4 ml^{-1}. The accompanying mixed bacterial assemblage reached densities of 5.3×10^6 to 10.9×10^6 ml^{-1}. The outflow from this first-stage vessel was pumped into three parallel second stages (flow rates, 0.25 to 0.28 day^{-1}). When the algal concentration in all vessels was stable for several days, one second-stage vessel (referred to as stage II/*Bodo*) was inoculated with the bacterivorous nanoflagellate *Bodo saltans*. Protists were separated from accompanying bacteria prior to inoculation by repeated rinsing with prefiltered (0.2-μm-pore-size filters) tap water in an autoclaved filtration apparatus (for details, see reference 36). One second-stage vessel (stage II/control) without protists served as a control. The cultivation system was maintained at 20°C and under a 16:8-h (light:dark) illumination regime. Samples were taken for 12 days from all vessels at 24- to 48-h intervals.

Bacterial and *B. saltans* abundances and protistan grazing rates. Five milliliters of subsample for counts of bacteria and *B. saltans* was fixed with 2% formaldehyde, filtered on black membrane filters (Poretics; 0.2-μm pore size; diameter, 25 mm), stained with 4′,6′-diamidino-2-phenylindole (DAPI) (0.2% [wt/vol]), and enumerated by epifluorescent microscopy (25). At least 400 bacteria and 100 protists were counted per sample (magnification, ×1,250). Threadlike bacteria (>10 μm) (400 per sample) were counted separately at a magnification of ×640.

The uptake of bacteria by *B. saltans* was estimated in short-term (10 to 30 min) experiments with fluorescently labeled bacteria (FLB) (32). FLB were produced from bacteria from the first stage of the cultivation system (for details of the procedure see reference 36). Hourly uptake rates were estimated from ingested tracer cells and the feeding period, assuming a linear ingestion rate. Total protozoan uptake of tracer particles was calculated as the product of flagellate abundance and their average uptake rates. The hourly loss of bacteria from the alpha and beta subdivisions of the phylum *Proteobacteria* (α- and β-Proteobacteria) was estimated from the percentage of tracer addition and the relative proportions of the two subgroups of all DAPI-stained bacteria, assuming density-dependent grazing. The fraction of threadlike bacteria was subtracted from the total number of β-Proteobacteria, as these filaments could not be grazed upon by *B. saltans.*

Whole-cell fluorescent in situ hybridization on membrane filters. Twenty milliliters of sample from each variant of the cultivation system was prefixed with a drop of Lugol's solution and immediately decolorized with sodium thiosulfate. This procedure prevented algal and flagellate cells from disruption. Samples were filtered on white 0.2-μm-pore-size polycarbonate membrane filters (Poretics Corp.; 50-mm diameter) in Nalgene plastic filter holders. Postfixation was performed by overlaying the filters with 4% paraformaldehyde in phosphate-buffered saline (pH 7.2) for 30 min at room temperature. The fixative was removed by gentle vacuum and the filters were rinsed with double-distilled water prior to removal from the filtration apparatus. Postfixation helped to attach bacteria, algae, and flagellates firmly to the filters during the hybridization procedure. Filters were air-dried and stored at −20°C until further processing.

Hybridization with three different oligonucleotide probes was used in the context of this study: ALF, complementary to the 16S rRNA (positions 19 to 35) of α-Proteobacteria, BET for β-Proteobacteria (23S rRNA; positions 1027 to 1043), and EUB for *Bacteria* (16S rRNA; positions 338 to 355) (3). The probes were fluorescently labeled with the indocarbocyanine dye cyanine CY3 (BDS, Pittsburg, Pa.) (3) and stored at −20°C. Filters were cut in smaller sections before hybridization (0.5 to 1 cm^2). Small plastic jars with slip-on lids (Brand, Wertheim, Germany) were used as hybridization chambers. A piece of blotting paper soaked in hybridization buffer was put into the jars in order to prevent the filter sections from drying out during the hybridization process. On this we placed a piece of a microscopic slide, which carried the filter section to be hybridized. The filter sections were covered with 16 μl of hybridization buffer containing 2 μl (50 ng ml^{-1}) of the respective fluorescent probe and hybridized inside the sealed jars at 46°C for 90 min. Hybridization buffers consisted of 0.9 M NaCl, 20 mM Tris-HCl (pH 7.4), 0.01% sodium dodecyl sulfate (SDS), and a variable concentration of formamide (EUB, 0%; ALF, 20%; and BET, 35%). After hybridization, the filter sections were incubated in 20 ml of prewarmed washing buffer at 48°C for 15 min, rinsed with particle-free distilled water, and air-dried. The washing solution consisted of 20 mM Tris-HCl (pH 7.4), 5 mM EDTA (for probes ALF and BET), 0.01% SDS, and NaCl (EUB, 0.9 M; ALF, 0.225 M; and BET, 80 mM). To determine total bacterial abundance, the dried filter sections were placed on a microscopic slide and stained for 5 min with DAPI (final concentration, 2.5 μg/ml). Filter sections were mounted in glycerol medium (Citifluor, University of Kent, Canterbury, England) and inspected by epifluorescence microscopy (Zeiss Axioplan). Filter sets for DAPI were 365/395/397, and those for CY3 were 535-550/565/610-675. Between 500 and 1,200 DAPI-stained bacterial cells were counted on each hybridized filter (magnifications, ×1,000 to 1,250). The fraction of cells stained with the respective fluorescent probe was enumerated from the same microscopic fields by switching between the two filter sets. We also inspected several unhybridized, DAPI-stained filter sections in order to check for other red fluorescent bacterium-sized particles (e.g., algal detritus). The reproducibility of the method was tested by repeated hybridization of sections from the same filter on different occasions. To determine the number of DAPI-stained cells that need to be enumerated for an accurate estimate of the hybridized bacterial fraction, we tested how the fraction of hybridized cells changed with increasing cell counts. Data from these counts were normalized by calculating

$$d = abs\left(\frac{x_{\mathrm{probe}}}{x_{\mathrm{DAPI}}} - \frac{y_{\mathrm{probe}}}{y_{\mathrm{DAPI}}}\right) \times 100\ [\%] \tag{1}$$

where d is the percent deviation of the hybridized fraction between x and y counted cells, x is the lower count of CY3-stained (x_{probe}) or DAPI-stained (x_{DAPI}) cells, and y is the higher count of CY3-stained (y_{probe}) or DAPI-stained (y_{DAPI}) cells. Differences between x_{DAPI} and y_{DAPI} were in the range of 40 to 120 counted cells.

To gain information about the staining efficiency of the method, hybridizations with the eubacterial probe EUB were performed for all dates and treatments.

Flagellate ingestion of different bacterial taxa. In order to estimate the fraction of protists with ingested α- and β-Proteobacteria, two additional series of filter sections from the treatment containing *B. saltans* were hybridized with probes ALF and BET as described above. Samples were stained with DAPI in order to locate protistan nuclei. At least 200 randomly selected flagellates per sample were inspected for CY3-stained bacteria inside food vacuoles. Care was taken to distinguish between ingested bacteria and cells attached to the surface of flagellates by switching between the two filter sets.

Estimation of bacterial growth rates. Bacterial growth rates in the first stage of the cultivation system were calculated as

$$\Delta = \frac{\ln N_1 - \ln N_0}{t_1 - t_0}\ [\mathrm{h}^{-1}] \tag{2}$$

$$\mu_{\mathrm{st1}} = \Delta + D_{\mathrm{st1}}\ [\mathrm{h}^{-1}] \tag{3}$$

where Δ is apparent growth from density changes between two consecutive samplings, N_O is the abundance of α- or β-Proteobacteria at time t_0 (bacteria per milliliter), N_1 is the abundance of α- or β-Proteobacteria at time t_1 (next sampling date) (bacteria per milliliter), μ_{st1} is the growth rate of α- or β-Proteobacteria in the first stage, and D_{st1} is the dilution rate of the first stage (per hour).

In the second-stage treatments we modified the above formula to include the various gain and loss processes.

$$M = \frac{\mathrm{TGR} + D_{\mathrm{st2}} \times N_{\mathrm{st2}} - D_{\mathrm{st2}} \times N_{\mathrm{st1}}}{N_{\mathrm{st2}}}\ [\mathrm{h}^{-1}] \tag{4}$$

$$\mu_{\mathrm{st2}} = \Delta + M\ [\mathrm{h}^{-1}] \tag{5}$$

where M is the total loss rate (mortality) of α- or β-Proteobacteria, D_{st2} is the dilution rate of the respective second stage (per hour), TGR is the flagellate total grazing rate on α- or β-Proteobacteria (bacteria per milliliter per hour) (in stage II/*Bodo*), N_{st2} is the abundance of α- or β-Proteobacteria in the second stage (bacteria per milliliter), N_{st1} is the abundance of α- or β-Proteobacteria, in the first stage (bacteria per milliliter), and μ_{st2} is the growth rate of α- or β-Proteobacteria in the second stage.

For the calculation of feeding rates on α- and β-Proteobacteria from FLB uptake rates, we assumed that *B. saltans* feeds unselectively on different bacterial taxa, i.e., according to their relative abundances in the system, if bacteria are within the edible size range. This assumption will be discussed later. Growth rate calculations for the second stages must be regarded as rough estimates only, as the studied system was not in steady state, so that simple chemostat theory cannot describe it exactly. In addition, we tried to calculate precision intervals, using, if possible, the known error ranges of the various methods and assuming multiplicative error propagation.

RESULTS

Bacterial and protistan abundances. Initial bacterial numbers were about 6×10^6 ml^{-1} in all treatments. After the introduction of *B. saltans*, a 50% drop in bacterial abundance was observed. This was accompanied by the appearance of

TABLE 1. Percentages of DAPI-stained bacteria detectable with probe EUB

Day	% Bacteria detected		
	Stage 1	Stage II	
		Control	*Bodo*
1	86.2	81.3	90.1
2	88.1	96.2	97.6
3	88.0	92.6	80.2
4	94.1	90.3	92.2
5	97.4	88.7	88.5
6	85.1	89.5	92.3
8	70.3	80.2	54.5
10	84.1	97.1	82.4
12	84.3	78.1	84.1
Mean	86.4	88.2	84.7

large, inedible filaments (10 to >100 μm), representing between 1.5 and 4% of total bacterial numbers. In the other treatments bacterial numbers increased to final values of between 10.9×10^6 (first stage) and 11.58×10^6 ml^{-1} (stage II/control), and no threadlike bacteria appeared. *B. saltans* increased from 0.25×10^5 to 1.64×10^5 cells ml^{-1} within 5 days after inoculation. Afterwards, abundances stagnated and decreased to 1.05×10^5 ml^{-1} at the last sampling date.

In situ hybridization and bacterial community composition, and protistan grazing. When more than 500 DAPI-stained cells were counted, the observed difference in the fraction of hybridized cells between two counts was <5%. In repeated hybridizations of the same filter sections the differences were between 1 and 14% (mean, 8.2%; $n = 10$). The detectability of bacteria in the cultivation system with probe EUB was usually between 78.1 and 97.6% of all DAPI-stained bacteria (Table 1). On one sampling date, however, only 70.3% of all cells stained with EUB in the first stage, and 54.5% stained in stage II/*Bodo*.

The abundances of α-Proteobacteria in stage II/*Bodo* increased almost threefold after the addition of the flagellate and then decreased to around 1.5×10^6 ml^{-1} and remained fairly stable during the following four days (Fig. 1a). Afterwards, a drop below the original densities was observed. β-Proteobacteria decreased by almost 1 order of magnitude within 2 days after inoculation with the predator and remained low (0.23×10^6 to 0.65×10^6 ml^{-1}) thereafter (Fig. 1a). Between 8.7 and 34% of β-Proteobacteria formed large threads of >10 μm in length (Fig. 4a and b).

Within 1 day, the total grazing rate of the *B. saltans* population increased from 0.34×10^6 to about 1×10^6 bacteria ml^{-1} h^{-1} on day 3 (maximum, 1.7×10^6 on day 8). Uptake rates ranged from 7.9 to 13.6 bacteria flagellate^{-1} h^{-1}. Figure 1b shows the estimated hourly standing stock loss of α- and β-Proteobacteria when strictly density-dependent grazing is assumed.

Hybridized bacteria inside flagellate food vacuoles were clearly visible, and their fluorescence was usually as bright as the staining of bacteria hybridized on membrane filters. The majority of flagellates were found with one or several ingested particles that hybridized with probe ALF (Fig. 4c and d). During the second half of the investigation, this fraction decreased from >90 to about 70% of the total *B. saltans* population. On the other hand, only 26% of flagellates contained on average one BET-stainable particle on the first day after predator addition, but during the following days, this proportion was only around 10 to 15% (Fig. 2).

In the first stage, the growth rates of α- and β-Proteobacteria were almost identical, reflecting the constant proportions between these two taxonomic groups in the cultivation system. Growth rates in the second-stage control vessel were usually around zero. In stage II/*Bodo* α-Proteobacteria showed signif-

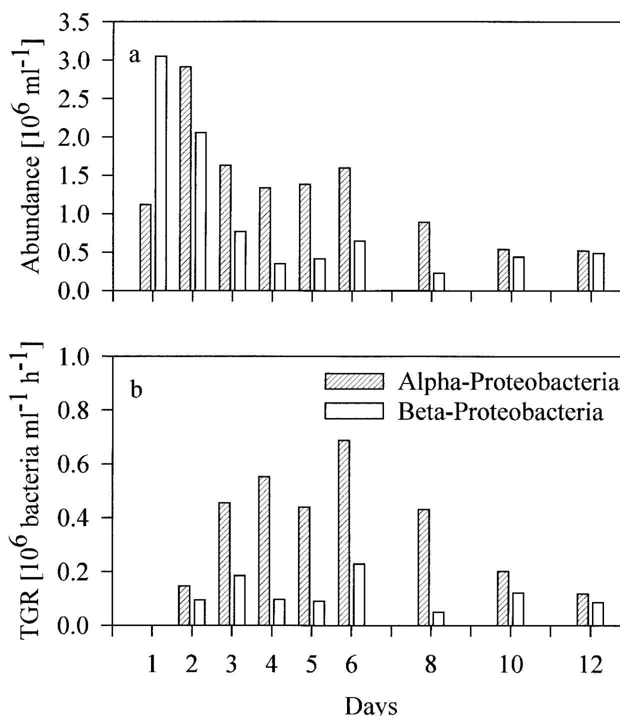

FIG. 1. (a) Abundance of α- and β-Proteobacteria in the continuous-culture system after the addition of *B. saltans* (on day 1). (b) Estimated total grazing rate (TGR) of the *B. saltans* population on α- and β-Proteobacteria, assuming predation to be strictly proportional to prey abundance.

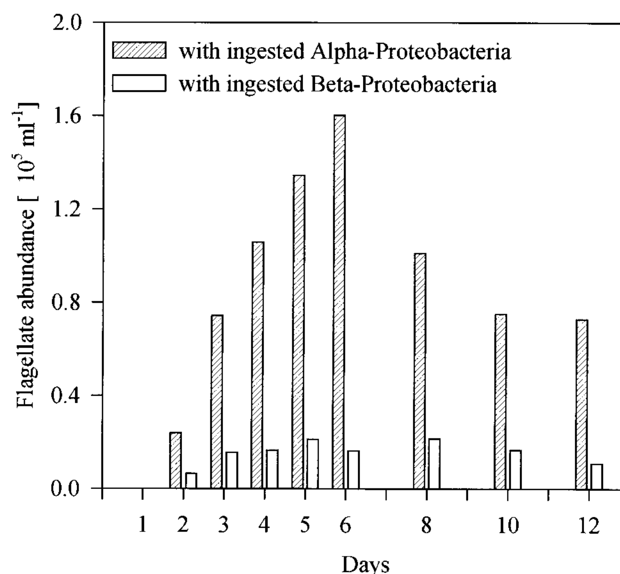

FIG. 2. Abundance of flagellates with at least one ingested cell from α- or β-Proteobacteria.

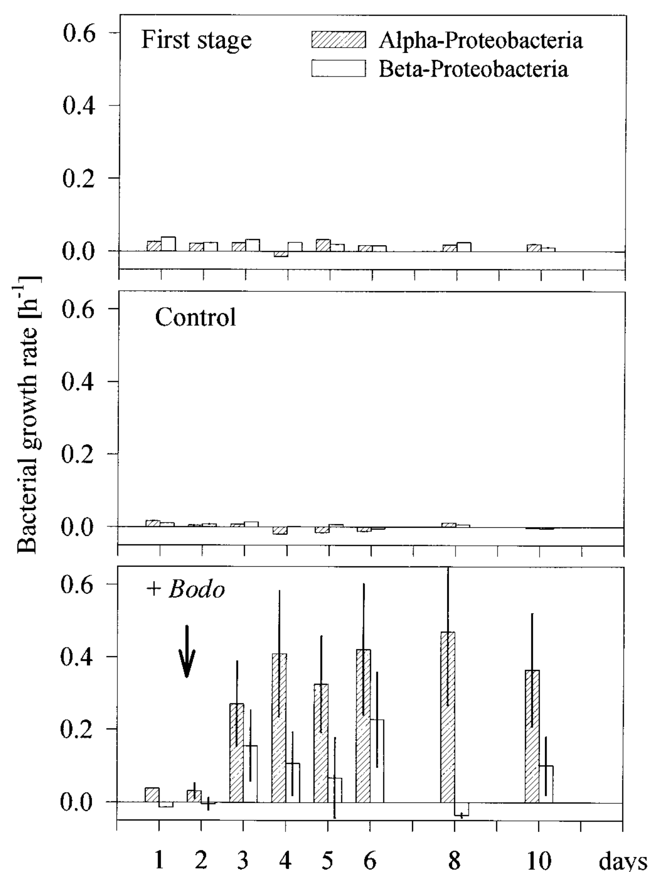

FIG. 3. Estimated growth rates of α- and β-Proteobacteria in the different treatments of the continuous-culture system. Error bars are error estimates derived from individual precision levels of the parameters used for growth rate calculations, assuming multiplicative error propagation.

icantly enhanced growth rates (Fig. 3). Growth rates of β-Proteobacteria after the addition of flagellates were clearly higher than in the second-stage control vessel. However, regarding the large error intervals of the calculation method, those rates cannot be distinguished from growth rates in the first stage in six out of eight cases (Fig. 3).

DISCUSSION

The growth rate estimates for the two bacterial subgroups (Fig. 3) are an attempt to quantify the counterintuitive observations that α-Proteobacteria were proportionally and absolutely increasing in the assemblage after inoculation with *B. saltans* (Fig. 1a) but at the same time were subject to much more intensive predation (Fig. 1b and 2). The estimated cell division rate of α-Proteobacteria increased by 1 to 2 orders of magnitude in the presence of the predator. This means that these cells went through 50 to 75 successive generations during the 12 days, whereas there was practically no growth in the parallel control vessel (Fig. 3). These calculations can be criticized for the assumption of density-dependent flagellate grazing on bacteria from the two subgroups. However, as over 80% of flagellates contained particles that hybridized with probe ALF (Fig. 4c and d), grazing on α-Proteobacteria must have been at least proportional to the abundance of cells from this taxonomic group. *B. saltans* showed high total grazing rates on fluorescently labelled prey and steeply increased in abundance during the first days of the experiment (36). We thus conclude that the ingested α-Proteobacteria were nutritionally adequate to support high flagellate growth. Our results agree with the conclusions of González et al. (15) that heterotrophic flagellates preferably feed on growing bacterial cells.

β-Proteobacteria, on the other hand, were ingested by a small fraction of the *B. saltans* population (Fig. 2) but strongly decreased in numbers. This can be explained by a low cell division rate of some strains within this group. About one-fifth of the β-Proteobacterial community was able to form long protist-inedible threads. Within the remaining 80%, at least one additional mechanism of grazing avoidance might have been present: we found about 15% of *B. saltans* cells (which corresponded to 2×10^4 flagellates) containing generally one particle that hybridized with BET. In the case of density-dependent flagellate feeding, the *B. saltans* population should have consumed almost 10 times as many β-Proteobacteria per hour, i.e., 1×10^5 to 1.5×10^5 ml^{-1} (Fig. 1b), which would imply a particle digestion time of 5 to 10 min. As this is much lower than published digestion rates of bacterivorous flagellates at comparable temperatures (14, 31), we suggest that flagellates were consuming β-Proteobacteria at a lower rate than in the case of random encounter foraging. A varying preference for different bacterial strains by *B. saltans* has been observed before (20).

Threadlike β-Proteobacteria constituted over 50% of total bacterial biomass in stage II/*Bodo* during the second half of the investigation (Fig. 4c and d; see reference 36). The rapid production of biomass by filamentous bacteria after the introduction of flagellates suggests that the presence of the predator stimulated a different type of physiological shift-up, i.e., cell elongation. It is remarkable that the formation of filaments from rod-shaped β-Proteobacteria occurred within 24 h after the addition of *B. saltans*, whereas no more increase in abundance or biomass of threadlike cells was observed during the remaining study period (36).

The sudden increase in bacterial cell size (36) or division rate may have been indirectly favored by protistan grazing through a recycling of nutrients (29). One possible explanation lies in the elemental composition of the respective organic material released by grazing. Bacteria contain more phosporus per unit carbon than algae (38), and Bloem et al. (5) found that bacterivorous flagellates greatly enhanced phosphorus remineralization in a comparable system. Phosphorus was the limiting factor for algal growth in our cultivation system (36), and bacterial growth was probably directly or indirectly limited by this nutrient, too.

Several studies describe a variety of bacterial physiological shift-ups induced by protist predation. The nitrifying activity of *Nitrosomonas europaea* was enhanced when it was selectively grazed upon by the flagellate *Adriamonas peritocrescens* (39). In a two-stage continuous cultivation system, bacterial [^{3}H]thymidine incorporation per cell was twofold higher during intense predation by the flagellate *Monas* sp. (5). Shikano et al. (33) observed cell elongation of an unknown bacterial strain into long threads in the presence of the bacterivorous ciliate *Cyclidium* sp. Grazing by heterotrophic flagellates enhanced bacterial enzymatic digestion of *Peridinium* cell walls in lakewater (30). Gurijala and Alexander (16) found that within a mixture of several different bacteria those species survived in the presence of a *Tetrahymena* sp. "that grew quickly before the onset of active predation," which agrees with the observed overshoot of α-Proteobacterial abundance in our culture system immediately after the addition of *B. saltans* (Fig. 1a). The rapid physiological responses of bacteria from both taxonomic groups to the presence of *B. saltans* raise the question of

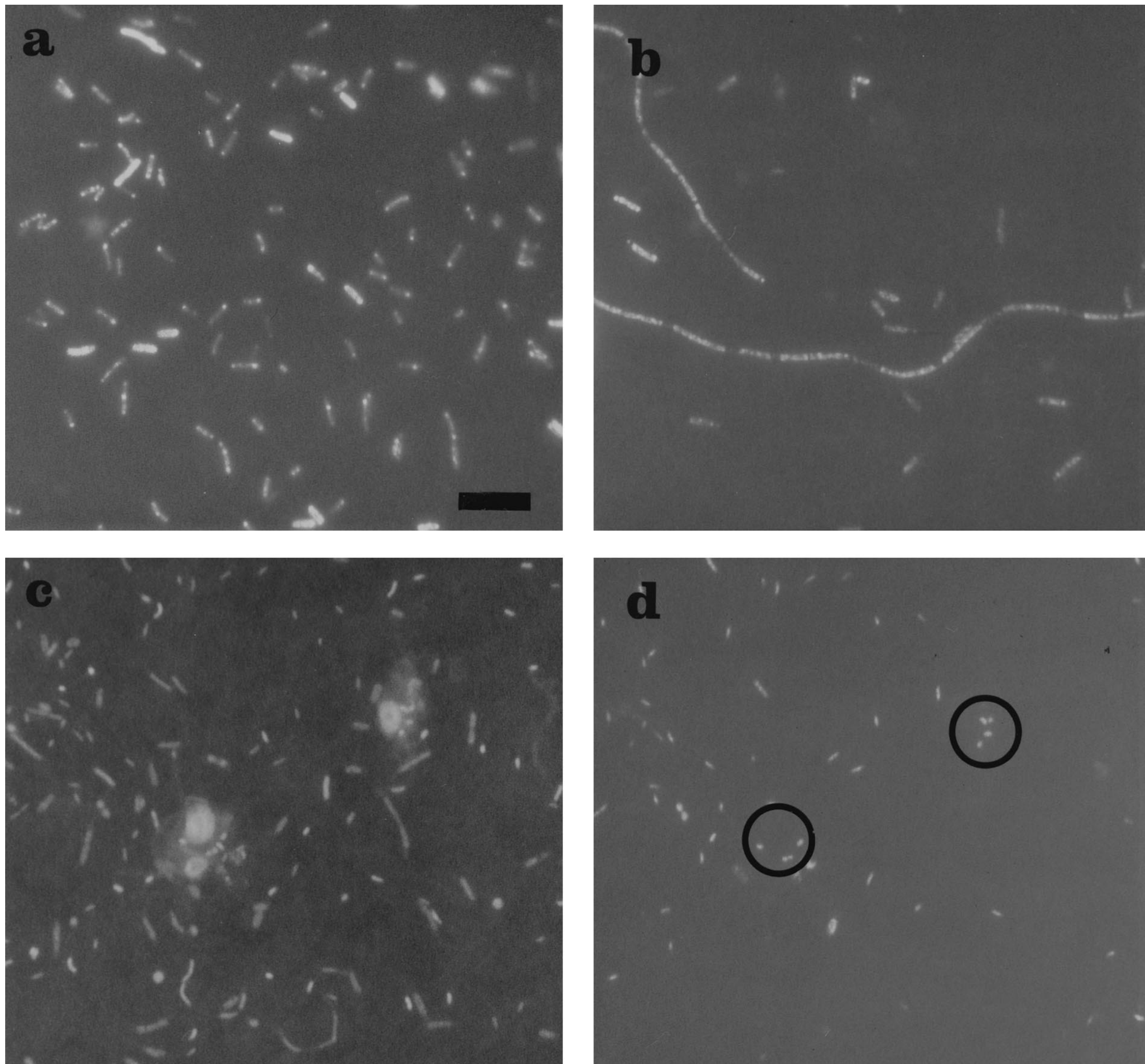

FIG. 4. Epifluorescence micrographs. (a) Bacteria hybridizing with probe BET before the addition of *B. saltans*; (b) threadlike β-Proteobacteria 24 h after inoculation with flagellates (green excitation; CY3 staining); (c and d) α-Proteobacteria ingested by *B. saltans* (encircled); (c) UV excitation (DAPI staining); (d) same microscopic field (green excitation; CY3 staining (hybridized cells). Magnification, ×1,250. Bar, 8 μm.

feedback mechanisms between predator and prey. We hypothesize that there might be one or several morphogenetic factors released by flagellates, stimulating, e.g., bacterial cell size increase or cell division. Such chemical cues are well-described for other planktonic predator-prey systems (9, 19, 37).

Our continuous-culture system does not resemble a pelagic microbial community in general but probably mimics a very distinct period during seasonal succession: it describes in a simplified way the simultaneous effects of substrate input and the sudden development of a predator population on a mixed bacterial community, which is, e.g., the case during the spring phytoplankton bloom in temperate lakes (41). A phenotypic community shift within a natural bacterioplankton assemblage during the spring phytoplankton bloom caused by flagellate predation has been described recently (24), and such shifts could also be artificially induced via food web manipulation inside limnocorrals (18). The appearance of filamentous bacteria in response to predation (17) might thus reflect the rise of specific genotypes of β-Proteobacteria also within natural planktonic communities. On the other hand, some species within the bacterioplankton can be expected to adopt a "high division rate strategy" as a response to grazing pressure (Fig. 3), as postulated by Simek et al. (36). The observed physiological strategies, together with other ones, such as cell size reduction (21, 35), toxicity, or motility (15, 21), increase the complexity of possible ecological relationships between different bacterial populations and their predators in natural planktonic communities.

In situ hybridization of bacteria inside protistan food vacuoles offers a new possibility for analyzing predator effects on the composition of mixed bacterial assemblages. This additional information may be limited, but on the other hand, no prior manipulation of either predator or prey is required. This method may allow much more detailed studies, e.g., about the fate of artificially introduced, pathogenic, or genetically engineered microorganisms in aquatic ecosystems. In addition, liv-

ing bacteria detectable by specific oligonucleotide probes could be added in tracer amounts to mixed bacterial assemblages for short-time uptake experiments, as described previously for fluorescently labelled prey (32). Fluorescent oligonucleotide probes with higher taxonomic specificity are, however, required for future studies on predator-prey interactions in mixed bacterial assemblages.

ACKNOWLEDGMENTS

We thank P. Hartman for assistance during sampling and A. Alfreider, M. Macek, and R. Sommaruga for helpful comments on the manuscript. Protistan cultures were kindly provided by H. Müller and D. Springmann.

J.P. was financed by a grant from the Fonds zur Förderung der wissenschaftlichen Forschung (Project P-10184-MOB awarded to R.P.). The study was also supported by a GA CR research grant (206/960012) awarded to K.Š. and by an AC CR grant, "Biodiversity" no. 21/96/K.

REFERENCES

1. **Alfreider, A., J. Pernthaler, R. Amann, B. Sattler, F.-O. Glöckner, A. Ville, and R. Psenner.** 1996. Community analysis of the bacterial assemblages in the winter cover and pelagic layers of a high mountain lake using in situ hybridization. Appl. Environ. Microbiol. **62:**2138–2144.
2. **Amann, R. I., L. Krumholz, and D. A. Stahl.** 1990. Fluorescent oligonucleotide probing of whole cells for determinative, phylogenetic and environmental studies in microbiology. J. Bacteriol. **172:**762–770.
3. **Amann, R. I., W. Ludwig, and K. H. Schleifer.** 1995. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol. Rev. **59:**143–169.
4. **Amann, R. I., N. Springer, W. Ludwig, H.-D. Görz, and K. H. Schleifer.** 1991. Identification in situ and phylogeny of uncultured bacterial endosymbionts. Nature **351:**161–164.
5. **Bloem, J., M. Starink, M.-J. B. Bär-Gilisen, and T. E. Cappenberg.** 1988. Protozoan grazing, bacterial activity, and mineralization in a two-stage continuous culture. Appl. Environ. Microbiol. **54:**3113–3121.
6. **Cole, J. J., G. E. Likens, and D. L. Strayer.** 1982. Photosynthetically produced dissolved organic carbon: an important carbon source for planktonic bacteria. Limnol. Oceanogr. **27:**1080–1090.
7. **del Giorgio, P. A., and G. Scarborough.** 1995. Increase in the proportion of metabolically active bacteria along gradients of enrichment in freshwater and marine plankton: implications for estimates of bacterial growth and production rates. J. Plankton Res. **17:**1905–1924.
8. **DeLong, E. F., D. G. Franks, and A. L. Allredge.** 1993. Phylogenetic diversity of aggregate-attached vs. free-living marine bacterial assemblages. Limnol. Oceanogr. **38:**924–934.
9. **Dodson, S.** 1988. The ecological role of chemical stimuli for the zooplankton: predator-avoidance behaviour in *Daphnia*. Limnol. Oceanogr. **33:**1431–1439.
10. **Dufour, P., J.-P. Torreton, and M. Colon.** 1990. Advantages of distinguishing the active fraction in bacterioplankton: some examples. Hydrobiologia **207:** 295–301.
11. **Fuhrman, J. A., and F. Azam.** 1980. Bacterioplankton secondary production estimates for coastal waters of British Columbia, Antarctica, and California. Appl. Environ. Microbiol. **39:**1085–1095.
12. **Giovannoni, S. J., T. B. Britschgi, C. L. Moyer, and K. G. Field.** 1990. Genetic diversity in Sargasso Sea bacterioplankton. Nature **345:**60–63.
13. **Glöckner, F. O., R. Amann, A. Alfreider, J. Pernthaler, R. Psenner, K. H. Trebesius, and K. H. Schleifer.** 1996. An in situ hybridization protocol for detection and identification of planktonic bacteria. Syst. Appl. Microbiol. **19:**403–406.
14. **González, J. M., J. Iriberri, L. Egea, and I. Barcina.** 1990. Differential rates of digestion of bacteria by freshwater and marine phagotrophic protozoa. Appl. Environ. Microbiol. **56:**1851–1857.
15. **González, J. M., E. B. Sherr, and B. F. Sherr.** 1993. Differential feeding by marine flagellates on growing versus starving, and on motile versus nonmotile, bacterial prey. Mar. Ecol. Prog. Ser. **102:**257–267.
16. **Gurijala, K. R., and M. Alexander.** 1990. Effect of growth and hydrophobicity on bacteria surviving protozoan grazing. Appl. Environ. Microbiol. **56:** 1631–1635.
17. **Jürgens, K., and H. Güde.** 1994. The potential impact of grazing-resistant bacteria in planktonic systems. Mar. Ecol. Prog. Ser. **112:**169–188.
18. **Jürgens, K., H. Arndt, and K. O. Rothhaupt.** 1994. Zooplankton-mediated changes of bacterial community structure. Microb. Ecol. **27:**27–42.
19. **Kuhlmann, H. W., and K. Heckmann.** 1985. Interspecific morphogens regulating prey-predator relationships in protozoa. Science **227:**1347–1349.
20. **Mitchell, G. C., J. H. Baker, and M. A. Sleigh.** 1988. Feeding of a freshwater flagellate, *Bodo saltans*, on diverse bacteria. J. Protozool. **35:**219–222.
21. **Monger, B. C., and M. R. Landry.** 1992. Size-selective grazing by heterotrophic nanoflagellates: an analysis using live-stained bacteria and dual-beam flow cytometry. Arch. Hydrobiol. Beih. Ergebn. Limnol. **37:**173–185.
22. **Pace, M. L., and J. J. Cole.** 1994. Comparative and experimental approaches to top-down and bottom-up regulation of bacteria. Microb. Ecol. **28:**181–193.
23. **Pedrós-Alió, C., and R. Guerrero.** 1994. Protokaryology for the limnologist. *In* R. Margalef (ed.), Limnology now: a paradigm of planetary problems. Elsevier Science B.V., Amsterdam, The Netherlands.
24. **Pernthaler, J., B. Sattler, K. Šimek, A. Schwarzenbacher, and R. Psenner.** 1996. Top-down effects on the size-biomass distribution of a freshwater bacterioplankton community. Aquat. Microb. Ecol. **10:**255–263.
25. **Porter, K. G., and Y. S. Feig.** 1980. The use of DAPI for identifying and counting aquatic microflora. Limnol. Oceanogr. **25:**943–948.
26. **Proctor, L. M., and J. A. Fuhrman.** 1992. Mortality of marine bacteria in response to enrichments of the virus-size fraction from seawater. Mar. Ecol. Prog. Ser. **87:**283–293.
27. **Psenner, R., and R. Sommaruga.** 1992. Are rapid changes in bacterial biomass caused by shifts from top-down to bottom-up control? Limnol. Oceanogr. **37:**1092–1100.
28. **Sanders, R. W., D. A. Caron, and U.-G. Berninger.** 1992. Relationship between bacteria and heterotrophic nanoplankton in marine and fresh waters: an inter-ecosystem comparison. Mar. Ecol. Prog. Ser. **86:**1–14.
29. **Sherr, B. F., E. B. Sherr, and T. Berman.** 1982. Decomposition of organic detritus: a selective role for microflagellate protozoa. Limnol. Oceanogr. **27:**765–769.
30. **Sherr, B. F., E. B. Sherr, and J. McDaniels.** 1992. Effect of protistan grazing on the frequency of dividing cells in bacterioplankton assemblages. Appl. Environ. Microbiol. **58:**2381–2385.
31. **Sherr, B. F., E. B. Sherr, and F. Rassoulzadegan.** 1988. Rates of digestion of bacteria by marine phagotrophic bacteria: temperature dependence. Appl. Environ. Microbiol. **54:**1091–1095.
32. **Sherr, E. B., and B. F. Sherr.** 1993. Protistan grazing rates via uptake of fluorescently labelled prey, p. 695–701. *In* P. Kemp, B. F. Sherr, E. B. Sherr, and J. Cole (ed.), Handbook of methods in aquatic microbial ecology. Lewis Publishers, Boca Raton, Fla.
33. **Shikano, S., L. S. Luckinbill, and Y. Kurihara.** 1990. Changes of traits in a bacterial population associated with protozoal predation. Microb. Ecol. **20:** 75–84.
34. **Šimek, K., J. Bobková, M. Macek, J. Nedoma, and R. Psenner.** 1995. Ciliate grazing on picoplankton in a eutrophic reservoir during the summer phytoplankton maximum: a study at the species and community level. Limnol. Oceanogr. **40:**1077–1090.
35. **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.
36. **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.
37. **Stemberger, R. S., and J. J. Gilbert.** 1984. Spine development in the rotifer *Keratella cochlearis*: induction by cyclopoid copepods and *Asplanchna*. Freshwater Biol. **14:**639–647.
38. **Vadstein, O., and A. Jensen.** 1988. Growth and phosphorus status of limnetic phytoplankton and bacteria. Limnol. Oceanogr. **33:**489–503.
39. **Verhagen, F. J. M., and H. J. Laanbroek.** 1992. Effects of grazing by flagellates on competition for ammonium between nitrifying and heterotrophic bacteria in a chemostat. Appl. Environ. Microbiol. **58:**1962–1969.
40. **Vrba, J., K. Šimek, J. Pernthaler, and R. Psenner.** 1996. Evaluation of extracellular, high-affinity beta-*N*-acetylglucosaminidase measurements from freshwater lakes: an enzyme assay to estimate protistan grazing on bacteria and picocyanobacteria. Microb. Ecol. **32:**81–99.
41. **Weisse, T., H. Müller, R. M. Pinto-Coelho, A. Schweizer, D. Springman, and G. Baldringer.** 1990. Response of the microbial loop to the phytoplankton spring bloom in a large prealpine lake. Limnol. Oceanogr. **35:**781–794.