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MiniReview

The ecology of *Cytophaga–Flavobacteria* in aquatic environments

David L. Kirchman *

College of Marine Studies, University of Delaware, Lewes, DE 19958, USA

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1. Introduction

Culture-dependent and -independent studies have found that prokaryotic assemblages are quite diverse in aquatic habitats and contain representatives of virtually all of the roughly 40 divisions of bacteria and the major archaeal groups found so far in the biosphere [1]. Fortunately, not all of these prokaryotic groups are abundant in the plankton nor important in all biogeochemical cycles. Autotrophic and heterotrophic bacteria dominate the prokaryotic biomass in surface waters, as Archaea appear to be abundant only in the plankton of the deep oceans [2]. Among the heterotrophic bacteria, the two most abundant groups are often the *Proteobacteria* and the subject of this review, the *Cytophaga–Flavobacteria* cluster (Table 1).

This paper reviews recent studies that have applied molecular methods to examine uncultured *Cytophaga–Flavobacteria* in freshwaters and the oceans, with the ultimate goal of using this information to better understand the role of heterotrophic bacteria in carbon cycles and other biogeochemical processes. The importance of heterotrophic bacteria in biogeochemical processes is now well appreciated, but until recently geochemists and field-orientated microbial ecologists considered these microbes as if they were a single group, even though microbiologists have been accumulating for several years information about the taxonomic and phylogenetic make up ('community structure') of heterotrophic bacterial communities. Only recently, however, have microbial ecologists been able to link community structure with specific biogeochemical processes ('function') [3]. Here I summarize briefly our progress in these areas while discussing what we know about *Cytophaga–Flavobacteria* in aquatic habitats. This microbial group is a natural starting point because of its high abundance in many freshwater and marine systems.

2. Taxonomy of *Cytophaga–Flavobacteria*

The *Cytophaga–Flavobacteria* cluster belongs to a diverse bacterial division that has been labeled differently over the years. Hugenholtz et al. [1] and other authors have used '*Cytophagales*' while Hamana and Nakagawa [4] recently stated that the division name was '*Flavobacterium–Flexibacter–Bacteriodes–Cytophaga*'. The latter name does give some indication of the diversity of this division and highlights some of the major genera (Fig. 1). The label used in the most recent edition of Bergey's Manual of Systematic Bacteriology [5] is simply '*Bacteroidetes*', which will be used in this review. A more common label, however, is *Cytophaga–Flavobacteria–Bacteriodes* (or *Flavobacterium–Bacteriodes–Cytophaga* and other permutations), similar to the label (*Cytophaga–Flexibacter–Bacteriodes*) originally used by Woese and colleagues [6]. Other genera, which are prominent because of their association with humans, include *Prevotella* and *Porphyromonas* [7].

The *Cytophaga–Flavobacteria* cluster can be characterized by both phenotypic traits and 16S rRNA sequence data. As first described by Winogradsky, *Cytophaga*-like bacteria are unicellular, gliding, nonspore-forming Gram-negative rods, although shape can vary [8]. Colonies of many *Cytophaga*-like bacteria are pigmented due to flexirubin-type pigments found only in these bacteria and flavobacteria. Most notably, *Cytophaga–Flavobacteria* are chemoorganotrophic and are especially proficient in degrading various biopolymers such as cellulose, chitin, and pectin [8] (see also references in [9]). Another distinguishing feature is that *Cytophaga–Flavobacteria* are

* Tel.: +1 (302) 645 4375; Fax: +1 (302) 645 4028.
E-mail address: kirchman@udel.edu (D.L. Kirchman).

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mainly aerobic, although some anaerobic strains are currently classified as *Cytophaga* [8]. In contrast, the bacteria within the *Bacteroides* subgroup are all anaerobes and are found most notably in the microflora of the human colon, making up 30% of total fecal isolates (see references in [9]).

In his early review of bacterial systematics, Woese [10] did not use the term '*Cytophaga–Flavobacteria* cluster', but he did divide 19 16S rRNA sequences from *Bacteroidetes* bacteria into two clusters, the *Bacteroides* and the *Flavobacterium* subdivisions. The number of *Bacteroidetes* sequences has increased by over 10-fold since Woese's review, so there are now several other groups in the *Bacteroidetes* division, but most of the *Cytophaga* and *Flavobacteria* species still cluster together (Fig. 1). However, one sign of problems in *Bacteroidetes* systematics is that the genus name *Cytophaga* is scattered throughout the entire *Bacteroidetes* phylogenetic tree, as illustrated by the tree presented recently by Weller et al. [7]. It seems that the call by Nakagaw and Yamasato [11] to divide *Cytophaga* into several genera and higher taxa has not been fully heeded.

The large diversity in rRNA sequences complicates several molecular methods for examining members of *Bacteroidetes* and other bacterial groups, but its impact on one method, fluorescence in situ hybridization (FISH) with oligonucleotide probes, is critical for this review. By far the most widely used FISH probe for *Cytophaga–Flavobacteria* is CF319a [9]. Although often termed a probe for *Cytophaga–Flavobacteria*, CF319a also hybridizes to some members of the *Bacteroides–Prevotella* cluster [7]. What is also noteworthy is that CF319a does not recognize several *Cytophaga–Flavobacteria* [7], implying that studies using this probe have underestimated *Cytophaga–Flavobacteria* abundance. One strain not recognized by the CF319a probe is *Cytophaga hutchinsonii*; the genome of this bacterium is now being sequenced by the Joint Genomic Institute (http://www.jgi.doe.gov/). Even though CF319a misses several *Bacteroidetes* genera, it remains the most general probe for the *Bacteroidetes* division [7]. Rather than replacements for CF319a, new probes are needed to examine clusters within the *Bacteroidetes* division, including those recognized by the CF319a probe. Weller et al. [7] suggested some new probes, but these need to be applied to environmental samples. Eilers et al. [12] used new probes to count culturable *Cytophaga–Flavobacteria* in the North Sea; the FISH data indicated that these strains were about 6% of total prokaryote abundance (DAPI counts) in April through September, which is a large fraction for a single bacterial lineage. Weller et al. [7] mentioned that they were unable to design habitat-specific probes that recognize bacteria found uniquely in freshwaters, the oceans, or soils.

3. The abundance of *Cytophaga–Flavobacteria* in nature, clone libraries and data bases

Bacteria in the *Bacteroidetes* division can be found in just about every habitat in the biosphere, including kusaya [13] (a Japanese delicacy consisting of putrid fish), rumens [14], hydrothermal vents [15], rocks and sea-ice in Antarctica [16], and sediments of lakes and the oceans [17]. *Cytophaga–Flavobacteria* seem particularly common in the oceans, according to FISH. In fact, in many oceanic habitats, the *Cytophaga–Flavobacteria* cluster is the most abundant of all bacterial groups (Fig. 2), accounting for as much as half of all bacteria potentially identified by FISH. Generally, β-proteobacteria are the most abundant

Table 1
The relative abundance of the major heterotrophic bacterial groups in aquatic ecosystems, as determined by FISH with oligonucleotide probes

System	Location	% of Total Prokaryotes[a]					
		Bacteria[b]	α	β	γ	CF	Reference
Lakes	Alpine lake	55	21	24	<2	4	[71]
	Lago di Cadagno	46	0	21	0	3	[72]
	Grosser Ostersee	46	3	14	1	2	[72]
	Lake Baikal	44	1	4	0	4	[72]
	Lake Constance aggregates (50 m)	50–70	10–20	40–60	<5	<5	[38]
Rivers	River Ter	50–90	5–7	10–33	1–4	20–65	[20]
	Elbe River detritus	75	5–10	50	30	10–30	[37]
	Spittelwasser biofilm	77	12–22	20–50	<5	<10	[73]
Oceans	Several oceanic sites	39–96	4–14	0–4	0–9	2–72	[72]
	Southern Ocean	60–95	5–10	0	<5–10	25–70	[74]
	North Sea	31–71	3–25	0	5–9	<1–30	[27]
	California coast	54–88	1–34	0–3	7–35	22–38	[24]
	Delaware coast	80	28	5	10	14	[47]
	Wadden Sea sediments	40–73	1.5–3.5	1.0–4.1	0.8–3.6	7.5–18	[17]

The table includes only studies using probes for at least three proteobacterial subdivisions (alpha, beta and gamma) and for the *Cytophaga–Flavobacteria* cluster (CF). Ranges are given when the study reports several values taken over time or within a habitat. When more than depth was sampled, the surface value is reported unless noted otherwise.

[a]All DAPI-positive microbes, probably nearly all bacteria with a few Archaea and fewer (to none) Eukarya.

[b]Percent of DAPI-positive microbes that are recognized by the Eub338 probe.

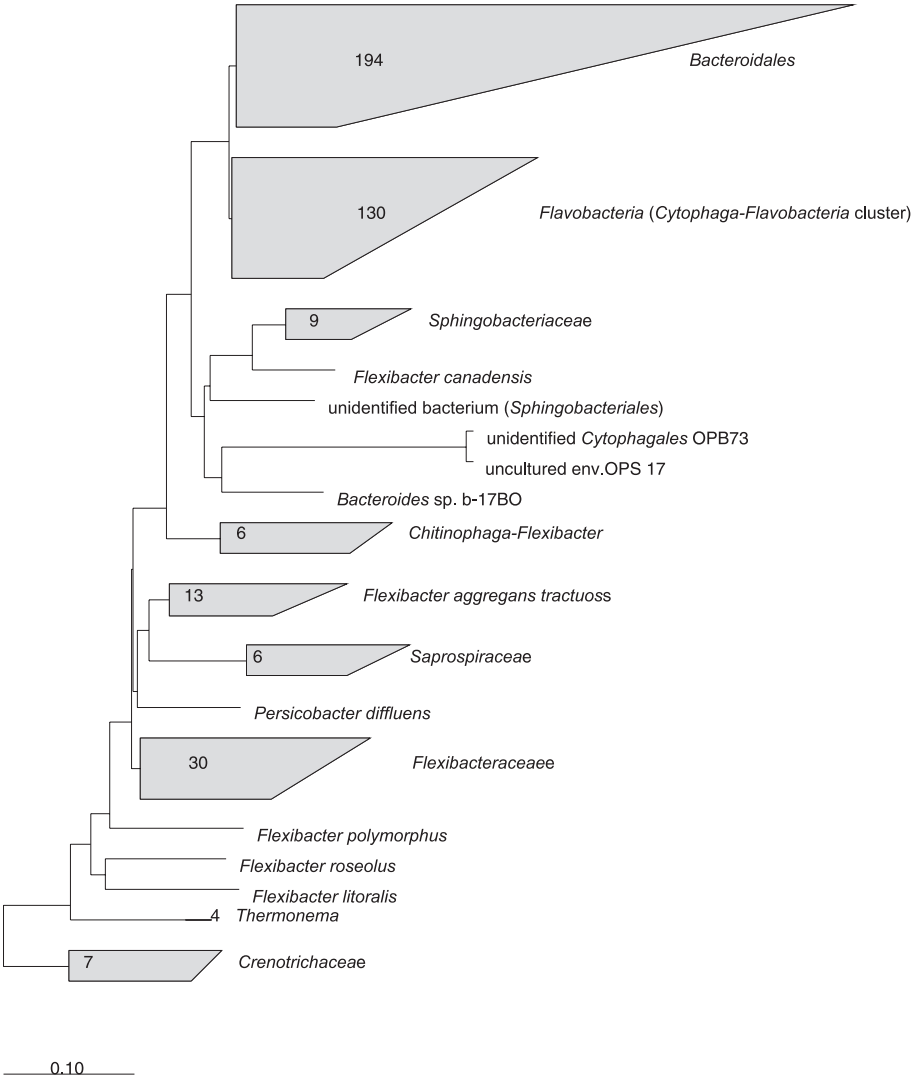

Fig. 1. A phylogenetic tree of the *Bacteroidetes* division, illustrating the *Cytophaga–Flavobacteria* cluster, based on nearly complete 16S rRNA sequences. The tree was constructed using ARB and the latest data set (May 2001) available at http://www.arb-home.de/. The numbers refer to the number of strains grouped together.

group in freshwater systems (Table 1), although *Cytophaga–Flavobacteria* can dominate mature freshwater biofilms [18], and they were abundant in two Japanese rivers [19] and a river-reservoir system in Spain [20]. *Cytophaga–Flavobacteria* can be abundant in sediments [21], and in one study of Wadden Sea sediments, CF319a-positive bacteria were the most abundant group even in anoxic layers [17].

In contrast to the FISH results, few *Cytophaga–Flavobacteria* sequences have been found by most studies of clone libraries of 16S rRNA genes from free-living bacterial assemblages. In one review of these studies, Giovonnoni and Rappé [22] noted that clone libraries from marine systems were dominated by the following heterotrophs, in descending order: three groups of α-proteobacteria (SAR11, *Roseobacter*, and SAR116); a γ-proteobacterial group (SAR86); and *Actinobacteria*. The *Cytophaga–Flavobacteria* cluster was not in this list. Several *Cytophaga–Flavobacteria* sequences have been retrieved from particulate detritus communities [23] but few from free-living microbial assemblages, which usually make up $>90\%$ of total microbial abundance in the oceans.

There are two possible explanations for the difference between the FISH and clone library results. One is simply that all clone libraries to date have been constructed from sites or at times when *Cytophaga–Flavobacteria* were relatively sparse. After all, bacterial community structure varies greatly among habitats and over time. Another explanation is that clone-library construction is biased against the *Cytophaga–Flavobacteria* cluster. This latter explanation appears to the case.

Two studies have compared FISH and clone libraries of 16S rRNA genes from the same water sample. FISH results indicated that the *Cytophaga–Flavobacteria* cluster was the most abundant bacterial group along a transect off the coast of California [24], although a few stations had large numbers of α-proteobacteria. In contrast to

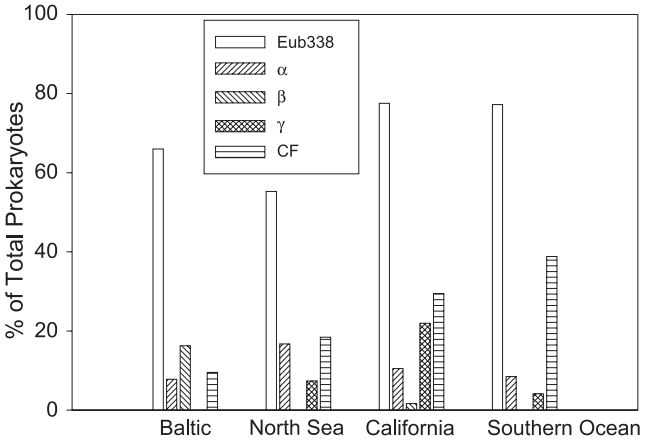

Fig. 2. Summary of FISH results for the Baltic [72], North Sea [27], California [24], and the Antarctic circumpolar current in the Southern Ocean [74]. The Greek letters indicate the subdivisions of proteobacteria and 'CF' is *Cytophaga–Flavobacteria*. See Table 1 for other environments. The error on FISH data is on the order of 20%.

the FISH results, libraries from two sites of this transect were dominated by 16S rRNA genes from α-proteobacteria, and only five of 169 clones were from *Cytophaga–Flavobacteria*. Similar to other studies [22], the α-proteobacterial group SAR 11 was abundant in one of the libraries (12% of all clones), but SAR 11-like bacteria were $<1\%$ of total bacterial abundance according to FISH. Clones from the γ-proteobacterial group SAR 86, which has attracted much attention recently [25,26], were also abundant in the libraries (about 10%), but these bacteria averaged only 2% of total abundance according to FISH. Eilers et al. [27] also found discrepancies between FISH and clone-library results. Specifically, these authors did not find any 16S rRNA *Cytophaga–Flavobacteria* clones in a library of 54 clones from a North Sea sample, yet this bacterial group was the most abundant one as identified by FISH. In short, the *Cytophaga–Flavobacteria* cluster is under-represented and the α-proteobacteria over-represented in clone libraries, compared with the FISH data (Fig. 3).

Of course, FISH is not perfect [28]. One problem is that a relatively large fraction (ca. 50%) of the prokaryotic cells sometimes cannot be probed with any oligonucleotide probe, including the Eub338 probe, which should recognize all bacteria (but see reference [29]). However, Cottrell and Kirchman [24] argued that, for their samples, the composition of clone libraries would still overestimate the α-proteobacterial abundance as determined by FISH, even if all bacteria not detected by FISH were α-proteobacteria. Nonspecific hybridization with CF319a to proteobacteria, which would lead to overestimating *Cytophaga–Flavobacteria* abundance, has not been observed to date (R. Amann, pers. comm.). On the contrary, it seems likely that CF319a misses members of the *Cytophaga–Flavobacteria* cluster (see above), thus leading to underestimates of their numbers.

The PCR step is an obvious place to look for biases against the *Cytophaga–Flavobacteria* cluster. Suzuki et al. [30] pointed to the mismatches between EubA (the reverse primer used by Cottrell and Kirchman [24,47]) and the 16S rRNA sequences from their *Bacteroidetes* clones. However, these mismatches seem insufficient to explain the discrepancy, because clone libraries made with other primer pairs also have few *Cytophaga–Flavobacteria* 16S rRNA genes [24]. For example, a library of 190 clones made with primers 27F and LSU1933R had only three *Bacteroidetes* clones compared with 67 from α-proteobacteria [30]. The library of Eilers et al. [27] made with primers 8f and 1542r had four α-proteobacterial clones (and 49 from the γ-proteobacterial subdivision) but none from the *Bacteroidetes* division. It seems unlikely that primer mismatches explain the paucity of *Bacteroidetes* clones in all of these libraries. Biases other than primer mismatches need to be considered.

Regardless of the explanation, relatively few 16S rRNA sequences from the *Cytophaga–Flavobacteria* cluster are in the databases. According to the RDP database (http://www.cme.msu.edu/rdp; June 2000 release), the entire *Bacteroidetes* division is represented by 781 sequences or about 5% of all 16S rRNA sequences, almost an order of magnitude less than the nearly 7000 sequences from the *Proteobacteria* division. The discrepancy is even larger when sequences of only uncultured bacteria are considered. A search of GenBank (May 2001) turned up 93 16S rRNA gene sequences when 'Cytophaga' and 'uncultured' were used as keywords. An analogous search for uncultured proteobacterial sequences yielded over 1700 entries. Glöckner et al. [31] reported new *Cytophaga–Flavobacteria* 16S rRNA sequences from several freshwater habitats, but more sequence data are still needed.

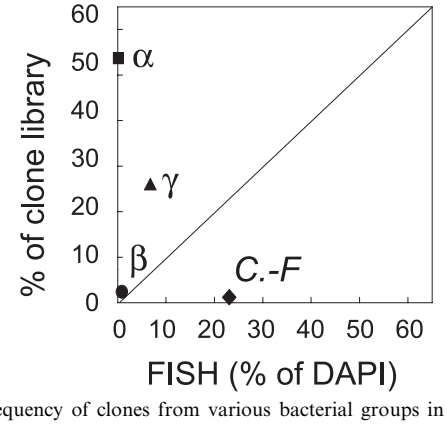

Fig. 3. Frequency of clones from various bacterial groups in libraries of 16S rRNA genes compared with their relative abundance as estimated by FISH in California coastal waters. The Greek letters indicate the subdivisions of proteobacteria and 'CF' is *Cytophaga–Flavobacteria*. Data from Cottrell and Kirchman [24].

4. Degradation of organic material by *Cytophaga–Flavobacteria*

Heterotrophic bacteria are the dominant organisms in aquatic habitats using dissolved organic material (DOM), which is one of the largest pools of organic carbon in the biosphere, and thus is critical in carbon budgets and cycles. Uptake of DOM is the first step in the microbial loop that ultimately mineralizes about 50% of primary production [32]. Microbial ecologists interested in carbon cycling would like to know the role of various bacterial groups in taking up DOM. The *Cytophaga–Flavobacteria* cluster is one obvious starting point because of its high abundance, but also because these bacteria may have a specialized role in DOM uptake and degradation. Cultured isolates of *Cytophaga–Flavobacteria* are well known to be proficient in degrading biopolymers such as cellulose and chitin, part of the high molecular mass (HMW) fraction of DOM. This DOM fraction is being intensively examined by organic geochemists because concentrations and fluxes of HMW DOM are high, especially in the oceans [33].

Two lines of evidence indicate that *Cytophaga–Flavobacteria* have a special role in using HMW DOM in aquatic environments, not just in laboratory cultures. First, several studies have demonstrated that *Cytophaga–Flavobacteria* are enriched on particulate organic detritus in marine habitats [23,34–36]. In contrast, detritus in the Elbe River [37] and in Lake Constance [38] was always dominated by β-proteobacteria, although *Cytophaga–Flavobacteria* became more abundant over time on artificial organic aggregates made from riverine as well as marine DOM [39,40]. One major phenotypic property of *Cytophaga–Flavobacteria*, use of HMW organic material, would help explain the success of these bacteria on both particulate detritus and in free-living assemblages.

It could be argued that the high abundance of *Cytophaga–Flavobacteria* in the free-living assemblage is not due to use of HMW organic material (or any DOM component) but rather the release of these bacteria from detritus-associated communities. However, concentrations and fluxes of particulate detritus are usually too small, much less than those of DOM in most aquatic habitats, to support the large, detritus-free *Cytophaga–Flavobacteria* biomass observed at least in the oceans (Table 1). Detritus-associated microbial communities certainly release *Cytophaga–Flavobacteria*, but these cells must be able to survive in the free-living community in order to result in their high abundance. Similarly, the observation that similar ribotypes of *Cytophaga–Flavobacteria* are found free-living and detritus-associated communities [41] can be explained by use of organic compounds available to both communities, that is, HMW organic material, rather than exchange between these two communities.

The detritus examined by previous studies was either macroscopic material collected by scuba divers or suspended material caught on filters with large pore sizes. Little is known about the microbes associated specifically with other forms of detritus, such as gels [42], transparent polymers [43] and submicron particles [44]. The boundary between the free-living community and microbes associated with these detrital forms is probably not sharp.

The second line of evidence for HMW DOM use by uncultured *Cytophaga–Flavobacteria* comes from a new approach that combines microautoradiography and FISH [45,46]. Using a version of this approach ('Micro-FISH'), Cottrell and Kirchman [47] examined uptake of protein and chitin and monomers of these two biopolymers by bacterial assemblages in coastal waters of Delaware (USA). Bacteria belonging to the *Cytophaga–Flavobacteria* cluster were the most abundant microbes using protein and chitin (Fig. 4 illustrates chitin use), but surprisingly few of these bacteria were among the microbes using amino acids, although a large fraction using *N*-acetylglucosamine were also *Cytophaga–Flavobacteria*. The Micro-FISH data suggest that amino acid uptake was dominated by α-proteobacteria in these coastal waters. Consistent with these results, Schweitzer et al. [38] found net amino acid uptake when lake snow aggregates were dominated by α-proteobacteria, but net release of amino acids when α-proteobacteria declined and the abundance of *Cytophaga–Flavobacteria* and β-proteobacteria increased.

These data do not indicate that *Cytophaga–Flavobacteria* are incapable of using amino acids and other monomers. Since compounds larger than ca. 500 Da cannot be directly transported across cell membranes, even biopolymer-degrading bacteria need to be able to take up monomers or small oligomers resulting from the hydrolysis of HMW DOM. However, biopolymer-degrading bacteria may be less proficient than other bacteria in using low molecular mass compounds. Conceivably, local concentra-

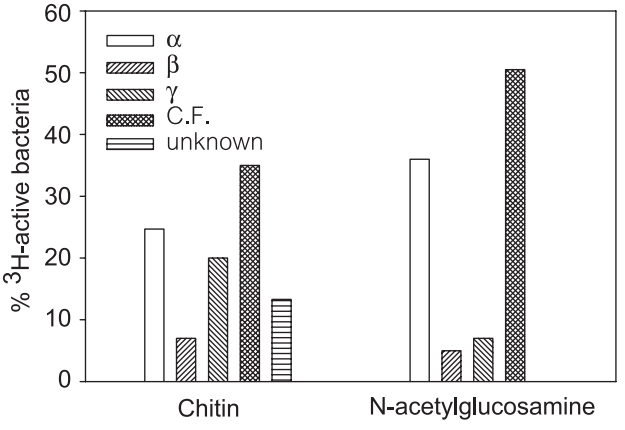

Fig. 4. Uptake of chitin and *N*-acetylglucosamine by bacterial groups in Delaware coastal waters, as determined by Micro-FISH. The Greek letters indicate the subdivisions of proteobacteria. The bar labeled with '?' represents those bacteria not identified by any of the group-specific probes. Data taken from Cottrell and Kirchman [47]. The error on FISH data is on the order of 20%.

tions of low molecular mass byproducts of HMW DOM hydrolysis are sufficiently high to alleviate the need for biopolymer-hydrolyzing bacteria to maintain high-affinity transport systems for monomers. Uptake by bacteria with low-affinity transport systems is probably not detected by the Micro-FISH method, which is designed to assay the most active cells.

Another approach for exploring the role of *Cytophaga–Flavobacteria* in using HMW DOM would be to follow the abundance of specific bacterial groups during times or locations when the input of HMW DOM is high, such as during phytoplankton blooms. A few studies have tried to follow *Cytophaga–Flavobacteria* abundance during blooms in marine [48–51] and freshwater habitats [52], but it is difficult to draw any general conclusions with these data. Eilers et al. [12] did observe large maxima in *Cytophaga–Flavobacteria* during short-lived phytoplankton blooms in the North Sea; this bacterial group was the most abundant through most the year but other bacterial groups also increased when *Cytophaga–Flavobacteria* increased. Nonetheless, it is intriguing to note that *Cytophaga–Flavobacteria* responded more than other bacteria when cyanobacterial biomass was added to anoxic sediments [53], and *Cytophaga–Flavobacteria* increased six- to eight-fold in a glacial stream following a storm that washed in allochthonous organic material [54]. Of course, factors other than DOM uptake will affect the success of *Cytophaga–Flavobacteria* and other bacteria in natural environments.

The great interest in uncultured *Cytophaga–Flavobacteria*, evident in the papers reviewed above, has not been matched by relevant work on cultured representatives. Biopolymer degradation by cultured *Cytophaga–Flavobacteria* was examined by studies published over 20 years ago (see review by [8]), and other studies of interest to ecologists are nearly as old [55]. More current research seems lacking. One specific gap is the paucity of sequence data on enzymes catalyzing biopolymer hydrolysis. Even though biopolymer hydrolysis by *Cytophaga–Flavobacteria* is well known, GenBank has only three cellulase and no chitinase sequences from the entire *Bacteroidetes* division; over 70% of the cellulase sequences are from Gram-positive bacteria while the chitinases sequences are mainly from γ-proteobacteria. The whole genome sequencing of *C. hutchinsonii* already has found several genes for presumptive endoglucanases, but more sequence data are needed to elucidate the ecology and physiology of these bacteria.

5. Growth rates of *Cytophaga–Flavobacteria* and other bacterial groups

Data on population growth rates are essential in examining the autecology and biogeochemical role of bacterial groups in natural environments. More specifically, growth rate data may help explain the abundance of *Cytophaga–Flavobacteria* in some environments. One of the rare studies with relevant data is that of Jürgens et al. [56]. These authors used FISH to follow over time the abundance of several bacterial groups after filtration of lake water through a 250-μm mesh net. Even though small protist grazers and viruses were not removed, bacterial abundance increased in these experiments because the screen removed the crustacean zooplankton *Daphnia*, a major bacterivore in lakes. Jürgens et al. found that the net growth rate of *Cytophaga–Flavobacteria* was nearly two-fold faster than that of the other bacterial groups they examined (Fig. 5). These rates are some evidence that the high abundance of *Cytophaga–Flavobacteria* is due to high growth rates.

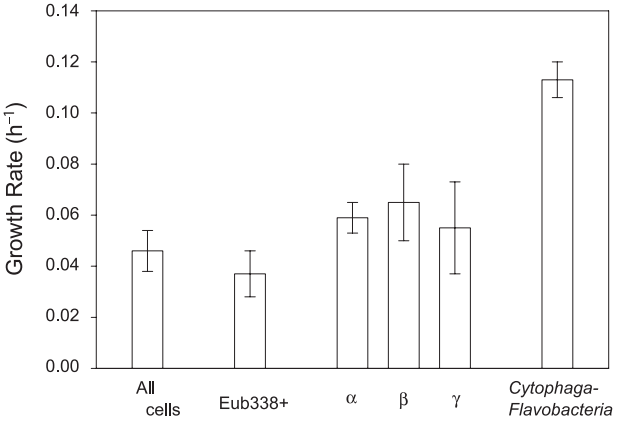

Fig. 5. Growth rates of bacterial groups in *Daphnia*-free water. 'All cells' refers to the growth rate of all DAPI-positive microbes. 'Eub338+' is the growth rate of the bacteria recognized by the Eub338 probe. The Greek letters indicate the subdivisions of proteobacteria. Data taken from Jürgens et al. [56].

However, other experiments indicate that *Cytophaga–Flavobacteria* may not always grow faster than other bacteria. In experiments similar to those conducted by Jürgens et al., but with 0.8-μm filtration, Šimek et al. [57] found that *Cytophaga–Flavobacteria* (those recognized by the CF319a probe) grew the slowest in a reservoir in South Bohemia, although only marginally more slowly than β-proteobacteria recognized by the BET42a probe. A subgroup of β-proteobacteria grew the fastest. In experiments with samples from the Plymouth Sound and English Channel, Fuchs et al. [58] found that both γ- and α-proteobacteria grew faster than *Cytophaga–Flavobacteria* in dilution cultures in which microbes were diluted 10-fold with filter-sterilized water.

The growth rates in the dilution culture experiments conducted by Fuchs et al. [58] were consistent with flow cytometric data. Fuchs et al. found that the proteobacterial subdivisions dominated the high-protein cells sorted by flow cytometry, whereas cells in the low-protein cells mostly belonged to the *Cytophaga–Flavobacteria* cluster. Zubkov et al. [59] used leucine incorporation to estimate growth rates of three groups of bacteria sorted by DNA

content. These authors found that the group dominated by *Cytophaga–Flavobacteria* had the lowest growth rate in the mixed layer of the Celtic Sea. Growth rates of the three sorted groups were similar in the pycnocline and deep layer.

6. Impact of grazing on *Cytophaga–Flavobacteria*

The abundance of the total bacterial community and perhaps of specific bacterial groups is relatively constant over short time periods (day time scale) in most aquatic habitats, because growth of these microbes is matched by mortality due to both viral lysis and grazing. Generally, grazing accounts for most of bacterial mortality [60], but the impact of grazing and viral lysis on individual bacterial groups is largely unknown. Phages of *Cytophaga–Flavobacteria* have been examined in laboratory experiments [61,62], but nothing is known about the impact of viral lysis on natural communities of *Cytophaga–Flavobacteria*. Turning the tables, *Cytophaga*-like bacteria are known to lyse cyanobacteria [63], but the significance of this process in nature is unclear. More is known about the impact of grazing, a topic that was reviewed recently by Hahn and Höfle [64].

The impact of grazing can be deduced by following the abundance of bacterial groups in incubations with and without bacterivores. The experiments conducted so far have been with freshwater assemblages. In these experiments, the abundance of either α- or β-proteobacteria increased after removal of grazers, but in all experiments the absolute and relative abundance of *Cytophaga–Flavobacteria* increased substantially, more so than observed in the controls [56,65,66]. These data imply that *Cytophaga–Flavobacteria* and, less consistently, one of the proteobacterial subdivisions are preferentially grazed by bacterivores.

This 'preference', however, may simply result from the high growth rates of these bacterial groups. Since grazing rates increase with prey density, rapidly growing *Cytophaga–Flavobacteria* will in effect be grazed on more heavily than slow-growing bacteria [67,68]. However, growth rate could affect cell properties that do attract heavy grazing activity independent of prey density.

One such cell property is cell size. Since grazing pressure increases with cell size, large, fast growing cells may be grazed on more heavily than small, slow-growing cells. There is little information about growth rates (except for the data reviewed before) and cell size of *Cytophaga–Flavobacteria* and other uncultured bacteria in nature. Some experiments have examined an extreme case where the bacterial prey becomes too large to be eaten by protist grazers. In laboratory experiments of simple predator–prey systems, bacteria can form long filaments when a bacterivore is present [64], and thus escape being eaten. The capacity to form these filaments seems to be common among the bacterial groups examined to date, and the morphology of both proteobacteria and *Cytophaga–Flavobacteria* can shift to filaments under grazing pressure in simple laboratory experiments [64].

Cell properties other than size also may affect grazing. In cultures, heterotrophic protists often grow on some bacterial species better than on others, and grazing rates are often higher on live than on heat-killed bacteria [60]. Cell size does not explain these observations. We know little about how grazer-sensitive cell properties vary among the major bacterial groups.

7. How much do we need to know about bacterial diversity?

This review has discussed the ecology of the entire *Cytophaga–Flavobacteria* cluster and of three subdivisions of proteobacteria, as if these bacterial groups were simple collections of a few microbes, which of course they are not. In part, we have little choice but to use these broad phylogenetic labels because of limitations in our understanding of bacterial diversity in aquatic environments. These limitations in turn affect our methods (e.g. designing new FISH probes) for examining aquatic bacteria. In particular, we still know little about the diversity of *Cytophaga–Flavobacteria* in aquatic habitats and about which clades within this cluster we should focus on. In spite of these limitations, however, I suggest that the studies reviewed here were able to make useful generalizations about the ecology of these bacteria. In spite of great differences among the aquatic habitats discussed here, we can see some consistent patterns in how *Cytophaga–Flavobacteria* and other bacterial groups respond to experimental manipulations and vary with locations and the seasons. But, in other cases, we may need more data about subgroups within the *Cytophaga–Flavobacteria* cluster. The question then becomes, what phylogenetic level is most appropriate for examining the biogeochemical roles of *Cytophaga–Flavobacteria* and other uncultured bacterial groups?

The appropriate level will depend on the specific question. At one extreme, we already know that a strain-specific approach is needed to examine pathogenicity or antibiotic production by bacteria (but see [69]). At the other extreme, it may be adequate to consider all heterotrophic bacteria together as a single functional unit when examining DOM uptake and mineralization in aquatic habitats. An argument against lumping all heterotrophic bacteria together is that we now know that uncultured *Cytophaga–Flavobacteria* and other bacterial groups differ in their use of DOM. Still, before diving into studies of DOM use by individual species (or finer phylogenetic levels), which may number in the hundreds to thousands in lakes and the oceans, it may be useful to keep in mind that current models of the carbon cycle have a single compartment for all heterotrophic bacteria (and Archaea) using DOM [70]. So, even adding four groups (the *Cytophaga–Flavo-*

bacteria and three proteobacterial subdivisions) would drastically change (and complicate) current models.

Although microbial ecologists should not be limited by models, keeping them in mind may help ensure that information about microbial community structure will be useful in examining larger issues, such as the role of microbes in carbon cycles and global climate change. Elucidating the links between community structure and function is likely to enrich our understanding of not only microbial processes but also of the diversity of microbial communities in natural environments. We are still in the beginning stages of examining structure–function links, but even now it seems clear that *Cytophaga-Flavobacteria* are important members of the bacterial community in many aquatic environments.

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