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Predatory Prokaryotes: Predation and Primary Consumption Evolved in Bacteria

Lynn Margulis, *University of Massachusetts - Amherst*
Ricardo Guerrero
Carlos Pedrós-Alió
Isabel Esteve
Jordi Mas, et al.

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Predatory prokaryotes: Predation and primary consumption evolved in bacteria

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RICARDO GUERRERO*, CARLOS PEDRÓS-ALIÓ*, ISABEL ESTEVE*, JORDI MAS*, DAVID CHASE†, AND LYNN MARGULIS‡

*Department of Microbiology and Institute for Fundamental Biology, Autonomous University of Barcelona, Bellaterra (Barcelona), Spain; †Cell Biology Laboratory (151B5), Sepulveda Veterans Administration Hospital, Sepulveda, CA 91343; and ‡Department of Biology, Boston University, Boston, MA 02215

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ABSTRACT **Two kinds of predatory bacteria have been observed and characterized by light and electron microscopy in samples from freshwater sulfurous lakes in northeastern Spain. The first bacterium, named *Vampirococcus*, is Gram-negative and ovoidal (0.6 μm wide). An anaerobic epibiont, it adheres to the surface of phototrophic bacteria (*Chromatium* spp.) by specific attachment structures and, as it grows and divides by fission, destroys its prey. An important *in situ* predatory role can be inferred for *Vampirococcus* from direct counts in natural samples. The second bacterium, named *Daptobacter*, is a Gram-negative, facultatively anaerobic straight rod (0.5 × 1.5 μm) with a single polar flagellum, which collides, penetrates, and grows inside the cytoplasm of its prey (several genera of Chromatiaceae). Considering also the well-known case of *Bdellovibrio*, a Gram-negative, aerobic curved rod that penetrates and divides in the periplasmic space of many chemotrophic Gram-negative bacteria, there are three types of predatory prokaryotes presently known (epibiotic, cytoplasmic, and periplasmic). Thus, we conclude that antagonistic relationships such as primary consumption, predation, and scavenging had already evolved in microbial ecosystems prior to the appearance of eukaryotes. Furthermore, because they represent methods by which prokaryotes can penetrate other prokaryotes in the absence of phagocytosis, these associations can be considered preadaptations for the origin of intracellular organelles.**

Although symbiotic bacteria have been extensively studied and their evolutionary importance in the origin of eukaryotic cells has been recognized (1, 2), predatory behavior in bacteria is known only for *Bdellovibrio* (3, 4) and *Vampirovibrio* (5, 6). Antagonistic relationships among large organisms are considered to be properties of ecosystems and integrated into ecological theory (7); however, such behavior (e.g., primary consumption, predation, and scavenging) attributed only to animals and plants (8) has been ignored in microorganisms.

Techniques for measuring ecological variables at the microbial level have been developed in the last 20 years. We are now able to perform experiments and observations to see whether general ecological principles are applicable to microbial ecosystems. Studying microbial ecosystems not only takes us down the scale to the very small, it may also transport us back in time to the Archean and Proterozoic Eons (from 3400 until 570 millions of years ago), when microbes were the only inhabitants of the Earth. The study of microbial ecosystems not only helps us to interpret early stages of life on Earth but also reveals aspects of the evolution of ecological relationships as well.

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We report here bacterial scavenging and predation by two new bacteria, one epibiotic (*Vampirococcus*) and the other cytoplasmic (*Daptobacter*). *Vampirococcus* attacks different species of the genus *Chromatium*, a purple sulfur bacterium (9). It does not penetrate its prey cells and remains attached to the *Chromatium* cell wall. *Vampirococcus* reproduces while "sucking" the innards of its prey in a fashion reminiscent of vampires (thus its name). The second type of predatory bacteria is *Daptobacter*. *Daptobacter* penetrates and degrades the cytoplasm of its prey, several genera of Chromatiaceae (purple sulfur phototrophic bacteria); *Daptobacter* grows and divides inside the cytoplasm, leaving only the cell wall. *Vampirococcus* and *Daptobacter* have been found in several karstic lakes in which the anaerobic photic zone is extensive and dense populations of purple sulfur bacteria develop. Of the several lakes in which these bacteria were found, two were studied in detail. We describe the two environments where samples were taken. Our observations distinguished the two new bacteria from *Bdellovibrio* by their morphology, prey range, response to oxygen, and modes of feeding and reproduction.

MATERIALS AND METHODS

Studies were conducted in Lake Estanya (42° 02′ N, 0° 32′ E) and Lake Cisó (42° 08′ N, 2° 45′ E) in northeastern Spain. Both lakes are sinkholes formed in karstic areas, rich in calcium sulfate as gypsum and anhydrite. They receive most of their water inputs through seepage. The water conductivity, about 1800 $\mu S \cdot cm^{-1}$ for Lake Estanya and 1300 $\mu S \cdot cm^{-1}$ for Lake Cisó, is high, primarily as a consequence of dissolved salts as sulfates (siemens are reciprocal ohms; $S = 1/\Omega$). From 7 to 10 mM sulfate is present in solution in the hypolimnia of both lakes. Lake Estanya, figure-eight shaped, has two basins 12 and 20 m deep, respectively. They are separated by a 2-m-deep sill (10). Lake Cisó, an almost semispherical basin, is 9 m deep and 25 m in average diameter at the surface. Because of high production of hydrogen sulfide in the sediments, it is completely anoxic during mixing (11). Details of lake ecology and methods of study have been published (12–14). In both lakes light penetrates down to the thermocline, and in both during stratification, hydrogen sulfide is abundant in the hypolimnia. Thus, phototrophic sulfur bacteria, which are anaerobic and anoxigenic, reach large population densities in horizontal layers where adequate amounts of light and sulfide are present simultaneously. These thick, purple-colored layers (15, 16), sometimes called "bacterial plates," are easily detected by a large decrease in the transmittance of light. The samples for microscopic observation of the predatory bacteria were taken from various depths in the bacterial layer. Values for light, turbidity, hydrogen sulfide, oxygen, and temperature are shown for Lake Estanya (Fig. 1). The vertical distribution of these values in Lake Cisó is very similar (15) except that the

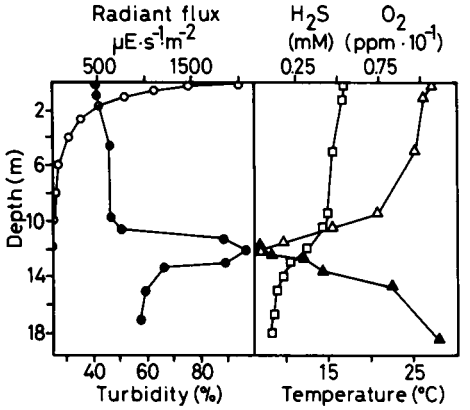

FIG. 1. Vertical distribution of physicochemical parameters in Lake Estanya. (*Left*) Radiant flux as microeinsteins per sec/m² (○) and percent turbidity (●) as a function of depth. (*Right*) Hydrogen sulfide concentrations (▲), oxygen from undetectable quantities to 10.2 ppm (△), and temperature (□) as a function of depth. Air was taken to be 0% turbidity.

maximal biomass (corresponding to highest turbidity) occurs at 2 m in Cisó rather than at 12 m in Estanya.

Samples of water were collected from the depths indicated for both lakes in Table 1. Portions of these samples were reserved for epifluorescence and phase-contrast light microscopic observations of live material. Others were prepared for electron microscopy by fixation in 2.5% glutaraldehyde in sodium cacodylate buffer (pH 7.1) and postfixation in 1% osmium tetroxide; these samples were dehydrated and embedded in epoxy resin. Silver sections were cut on a Sorvall MT2B Microtome with a diamond knife (Dupont) and photographed with a Philips EM 201 electron microscope.

RESULTS AND DISCUSSION

The prokaryotic communities forming such layers in both lakes are dominated by phototrophic purple sulfur bacteria. In Lake Estanya three species of *Chromatium* could be found reaching concentrations up to 10^6 cells per ml at a 12.25-m depth (Table 1). In Lake Cisó the community consisted of concentrated populations (*ca.* 7×10^5 cells per ml between 2 and 2.5 m) of the single-celled *C. minus* and the aggregate-forming purple phototrophic bacterium *Lamprocystis* sp. (15) (Table 1).

By light microscopy many of the *C. minus* cells were observed to have smaller bacteria attached to them. These attached bacteria were especially abundant during the autumn and in the deeper parts of the bacterial layer. Cell counts for both lakes on particular dates are shown in Table 1. The number of epibionts increased with depth in parallel with decreasing viability of *Chromatium* (16). Viability of *Chromatium* diminishes with depth because of decreasing amounts of available sunlight. Thus, we propose that the epibiontic bacterium is an opportunistic scavenger taking advantage of unfavorable environmental conditions for *Chromatium* at the bottom of the layer. The epibiontic bacteria have been tentatively named *Vampirococcus*, from "vampire" (Serbian: vampir, blood-sucker) and "coccus" (Greek: coccus, a grain or berry). Although the name *Vampirococcus* has not been formally described, we use it for convenience. *Vampirococcus* has resisted attempts to grow it in axenic culture.

A characterization of its relationship with *Chromatium* in natural samples was done by electron microscopy and is illustrated in Fig. 2 *A–D*. A conspicuous attachment structure binds *Vampirococcus* to *Chromatium* (Fig. 2 *A* and *B*). From one to six *Vampirococcus* cells can attach to a single *Chromatium* (Fig. 2*C* and Table 1). The *Vampirococcus* cells apparently persist freely suspended in the water but were only seen to multiply when attached to their prey. Note the cross walls forming in the process of cell division of *Vampirococcus* (Fig. 2 *A–C*). As many as three offspring *Vampirococcus* cells can be seen, suggesting that it has a tendency to become multicellular (Fig. 2*C*). The beginning of the degradation of the prey cytoplasm can be clearly seen in Fig. 2*B*. All that remains of the prey, after degradation is complete, is the cell wall, cytoplasmic membrane, and some intracytoplasmic inclusions (Fig. 2*D*).

The study of some of the enrichment cultures by light microscopy revealed the presence of another type of bacteria. Small, rod-shaped, and free-swimming, they frequently collided with the *C. minus* cells. Samples were then prepared for electron microscopy as described above. In thin sections, the small bacteria could be seen attaching to the prey, penetrating through both cell wall and cell membrane into the *C. minus* cytoplasm, and degrading its content. This bacterium, capable of penetrating and dividing in the cytoplasm of its prey, has been called *Daptobacter* from "dapto" (Greek: devour, gnaw) and "bacter" (Latin from Greek: rod). A complete description of *Daptobacter* in the bacteriological literature is underway by I.E. and her colleagues. This

Table 1. Vertical distribution of Chromatiaceae and predatory bacteria in Lakes Estanya and Cisó

Lake Estanya, October 13, 1984								Lake Cisó, July 6, 1982				
Depth, m	Population density of *Chromatium* species, cells × 10^{-4} per ml			*Vampirococcus* on				Depth, m	Population density, cells × 10^{-4} per ml		*Vampirococcus* on *C. minus*	
				C. minus		*C. vinosum*						
	okenii	*minus*	*vinosum*	%I*	n_{avg}†	%I*	n_{avg}†		*C. minus*	*Lamprocystis* sp.	%I*	n_{avg}†
5.00	0	0	0	—	—	—	—	1.25	0	0	—	—
10.00	0	0	0	—	—	—	—	1.50	0	0	—	—
12.00	5.2	19.0	56.0	38.3	1.39	21.7	1.00	1.75	2.3	0	2.0	2.00
12.25	3.0	19.0	110.0	67.7	2.16	44.6	1.14	2.00	66.1	48.1	1.9	1.42
12.50	1.8	4.3	24.0	85.0	3.78	35.0	1.24	2.50	47.3	74.7	6.5	3.26
13.00	0.7	5.8	8.7	77.5	2.71	40.0	1.44	3.00	16.6	21.5	23.5	2.49
15.00	0.1	0.9	2.6	93.5	3.14	40.0	1.38	4.00	9.6	10.2	31.4	3.65
20.00	0	1.9	2.6	80.0	3.50	13.3	1.00	5.00	6.7	6.1	41.3	3.51

All counts were performed by epifluorescence microscopy. *Vampirococcus* did not attack *C. okenii* in Lake Estanya or *Lamprocystis* in Lake Cisó.

*%I = percentage of infected cells.

†n_{avg} = average number of *Vampirococcus* per infected cell.

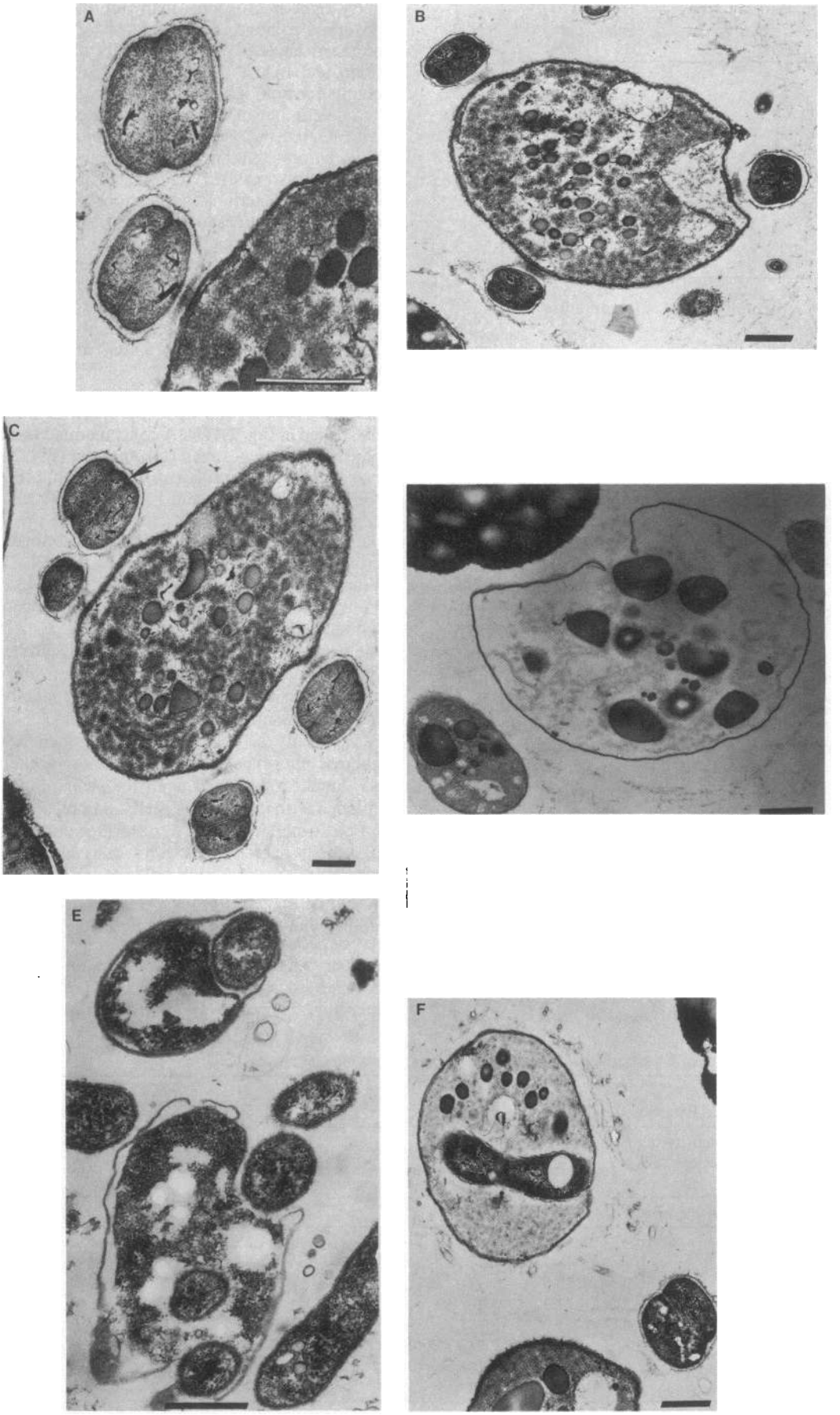

FIG. 2. Transmission electron micrographs of thin sections of *Vampirococcus* (*A–D*) and *Daptobacter* (*E, F*) from samples taken in Lake Estanya. (*A*) An early stage in the attachment to *Chromatium* by *Vampirococcus*. (*B*) The attachment structure: dense material appears to attach *Vampirococcus* to *Chromatium*. Note the breach in the outer membrane of *Vampirococcus* and the plaque of dense material at the attachment site. (*C*) Four

Table 2. Comparison of three types of predatory prokaryotes

	Bdellovibrio	*Vampirococcus*	*Daptobacter*
Source	Seawater, freshwater, or soil	Freshwater	Freshwater
Morphology	Curved rod, 0.35 × 1.2 μm	Ovoidal, 0.6 μm	Straight rod, 0.5 × 1.5 μm
Motility	Motile, by single polar sheathed flagellum	No motile forms have been found	Motile, by single polar unsheathed flagellum
Site in prey cell where reproduction occurs	Periplasmic: periplasmic space; segmentation from parent cell	Epibiotic: attached to the cell wall; binary fission	Endobiotic: cytoplasm; binary fission
Prey range	Heterotrophs: many Gram-negative bacteria	Phototrophs: several species of *Chromatium*	Phototrophs: several genera of Chromatiaceae
Response to oxygen	Aerobe	Anaerobe	Facultative anaerobe
Host dependency*	Obligate	Obligate	Facultative

*Although slow growing, mutants of *Bdellovibrio* capable of reproduction in the absence of live prey have been isolated. The wild-type requires live prey. On the other hand, *Daptobacter* grows easily axenically but *Vampirococcus* has never been grown in culture.

rod-shaped, Gram-negative bacterium attaches perpendicularly to the prey cell, eventually penetrating its interior, where it degrades the cytoplasm and then divides. Several *Daptobacter* can be seen at a time inside a single prey cell (Fig. 2*E*). Finally, only the cell wall, cytoplasmic membrane, and storage granules remain (Fig. 2*F*). *Daptobacter* has been isolated in axenic culture, where it is able to grow both aerobically and anaerobically. Consequently we note here that *Daptobacter* can be distinguished clearly from the well-known predatory bacterium *Bdellovibrio* on the basis of its morphology, physiology, type of flagellum, location in the prey cell, and range of prey (Table 2).

With our observations on the two new genera, there are now three types of predatory relationships known among prokaryotes, shown schematically in Fig. 3 and summarized in Table 2. *Bdellovibrio* spp. are aerobic, Gram-negative, curved rods, predatory on other Gram-negative bacteria, penetrating the periplasmic space and dividing there (3). *Daptobacter* shares with *Bdellovibrio* the property of penetrating the prey cell, but, unlike the latter, *Daptobacter* goes through the cell membrane right into the cytoplasm of the prey and degrades it both under aerobic and anaerobic conditions. The newly reported *Vampirococcus* shares many of its characteristics with the previously described *Vampirovibrio* (5, 6). Unlike *Vampirovibrio*, which attacks the eukaryote *Chlorella*, *Vampirococcus* attacks several species of *Chromatium* and develops high population densities in nature (Tables 1 and 2). Furthermore, as implied by the environmental data, *Vampirococcus* is able to grow under anaerobic conditions; thus, it differs from *Bdellovibrio* and *Vampirovibrio*, both of which are strict aerobes (3, 6).

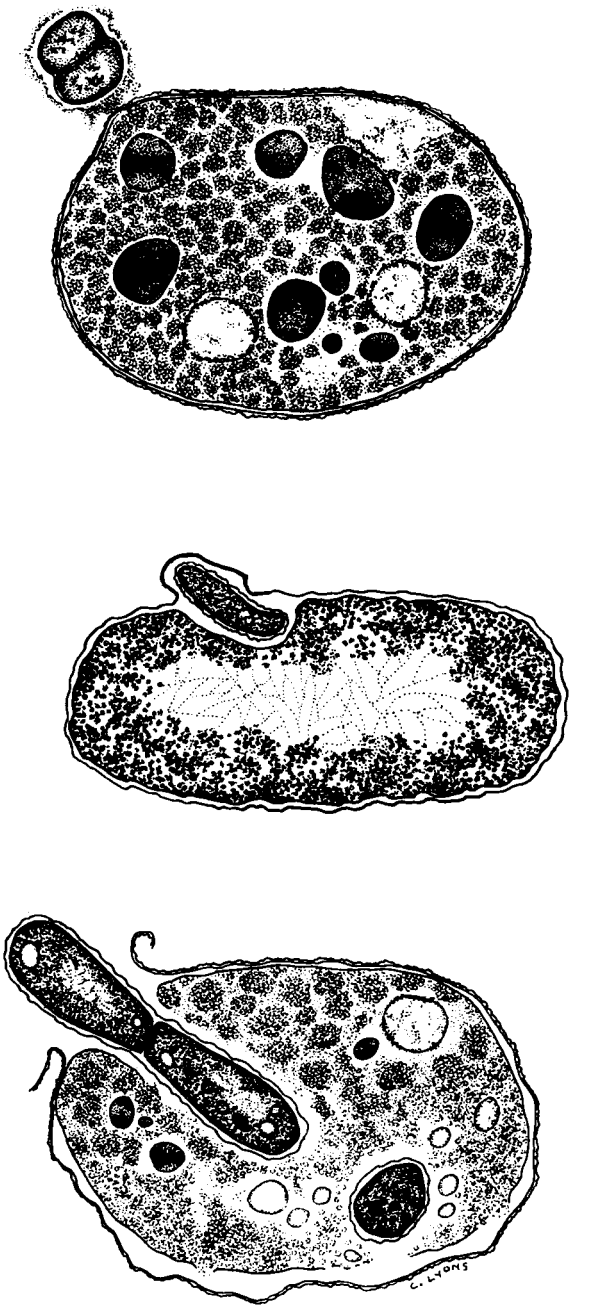

FIG. 3. Topological relations among predatory bacteria. (*Top*) *Vampirococcus* attaches to the cell wall of several species of *Chromatium* and divides epibiotically while degrading the host cytoplasm. (*Middle*) *Bdellovibrio* attacks Gram-negative heterotrophic bacteria and grows, making a long parental cell that segments to form several linearly aligned *Bdellovibrio* in the periplasmic space of its prey. (*Bottom*) *Daptobacter* penetrates the cytoplasm of several members of Chromatiaceae and divides by binary fission within the cytoplasm. (Drawings by Christie Lyons.)

The first conclusion from an analysis of Table 2 is that existing terminology is not appropriate for prokaryotic organisms. *Vampirococcus* and *Daptobacter*, although bacterivo-

Vampirococcus attached to one *Chromatium*; several of the *Vampirococcus* have cross walls. Arrow points to second crosswall. (*D*) Dissolution of the cytoplasmic matrix of *Chromatium* is shown. Only ruptured outer membrane and inclusions remain. Clearly, this is a terminal stage in the interaction. (*E*) *Daptobacter* penetrates both membranes of the prey cell walls and reproduces in their degrading cytoplasm. Five *Daptobacter* can be seen here associated with the degradation of one prey's cytoplasm. (*F*) *Daptobacter* dividing in partially degrading cytoplasm of a *Chromatium* cell. A *Vampirococcus* can be seen attacking another *Chromatium* cell in the lower right. (Bar = 0.5 μm.)

rous, might be considered primary consumers or "herbivores," since they exclusively attack phototrophic bacteria which are primary producers. Because of their size, one would tend to consider them parasites. The attacked Chromatiaceae might thus be called either host or prey cells, while they are actually primary producers. Such confusion in the standard ecological terminology when applied to microbes has also been observed in the literature of *Bdellovibrio*, which has been alternatively called a parasite or a predator (3). *Bdellovibrio* actually is a necrotrophic endobiont (17) that reproduces in the periplasm of a wide range of heterotrophic bacteria. But since *Bdellovibrio* only attacks heterotrophs, it is a secondary consumer. Hopefully, further work will clarify current ecological terminology by using more meaningful references to nutritional modes and topological relationships, thereby indicating what is accessory and what is essential in the standard jargon.

Even though many eukaryotic organisms such as crustaceans, rotifers, and ciliates normally feed on bacteria, none of them seems to be important in consuming bacterial biomass in fresh-water planktonic ecosystems (18–20). These bacteriovores are apparently of greater ecological significance in the ocean (21, 22) and in soils and sediments (18, 21). The only noneukaryotic bacterial killers known until now are *Bdellovibrio* and bacteriophages. Both have been considered unimportant in controlling natural populations of bacteria (19, 21). The first indication of significant predation by one prokaryote upon another in a natural system is shown in Table 1, columns 5, 7, and 12—e.g., by the high percentage of prey under attack. The large populations and high percentages of prey cells affected argue for a significant role of these predators in nature. These predatory relationships have been elusive in natural habitats because of their inconspicuous morphology as well as the high physiological and molecular diversity of bacteria, which is refractory to direct observation. Furthermore, *Bdellovibrio* and the highly specific predators described here tend to be in low concentrations in most communities. Predatory prokaryotes are expected to be easily observed only in specific habitats where high numbers of microbial prey species dominate the community.

Not only do the phototrophic bacterial layers in the lake water harbor predatory prokaryotes, but also we suspect that termite guts and microbial mats do as well (these communities also harbor high population densities of potential prey). The bacterial plates of lakes, microbial mats (23, 24), and termite hindguts (ref. 23; ref. 25, figure 10C) are all elaborate, highly structured, strictly microbial communities inhabited by a characteristic set of dominant genera. Relationships analogous to those described here for *Vampirococcus* and *Daptobacter* have been observed on two occasions by *in situ* electron microscopy in both microbial mats and termite hindguts (ref. 25; ref. 26, figure 13E).

A major criticism of the proposed origin of undulipodia and mitochondria by bacterial symbiosis is the absence of any mechanism of incorporation of prokaryotic cells by other prokaryotes (1). If phagocytosis and pinocytosis are entirely absent in prokaryotes, how did the bacteria that became organelles come to reside inside their hosts? The periplasmic location of *Bdellovibrio* (analogous to the mitochondria) and cytoplasmic location of *Daptobacter* (analogous to undulipodia) indicate that bacteria have the potential to penetrate other bacteria, a process suggested to have occurred in the origin of eukaryotic organelles by symbiosis.

Note Added in Proof. It has been pointed out to us by Hans G. Trueper that these predatory bacteria were most likely seen in cultures of *Chromatium*. Although misinterpreted as "buds" or "connection stages," the possibility that they were bacterial parasites was raised by H. Potthoff (see figure 8 on page 93 in ref. 27.)

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