April, 1986

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.

Available at: https://works.bepress.com/lynn_margulis/98/
Predatory prokaryotes: Predation and primary consumption evolved in bacteria

**ABSTRACT**

Two kinds of predatory bacteria have been observed and characterized by light and electron microscopy in samples from freshwater sulfuric 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.

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 inner cells 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 lake water conductivity, about 1800 μS cm⁻¹ for Lake Estanya and 1300 μS cm⁻¹ for Lake Cisó, is high, primarily as a consequence of dissolved salts as sulfates (siemens are reciprocal ohms; S = 1/Ω). 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 hypolimnus. Thus, phototrophic sulfur bacteria, which are anaerobic and anoxicigenic, 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 layers. 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
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 10⁸ cells per ml at a 12.25-m depth (Table 1). In Lake Cisó the community consisted of concentrated populations (ca. 7 × 10⁵ 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).

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

<table>
<thead>
<tr>
<th>Depth, m</th>
<th>Lake Estanya, October 13, 1984</th>
<th>Lake Cisó, July 6, 1982</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Population density of Chromatium species, cells × 10⁻⁴ per ml</td>
<td>Population density, cells × 10⁻⁴ per ml</td>
</tr>
<tr>
<td></td>
<td>okenii</td>
<td>minus</td>
</tr>
<tr>
<td>5.00</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10.00</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>12.00</td>
<td>5.2</td>
<td>19.0</td>
</tr>
<tr>
<td>12.25</td>
<td>3.0</td>
<td>19.0</td>
</tr>
<tr>
<td>12.50</td>
<td>1.8</td>
<td>4.3</td>
</tr>
<tr>
<td>13.00</td>
<td>0.7</td>
<td>5.8</td>
</tr>
<tr>
<td>15.00</td>
<td>0.1</td>
<td>0.9</td>
</tr>
<tr>
<td>20.00</td>
<td>0</td>
<td>1.9</td>
</tr>
</tbody>
</table>

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.

*ₙavg = average number of Vampirococcus per infected cell.

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 epibiotic bacterium is an opportunistic scavenger taking advantage of unfavorable environmental conditions for Chromatium at the bottom of the layer. The epibiotic 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. 2B. 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 "dapt" (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.
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

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

*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. 2E). Finally, only the cell wall, cytoplasmic membrane, and storage granules remain (Fig. 2F). 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).

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

---

Vampirovibrio attacked 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 “herbi-
vores,” 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 mi-
crobes has also been observed in the literature of Bdel-
lovibrio, 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,
without further work will clarify current ecological terminology by using more meaningful references to nutritional modes and
topological relationships, thereby indicating what is acces-
sory and what is essential in the standard jargon.

Even though many eukaryotic organisms such as crusta-
cceans, rotifers, and ciliates normally feed on bacteria, none
of them seem to be important in consuming bacterial
biomass in fresh-water planktonic ecosystems (18–20). These
bacteriovores are apparently of greater ecological signifi-
cance in the ocean (21, 22) and in soils and sediments (18, 21).
The only noneukaryotic bacterial killers known until now are
Bdellovibrio and bacteriovores. 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 per-
centages 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 inconspicu-
ous morphology as well as the high physiological and mo-
lecular diversity of bacteria, which is refractory to direct
observation. Furthermore, Bdellovibrio and the highly spe-
cific predators described here tend to be in low concentra-
tions in most communities. Predatory prokaryotes are ex-
pected to be easily observed only in specific habitats where
high numbers of microbial prey species dominate the com-
munity.

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 communi-
ties 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 undulip-
odia) 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.
Truiper 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 paras-
ites was raised by H. Potthoff (see figure 8 on page 95 in ref. 27.)

We thank Núria Gaju and Josep M. Gasol for help with bacterial
counts and chemical measurements, Christie Lyons for drawing Fig.
3, and Carmen Chica and Geraldine Kline for manuscript prepara-
tion. This work was supported by Grant 875/81 from Comisión Asesora de Investigación Científica y Técnica (Spain) to R.G.
and Grant NGR 004-025 from the National Aeronautics and Space
Administration and the Lounsbery Foundation to L.M. The support
of the National Aeronautics and Space Administration Planetary
Biology and Microbial Ecology program (1984, San Jose State
University) is gratefully acknowledged.

Francisco).
H., Trüper, H. G., Balows, A. & Schlegel, H. G. (Springer,
Microbiol. 24, 1387–1394.
5. Gromov, B. V. & Mammeka, K. A. (1978) Tsiologiya 14,
256–260.
7. Lotka, A. J. (1956) Elements of Mathematical Biology (Dover,
New York).
ogy (Yale Univ. Press, New Haven).
Microb. Ecol. 9, 57–64.
10. Ávila, A., Burrell, J. L., Domingo, A., Fernández, E., Godall,
15. Guerrero, R., Montesinos, E., Pedrós-Aliò, C., Esteve, I.,
Mas, J., Van Gemerden, H., Hofman, P. A. G. & Bakker,
16. Van Gemerden, H., Montesinos, E., Mas, J. & Guerrero, R.
261–280.
499–505.
37, 774–778.
160–170.
Cohen, Y., Castenholz, R. W. & Halvorson, H. O. (Liss, New
BiOSystems 13, 109–137.
27. Bauendamm, W. L. (1924) Die Farblosen und Roten
Schwefelbakterien des Säss- und Salzwassers (Gustav Fischer
Verlag, Jena, G.D.R.).