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Antagonistic Association of the Chlorellavorus Bacterium ("*Bdellovibrio*" *chlorellavorus*) with *Chlorella vulgaris*

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Abstract. The chlorellavorus bacterium (*Bdellovibrio chlorellavorus* Gromov and Mamkaeva 1972) attaches to (but does not enter) cells of the unicellular green alga, *Chlorella*, which is killed and the cell contents of which are digested. The bacterium is pleomorphic (vibrios 0.3 μm wide; cocci 0.6 μm wide), and it has a Gram-negative cell wall structure, pili, and a single, unsheathed, polar flagellum. Division may occur only in bacterial cells attached to algal cells, an attachment mediated by a pad (245 × 36 nm) of unknown composition. Bacterial growth occurs only in the presence of live *Chlorella* cells, and not on various bacteriological culture media, killed *Chlorella* cells, 4 strains of *Prototheca*, or 24 strains of Gram-negative bacteria. The chlorellavorus bacterium may not require algal protein synthesis, since the bacterium grows on algae in the presence of cycloheximide (30 μg/ml). Although the DNA base composition of the chlorellavorus bacterium (50 mol % G + C) is in the same range as *Bdellovibrio bacteriovorus*, its ultrastructure, developmental cycle, host range, and format of its intermicrobial association all distinguish the chlorellavorus bacterium from members of the genus *Bdellovibrio*.

Bdellovibrio is a genus of Gram-negative bacteria with several unusual properties. Chief among their unique features are (i) a dimorphic life-cycle (alternation of tiny, vibrioid, actively motile swarmers—each provided with a sheathed flagellum—and long, helical, nonflagellated cells that undergo multiple fragmentation and flagellar development to yield swarmers); and (ii) a capacity to associate antagonistically with other Gram-negative bacteria by a swarmer's entering the periplasm of a bacterial associant, maturing there into the helical form, digesting the cytoplasm and nucleoplasm of the entered associant in an exquisitely regulated manner, and fragmenting into several swarmers, which then leave the ghosted remnants of the victim and initiate a new cycle. Details about these and other features of *Bdellovibrio* can be found elsewhere [10,11,12, 13,14,15,16].

Four species have been assigned to the genus *Bdellovibrio:* the type species, *B. bacteriovorus*, by Stolp and Starr [16]; *B. stolpii* and *B. starrii* by Seidler, Mandel, and Baptist [8]; and *B. chlorellavorus* by Gromov and Mamkaeva [2]. It is this latter organism—the so-called *Bdellovibrio chlorellavorus*—that concerns us here. Since there is some question [11,13] about the generic assignment of this antagonistic bacterial associant [10,11] of the unicellular green alga *Chlorella vulgaris*, we will generally use here the noncommittal vernacular "chlorellavorus bacterium" to refer to this fascinating creature, which was isolated from "a mass culture of *Chlorella* from reservoirs in the Ukraine" [2]. The present report summarizes our initial study of the chlorellavorus bacterium, including its ultrastructure, morphological aspects of its association with *Chlorella*, recital of our vain attempts to grow the bacterium axenically, and our current notions about its systematic position.

Materials and Methods

Cultures. The strain of *Bdellovibrio chlorellavorus* was received from B. V. Gromov, one of its describers [2], as a "lysate"—a two-membered system with *Chlorella vulgaris*. We believe that this preparation is the "lysate 2" of Mamkaeva [6]; the two-membered system has been deposited in culture collections (ICPB 3707, NCIB 11383, ATCC 29753). We also received from B. V. Gromov an axenic culture of the same *Chlorella vulgaris* strain that was part of the lysate, his strain 157 (ICPB 4049, NCIB 11384, ATCC 30821). *Chlorella vulgaris* var. *viridis* UTEX 396, *C. pyrenoidosa* UTEX 1230, and *C. pyrenoidosa* UTEX 395 were kindly provided by N. J. Lang. *Prototheca* strains 60-47, 66-2, 66-6, and

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66-10 were obtained through the generosity of H. J. Phaff. The control cultures—*Bdellovibrio bacteriovorus* 109D (ICPB 3454) and its associant *Escherichia coli* (ICPB 2262)—came from the International Collection of Phytopathogenic Bacteria.

Media and culture conditions. Cultures of *Chlorella* and *Prototheca* were maintained on PGY agar, which contained (wt/vol) 1.0% Difco peptone, 1.0% glucose, 0.5% Difco yeast extract, and 1.5% Bacto agar. This medium (and all others) was sterilized by autoclaving for 15 min at 121°C. *B. bacteriovorus* 109D and *E. coli* 2262 were maintained as described by Starr and Stolp [14]. The chlorellavorus bacterium was grown in liquid cultures containing *C. vulgaris* 157 suspended in Medium 2, which contains Tris buffer (5 mM, pH 7.1) supplemented with 0.05% $CaCl_2$ and 0.33% $MgCl_2$. Medium 2 was also used as the suspending and washing medium, except where noted. Inoculation of 20 ml of *Chlorella* suspension (about 0.4 mg algal dry weight per ml) in Medium 2 was with 0.5 ml of a mature culture ("mature" is defined here as marked clumping of the algae in the suspension, a change in color from grass-green to yellow-green, the appearance of a large number of motile, vibrioid bacterial cells, and the presence of numerous bacteria attached to the outside of almost all of the algal cells remaining in suspension). Following incubation in the dark at room temperature (about 24°C) on a shaker at 200 rpm, a mature culture is obtained in 4 to 5 days. When necessary, bacteria were separated from algal cells in the culture by centrifugation for 5 min at 5,000 × *g*, followed by filtration of the supernatant through a glass fiber prefilter and a 1.2-μm pore-diameter membrane filter (Millipore).

Attempts to cultivate the chlorellavorus bacterium axenically. The ability of the chlorellavorus bacterium to grow axenically (i.e., apart from the *Chlorella*) was examined in many complex culture media, both solid and liquid. Most of these were commercial dehydrated media, reconstituted according to manufacturer's directions except that normally acidic media were adjusted to pH 7.1. For solid media, duplicate sets of plates were streaked with heavy suspensions of the chlorellavorus bacterium separated from algal cells as described above; for liquid media, duplicate sets of flasks were inoculated with 0.5 ml of the same bacterial suspension; *C. vulgaris* 157 suspended in Medium 2 served as a control for viability of the inoculum. One set was incubated at room temperature (about 24°C) and another set at 30°C; liquid media were incubated at the same temperatures on a shaker. After at least two weeks, the preparations were scored for colonies or turbidity.

The ability of the chlorellavorus bacterium to grow on dead or disrupted cells of *C. vulgaris* 157 was also examined. *Chlorella* cells suspended in Medium 2 were killed by heating at 60°C for 10 min, disrupting by two or more freezing-and-thawing cycles, or breaking with glass beads in a Braun tissue homogenizer cooled with a continuous stream of CO_2 to prevent warming. Triplicate preparations from each treatment were incubated with an algae-free suspension of the chlorellavorus bacterium on a shaker at room temperature in the dark, and periodically examined for qualitative changes in color, clump formation, and turbidity. The incidence of bacteria attached to the outsides of cells or to cellular debris, and of bacteria free in the medium, was judged qualitatively using phase-contrast or Nomarski interference-contrast microscopy.

Host range. Growth of the chlorellavorus bacterium on the other *Chlorella* strains as well as on the *Prototheca* strains was examined in triplicate in the same manner as was *C. vulgaris* 157; the latter strain was always included as a control of viability of the bacterial inoculum. A limited survey (G. E. Buchanan and M. P. Starr, personal communication) has been made regarding the ability of heavy algae-free suspensions of the chlorellavorus bacterium to form plaques on lawns of 24 strains of Gram-negative bacteria on double-layer plates of YPSC agar [14], as well as on lawns of *Chlorella vulgaris* 157. The plaque-forming ability of *B. bacteriovorus* 109D was tested on the same lawns.

Growth at different temperatures. Duplicate two-membered cultures in Medium 2 of the chlorellavorus bacterium with *C. vulgaris* 157 were incubated at 15, 25, 30, 37, and 44°C on a shaker in the dark, and scored as described above.

Scanning electron microscopy (SEM). Samples of a mature culture were prepared for SEM by the method of Marchant [7]. Material fixed for transmission electron microscopy (see next paragraph) also was examined by SEM. Filters were sputter-coated with gold-paladium or evaporative-coated with carbon and gold, and viewed in a Cambridge Stereoscan Mark IIA SEM or a Japan Electron Optics Ltd. (JEOL) JSM-2 SEM.

Transmission electron microscopy (TEM). Using sodium cacodylate buffer (50 mM, pH 7.1) at each step, mature cultures of the chlorellavorus bacterium or axenic *C. vulgaris* 157 control cultures were washed with buffer, suspended in 2.5% glutaraldehyde in buffer, and treated 30 sec later with an equal volume of 2% OsO_4 in buffer. Cells were fixed 45 min at room temperature, washed three times with buffer, and mixed with an equal volume of 2% Noble agar (Difco). The gelled agar was cut into small blocks, dehydrated in a graded series of water:acetone, and embedded in Spurr's resin. Sections were collected on Formvar-coated grids, stained with saturated uranyl acetate in methanol and Reynolds' lead citrate, and viewed in a JEOL 100B TEM.

Negatively stained material was prepared by the method of Allen and Baumann [1], using 0.5% (wt/vol) uranyl acetate; sometimes 100 μg/ml bacitracin was used as wetting agent. Some material was fixed with 1% formaldehyde, which preserves the flagellar sheath of *Bdellovibrio bacteriovorus* [9]; *B. bacteriovorus* 109D served as a positive control. All negatively stained material was viewed in a Zeiss EM-9A TEM.

Results and Discussion

Mamkaeva [6] and Gromov and Mamkaeva [2] report that the coinoculation of *Chlorella vulgaris* 157 and the chlorellavorus bacterium results in the destruction of the *Chlorella* cell contents after 5 to 7 days in the dark. They characterized the association as the clumping of algal cells, a change in color of the culture from green to yellow-green to gray-green to colorless, formation of refractile bodies within the degraded algal cells, the presence of motile vibrioid bacteria attached to the algal cells, and the death of all the algal cells. Our findings generally corroborate these findings, except for color changes and algal viability. We found a change in color from grass-green to yellow-green or gray-green to occur regularly, but some color was always retained for several months if the cultures were exposed to ambient room light for a few minutes each day; cultures became colorless only if they were fully exposed to ambient

room light. Moreover, killing of the algal cells was not complete: some algal cells capable of growth on PGY agar always persisted. We do not know the basis for this discrepancy. Perhaps this residual viability stems from our use of *Chlorella* suspensions denser than those used by Mamkaeva [6] (though comparable densities were used in some experiments), or possibly the population contained individual algal cells resistant for some unknown reason to attack by the bacterium. The latter situation obtains when *B. bacteriovorus* associates with its bacterial symbionts; there are always survivors that, when isolated and grown separately, show the usual susceptibility to the bdellovibrios [11,13].

Clumping of algal cells is a characteristic and unusual property of the association; no such clumping was ever observed in the axenic *Chlorella* cultures. The process, which occurs gradually, becomes noticeable about two days after coinoculation, when the culture assumes a finely granular texture. This change is followed a day or two later by the formation of small clumps, which may aggregate further into a floc several millimeters in diameter—resulting in a partial clarification of the medium. Some of the floc can be broken by vigorous shaking, but it can never be completely resuspended.

Nomarksi interference-contrast microscopy of the floc shows algal and bacterial cells imbedded in a hyaline matrix. By SEM, the matrix appears as a fibrous network (Fig. 1a), which may be an artifact of drying. It is not yet clear whether production of the hyaline matrix is a result of bacterial metabolism, algal metabolism, or an interaction of the two. However, algal protein synthesis is apparently not required for floc formation since *Chlorella* cells preincubated in cycloheximide (24 h; 30 μg/ml) still form clumps in association with the bacterium.

Scanning electron micrographs (Fig. 1c) show that the chlorellavorus bacteria are attached at numerous sites on the surfaces of the algal cells. Negative staining, which preserves flagella, reveals that the bacteria usually attach to algal cells by the end opposite the bacterial flagellum. There appears to be no specificity for new or old algal cells; the bacteria attach to dividing or recently divided algae, as well as to aged algal cells.

Thick filaments, looking like bundles of pili in SEM [4], are seen between bacterial and algal cells in Fig. 1c. In negatively stained preparations examined by TEM (Fig. 1b), the filamentous bundle is resolved into fibrils whose widths (6.5 nm)—and perhaps attachment function—also are similar to those of pili [5].

In ultrathin sections (Fig. 1f–i), bacteria are always connected in a subpolar orientation to algal cells by a discrete pad (245 nm wide and 36 nm thick) of unknown composition. The texture and density of the attachment pad vary with the plane of the section, being either diffuse and fibrous or electron-dense and stratified. In some sections, no breaks are seen in the outer membrane of the bacterial cell wall at the point of contact with the outer surface of the attachment pad. The bacteria alone seem able to synthesize the pad, since it is also present when the chlorellavorus bacterium attaches to algal cell wall remnants. Gromov and Mamkaeva [2] describe a similar structure, which they state is derived from the cell wall of the bacterium. Though the ultrastructural evidence they present supports this view, the structures we have observed are clearly not a widening of the outer membrane of the bacterial cell wall. Other cases are known [3] of bacterial adhesion mediated by extracellular bacterial biopolymers, which also do not involve a dissolution or substantial modification of the bacterial cell wall.

Complete penetration and entrance of the entire cell of the chlorellavorus bacterium into the periplasmic space of the algal cell were never observed, although this is the typical locus in the interbacterial bdellovibrio association [12]. Although the bacterium-alga association being considered here is essentially exhabitational [10], a breach of the algal cell wall is suggested in some sections, where a "spike" of electron-dense material of unknown composition and function, penetrating the algal cell wall and pushing in the cytoplasmic membrane, can be seen (Fig. 1i). In this case, the spike crosses two layers of algal cell wall (walls of parent and progeny cells); such behavior may have a bearing on the apparent lack of preference by the chlorellavorus bacterium for algal cells of a particular age or developmental state.

The harmful effects of the chlorellavorus bacterium on *Chlorella*, observed at the macroscopic level, are fully substantiated at the ultrastructural level. Comparison of sections of axenically grown algae (Fig. 1d) with sections of algae from a mature lysate (Fig. 1e) shows that the latter are almost devoid of cytoplasmic contents and contain only membranous structures—presumably remains of organelles—and large vacuolate areas. Some of these membranous structures may correspond to the "peripheral vacuoles" described by Gromov and Mamkaeva [2] as being present in Ryter-Kellenberger fixed material. We have examined the effects of three other fixation procedures (including the one Gromov and Mamkaeva used). We have found that the Ryter-

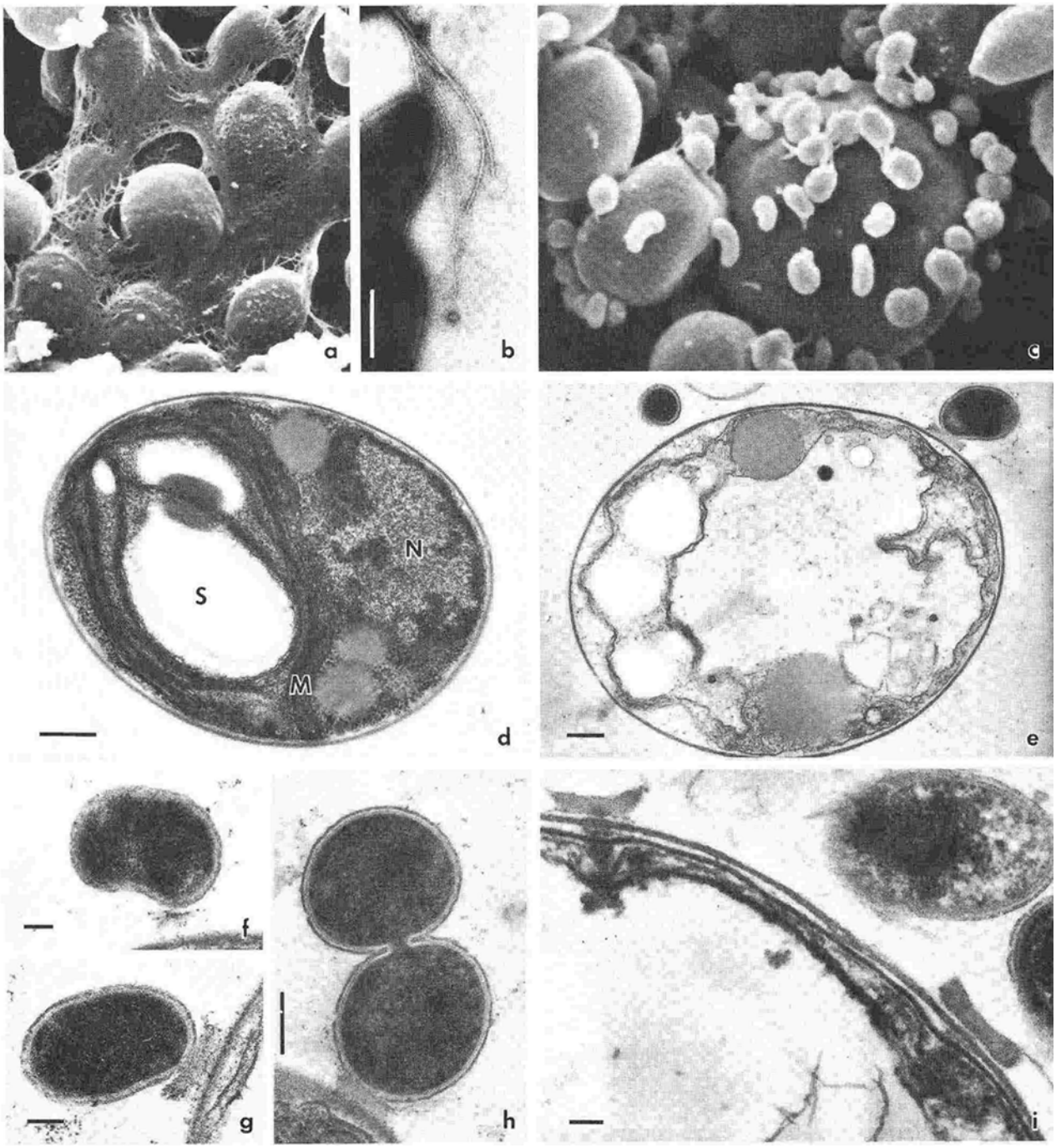

Fig. 1. The association between the chlorellavorus bacterium ("*Bdellovibrio*" *chlorellavorus*) and *Chlorella vulgaris* 157. (a) Clumps of *Chlorella vulgaris* 157 and chlorellavorus bacteria embedded in a matrix. SEM; evaporative coated; 12.4-μm field width. (b) Thin fibrils (like pili) near two bacterial cells; the thicker fibrils are flagella. Negatively stained; bar indicates 0.25 μm. (c) Chlorellavorus bacteria attached to *Chlorella vulgaris* 157. Note pleomorphism of bacterial cells, short fibrils between bacteria and between bacteria and algae. SEM: sputter-coated; 11.3-μm field width. (d) Section of axenic *Chlorella vulgaris* 157 with chloroplast containing starch grains (S), nucleus (N), and mitochondrion (M). Bar indicates 0.25 μm. (e, f, g, h) Sections of chlorellavorus bacteria attached to *Chlorella vulgaris*. Note subpolar attachment pads of bacteria (f, g). Contrast degraded algal cytoplasmic contents (e) with axenic control (d). Note Gram-negative cell wall structure in dividing cell (h). Bars indicate 0.25 μm (e), 0.1 μm (f), 0.1 μm (g), and 0.25 μm (h). (i) Section of *Chlorella vulgaris* 157 and detached chlorellavorus bacteria: attachment pads remain on algal cell wall (top left and bottom right). "Spikes" of electron-dense material extend from the pads through two layers of algal cell wall to the plasmalemma. Bar indicates 0.1 μm.

Kellenberger fixation results in more extensive vacuole formation than the procedure reported here, which provides what we believe to be the most accurate representation of the cellular organization.

Though the destruction of the algal cell contents appears massive, breakage or lysis of the algal cells—in the strict sense that the walls are disrupted—never occurs. Mamkaeva [6] reported seeing ruptured algal cell walls, but these are probably nothing more than the empty parental walls that result from normal cell division in *Chlorella*.

The cell wall ultrastructural type and mode of division of the chlorellavorus bacterium are those typical of Gram-negative bacteria (Fig. 1h). Cell shape and width vary with the life cycle. Unattached cells range from vibrios to stout, curved rods (about 0.3–0.5 μm wide). Bacteria with similar shapes are seen attached to algal cells (Fig. 1c,e–h), but—in this associative locus—the bacterial cell shapes range from vibrios (about 0.4 μm wide) to cocci (about 0.6 μm wide), illustrating the morphological changes that occur during cell division. Some unattached dividing cells are occasionally seen, but these were apparently dislodged during preparation. We believe that the chlorellavorus bacterium grows and divides only when attached to an algal cell, probably because *Chlorella* provides the only source of bacterial nutrients in the nonnutritive medium used. Though Gromov and Mamkaeva [2] speculate that compounds may be released into the medium as algal degradation proceeds, they cite the work of others who had noted that soluble nitrogen-containing compounds do not increase under these conditions. Hence, there may be insufficient leakage of nutrients from the algae to support development of unattached chlorellavorus bacteria.

Extracellular appendages of the chlorellavorus bacterium include the fibrils mentioned above, and a single unsheathed polar flagellum. In well-preserved specimens, the shape of the flagellum approximates a damped sine wave, and it is about 15 nm wide (Fig. 1b). The flagellum is presumably fragile, since it is seen most often in a broken or irregular state. This fragility may be related to the observation that the bacterium is a rather clumsy swimmer. It rarely moves in straight lines, but mostly "twiddles" about—though oftentimes vigorously. Two other flagellar shapes have been illustrated: flagella with a regular (not damped) periodic structure [6], and a distorted flagellum [2]. In this connection, two different lysates (strains?) of the chlorellavorus bacterium are known [6], and the two may have different flagellar structures. The cited authors did not identify the lysate number of the material on which their micrographs are based; hence, we do not know whether they used the lysate 2 described by Mamkaeva [6] (as we assume we did). Hence, the discrepancies between our respective micrographs may simply reflect differences between the bacteria, including the fragility of the flagella, in the two lysates.

Though the shape of the flagellum of the chlorellavorus bacterium resembles that of *Bdellovibrio bacteriovorus* [9], its width is narrower, reflecting in part the lack of a flagellar sheath. That the absence of a sheath might be a preparative artifact was investigated using formaldehyde as a fixative [9] and *B. bacteriovorus* 109D as a positive control. Though a flagellar sheath [9] is commonly seen in preparations of *Bdellovibrio*, none has ever been observed in the chlorellavorus bacterium; we always observed sheaths in the *B. bacteriovorus* 109D culture run at the same time.

Mamkaeva [6] reported that the chlorellavorus bacterial lysate 2 was able to cause degradation ("lysis") of 25 out of the 48 *Chlorella* strains tested, but that it was unable to degrade cultures of other eukaryotic algae or the cyanobacterium, *Anacystis*. We have observed growth of the chlorellavorus bacterium on suspensions of all of the *Chlorella* strains we examined, but not on the 4 *Prototheca* cultures. The chlorellavorus bacterium did not form plaques on the lawns of the 24 Gram-negative bacteria on which *B. bacteriovorus* 109D did form plaques; moreover, neither the chlorellavorus bacterium nor *B. bacteriovorus* 109D forms plaques on lawns of *C. vulgaris* 157 (G. E. Buchanan and M. P. Starr, personal communication). In fact, despite repeated attempts, we have never observed the plaque formation by the chlorellavorus bacterium on *Chlorella* lawns reported and illustrated by others [2,6].

The chlorellavorus bacterium is unable to grow on any of the numerous solid or liquid complex media we have tried. Similar results were reported by Gromov and Mamkaeva [2], including lack of growth on boiling-water extracts or heat-killed cells of *C. vulgaris* 157. We have found, moreover, that neither heat-killed cells of *C. vulgaris* 157 nor algal cells killed by freezing-and-thawing or by breaking with glass beads support growth of the chlorellavorus bacterium. No substances toxic to the chlorellavorus bacterium seem to be released after such breakage of the algal cells, since the addition of live algal cells to such preparations permits renewed growth of the chlorellavorus bacterium. Thus, despite our efforts at axenic cultivation (only partly summarized here), it appears that the chlorellavorus

bacterium can at present be grown only symbiotically [10,11]—i.e., in association with live cells of certain *Chlorella* strains. In this connection, we have found growth of the two-membered culture to occur at 15, 25, 30, and 37°C, but not at 44°C. At 44°C, most of the algae are dead; hence, the lack of bacterial growth might be related either to temperature per se or, more likely, to lack of the viable algae for which the chlorellavorus bacterium seems to have an obligate requirement.

This need for viable algae suggests that algal metabolism may be required for bacterial development. However, cycloheximide at sufficient concentration (30 μg/ml) to completely inhibit algal growth, has no noticeable effect on growth of the chlorellavorus bacterium. Residual protein synthesis in the presence of cycloheximide [17] might satisfy some bacterial needs, but there may be an additional requirement for intact algal cells (which sequester pools of preexisting metabolites) or algal storage products (which the bacterium consumes). The chlorellavorus bacterium seems not to need whole algal cells for attachment, since the bacteria will commonly attach to remnants of algal cell walls.

Our present opinion about the systematic position of the chlorellavorus bacterium is that it probably is not a member of the genus *Bdellovibrio* Stolp and Starr 1963. Although the DNA of the chlorellavorus bacterium contains 50 mol % G + C (M. Mandel, personal communication), precisely in the range of *B. bacteriovorus* [8], the lack of the typical *Bdellovibrio* (i.e., vibrios⟷large helices) dimorphic life cycle in the chlorellavorus bacterium, its unsheathed flagellum and clumsy motility, its inability to associate antagonistically with other Gram-negative bacteria, its seemingly obligate requirement for live *Chlorella* cells, and its essentially extracellular (exhabitational) locus vis-à-vis the alga all speak for a lack of relationship to the bdellovibrios.

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Literature Cited

1. **Allen, R. D., Baumann, P.** 1971. Structure and arrangement of flagella in species of the genus *Beneckea* and *Photobacterium fischeri*. Journal of Bacteriology **107**:295–302.
2. **Gromov, B. V., Mamkaeva, K. A.** 1972. [Electron microscopic examination of *Bdellovibrio chlorellavorus* parasitism on cells of the green alga *Chlorella vulgaris*.] Tsitologiya **14**:256–260. [In Russian.]
3. **Harris, R. H., Mitchell, R.** 1973. The role of polymers in microbial aggregation. Annual Review of Microbiology **27**:27–50.
4. **Kraus, S. J., Glassman, L. H.** 1974. Scanning electron microscope study of *Neisseria gonorrhoeae*. Applied Microbiology **27**:584–592.
5. **Jephcott, A. E., Reyn, A., Birch-Andersen, A.** 1971. *Neisseria gonorrhoeae*. III. Demonstration of presumed appendages to cells from different colony types. Acta Pathologica et Microbiologica Scandinavica Section B **79**:437–439.
6. **Mamkaeva, K. A.** 1966. [Observations on the lysis of cultures of the genus *Chlorella*.] Mikrobiologiya **35**:853–859. [In Russian.]
7. **Marchant, H. J.** 1973. Processing small delicate biological specimens for scanning electron microscopy. Journal of Microscopy **97**:369–371.
8. **Seidler, R. J., Mandel, M., Baptist, J. N.** 1972. Molecular heterogeneity of the bdellovibrios: Evidence of two new species. Journal of Bacteriology **109**:209–217.
9. **Seidler, R. J., Starr, M. P.** 1968. Structure of the flagellum of *Bdellovibrio bacteriovorus*. Journal of Bacteriology **95**:1952–1955.
10. **Starr, M. P.** 1975. A generalized scheme for classifying organismic associations. Symposia of the Society for Experimental Biology **24**:1–20.
11. **Starr, M. P.** 1975. *Bdellovibrio* as symbiont: The association of bdellovibrios with other bacteria interpreted in terms of a generalized scheme for classifying organismic associations. Symposia of the Society for Experimental Biology **24**:93–124.
12. **Starr, M. P., Baigent, N. L.** 1966. Parasitic interaction of *Bdellovibrio bacteriovorus* with other bacteria. Journal of Bacteriology **91**:2006–2017.
13. **Starr, M. P., Seidler, R. J.** 1971. The bdellovibrios. Annual Review of Microbiology **25**:649–678.
14. **Starr, M. P., Stolp, H.** 1976. *Bdellovibrio* methodology. Methods in Microbiology **9**:217–244.
15. **Stolp, H.** 1973. The bdellovibrios: Bacterial parasites of bacteria. Annual Review of Phytopathology **11**:53–76.
16. **Stolp, H., Starr, M. P.** 1963. *Bdellovibrio bacteriovorus* gen. et sp. n., a predatory, ectoparasitic, and bacteriolytic microorganism. Antonie van Leeuwenhoek **29**:217–248.
17. **Wanka, F., Schrauwen, P. J. A.** 1971. Selective inhibition by cycloheximide of ribosomal RNA synthesis in chlorella. Biochimica et Biophysica Acta **254**:237–240.