

Ultrastructure and Cell Division of a Facultatively Parasitic Strain of *Bdellovibrio bacteriovorus*¹

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Received for publication 12 December 1969

Some aspects of cell development and division of *Bdellovibrio bacteriovorus* strain UKi2 were examined by use of electron microscopic techniques. Under saprophytic and parasitic conditions of growth, the comma-shaped cells enlarge, elongate, and form helical filaments. The mechanism of division appears to consist of an asymmetrical constriction of the filamentous cell by the cytoplasmic membrane, accompanied by a breakdown of the outer layers of the cell wall in the division region. During regeneration of the cell wall, the flagellum and flagellar sheath are formed. The development of the flagellum of the daughter cell is initiated prior to separation of the newly formed cells from the filament. Observations of *B. bacteriovorus* UKi2 grown under saprophytic and parasitic conditions indicate that development and ultrastructure are similar in both modes of growth.

Cell development of obligately parasitic bdellovibrios has been observed by electron microscopy and other techniques (5, 8, 10-12, 14, 15). These observations indicate that the comma-shaped bdellovibrio within a bacterial host cell develops into a coiled or spiral-shaped cell. The bdellovibrio spiral then divides by a constrictive process into a number of progeny cells (5, 8, 12).

A facultatively parasitic *Bdellovibrio bacteriovorus* (strain UKi2), i.e., a bdellovibrio which can grow endoparasitically in a bacterial host or saprophytically in a bacterial-free medium, was isolated and characterized in our laboratory (3).

Cell elongation, cell division, flagellar development, and fine structural changes, all of which are difficult to follow in obligately parasitic bdellovibrios because of the confining host bacteria, were observed in host-free environments and were related to parasitic development.

MATERIALS AND METHODS

Organisms. The isolation and characterization of *B. bacteriovorus* strain UKi2 has been described (3). The host bacterium employed was *Escherichia coli* B/r.

Cultural conditions. All cultures were grown in 100 ml of YP medium, consisting of 0.3% (w/v) yeast extract and 0.06% (w/v) peptone adjusted to pH 6.9, in 500-ml Erlenmeyer flasks on a Gyrotory shaker at 30 C (1). Routine procedures for maintenance of

both host and bdellovibrios have been described (3, 12, 15). The saprophytically growing bdellovibrios were transferred every 24 hr. Host-parasite interactions were followed by harvesting 18-hr cultures of *E. coli* and *B. bacteriovorus* by centrifugation and mixing the bdellovibrios with an excess number of host cells in YP medium. For continuous observation of cells with a phase-contrast microscope, a slide microculture utilizing YP medium containing 0.8% agar was employed (3).

Sampling procedure. Both parasitically and saprophytically growing cultures of bdellovibrios were sampled at appropriate intervals as determined by observations with a phase-contrast microscope.

Electron microscopy. Samples were mixed with glutaraldehyde [buffered at pH 6.1 with a modified Veronal acetate buffer (4)] in the culture flasks to yield a final aldehyde concentration of 1.0%. The cells were then centrifuged and suspended in 6% glutaraldehyde in Veronal acetate buffer for 24 hr. Subsequent procedures for fixation, dehydration, and embedding have been described (1).

Specimens were sectioned with a DuPont diamond knife on an LKB Ultratome and placed on 300-mesh copper grids (Ladd Research Ind., Burlington, Vt.). Sections were stained with uranyl acetate and lead citrate (6).

Specimens were negatively stained with 2% (w/v) potassium phosphotungstate adjusted to pH 5.3 with 1 N KOH. Samples for electron microscopy were examined with a Philips EM 200 electron microscope operated at 60 kv.

RESULTS

The growth of *B. bacteriovorus* UKi2 in YP medium in the absence of host cells involves the

¹ A portion of this investigation was presented at the 69th Annual Meeting of the American Society for Microbiology, 4-9 May 1969, Miami Beach, Fla.

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enlargement and elongation of individual cells into regular spiral forms (Fig. 1). These spiral cells usually attain a length of about 10 μm before fragmentation occurs, although some cells can reach 30 to 50 μm in length. Direct observation of 9- to 11-hr cells from both liquid culture and slide microculture with a phase-contrast microscope indicates that the dividing filaments occasionally form spheroplasts which eventually lyse (Fig. 2).

Figure 3 illustrates the appearance of a single cell of *B. bacteriovorus* UKi2 taken from a saprophytically grown culture at a stage in growth when the cells are actively motile and predacious. The motile cells are about 0.3 μm in diameter and approximately 0.8 μm long. The internal fine structure of the facultatively parasitic bdellovibrio shown in Fig. 3 is quite similar to that described for obligate parasites (1, 5, 8, 9, 12). The flagellum is sheathed by an extension of the outer layers of the cell wall and has an overall diameter of 28 nm. Although not shown in Fig. 3, the anterior end of the cell, i.e., the end opposite the flagellum, possesses a distorted region as previously observed in the obligately parasitic strains (1, 5, 8).

In general, actively motile cells have mesosomes located in the anterior region of the cell. As cells begin to elongate, a large number of mesosomes develop in other areas of the cell, particularly in those regions where septation will occur (Fig. 10).

The first observed morphological change in the vibrio-shaped motile cells during incubation in either liquid or solid medium is an increase in cell width (~20%) followed by a progressive elongation of the cell into a spiral-shaped filament (Fig. 4). Accompanying this initial develop-

ment of the bdellovibrio cell is a corresponding degeneration of the sheathed flagellum (arrow, Fig. 4), resulting in the loss of motility for elongated spiral-shaped filaments. The mature filamentous bdellovibrios are often tightly coiled (Fig. 5-7).

Some *B. bacteriovorus* UKi2 filaments have been observed which differ in fine structural organization from the normal saprophytic filamentous spiral seen in Fig. 5, 6, and 14. Figure 7 shows such an abnormal spiral cell which is enveloped by an outer cell wall; the spiral cell appears to be comprised of a filament within a continuous sheath. Numerous lamellar structures and regions of cytoplasmic disruption (arrows) can be observed in cross-section of the cylinder.

Under the conditions employed, fragmentation of the filaments usually occurs 10 to 11 hr after inoculation. This is evident from both structural observations and studies of the growth kinetics (3). Fragmentation does not occur simultaneously along the entire length of the helical filament, but appears to involve a sequential segmentation of the filament into daughter cells. Figure 8 shows a filament in an advanced stage of septation. Generally, the daughter cells are of about the same length and width, but occasionally small abnormal cells are formed (arrow, Fig. 8).

The division of saprophytically growing cells as regularly observed in negatively stained and sectioned preparations appeared to involve an asymmetric constriction of the cells (Fig. 9 and 10). The cytoplasmic membrane initially invaginates only from one side of the cell; accompanying this invagination is an apparent weakening of the outer cell wall layers in the region immediately adjacent to the invagination (Fig.

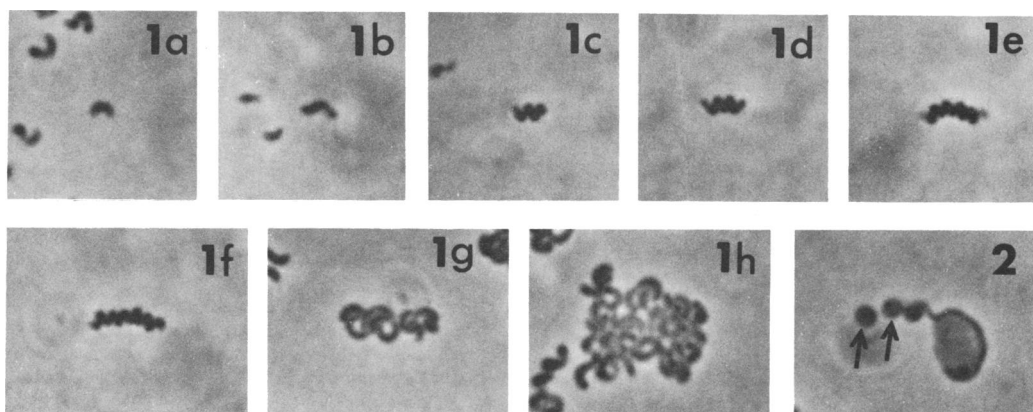


FIG. 1. Sequential phase-contrast micrographs of a saprophytically growing cell of *B. bacteriovorus* UKi2. The time (in hours) elapsed in microculture is: a, 2; b, 4; c, 6; d, 7; e, 9; f, 10.5; g, 12; h, 34. $\times 2,500$.

FIG. 2. Phase-contrast micrograph of a *B. bacteriovorus* UKi2 cell grown saprophytically for 12 hr. Presence of spheroplast-like cells is indicated (arrows).

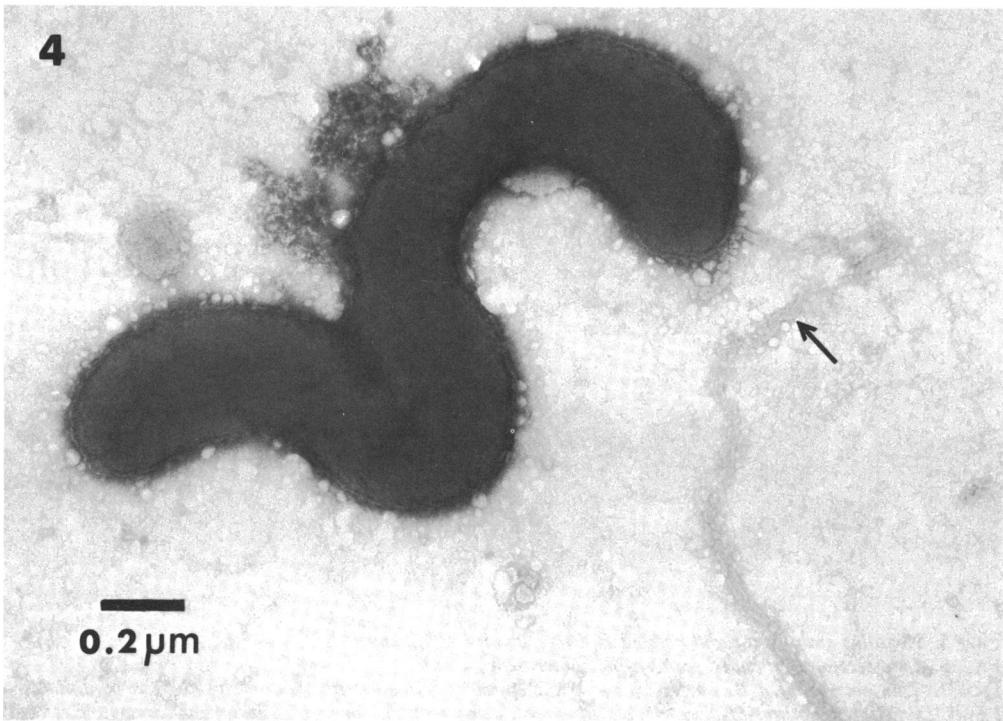
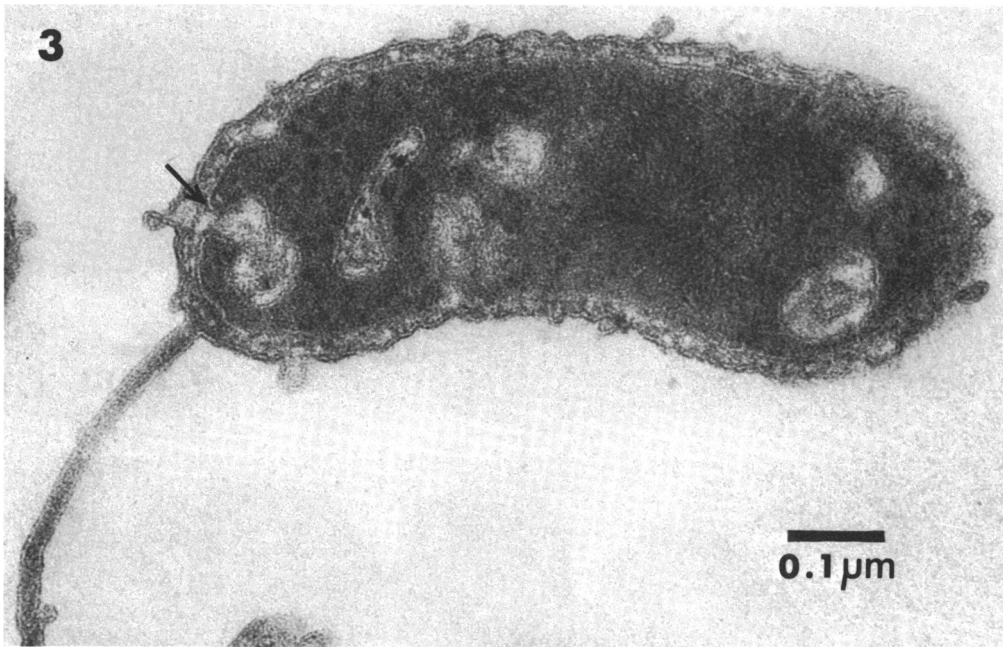


FIG. 3. Thin section through a *B. bacteriovorus* UKi2 cell; the flagellar core is ensheathed by a continuation of the outer layer of the cell wall. The cell envelope possesses an outer wall layer, a periplasmic layer, and a cytoplasmic membrane. The direct association of a mesosome with the cytoplasmic membrane is evident (arrow).

FIG. 4. Negative stain of a *B. bacteriovorus* UKi2 cell in an early phase of spiral formation. The sheathed flagellum (arrow) is in the process of disintegration.

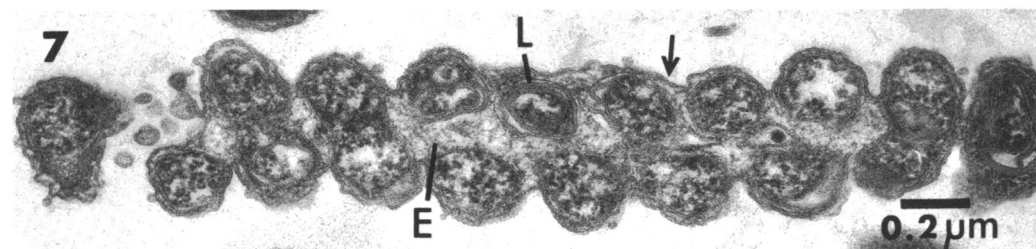
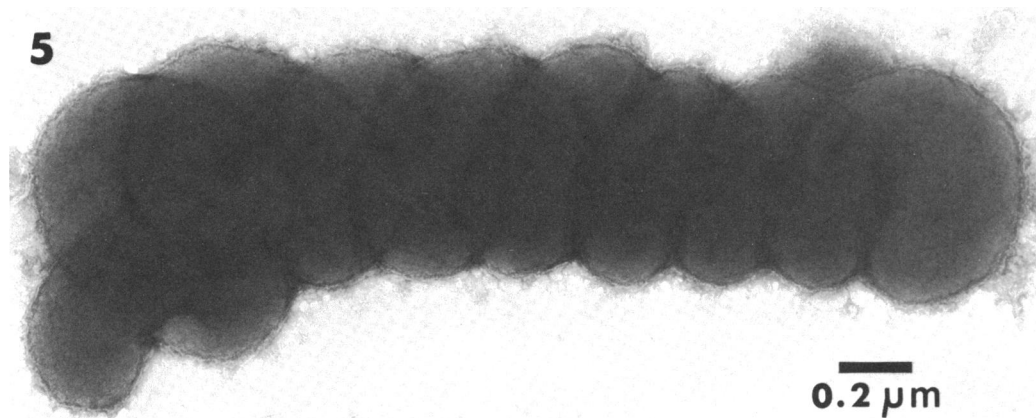


FIG. 5. Negative stain of a tightly coiled *B. bacteriovorus* UKi2 spiral.

FIG. 6. Thin section of a coiled spiral of *B. bacteriovorus* UKi2.

FIG. 7. Thin section of a *B. bacteriovorus* UKi2 spiral showing the filament enclosed by a continuous outer cell wall. Lamellar structures (L) are evident in several cross sections through the filament (arrow). Extracellular material (E).

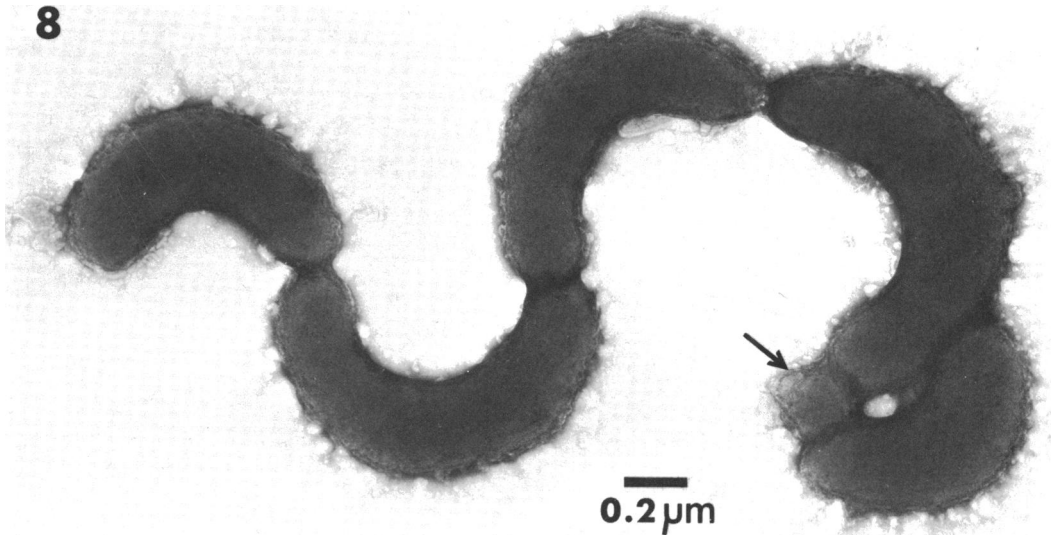


FIG. 8. Negative stain showing a short filament of *B. bacteriovorus* UKi2 after segmentation is complete. Progeny are of approximately the same width and length except for an "abnormal" small cell (arrow).

10a and 10b). Subsequently, constriction proceeds from both sides until the daughter cells in a filament are completely separated. The fine structure of the progeny cells is identical to that described for young motile bdellovibrios (Fig. 3).

Daughter segments often initiate flagellar development before they separate from the filament (Fig. 11, 12). The flagellum is always sheathed, but occasionally the core is seen to protrude slightly beyond the end of the sheath.

Since the posterior end of the bdellovibrio cell possesses a flagellum, adjacent daughter cells along a filament can be arranged in three ways: anterior to anterior; anterior to posterior; and posterior to posterior. Anterior-posterior juxtaposition (Fig. 12) seemed to be the dominant pattern.

The development of *B. bacteriovorus* UKi2 under parasitic conditions was also observed. The attachment and penetration of the facultatively parasitic bdellovibrio into the host appear similar to that described for the obligate strains (1, 5, 8, 12, 15, 16).

Figure 13 shows a *B. bacteriovorus* UKi2 cell developing inside an *E. coli* B/r host cell. This facultatively parasitic strain, in common with the obligately parasitic bdellovibrios, does not actively penetrate the cytoplasmic membrane of the host.

Although parasitically and saprophytically grown bdellovibrios differ in average filament length and time of development (parasitically

grown *B. bacteriovorus* UKi2 reaches a length of about 4 μ m in 4 hr), their rates of growth are similar.

DISCUSSION

Our observations support the conclusion that cell ultrastructure and development under saprophytic and parasitic conditions are closely parallel. Figure 14 emphasizes this by illustrating that saprophytic development of this bdellovibrio, i.e., the thickening, coiling, and fragmentation leading to new progeny, is similar to the development of the bdellovibrio within the confines of the host. It may be significant that coiling of the filament in the parasitic phase is not as great in the saprophytic phase. The observation that filament elongation is greater under saprophytic as compared to parasitic conditions may be explained by differences in availability of nutrients and other local environmental conditions.

The examination of saprophytically growing cells during the fragmentation period resulted in the observation that the division of this facultatively parasitic bdellovibrio was unlike that described for other gram-negative bacteria. Cell division and fragmentation were usually followed in the saprophytically grown cells because these processes were more readily observed in a host-independent culture.

The mechanism of cellular division described for this strain is a variation of the constriction mechanism previously described for other gram-

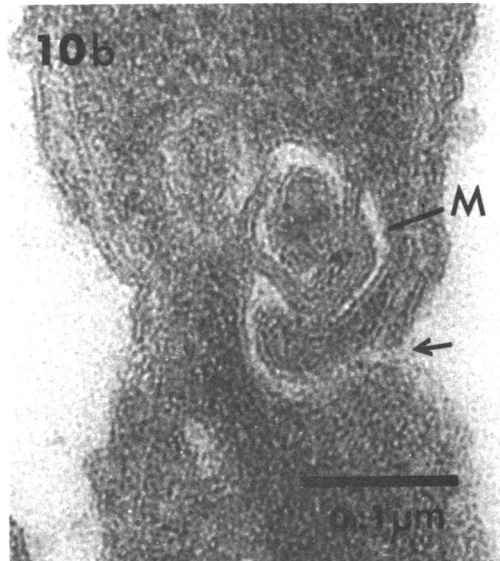
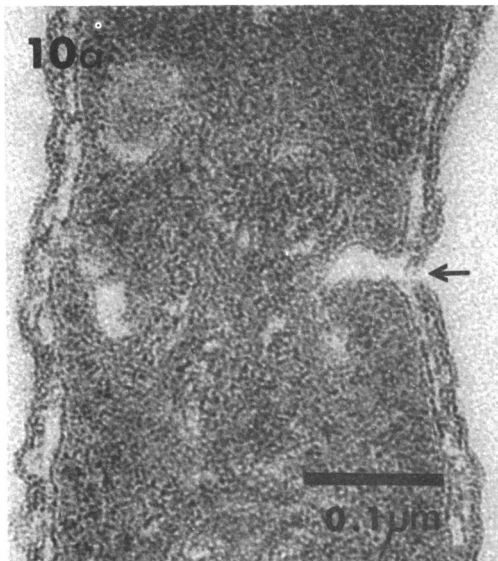
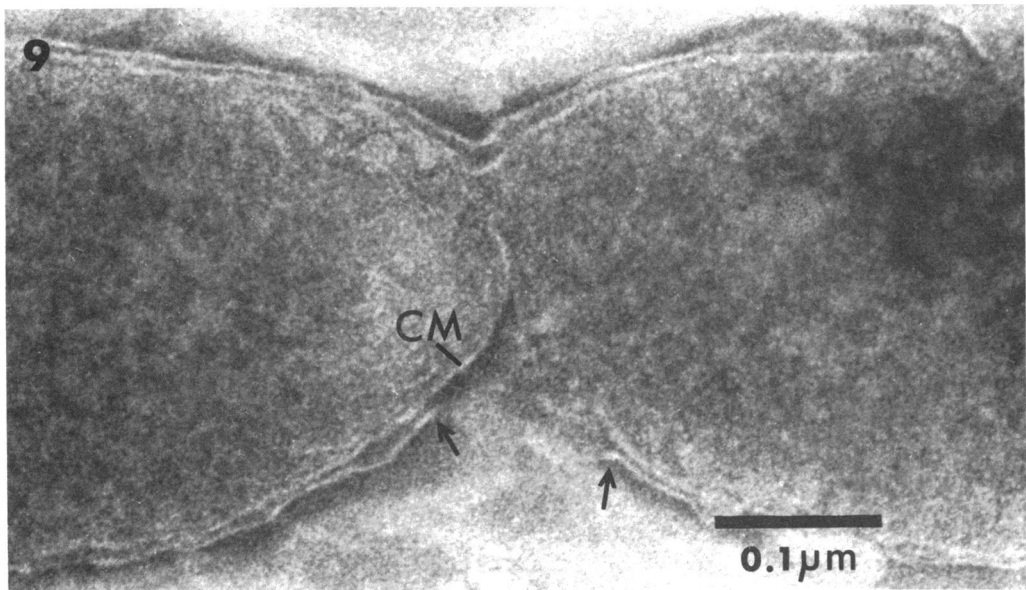


FIG. 9. Negative stain of *B. bacteriovorus* UKi2 at 10 hr showing the asymmetrical constriction of the cell by the cytoplasmic membrane (CM) and the localized breakdown of the cell-wall layers in the division region (arrow).

FIG. 10. Thin sections of *B. bacteriovorus* UKi2 at 10 hr showing membrane invaginations and apparently broken cell-wall layers (arrows). Mesosome (M).

negative bacteria (2, 13). The asymmetrical constriction of the cell by the cell membrane and the apparent weakening or breakdown of the cell wall at the region of septum formation are the unique features. In liquid cultures, the bdellovibrios are still able to maintain their rigidity throughout the division process despite the apparently localized breakdown of cell wall layers.

This suggests that the cell wall layers are not broken completely, but are weakened, as evidenced by tendency of some cells to spheroplast (Fig. 2) during this period.

The "distorted" region located at the anterior end of the cell (1, 5, 8) may be an artifact of preparative techniques. It may be that this region of the cell wall, being newly formed during cell

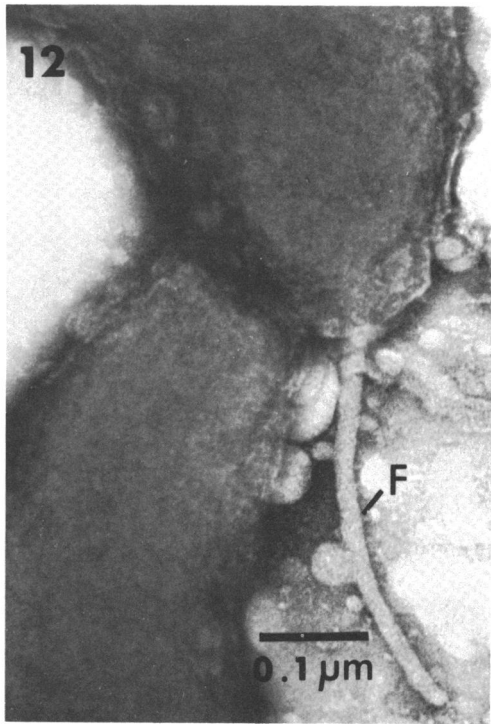


FIG. 11. Thin section of *B. bacteriovorus* UKi2 in the process of fragmenting into progeny cells. A flagellum (arrows) is developing from one of the daughter cells prior to the separation of the cells from the filament.

FIG. 12. Negative stain of dividing *B. bacteriovorus* UKi2 showing a sheathed flagellum (F) developing from one daughter cell prior to cell separation.

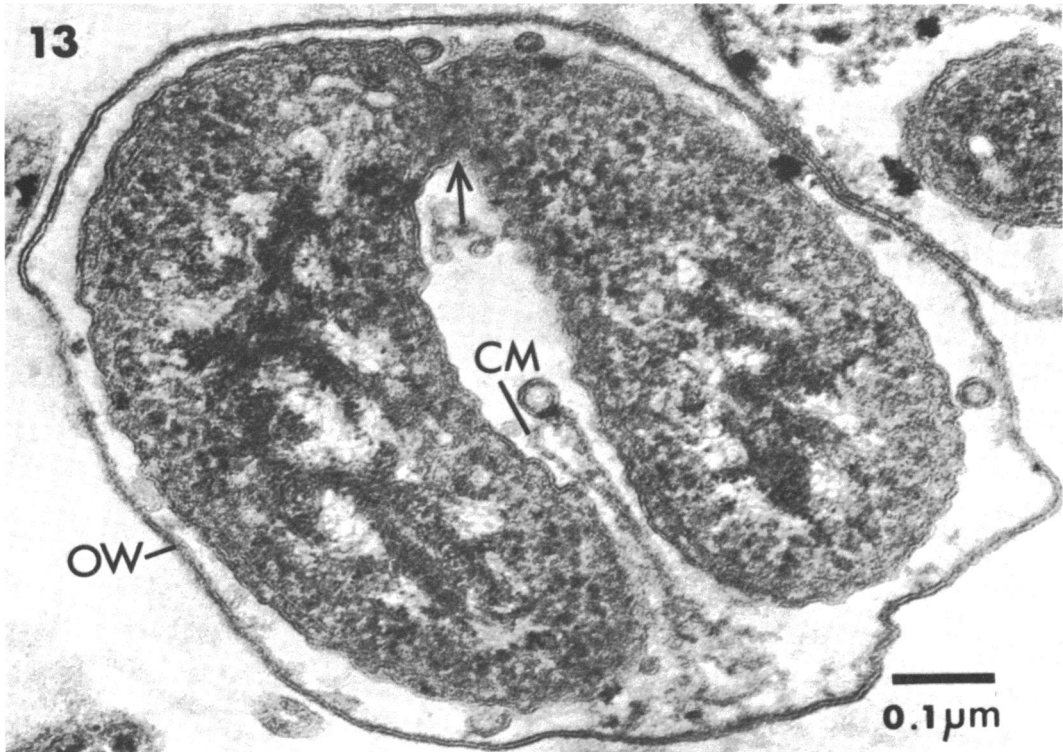


FIG. 13. Thin section of a *B. bacteriovorus* UKi2 cell in an *E. coli* B/r host cell. Arrow indicates possible constriction site. The enclosing outer cell wall (OW) and remnants of the cytoplasmic membrane (CM) of the host cell are evident.

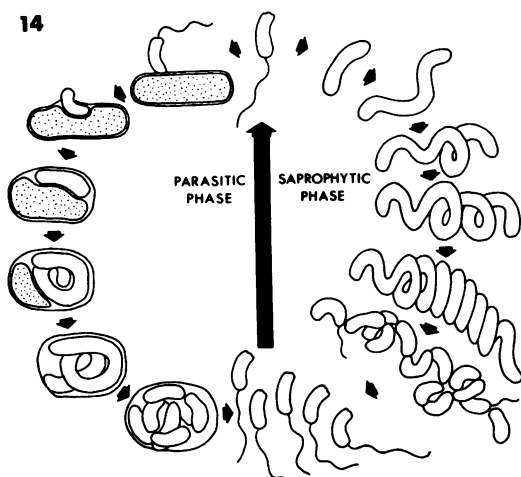


FIG. 14. Growth cycle of *B. bacteriovorus* UKi2 as constructed on the basis of our light and electron microscopic observations.

division, is not completely developed and thus more sensitive to distortion than the rest of the cell wall when exposed to fixation procedures.

The flagellum appears to develop in daughter cells prior to fragmentation of the filament. Subsequent synthesis and growth of the cell wall presumably result in ensheathment of the flagellum.

Mesosomes were observed at or near the sites where cell division occurred, and they may play a role in the cell division process (7). Further study of *bdellovibrio* mesosomes is required before a definitive hypothesis can be constructed correlating their strategic location with a function in division and parasitic penetration (1).

ACKNOWLEDGMENTS

We acknowledge the technical assistance of Bruce Tutein throughout the course of this study.

This investigation was supported by National Science Foundation grant GB7972. The senior author holds Postdoctoral Fellowship no. 7F02A137472-01A1.

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