JOURNAL OF BACTERIOLOGY, Jan. 1997, p. 148–156 Vol. 179, No. 1
0021-9193/97/$04.00+0

Fine-Structure Evidence for Cell Membrane Partitioning of the Nucleoid and Cytoplasm during Bud Formation in *Hyphomonas* Species

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Received 22 July 1996/Accepted 22 October 1996

***Hyphomonas* spp. reproduce by budding from the tip of the prosthecum, distal to the main body of the reproductive cell; thus, the chromosome must travel through the prosthecum to enter the progeny, the swarm cell. When viewed by electron microscopy, negatively stained whole cells, ultrathin-sectioned cells, and freeze-etched and frozen hydrated cells all had marked swellings of the cytoplasmic membrane (CM) in the prosthecum which are termed pseudovesicles (PV). PV were separated by constrictions in the contiguous CM. In replicating cells, PV housed ribosomes and DNA, which was identified by its fibrillar appearance and by lactoferrin-gold labeling. The micrographs also revealed that the CM bifurcates at the origin of the prosthecum so that one branch partitions the main body of the reproductive cell from the prosthecum and swarm cell. The results of this fine-structure analysis suggest models explaining DNA segregation and the marked asymmetric polarity of the budding reproductive cell.**

In most bacteria, the chromosome, ribosomes, and macromolecules travel a relatively short distance before they are partitioned in the adjacent dividing daughter cell. However, in one group of procaryotes, hyphomicrobia, the newly replicated chromosome and other cellular components must traverse a prosthecum of at least 1 μm before entering the progeny bud. Therefore, hyphomicrobia are interesting models for the study of DNA segregation and other parts of the metabolic machinery that the daughter cells inherit. Additionally, reproductive hyphomicrobial cells have pronounced asymmetric morphological and physiological polarity. It has not been shown how the main reproductive cell could separate its function from the less metabolically active swarm cell (9, 10, 22).

The biphasic cell cycle (Fig. 1) of *Hyphomonas* spp. has been described elsewhere (19, 25, 26). Briefly, as shown in Fig. 1, a spherical swarm cell loses its flagellum, metamorphoses to an adherent specialist, elaborates a prosthecum, which elongates to a length of 1.0 to 3.0 μm (remaining 0.2 μm in diameter) and forms a bud from the distal end, which matures apart from the reproductive cell. During the swarm cycle, DNA is synthesized from the interval of prosthecate formation through bud formation (Fig. 1C to E) (25); at the end of the process, the nucleoid appears condensed and centrally located in the bud (27).

A number of models for the segregation of the bacterial nucleoid have been proposed including the involvement of helicase-created tension (6), unspecified nuclear proteins (13), SpoIIIE protein during asymmetric cell division (30), and/or the cell membrane (29). In *Escherichia coli*, nucleoid movement was observed to be independent of DNA replication (28), as in hyphomicrobia where the DNA was thought to pass quickly through the prosthecum, probably as a compressed nucleoid (16). The subject of chromosome segregation has received much attention (8, 24), but the mechanism remains theoretical.

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Here we report that the *Hyphomonas* cytoplasmic membrane (CM) forms pseudovesicles (PV), many of which contain DNA and ribosomes, and that a portion of the CM partially separates the main body of the reproductive cell from the prosthecum. The probable functions of these structures are discussed.

(A portion of this research was presented at the 95th General Meeting of the American Society for Microbiology [31] and published in partial fulfillment of the requirements for a M.S. degree for P. Zerfas [32] from the University of Maryland, College Park, 1995.)

MATERIALS AND METHODS

Bacteria and media. The fine structure of two diverse strains of *Hyphomonas* sp. (15), VP-6 (ATCC 43869) and MHS-3 (ATCC 43965), was examined with cultures maintained in the University of Maryland Culture Collection since 1988. Cultures were grown at 37°C with aeration in marine broth 2216 (33) (37.4 g/liter) (MB) (Difco Laboratories, Detroit, Mich.) and harvested during mid-logarithmic growth. Synchronous cultures were prepared as previously described (25). Nalidixic acid (NA), which acts on DNA gyrase, was used to rapidly inhibit DNA replication (18). Determinations of the sensitivities of *Hyphomonas* strains to and growth on NA were done as described previously (26).

Chemicals. Polyethylene glycol (PEG) 20,000, glutaraldehyde, formaldehyde, and the lactoferrin-gold complex were obtained from Sigma Chemical Co., St. Louis, Mo. The lactoferrin-gold complex was stored at −20°C until it was diluted and then stored at 4°C. LR White, Spurr's medium, osmium tetroxide, grids (copper, nickel, and gold grids), and all other transmission electron microscopy materials were obtained from Electron Microscopy Services, Fort Washington, Pa. DNase was purchased from Boehringer-Mannheim, Indianapolis, Ind., and uranyl acetate and lead citrate were from Fluka, Ronkonkoma, N.Y.

Electron microscopy of whole cells. Twenty microliters of culture was placed onto a 400-mesh (carbon-stabilized) collodion-covered copper grid. After 1 min, excess culture was removed by touching the edge of the grid with a piece of filter paper. Cells adhering to the grid were stained by placing 20 μl of 1% uranyl acetate on the grid for 1 min, removing the excess by touching the edge of the grid with filter paper, and air drying.

Embedding and gold labeling. The cultures in one-third-strength MB (26) were harvested (8,000 × *g*) and washed two times in 3.5% saline. They were resuspended in 20 mM phosphate buffer (pH 7) with 3.5% saline (PB) supplemented with cold 3% formaldehyde and 0.1% glutaraldehyde, resuspended, and held for 16 h at 4°C. All subsequent steps were done at room temperature. Cells were repelleted and washed three times with 1.5 ml of PB, resuspended in 1.0 ml of a 1.0% sodium metaperiodate solution for 15 min, pelleted, and washed in 1 ml of PB. The pellet was resuspended in 1 ml of 50 mM ammonium chloride for 15 min, centrifuged, washed with 1 ml of distilled H_2O (dH_2O), resuspended in

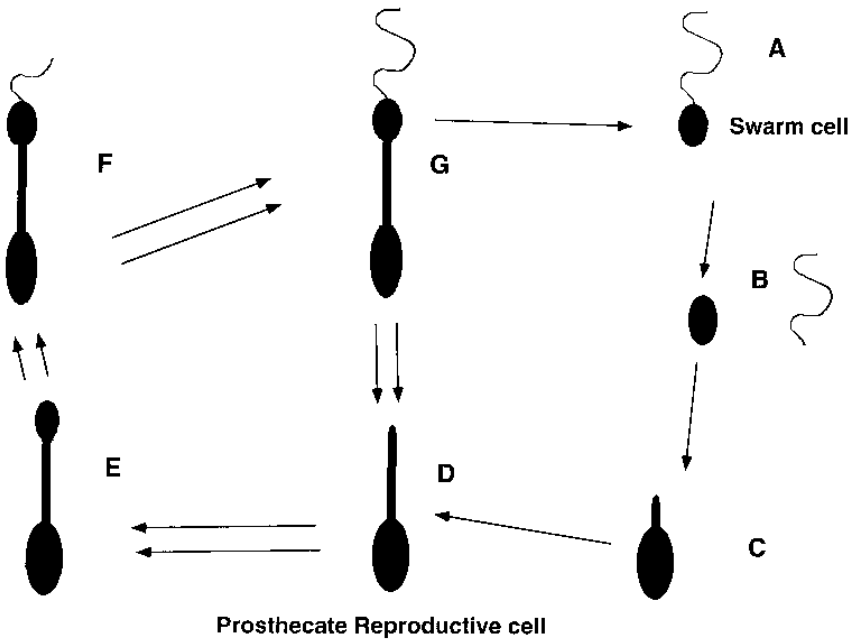


FIG. 1. Biphasic cycle of *Hyphomonas* depicting adherent reproductive stages and planktonic swarm cell maturation. A spherical swarm cell (A) loses its flagellum (B), metamorphoses to an adherent specialist (C), elaborates a prosthecum (D), which elongates and forms a bud from the distal end (E), which matures (F and G) apart from the reproductive cell. The swarm cycle (single arrows) and the reproductive cycle (double arrows) are indicated. The cells have a flagellum at stages A, F, and G. The cells have a capsule during stages C to G.

0.2 ml of 50% ethanol for 2 min at which time 0.4 ml of absolute ethanol was added. After 5 min, an additional 0.8 ml of absolute ethanol was added (5 min) and the mixture was centrifuged and resuspended in 100% absolute ethanol (5 min). The pellets were then resuspended in a 2:1 mix of ethanol-LR White, (1 h), centrifuged, resuspended in a 1:1 mix of ethanol-LR White (1 h), and then exposed to 100% LR White overnight, after which it was transferred to fresh LR White (1 h) and finally placed in gelatin capsules (filled with LR White). The capsules were polymerized in a vacuum oven at 50°C for 48 h. Ultrathin sections were placed on nickel grids, in lieu of copper grids which interfere with the binding of the lactoferrin-gold complex.

Lactoferrin-gold labeling. Labeling was carried out using substantial modifications of a previously reported procedure (4). Each thin section was floated on 1 drop of phosphate-buffered saline (PBS), (137 mM NaCl, 2.7 mM KCl, 4.3 mM $Na_2HPO_4 \cdot 7H_2O$, 1.4 mM KH_2PO_4; pH 7.3) with 10 mM $NaHCO_3$ and 0.2 mg of PEG 20,000 per ml (PBS-PEG) for 30 min, and the edge of the section was touched with a piece of filter paper to remove excess PBS-PEG. Each grid was then floated on 1 drop of lactoferrin-gold complex (10-nm-diameter gold particles) (diluted 1:5 with 0.04 M PBS) for 1 h, washed with several drops of dH_2O, touched with filter paper to remove excess water, air dried for 10 min, and stained for 30 s with 1% uranyl acetate.

Negative controls were prepared by exposing thin sections to DNase (10 U/μl), (diluted 1:10 in 0.04 M PBS) for 30 min (20°C) and washed with dH_2O. These specimens were treated with lactoferrin-gold as described above. Positive controls were purified *Hyphomonas* strain VP-6 DNA. Twenty microliters of DNA was placed on a collodion carbon-coated stabilized copper grid for 1 min, labeled with lactoferrin-gold complex, diluted 1:8 with PBS-PEG for 30 min, and washed by dripping several drops of dH_2O over the grid. Glutaraldehyde and formaldehyde, commonly used as fixatives prior to immunolabeling, did not reveal the PV as distinctly as those fixed by the protocol of Ryter and Kellenberger (21).

Visualization of fine ultrastructure. Cultures were pelleted, washed twice in 3.5% saline, suspended in a solution of 30 ml of 3.5% saline and 3 ml of Ryter-Kellenberger Veronal-acetate-buffered osmium tetroxide fixative (containing 0.4% osmium tetroxide), pelleted again, resuspended in a solution of 1.0 ml of Ryter-Kellenberger fixative (21) and 0.1 ml of 3.5% saline, and fixed for 16 h (20°C). The remainder of the procedure was done as described previously by Glauert (12). The cell suspension was diluted with 8 ml of Ryter-Kellenberger Veronal-acetate buffer, pelleted, and exposed to 0.5% uranyl acetate buffer (0.1 g of uranyl acetate dissolved in 20 ml of Ryter-Kellenberger [21] Veronal-acetate buffer) for 2 h (20°C). The pellets were serially dehydrated and embedded in Spurr's medium (23).

Preparation and staining of ultrathin sections with potassium permanganate. Ground $KMnO_4$ was diluted to a 1% solution in boiled ultrapure water. The stain was adjusted to pH 6.0 and centrifuged to remove particulate material. The supernatant was sealed with paraffin, stored at 4°C, and recentrifuged prior to use. Grids were floated on a drop of this $KMnO_4$ solution for 3 min and rinsed with ultrapure water.

Preparation and staining of ultrathin sections with uranyl acetate and lead citrate. The lead citrate stain was prepared immediately before use by dissolving 0.02 g of lead citrate into 10 ml of dH_2O with 0.1 ml of 10 N NaOH. Each thin section was floated on 1 drop of 4% uranyl acetate in 50% methanol for 5 min, rinsed in dH_2O, air dried, and then stained for 1.5 min with lead citrate. Specimens were rinsed sequentially in 0.02 N NaOH, dH_2O, and 0.02 N NaOH and then rinsed three times in dH_2O.

Freeze-etch. Cultures were washed two times in 3.5% saline. Half the cultures were resuspended in cryoprotectant (12% [vol/vol] glycerol and 12% [wt/vol] sucrose in PBS; pH 7.4) and the other half were resuspended in PBS. Specimens in their holders were placed into melted Freon 22 and transferred to the specimen stage (Denton DFE-2 Freeze-Etch unit; Denton Vacuum Inc., Morestown, N.J.), prechilled to a temperature of −140°C. Upon evacuation, specimens were warmed to −100°C until frost dissipated and then cooled to −180°C at which temperature the cells were fractured. Specimens were etched at −98°C (2 min). The etched surface was shadowed (−150°C) with platinum/carbon. Replicas were cleaned with chromic acid, rinsed in dH_2O, and picked up on freeze-fracture grids (Pelco, Tustin, Calif.) with Formvar films.

Ultrathin sectioning and observation. The ultrathin sections (Reichert-Jung FC-4E Ultracut E microtome [Reichert, Vienna, Austria]; diamond knife [Diatome Ltd., Bienne, Switzerland]) were placed on copper grids for staining with uranyl acetate and lead citrate, nickel grids for lactoferrin-gold staining, or gold grids for potassium permanganate staining. Specimens were viewed using the JEM-100CX II transmission electron microscope (JEOL Ltd., Tokyo, Japan) operating at an accelerating voltage of 80 kV. Micrographs were recorded on Kodak SO-163 emulsion film.

RESULTS

Separation of spheroid body from prosthecum. In whole cells stained with uranyl acetate, the main body of the reproductive cell is partially partitioned from the prosthecum by a continuation of the CM, which bifurcates at the origin of the prosthecum (Fig. 2). The region of this double-layered cell membrane that divides the cell, which we term membrane cap (MC), is revealed more clearly in thin sections (Fig. 3 and 4). Ribosomes are retained in the cytoplasm of the main body of the reproductive cell and in the PV (Fig. 5). The MC is visible in cells in all stages of the reproductive cycle (Fig. 1C to G).

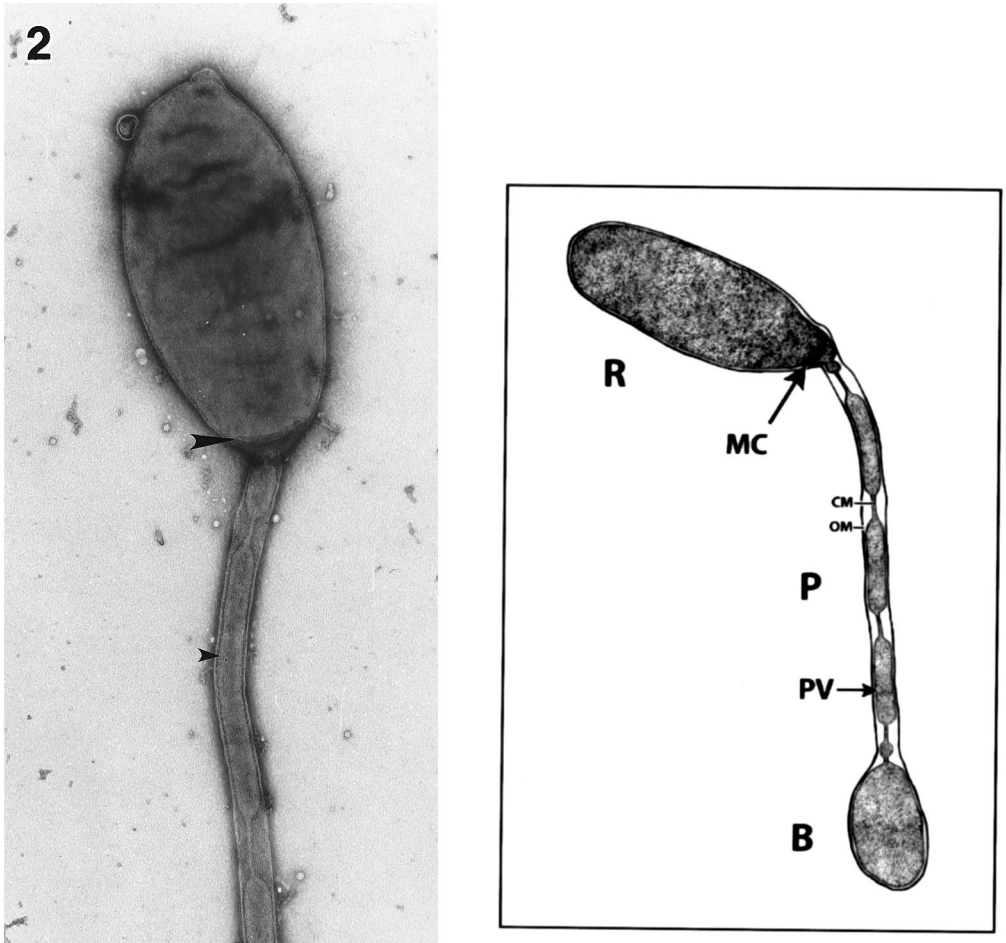


FIG. 2. Negatively stained (uranyl acetate) *Hyphomonas* strain MHS-3 growing logarithmically in MB. The wash step before fixation was omitted to reduce any plasmolysis. Fixative was added directly to the growth medium. The large arrow points to the membrane cap. (Right) Model of predicted CM complex structure in logarithmically growing reproductive cells (drawn from a micrograph similar to that shown in this figure). R, reproductive cell; P, prosthecum; B, bud; MC, membrane cap (large arrow); PV, pseudovesicle (small arrow); OM, outer membrane. Bar = 1.0 μm.

PV in prosthecae. PV, formed by invaginations and swellings of the cytoplasmic membrane, were observed in whole-cell preparations (Fig. 2) and in thin-sectioned specimens that were prepared by three different techniques: conventional embedding with ultrathin sectioning stained by either uranyl acetate plus lead citrate or potassium permanganate (Fig. 6 and 7, respectively), freeze-etching (Fig. 8a), and frozen hydrated (Fig. 8b). Figures 5 and 7 clearly show that PV are not closed but rather connected by the contiguous CM.

PV contain DNA. Evidence that the PV contain concentrations of ribosomes and DNA comes from ultrathin sections stained with potassium permanganate (Fig. 7) and from lactoferrin-gold labeling. In the Ryter-Kellenberger technique, uranyl acetate stabilizes DNA, resulting in a fibrillar appearance. Ribosomes are less concentrated in the PV in the prosthecum than in the main body of the mother cell. Most commonly, there appear to be two nucleoids in stage D (Fig. 1) reproductive cells, although without serial sectioning, the number of nucleoids cannot be determined unequivocally. Typically, one nucleoid is centrally located in the main body of the cell, and the second, also in the main body of the cell, is located adjacent to the MC (Fig. 6 and 7). The centrally located nucleoid occupies approximately three times the area of the chromosomal material in the PV.

Lactoferrin-gold, which has some specificity for single- and double-stranded DNA was used to label the nucleoid (2–4). Gold particles clustered in the PV, in the main body of the reproductive cell, and in the emerging bud over electron-transparent fibrillar regions (Fig. 9). For controls, purified *Hyphomonas* DNA, ultrathin sections treated with DNase and

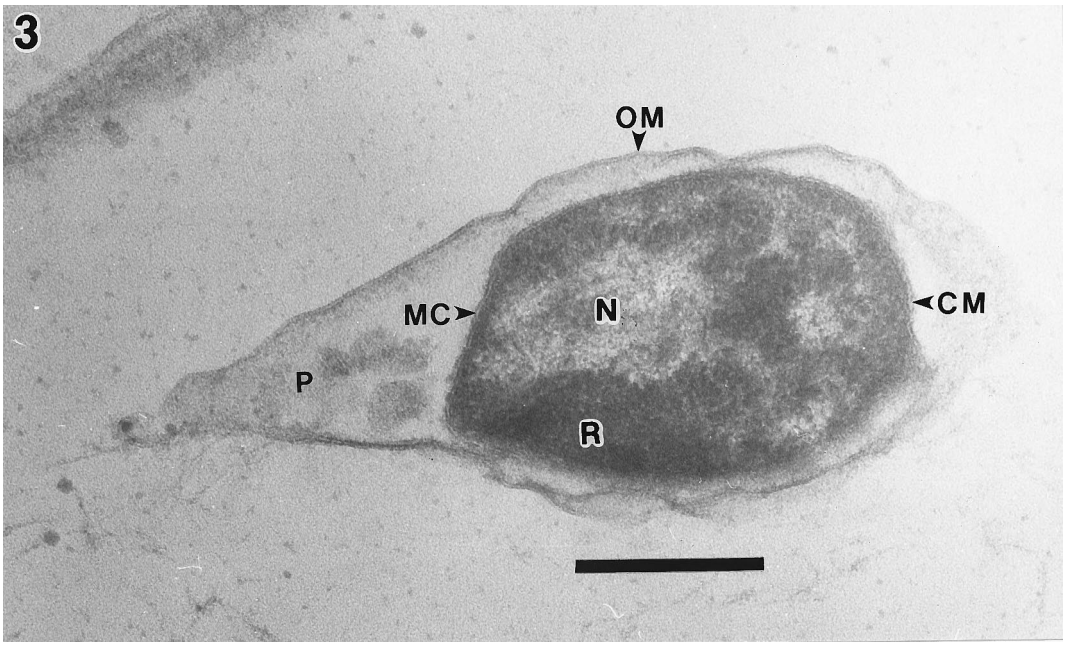


FIG. 3. Electron micrograph of *Hyphomonas* strain VP-6 showing the MC. Cells were fixed by the method of Ryter and Kellenberger (21), ultrathin sectioned, and stained with potassium permanganate. Note the MC at the point of constriction between the mother cell and prosthecum. The use of the method of Ryter and Kellenberger (21) imparts a trilaminar appearance to the membranes. Staining with $KMnO_4$ or uranyl acetate and lead citrate enhances this effect. DNA has a thread-like and electron-transparent appearance; the ribosomes appear as small dark bodies. CM, cell membrane, OM, outer membrane; P, prosthecum; R, ribosomes; N, nucleoid. Bar = 250 nm.

untreated cells were exposed to lactoferrin-gold. The fibrillar structure (DNA) of the control cells was labeled, as was the purified DNA. The ultrathin sections digested with DNase were not labeled. Additionally, other areas, presumably containing proteins and other macromolecules, were not labeled. The difference in gold labeling between control cells and those treated with DNase is significant ($P = 0.01$). The average number of particles labeling the control cells ($n = 36$) was 28 ($G\sigma_x = 4.2$); the DNase-treated cells ($n = 36$) contained an average of 7 ($G\sigma_x = 1.2$) gold particles.

PV in NA-treated cells do not contain DNA. NA-exposed cells elongated, generally without swarm cell separation as shown previously (26). PV were formed (Fig. 10), but no DNA was detected within them, either by fibrillar appearance or lactoferrin-gold labeling (negative results not shown). As above, the difference in gold labeling between control cells and those treated with NA is significant ($P = 0.01$). The average number of gold particles in PV in control cells ($n = 23$) was 7.5 ($G_{GX} = 0.6$); in cells treated with NA ($n = 21$), it was 1.8 ($G_{GX} = 0.3$).

DISCUSSION

This is the first report of extensive membranous structure in the genus *Hyphomonas*, a member of the prosthecate budding bacteria, having a relatively complex, biphasic life cycle. A MC has not been reported previously in any of the genera of budding bacteria in which the fine structure had been studied (5, 7, 11, 27). Examination of micrographs in this study revealed that the mother cell is partitioned from the prosthecum by the MC (we have not ruled out the possibility that there is a pore in the MC), that PV form in the prosthecum, that the PV are con-

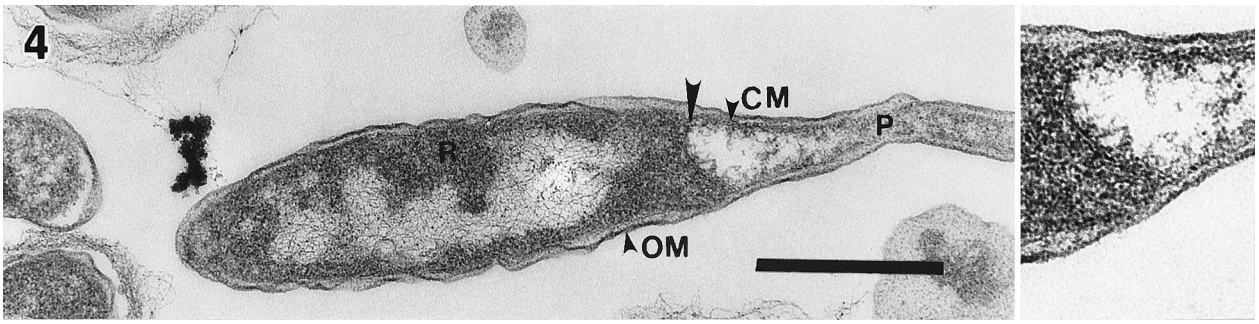


FIG. 4. Electron micrograph of *Hyphomonas* strain VP-6. Cells were embedded by the method of Ryter and Kellenberger, ultrathin sectioned, and stained with uranyl acetate and lead citrate. Note the MC (arrow) at the proximal pole of the mother cell. The nucleoid is centrally located within the mother cell, and ribosomes appear as darkened regions. CM, cell membrane; OM, outer membrane; P, prosthecum; R, ribosomes. Bar = 500 nm. (Right) Higher magnification of the MC. The cell membrane appears to bifurcate and extend across the cell, thereby separating the dense ribosome-filled mother cell cytoplasm from an area of electron-transparent DNA in the prosthecum. Magnification, ×93,000.

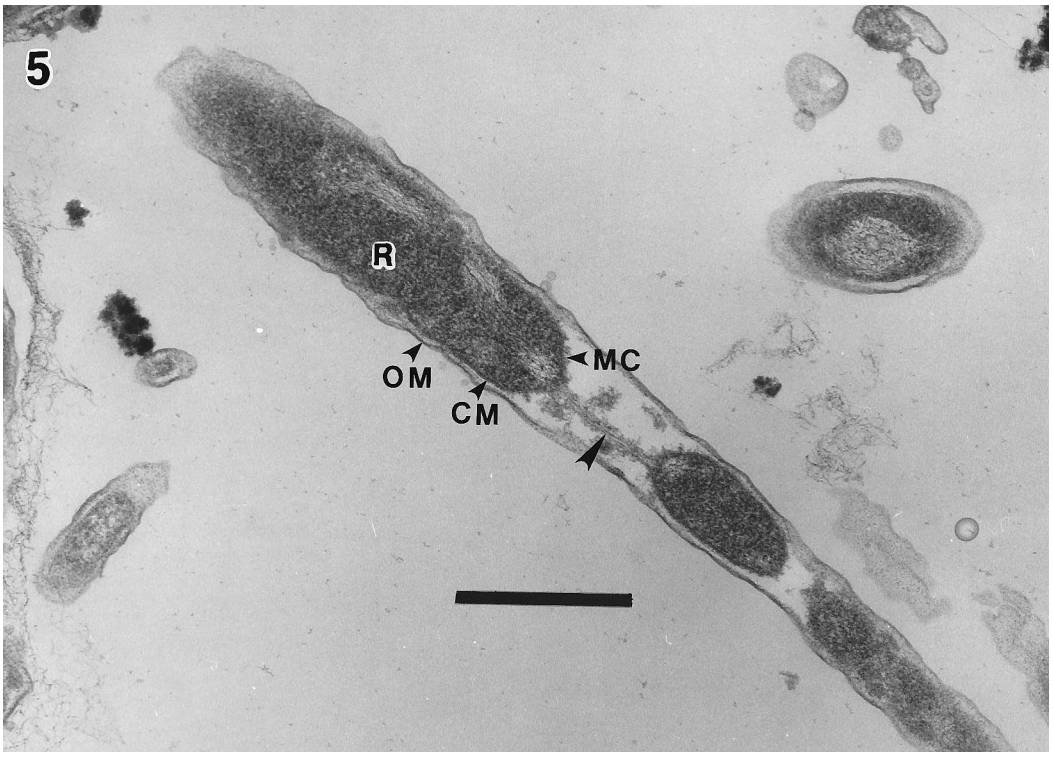


FIG. 5. Electron micrograph of *Hyphomonas* strain VP-6 showing the contiguous membrane. Cells were embedded by the method of Ryter and Kellenberger, ultrathin sectioned, and stained with uranyl acetate and lead citrate. Note the MC which retains the ribosomes that appear as dark bodies. The contigious membrane connecting the mother cell to the vesicle is indicated by the unlabeled arrowhead. R, ribosomes; CM, cell membrane; OM, outer membrane. Bar = 500 nm.

nected by membrane-bounded bridges, that the membrane forming the PV is an extension of the CM (model presented in Fig. 2), and that the PV normally contain DNA.

The micrographs clearly revealed a membrane (MC) that partitioned the mother cell from the prosthecum and daughter cell. This membrane was regularly visualized in negatively stained cells, thin-sectioned cells, and freeze-etched specimens. The MC is hypothesized to be a cytological adjunct to cell polarity which is highly asymmetrical in budding cells. For example, the main body of the reproductive cell of *Hyphomonas* strain MHS-3 alone is surrounded by capsular exopolysaccharide (20), while only the swarm cell has a flagellum (9). The MC would also partition cellular products, e.g., proteins that are unique to the reproductive or swarm cell (22). The MC may also have a role in DNA localization and segregation; i.e., the newly replicated nucleoid may be positioned near the MC (Fig. 5).

The CM, approximately 8.5 nm wide, forms the PV (e.g. Fig.

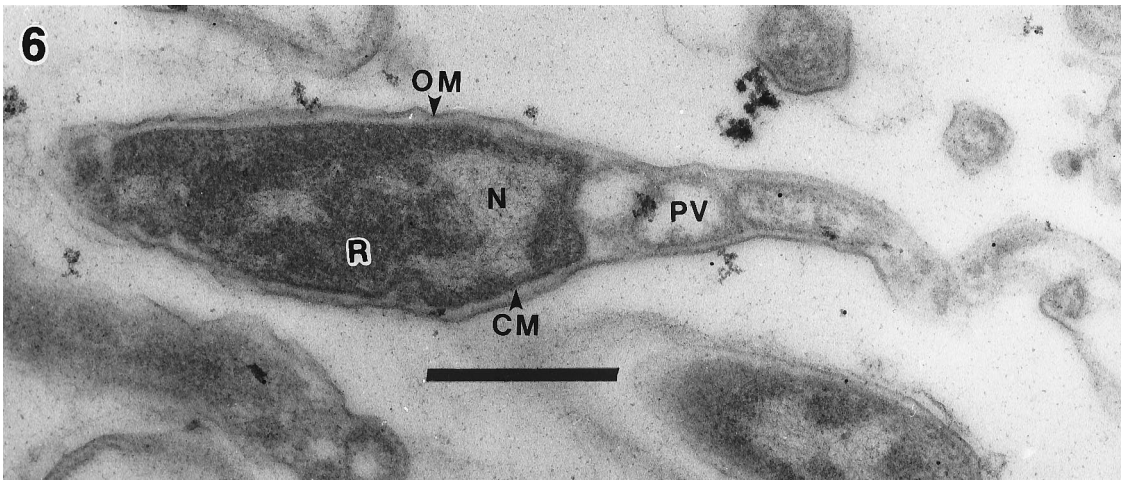


FIG. 6. Electron micrograph of *Hyphomonas* strain VP-6 showing DNA within the PV. Cells were embedded by the method of Ryter and Kellenberger, ultrathin sectioned, and stained with uranyl acetate and lead citrate. DNA in the PV is electron transparent. PV, pseudovesicle; CM, cell membrane; OM, outer membrane; R, ribosomes; N, nucleoid. Bar = 500 nm.

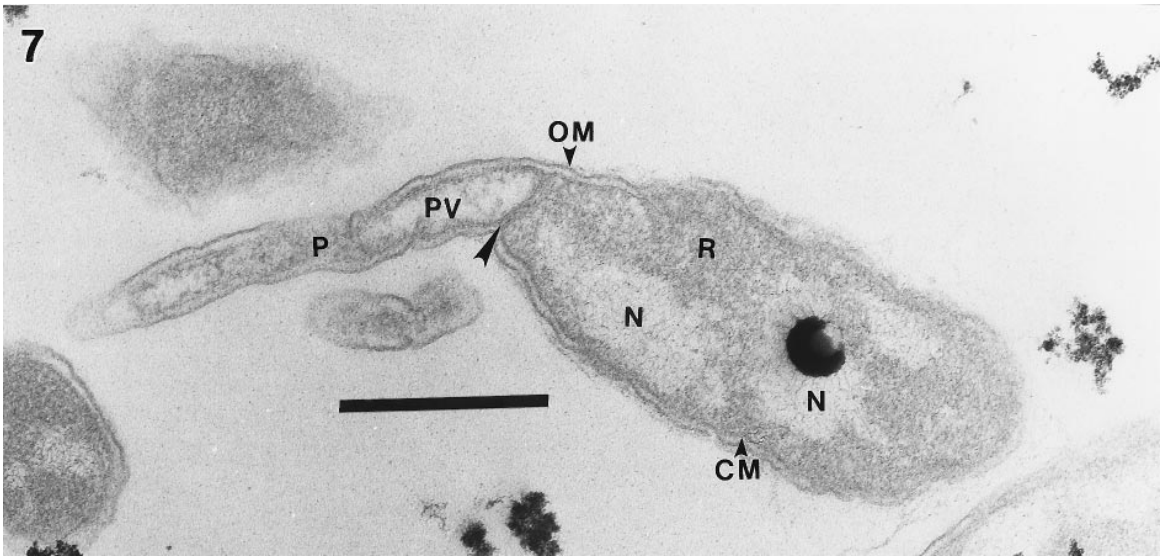
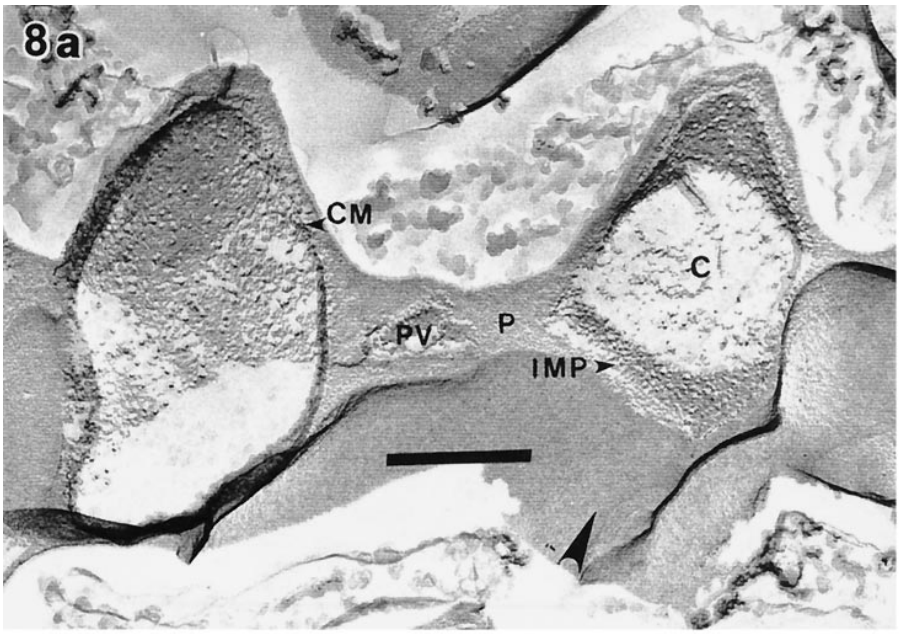


FIG. 7. Electron micrograph of *Hyphomonas* strain VP-6. Cells were fixed by the method of Ryter and Kellenberger, ultrathin sectioned, and stained with potassium permanganate. Note the MC (arrowhead) at the proximal pole of the mother cell. Two nucleoids are visualized within the mother cell. CM, cell membrane; OM, outer membrane; P, prosthecum; PV, pseudovesicle; R, ribosomes; N, nucleoid. Bar = 500 nm.



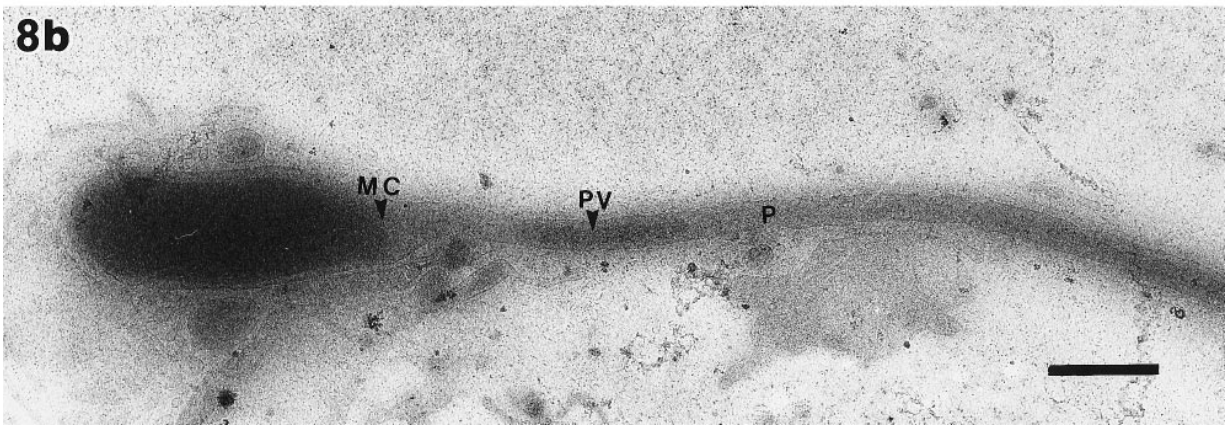


FIG. 8. (a) Freeze-etched *Hyphomonas* strain VP-6. A PV showing a P-face, is located in the prosthecum. The fracture plane was at the cell membrane. The large arrowhead indicates the shadowing direction. CM, cell membrane; P, prosthecum; PV, pseudovesicle; IMP, intramembranous particles; C, cytoplasm. Bar = 250 nm. (b) Frozen-hydrated preparation of *Hyphomonas* showing a darkly contrasting PV in the prosthecum (P). The MC is also clearly seen. Magnification, ×30,000. Bar = 500 nm. (Micrograph courtesy of F. Booy).

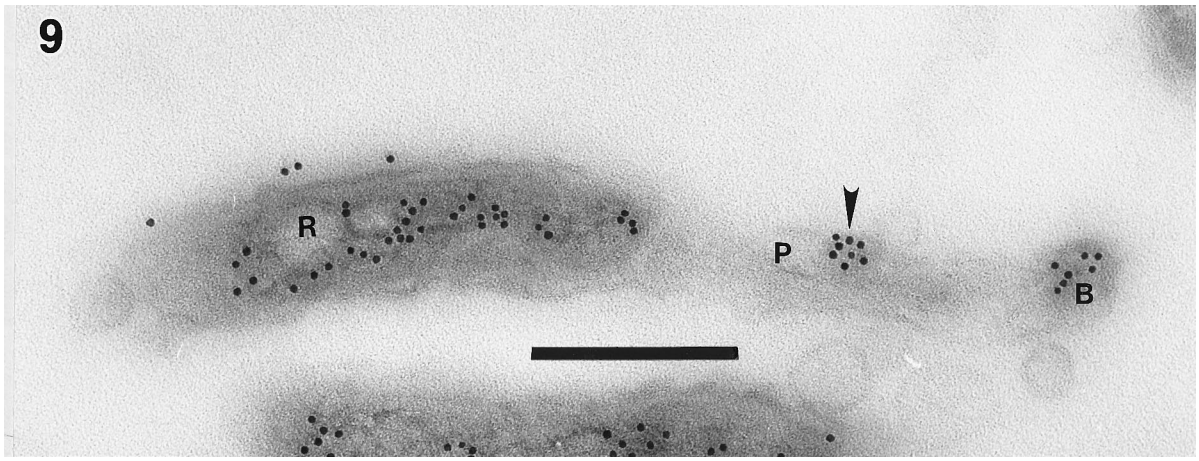


FIG. 9. *Hyphomonas* strain VP-6 labeled with lactoferrin-gold specific for DNA. The culture was synchronized, being started with young swarm cells (25). The specimen is a first-generation cell. Ultrathin sections were placed on nickel grids and incubated for 1 h with lactoferrin-gold diluted 1:5 in 0.04 M PBS and stained with uranyl acetate. Note the PV labeled with 10-nm-diameter gold particles (arrowhead) indicating the presence of DNA. R, reproductive cell; P, prosthecum; B, newly forming bud. Bar = 250 nm.

5 and 7), and careful analysis suggests that the CM does not completely enclose each PV; the CM alternately constricts and swells in the prosthecum so that the PV are essentially contiguous bodies formed by the CM. Similar structures in the prosthecum have been reported twice previously from the budding bacteria (5, 7), but not in any marine, nonphotosynthetic strains. While it was suggested that membranes of *Hyphomicrobium* strain B-522 did not enclose the nuclear region in the main body of the mother cell, the structure, in the prosthecum, was termed a membrane-bounded vesicle (7). In this report we show that the vesicle in *Hyphomonas* is not completely enclosed (PV). This is obvious by negative staining (Fig. 2) and in those few thin sections that slice through the narrow plane of the constricted part of the CM (e.g., Fig. 5 and 7). DNA segregation in conjunction with PV may be analogous to that in sporulation asymmetric cell division where DNA is thought to transverse a nearly completed septum (30).

The prosthecum is 200 nm wide, the PV is 185 nm wide, and the width of the constricted part of the membrane is approximately 19 nm. Unlike the prosthecum of *Caulobacter* spp., through which DNA does not pass, the budding, prothescate bacteria do not form crossbands (7). In eukaryotes, pores are 70 to 80 nm in diameter, and molecules 9 nm or less can pass freely between vesicular bodies (1). In *Hyphomonas*, the diameter of the constriction of the CM in the prosthecum should allow for free passage of proteins, but not ribosomes which are 20 nm in diameter. Therefore, the ribosomes should remain inside the PV during passage into the bud.

We have used several different methods of specimen preparation for electron microscopy, and by all of these we can

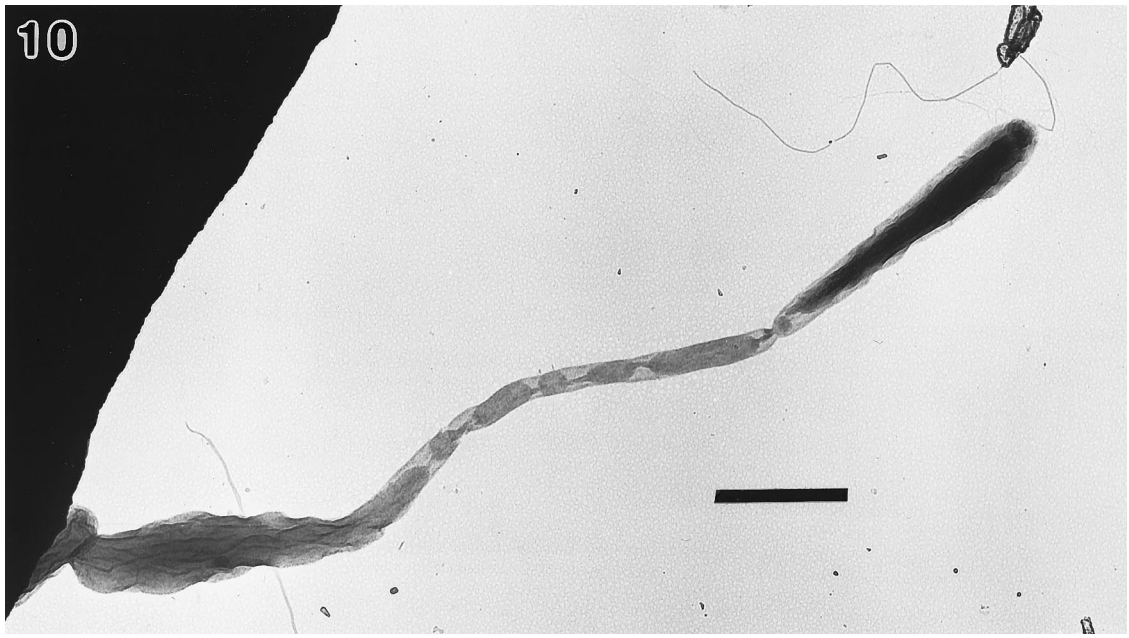


FIG. 10. *Hyphomonas* strain VP-6. A logarithmically growing culture was exposed to NA for 5 h and negatively stained (uranyl acetate). Note the large number of PV. Bar = 500 nm.

demonstrate the existence of the PV in the prosthecum of *Hyphomonas*. The CM which forms the PV was viewed when cells were prepared by both ultrathin sectioning and freeze-etching. Additionally, negatively stained and frozen hydrated whole cells, which are the optimal methods for preservation of cellular architecture, clearly demonstrate well-preserved PV in the prosthecum. Thus, the PV appear to be genuine structures.

Although the biogenesis of PV is not clear, their presence throughout the reproductive cycle of *Hyphomonas* has been established in this study. As suggested above, the PV may have a role in moving and partitioning DNA and ribosomes. From observations of ultrathin sections, one hypothesis is that the PV migrates into the newly formed bud, becoming the CM. This membrane would be synthesized in the reproductive cell. DNA may attach to it, as shown by lactoferrin-gold labeling in the vicinity of the MC. As reported for *E. coli* (13, 17), the attachment of DNA to the CM may function in chromosome segregation, i.e., in transversing the prosthecum.

Alternatively, the PV may represent a freeze-frame depiction of a dynamic wave of "peristaltic-like motion," moving macromolecules to the bud. However, if such movement were solely responsible for movement of the DNA into the progeny, the bud, which is a growth precursor cell (9) would have to synthesize its cell membrane de novo, a notion which is implausible.

The PV contain DNA, which is confirmed by both cytological appearance and lactoferrin-gold labeling. It is not known whether each PV carries a complete genome or whether two or more PV house the chromosome. The genomic DNA occupies three times the area in the main body of the cell than that in each PV, suggesting that more than one PV may house the genome; however, it must also be considered that the segregating DNA has been reported to be condensed (16).

Some information was obtained by observing the development of synchronously grown, first-generation prosthecate cells (because of the biphasic life cycle, synchrony can be maintained for only two budding cycles [25]). First-generation prosthecate cells contain several PV with DNA (31) (Fig. 9) but only two genomes (25, 26), also suggesting that a genome is packaged in more than one PV. PV are also clearly formed in cells that are blocked in DNA replication by NA; however, no DNA is then detected within the PV. Thus, PV formation is not stringently coupled with DNA replication. Synchronous cultures that are blocked at intervals in DNA replication would reveal whether DNA enters the PV as soon as it is replicated.

Lactoferrin-gold labeling resulted in 60 to 70 particles in the main body of the reproductive cell and 7 to 9 particles in each PV at densities of 14/mm^2 in the former and 2.5/mm^2 in the latter. The PV may attract less lactoferrin-gold due to the compact conformation of the nucleoid there, sterically hindering lactoferrin-gold binding. In fact, the label in the PV is clustered, indicative of aggregated DNA (4). Replicating DNA is more dispersed, which may account for the distribution of label in the main body of the reproductive cell. Lactoferrin-gold labeling does not appear to be sensitive enough to detect any putative DNA that may lie between the PV. The DNA in the PV and between them may represent one prosthecal chromosome. In any case, the fact that there are several DNA-containing PV in the prosthecum at any moment suggests that, contrary to a previous report (16), chromosome segregation in *Hyphomonas* is not rapid and does involve the CM. DNA replication has been reported to occur only in the main body of the mother cell (9, 16). The proximity of the chromosome to the membrane and particularly to the MC and PV is not inconsistent with current modifications (24) of the classical surface attachment model of Jacob et al. (14).

To summarize, in *Hyphomonas*, the CM surrounds the entire reproducing cell and is contiguous in the main body of the mother cell, prosthecum, and emerging bud. There is a branch point (e.g., Fig. 4) at the base of the prosthecum where part of the membrane surrounds the main body, forming the MC between it and the prosthecum. The MC may segregate adherent, reproductive cell function from pelagic swarm cell function (9). The other branch of the membrane continues into the prosthecum, alternately swelling and constricting, forming PV. The PV house DNA, ribosomes, and probably other macromolecules, probably directly or indirectly functioning in delivering these components to the budding swarm cell (Fig. 2). This structural arrangement is consistent with function.

ACKNOWLEDGMENTS

We thank S. Benson, J. Blankenship, T. Maugel, and W. Schafer for helpful discussions, Eric Erbe (USDA, Beltsville, Md.) for generously preparing the freeze-etched samples, and F. P. Booy (Laboratory of Structural Biology, National Institute of Arthritis, Musculoskeletal and Skin Diseases, NIH, Bethesda, Md.) for providing the micrograph of the frozen hydrated preparation.

This work was supported in part by grants from the Maryland Industrial Partnerships and Oceanix Corporation.

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