JOURNAL OF BACTERIOLOGY, Nov. 1977, p. 666-672

Vol. 132, No. 2
Printed in U.S.A.

Intracellular, Periodic Structures in the Gliding Bacterium *Myxococcus xanthus*

ANN C. BURCHARD, ROBERT P. BURCHARD,* AND JOHN A. KLOETZEL

Department of Biological Sciences, University of Maryland Baltimore County, Catonsville, Maryland 21228

Received for publication 10 August 1977

Electron microscopic observations of thin sections of *Myxococcus xanthus* vegetative cells revealed the presence of cytoplasmic bundles of 4- to 5-nm-diameter filaments, running longitudinally below the cell membrane and terminating in association with the envelope near one pole. Part of each bundle demonstrated a herringbone-like periodicity (~12-nm spacing). This structure was observed in cells from shake cultures and in gliding cells fixed by several methods. It is proposed that the structure may be attached to the envelope near both poles in gliding cells and that the motive force for motility may be provided by its contraction and relaxation. In one of four nongliding mutants examined, the periodicity was indistinct or lacking. In this mutant another structure, comprised of linearly arrayed beads, was observed in association with the filamentous bundle. Another structure, characterized by major, transverse bands (~34 nm apart), occurred in patches that may traverse the diameter of the wild-type cells in which the structure was observed.

The mechanism of gliding motility in procaryotes is unknown (10, 17). Several investigators have suggested cause-and-effect relationships between movement and structures observed in or on gliding cells. Fibrils have been observed in the envelopes of *Cytophaga columnaris*, *Sporocytophaga* spp., and *Oscillatoria princeps* (13-16, 21). The significance of these structures in *Cytophaga* has been questioned (5). Tubular structures have been observed in a variety of gliding procaryotes. Among these, rhapidosomes are in dispute (e.g., 8, 9, 14, 20, 22). Tubules, narrower than the microtubules of eucaryotes, have been observed in the cytoplasm of various species of myxobacteria and of the blue-green bacterium *Nostoc* (1, 18, 25, 26). Recently, cup-shaped subunits (goblets) have been observed on envelopes of *Flexibacter polymorphus* (23, 24). These may directionally secrete mucilagenous fibrils, thereby propelling the cells.

We initiated the fine-structure study reported here with a view to determining whether there is a structural correlate to the observation that treatment of *Myxococcus xanthus* with some proteolytic enzymes reversibly inhibits gliding motility (4). In addition to demonstrating a protease-induced morphological change, electron microscopic observations have revealed the presence of several unusual structures in both treated and untreated bacteria. These are described here.

MATERIALS AND METHODS

Bacterial strains. *M. xanthus* FB2 is similar in its characteristics to strain FB (11). Strain RB4 is a nongliding mutant, previously called NM (2). Strains DK306, 310, and 314, also nongliding mutants of *M. xanthus*, were provided by J. Hodgkin and D. Kaiser.

Media. Bacteria were cultured in PT-1 broth or on PTE agar at 30°C (3).

Protease treatment. Bacteria were centrifuged out of the culture medium (5,900 × *g*) and suspended in 0.01 M tris(hydroxymethyl)aminomethane-hydrochloride (pH 7.4) containing subtilisin (protease type VIII, Sigma Chemical Co.; 100 μg/ml). They were incubated at 37°C for 20 min.

Electron microscopy. Bacteria growing in shake cultures were fixed by direct addition of glutaraldehyde to a final concentration of 1%. After 3 h at 4°C, the cells were washed three times with 0.1 M s-collidine buffer (pH 7.5), postfixed with 3% OsO_4 in 0.01 M s-collidine buffer-0.01 M $CaCl_2$ for 1 h, dehydrated through an ethanol series, and embedded in Spurr resin. Plate-grown bacteria were fixed in situ with 1 or 4% cold glutaraldehyde in 0.01 M s-collidine buffer, followed by 3% OsO_4.

Single sections were mounted on bare copper grids (200/300 mesh). Serial sections were mounted on Formvar- and carbon-coated, single-hole grids. Sections were stained with uranyl acetate and lead citrate.

Microscopy was performed on a Hitachi HU-12 electron microscope operated at 75 kV.

RESULTS

Protease-treated cells. Many of the cells

treated with the protease subtilisin, which reversibly inhibits gliding (4), were characterized by a localized expansion of the periplasmic space or a separation of the protoplast from the outer layers of the cell envelope (Fig. 1). The significance of this morphological change is not known; it may account for the inability of treated cells to glide.

Intracellular structures. In longitudinal and oblique sections of normal cells and of cells treated with subtilisin, we observed 4- to 5-nm-diameter filaments arrayed in 40-nm diameter bundles running longitudinally and terminating subpolarly (Fig. 2). The bundles appeared to be closely associated with the envelope (Fig. 2 and 3). Examination of a complete series of sections through numerous cells indicated that one end of the bundle may be associated with or embedded in the cell envelope; the other end appears to fade out in the cytoplasm. We were able to find the filamentous bundle by starting with one section of a cell lacking the structure and tracing that cell through a series to a section containing it. The frequency of detection in such preparations was as high as 50%.

A herringbone-like periodicity (~12-nm spacing) characterized at least part of each filamentous bundle (Fig. 2 and 3). A through-focal series of electron micrographs indicated that the periodicity is not explicable as a positive phase artifact.

This structure has been observed in cells fixed while gliding on agar, in those grown in shake cultures, and in cells fixed by other methods (exposure to osmium tetroxide vapors followed by the standard procedure; fixation with a mixture of glutaraldehyde [2.5%] and formaldehyde [2%]; and fixation with 5% acrolein plus 0.25% glutaraldehyde).

If this structure is involved in motility, it may be expected to be absent or altered in some nongliding mutants. Preliminary observations of four such mutants revealed that one, *M. xanthus* DK306, contains a bundle of filaments comparable to that present in the wild-type. However, the periodicity is indistinct or lacking (Fig. 4). Cells of this mutant strain contain another structure generally observed in association with the bundle of filaments (Fig. 4B to D). It appears as a linear series of electron-dense beads sandwiched between electron-dense bars. The beads are electron dense in unstained sections also. The other nonmotile forms demonstrate apparently normal structures.

We saw the structure depicted in Fig. 5 infrequently. The major transverse bands are spaced approximately 34 nm apart. Alternating minor bands are sometimes seen. Examination of serial sections of a cell in which patches of bands are evident indicates that the structure may traverse the diameter of the cell (Fig. 6).

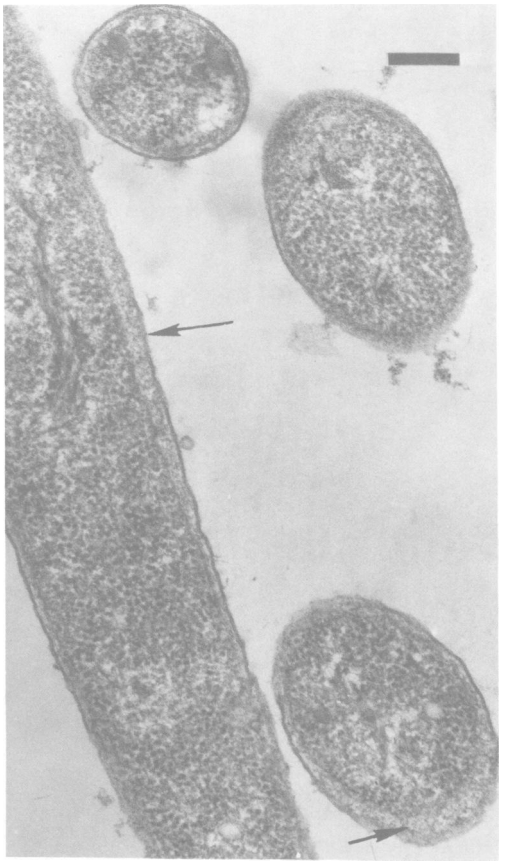

FIG. 1. *M. xanthus FB2 vegetative cells after treatment with subtilisin. Arrows indicate sites at which the protoplast has separated from the outer layers of the envelope. This and all subsequent bars represent 200 nm.*

DISCUSSION

The bundle of filaments depicted in Fig. 2 and 3 may be an organelle of gliding motility. If, for example, it were contractile and if it were attached to the semirigid envelope at or near both cell poles one could envision that translocation might occur by an inchworm-like process: the organelle and the cell alternately contract and relax or extend. This model also requires cycles of cell attachment to and release from the substrate on which it is gliding, at or near alternating poles. Although contractility theories have been proposed, gliding cells demonstrate no obvious contractions or changes in

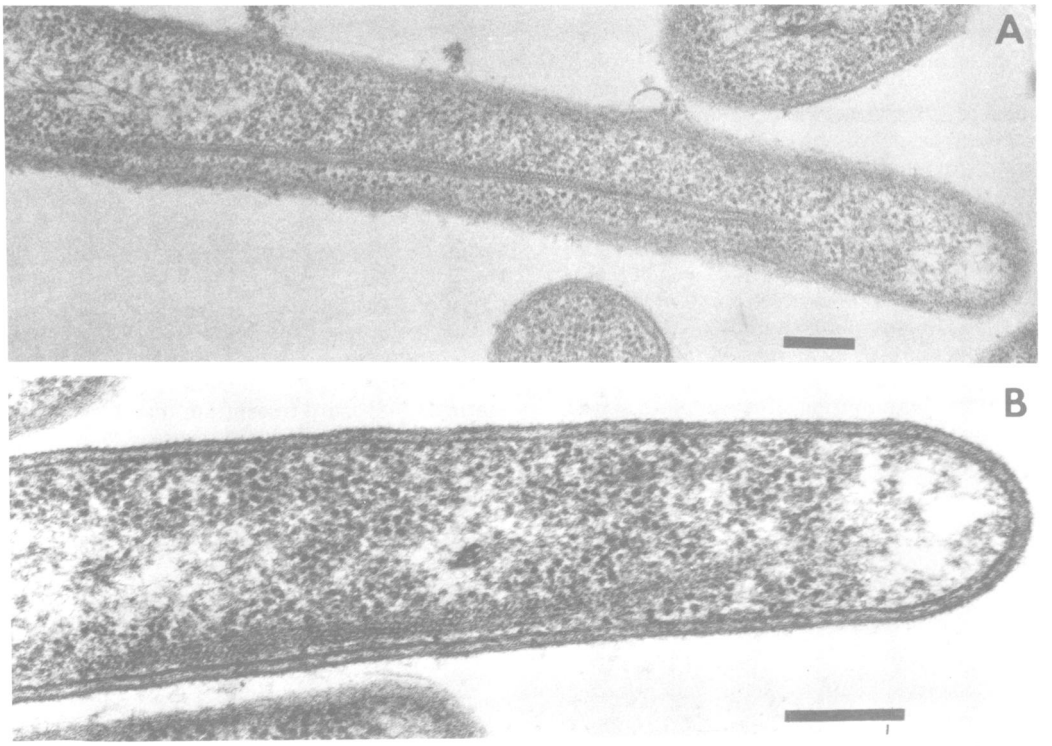

FIG. 2. *Longitudinal sections of M. xanthus FB2 cells demonstrating submembrane bundles of filaments with herringbone-like periodicity. No subtilisin treatment.*

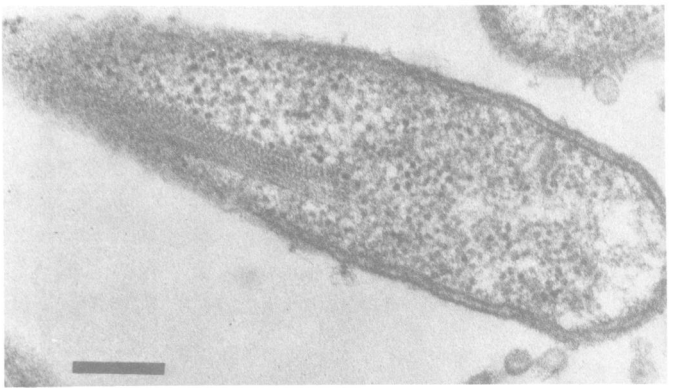

FIG. 3. *Oblique section of an M. xanthus FB2 cell demonstrating termination of bundle of filaments at or in the envelope. Filaments show partial periodicity. No subtilisin treatment.*

cell morphology (10, 27). If our inchworm hypothesis has any validity, gliding cells should appear to contract and extend to some degree. It may be that the frequency of this hypothetical contraction is high and/or its extent is submicroscopic. We are examining these possibilities at present.

To date we have been unable to clearly resolve the attachment site of the bundle of filaments to the envelope, possibly due in part to the relative geometry of the structures. Since the bundle runs almost parallel to the cell membrane, it may make contact with the membrane at a very shallow angle, or there may be lateral attachments. Another problem with the hypothesis is that we were not able to trace

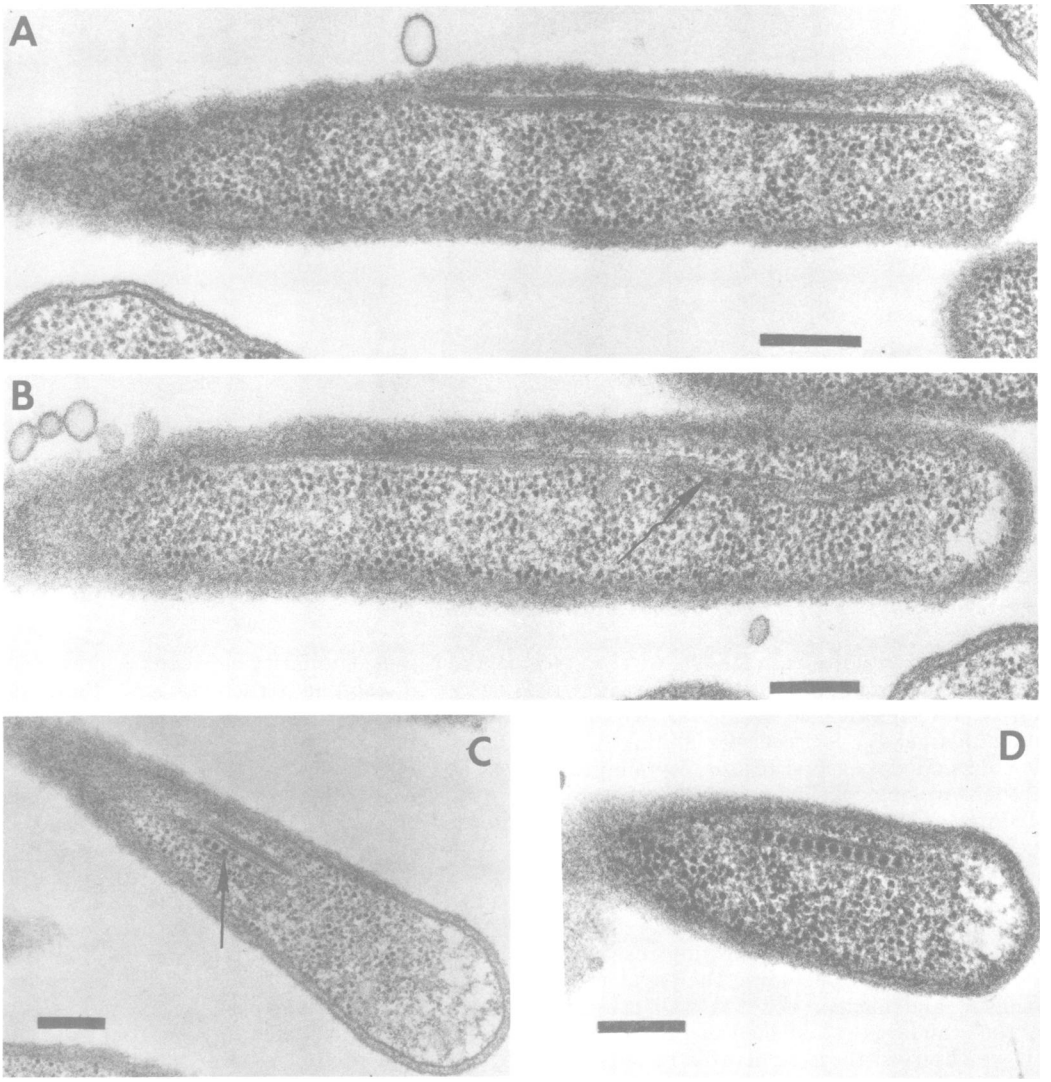

FIG. 4. *Longitudinal and oblique sections of M. xanthus DK306. The bundle of filaments demonstrates no obvious periodicity (A to C). The beaded structure (arrows) is observed to be associated with the bundle in some cells (B and C). No subtilisin treatment.*

the bundle of filaments to the second proposed attachment site. It is possible that the structure can only be visualized in a contracted (?) form after detachment from one or both sites of attachment. The proposed attachment site(s) may be particularly labile and may thus be affected by the preparative procedures we employed. This might account for the variation in the frequency of detection of the structure among our different preparations. If, in fact, the filamentous bundles we examined have detached from the site(s) of attachment, it is not surprising that they appear to fade into the cytoplasm.

If this structure were an organelle of gliding, we would expect it to be detectable in other gliding procaryotes and in an altered (or absent) form in some nongliding mutants. A substantial number of motility mutants have been isolated (2, 7, 13, 19; and J. Hodgkin and D. Kaiser, communicated at the Developmental Biology of Myxobacteria Meeting, 1976 [in press]). The structure in at least one of these mutants is altered (Fig. 4). In addition to the

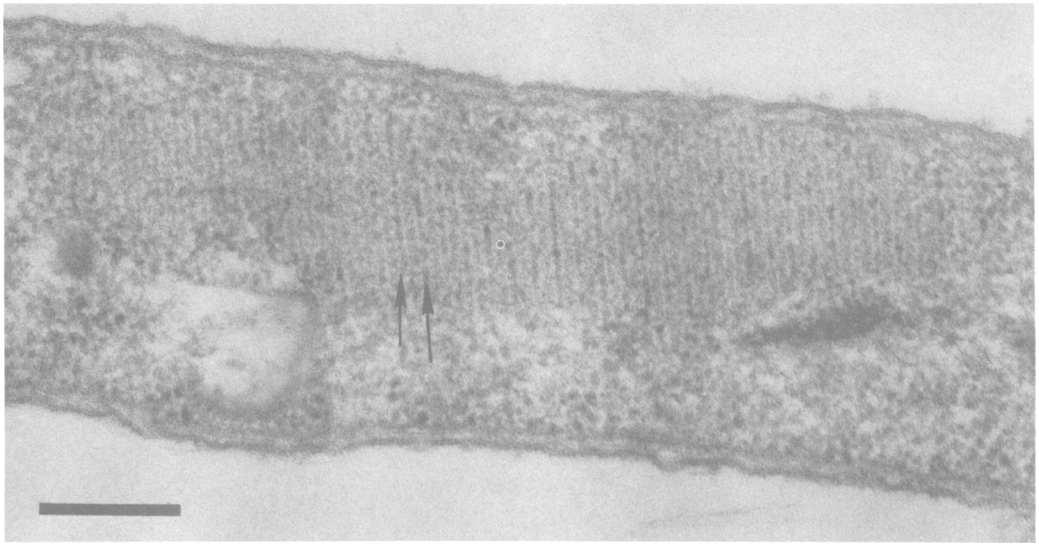

FIG. 5. *Longitudinal section of a subtilisin-treated M. xanthus FB2 cell containing a patch of regular, transverse bands. Two of the major bands are indicated by arrows.*

lack of periodicity in strain DK306, we observed a beaded organelle in association with the fibrils. Its significance is not known. Since numerous genetic loci code for gliding-related functions (Hodgkins and Kaiser, communicated at the Developmental Biology of Myxobacteria Meeting, 1976), it may not be surprising that the other nonmotile mutants we examined demonstrate an apparently unaltered bundle of filaments.

We are reasonably certain that this structure is not an artifact for the following reasons: (i) cell fine structure, including the envelope, cytoplasm, and nuclear body, is well preserved by the standards established in the literature; (ii) we observed these structures in cells fixed by other procedures, as indicated above.

With two possible exceptions, we are not aware that this structure has been observed before. Parallel arrays of 7.5- to 10-nm-diameter filaments lying in the polar regions of *Chondromyces crocatus* cells have been reported (18). Also, Schmidt-Lorenz and Kühlwein described bundles of 13- to 15-nm-diameter tubules that originate in granules located in the cytoplasm, extend the length of the cell, and emerge at one pole in *M. xanthus* and other myxobacteria (25, 26). MacRae and McCurdy have also described 15- to 19-nm-diameter tubules in *C. crocatus* cells (18). The structure we have described appears to be associated with the cell envelope at or near at least one pole. We did not observe terminal granules or emergence through the envelope. Although there is some similarity between the previously described tubular structures and those described here, we have no indication that the bundles are comprised of tubules. However, we observed short, 13- to 17-nm-diameter tubules in debris of cells disrupted by several procedures (Fig. 7).

The tubules described by Schmidt-Lorenz and Kühlwein were only observed in gliding cells harvested from agar (26). The bundle of filaments described above has been observed in both agar- and liquid-grown cells. Since the normal habitat of myxobacteria is terrestrial (12), we would not expect these organisms to have evolved a regulatory mechanism that would turn off synthesis or stop assembly of this organelle when they are grown in liquid culture. Also, shake culture-grown cells can glide on chloramphenicol (25 μg/ml)-containing agar, although they can not grow on it (6). Thus, they probably have an intact and functional gliding mechanism (assuming it is a proteinous structure). We cannot, however, exclude the possibility of assembly of a gliding organelle from preexistent subunits after the cells contact a solid substrate.

Further characterization of the structures described above should establish their function in gliding bacteria.

ACKNOWLEDGMENTS

This study was supported by National Science Foundation grant BMS 75-11915 to R.P.B. We thank J. Hodgkin and D. Kaiser for cultures of nongliding mutants.

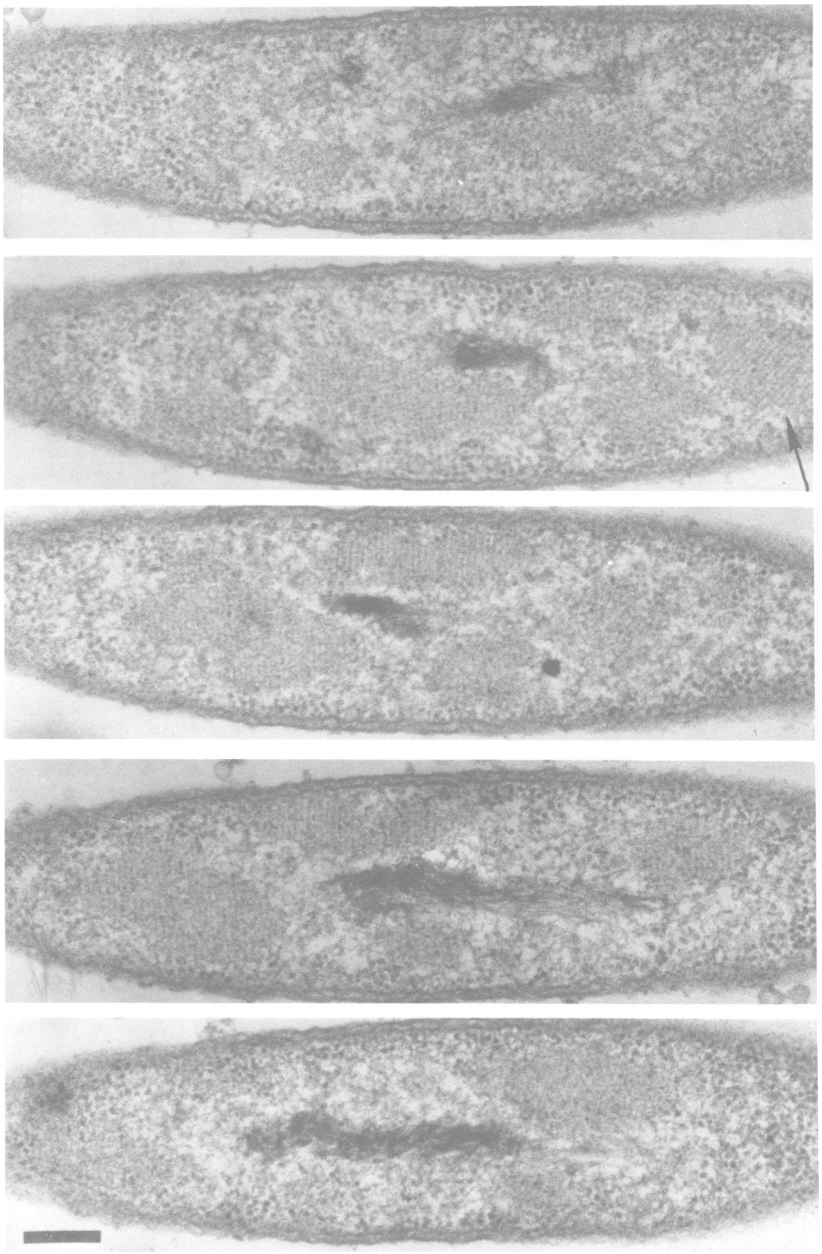

FIG. 6. *Serial, oblique sections through an M. xanthus FB2 cell demonstrating patches of transverse bands. In the second section, bands in one patch run diagonally (arrow). No subtilisin treatment.*

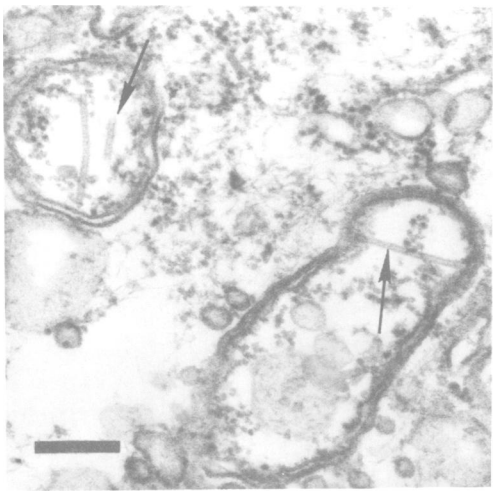

FIG. 7. *Freeze-thaw disrupted M. xanthus FB2 cells fixed with 1% glutaraldehyde. Tubules (arrows) are present in some of the envelope fragments.*

LITERATURE CITED

1. **Bisalputra, T., B. R. Oakley, D. C. Walker, and C. M. Shields.** 1975. Microtubular complexes in blue-green algae. Protoplasma 86:19-28.
2. **Burchard, R. P.** 1970. Gliding motility mutants of *Myxococcus xanthus*. J. Bacteriol. 104:940-947.
3. **Burchard, R. P.** 1974. Growth of surface colonies of the gliding bacterium *Myxococcus xanthus*. Arch. Microbiol. 96:247-254.
4. **Burchard, R. P.** 1974. Studies on gliding motility in *Myxococcus xanthus*. Arch. Microbiol. 99:271-280.
5. **Burchard, R. P., and D. T. Brown.** 1973. Surface structure of gliding bacteria after freeze-etching. J. Bacteriol. 114:1351-1355.
6. **Burchard, R. P., and J. H. Parish.** 1975. Chloramphenicol resistance in *Myxococcus xanthus*. Antimicrob. Agents Chemother. 7:233-238.
7. **Castenholz, R. W.** 1967. Aggregation in a thermophilic *Oscillatoria*. Nature (London) 215:1285-1286.
8. **Chang, H. Y. Y., and M. M. Allen.** 1974. The isolation of rhapidosomes from the blue-gree alga, *Spirulina*. J. Gen. Microbiol. 18:121-130.
9. **Delk, A. S., and C. A. Dekker.** 1972. Characterization of rhapidosomes of *Saprospira grandis*. J. Mol. Biol. 64:287-295.
10. **Doetsch, R. N., and G. J. Hageage.** 1968. Motility in procaryotic organisms: problems, points of view, and perspectives. Biol. Rev. 43:317-362.
11. **Dworkin, M.** 1962. Nutritional requirements for vegetative growth of *Myxococcus xanthus*. J. Bacteriol. 84:250-257.
12. **Dworkin, M.** 1966. Biology of the myxobacteria. Annu. Rev. Microbiol. 20:75-106.
13. **Glaser, J., and J. L. Pate.** 1973. Isolation and characterization of gliding motility mutants of *Cytophaga columnaris*. Arch. Mikrobiol. 93:295-309.
14. **Gräf, W.** 1965. Bewegungsorganellen bei Myxobakterien. Arch. Hyg. Bakteriol. 149:518-526.
15. **Halfen, L. N.** 1973. Gliding motility of *Oscillatoria*: ultrastructural and chemical characterization of the fibrillar layer. J. Phycol. 9:248-253.
16. **Halfen, L. N., and R. W. Castenholz.** 1970. Gliding in blue-green alga: a possible mechanism. Nature (London) 225:1163-1165.
17. **Henrichsen, J.** 1972. Bacterial surface translocation: a survey and a classification. Bacteriol. Rev. 36:478-503.
18. **MacRae, T. H., and H. D. McCurdy, Jr.,** 1975. Ultrastructural studies of *Chondromyces crocatus* vegetative cells. Can. J. Microbiol. 21:1815-1826.
19. **MacRae, T. H., and H. D. McCurdy, Jr.** 1976. The isolation and characterization of gliding motility mutants in *Myxococcus xanthus*. Can. J. Microbiol. 22:1282-1292.
20. **Pate, J. L., J. L. Johnson, and E. J. Ordal.** 1967. The fine structure of *Chondrococcus columnaris*. II. Structure and formation of rhapidosomes. J. Cell Biol. 35:15-35.
21. **Pate, J. L., and E. J. Ordal.** 1967. The fine structure of *Chondrococcus columnaris*. III. The surface layers of *Chondrococcus columnaris*. J. Cell Biol. 35:37-51.
22. **Reichenbach, H.** 1967. Die wahre Natur der Myxobakterien-"Rhapidosomen." Arch. Mikrobiol. 56:371-383.
23. **Ridgway, H. F., and R. A. Lewin.** 1973. Goblet-shaped sub-units from the wall of a marine gliding microbe. J. Gen. Microbiol. 79:119-128.
24. **Ridgway, H. F., R. M. Wagner, W. T. Dawsey, and R. A. Lewin.** 1975. Fine structure of the cell envelope layers of *Flexibacter polymorphus*. Can. J. Microbiol. 21:1733-1750.
25. **Schmidt-Lorenz, W.** 1969. The fine structure of the swarm cells of myxobacteria. J. Appl. Bacteriol. 32:22-23.
26. **Schmidt-Lorenz, W., and H. Kühlwein.** 1968. Intracelluläre Bewegungsorganellen der Myxobakterien. Arch. Mikrobiol. 60:95-98.
27. **Weibull, C.** 1960. Movement, p. 153-205. *In* I. C. Gunsalus and R. Y. Stanier (ed.), The bacteria, vol. 1. Academic Press Inc., New York.