JOURNAL OF BACTERIOLOGY, June 1973, p. 1351-1355

Vol. 114, No. 3
Printed in U.S.A.

NOTES

Surface Structure of Gliding Bacteria After Freeze-Etching

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Received for publication 9 March 1973

Ultrastructural studies of gliding bacteria demonstrate 10- to 11-nm beads on the inner surface of the outer bilayer of *Cytophaga columnaris*. These were not found in *Myxococcus xanthus*. On treatment with glutaraldehyde and ethanol, the beads appear in linear arrays.

The mechanism of gliding motility characteristic of some unicellular and trichome-forming prokaryotes has not been elucidated. Various hypotheses to explain gliding have been proposed (6, 8). Among these are models involving contractile waves propagated along the cell surface or on fibrils. Such mechanisms of gliding require a structural basis at some level of cellular organization. The presence of peripheral fibrils (about 10 nm in diameter) associated with the inner layer of the outer membrane of the glider *Cytophaga columnaris* (previously classified in the genus *Chondrococcus*) has been reported by Pate and Ordal (13). They suggested that these fibrils may be mechanistically involved in gliding. Other fine structure studies of *Myxococcus*, *Cytophaga*, *Sporocytophaga*, and *Stigmatella* (7, 15, 18, 19) revealed no fibrils in these gliding genera. A comparison of thin-section morphology of wild-type and nongliding mutants of *Myxococcus xanthus* demonstrated no differences in the surface structures of these strains (3).

To investigate the possibility that peripheral fibrils may be the result of the extensive pretreatment of cells prior to ultrathin sectioning (1, 16), we have used the technique of freeze-etching to examine the morphology of the gliding bacteria *C. columnaris* 1-R43 (13) and *M. xanthus* FB_t (4). The semimotile and nonmotile strains of *M. xanthus* (3) were also examined. This method does not require extensive pretreatment nor the loss of water encountered in standard fixation and dehydration procedures. Furthermore, freeze-etching allows observations of large areas of cell surface and of surface-associated structures.

M. xanthus strains were cultured on CT-1 agar and broth (4). *C. columnaris* 1-R43, supplied by J. Pate, was grown on cytophaga agar (13). Plates were incubated at 30 C.

Both *C. columnaris* and *M. xanthus* cells possessed a multilayered envelope (Fig. 1a and 3a) similar to that reported for other gram-negative bacteria (5, 11). The envelopes (60-nm thick) are composed of two bilayers, 20 nm apart. In *C. columnaris* the dense layer between the bilayers is more pronounced than it is in *M. xanthus* (9, 13, 19).

In *C. columnaris* cells, fibrillar structures with center-to-center spacing of 16 nm, similar to those described by Pate and Ordal (13), were observed in the intermembrane space associated with the outer bilayer (Fig. 1b, c, d). These were best resolved in partially lysed cells, representing less than 3% of the total population. We have not observed these structures in *M. xanthus*.

The multilayered structure of the gliding bacteria envelope observed in thin-sectioned preparations was confirmed by freeze-etching (Fig. 2 and 3b-d). The average thickness of the envelope in freeze-etched preparations was approximately 63 nm, which compares favorably with thin-section dimensions. The outer surface of the cells of both species was found to be

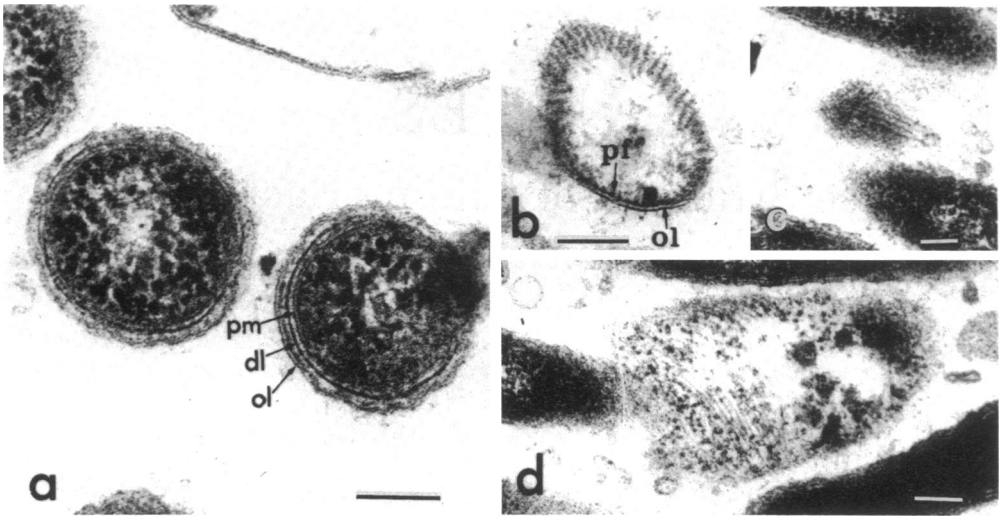
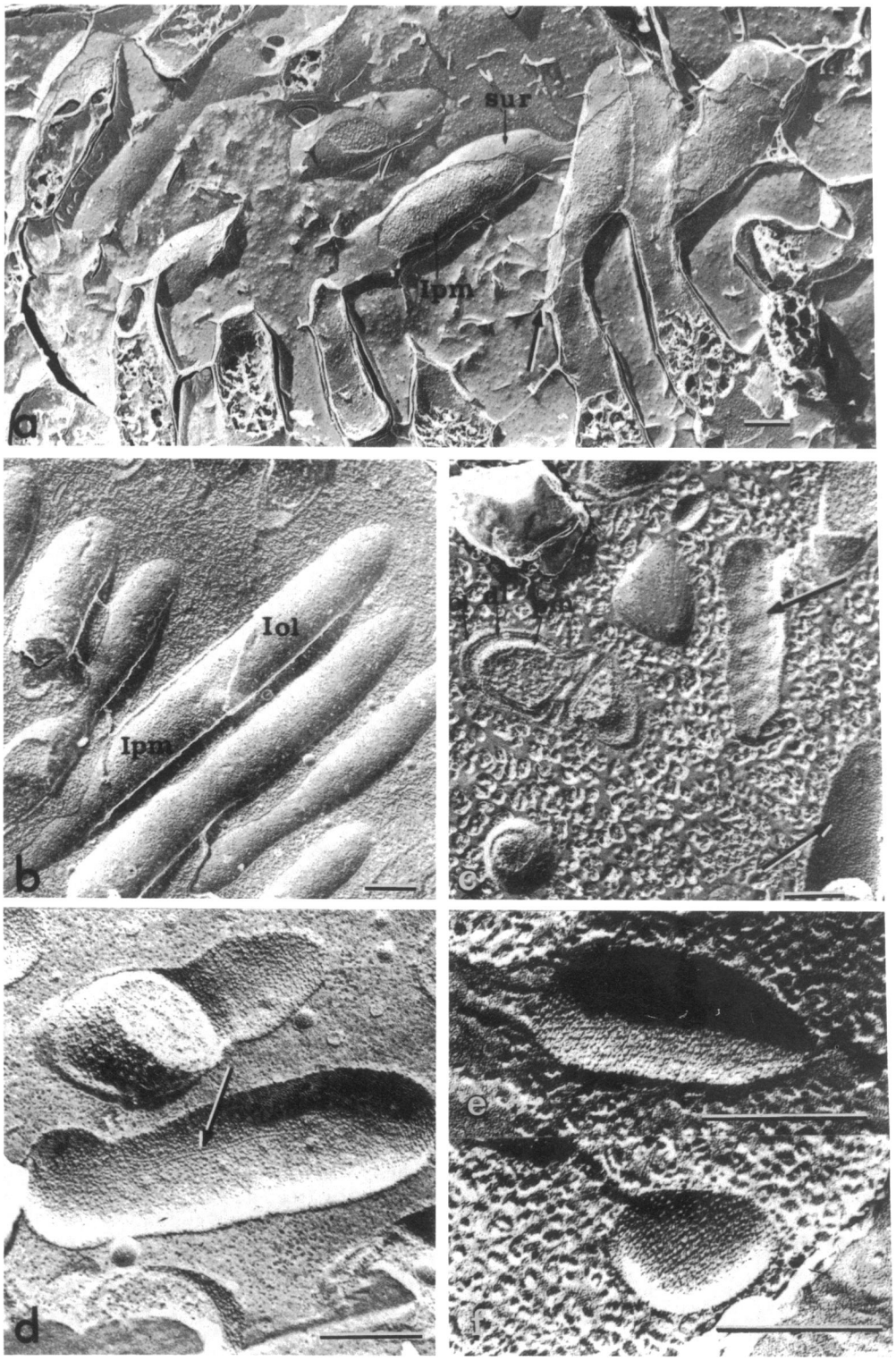


FIG. 1. *Electron micrographs of ultrathin sections of C. columnaris, embedded according to the procedure of Luft (10) and stained with uranyl acetate and lead citrate. a, Cross-sectioned cells. Abbreviations: pm, plasma membranes; dl, dense layer; ol, outer layer. b, Tangential section of partially lysed cell showing peripheral fibrils (pf) attached to the inner surface of the outer bilayer (ol). c and d, Peripheral fibrils at the tip of a cell (c) and in the envelope of a lysed cell (d). Bars represent 100 nm.*

FIG. 2. *C. columnaris cells after freeze-etching. The procedures used were those of Brown et al. (2). a, Cells after freeze-etching, without glycerol or fixation with glutaraldehyde. Abbreviations: sur, surface of cell; Ipm, interior surface of a cleaved plasma membrane. The large black and white arrow points out one of many filamentous extensions of the cell surface. b, Cells after freeze-etching in the presence of 30% glycerol. The marked cell is cleaved in such a way as to reveal the interior surfaces of the cleaved plasma membrane (Ipm) and outer layer (Iol). c, Cells after freeze-etching in the presence of 30% glycerol. The unmarked arrows point to an inwardly facing surface, possibly the inner surface of the outer bilayer. This surface is covered with a random array of particles, larger in size (11 nm) than those generally seen on the interior membrane surfaces (7 nm, Fig. 2b). These particles have a center-to-center spacing of approximately 16 nm. Another cell is seen in cross-fracture revealing a cleaved outer bilayer (ol), the dense layer (dl) and the cleaved plasma membrane (pm). d, Cells freeze-etched in 30% glycerol after pretreatment with 2% glutaraldehyde. The arrow points to 11-nm particles which are organized in fiber-like rows. e and f, Cells freeze-etched after pretreatment with glutaraldehyde and ethanol as prescribed for embedding in epoxy. The surfaces shown here correspond to those marked with black and white arrows in Fig. 2c and d. Many more 11-nm particles are seen organized in fiber-like rows similar to those shown in Fig. 2d. Bars represent 200 nm.*


Sur
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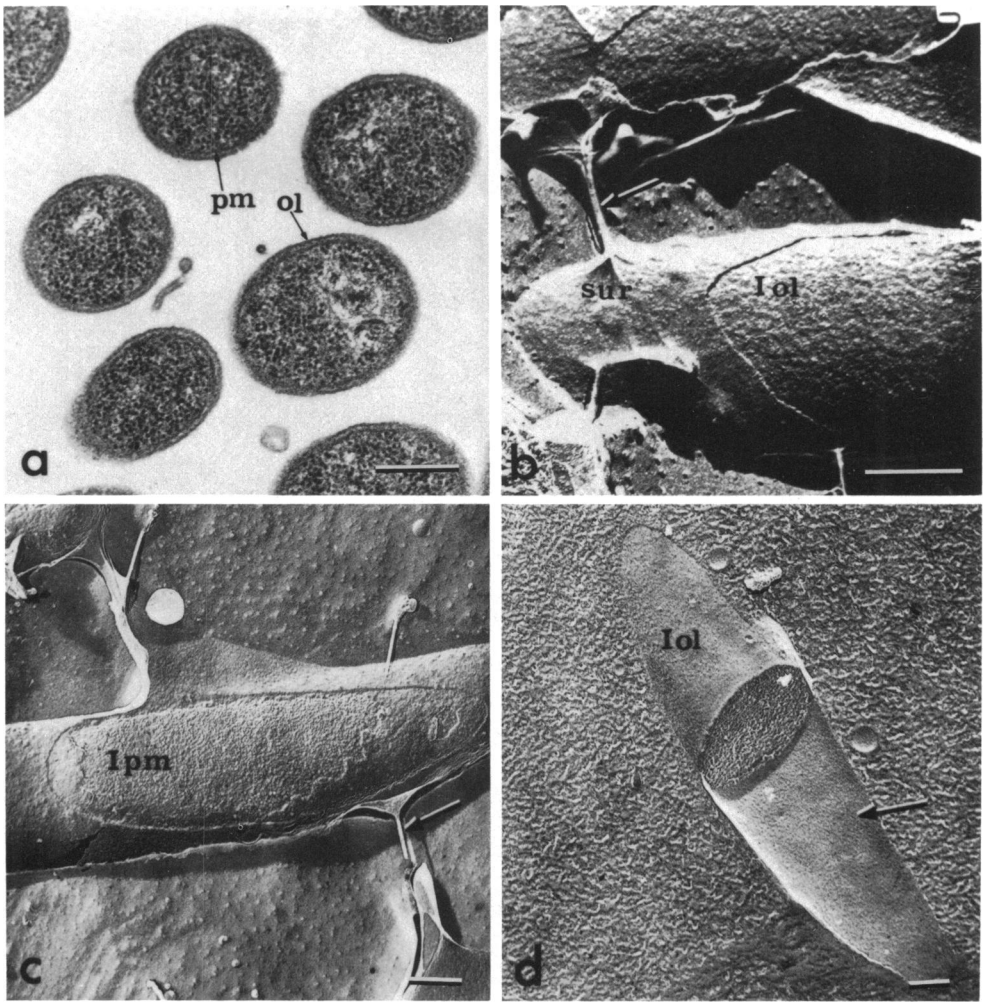

FIG. 3. *Morphology of M. xanthus. a, Cross-sectioned, epoxy-embedded cells. Abbreviations: pm, plasma membrane; ol, outer layer. b, c, and d, M. xanthus cells after freeze-etching, as in Fig. 2. b, Cell freeze-etched without glutaraldehyde or glycerol. Abbreviations: sur, surface of cell with filamentous deformation (black and white arrow); Iol, interior surface of outer layer. c, Cell freeze-etched as in Fig. 3b. Abbreviation: Ipm, interior surface of plasma membrane. Black and white arrow shows surface deformation. d, Cell freeze-etched in 30% glycerol after pretreatment with 2% glutaraldehyde. Abbreviation: Iol, interior surface of outer layer. The black and white arrow points out an inwardly facing surface similar to those in Fig. 2c, d. This surface lacks the 11-nm particles seen in C. columnaris. Bars represent 200 nm.*

smooth in appearance (Fig. 2a and 3b), similar to the outer surface of *Escherichia coli* cells (1, 12). Protrusions or filamentous extensions were observed on the envelopes of some cells, as though the outer surface were elastic and capable of being deformed (Fig. 2a and 3b, c).

Fracture planes split both the inner and outer bilayers of both organisms, revealing the presence of 6- to 10-nm intramembrane particles (14) (Fig. 2a, b, and 3b, c). Treatment of *C. columnaris* cells with 30% glycerol prior to freeze-etching resulted in a fracture plane which revealed 10- to 11-nm particles on what may be the inner face of the outer bilayer (Fig. 2c). These particles have not been observed in identically treated *M. xanthus* (Fig. 3d). When cells were fixed with glutaraldehyde, some of the 10- to 11-nm particles appeared to be organized linearly (Fig. 2d). Glutaraldehyde-fixed cells, after dehydration in a graded alcohol series identical to that used in embedding for thin sectioning and rehydration into a 30%

glycerol solution, demonstrated more distinct rows of particles in a greater percentage of *C. columnaris* cells (Fig. 2e, f).

The envelope fine structure of semimotile and nonmotile mutants of *M. xanthus* (3) was indistinguishable from that of the gliding wild type (data not shown).

We propose that the 10- to 11-nm particles observed in freeze-etched, glycerol-treated *C. columnaris* cells (Fig. 2c) occupy the same position as the peripheral fibrils reported by Pate and Ordal (13) (Fig. 1b). The appearance of linear arrays of particles after treatment with glutaraldehyde, ethanol, and freeze-etching suggests that these agents may cause particle reorganization into the peripheral fibrils observed in thin-sectioned cells. This observation is in agreement with Pate and Ordal (13), who stated that these fibrils are only detectable in aldehyde-fixed cells. Thus, these structures may result from cross-linking of 10- to 11-nm particles by aldehydes. The absence of the described fibrillar or particulate structures in *M. xanthus* FB_t suggests that they are not involved in gliding motility.

Intracellular filaments or microtubules observed in *Myxococcus* spp. were proposed as the gliding organelles (17). We have not detected similar structures in *M. xanthus* nor in *C. columnaris*.

ACKNOWLEDGMENTS

The expert technical assistance of Lilianna Visscher and Donald Filtzer is gratefully acknowledged. We thank Jack Pate for the *C. columnaris* strain.

This study was supported by National Science Foundation Grant GB-31111 to R.P.B. and an American Cancer Society, Maryland Division, grant to D.T.B.

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