Journal of General Microbiology (1982), **128**, 73–84. *Printed in Great Britain*

Ultrastructure of *Beggiatoa alba* Strain B15LD

By WILLIAM R. STROHL,*† KAREN S. HOWARD AND JOHN M. LARKIN

Department of Microbiology, Louisiana State University, Baton Rouge, Louisiana 70803, U.S.A.

(*Received 17 February 1981; revised 27 May 1981*)

The ultrastructure of *Beggiatoa alba* strain B15LD was examined in detail using thin section and freeze-etch techniques. The cell wall complex consisted of five discrete layers external to the cytoplasmic membrane. The surface layer was the most distinct, containing linearly arranged longitudinal fibrils, 10–12 nm in diameter. Sulphur inclusions observed in thin sections had a 12–14 nm thick pentalaminar envelope and were external to the cytoplasmic membrane. Similar pentalaminar envelope structures were observed in cells grown without hydrogen sulphide, but they were small, folded and probably lacked sulphur. After a few hours of exposure to hydrogen sulphide, large sulphur inclusions were present in the cells, presumably due to the expansion of the folded 'rudimentary' vesicles to accommodate the deposited sulphur. The location and morphology of the cytoplasmic membrane invaginations which surrounded the sulphur inclusions were elucidated by thin sectioning and freeze-etching. A three-dimensional model of the cell envelope structure is presented.

INTRODUCTION

Beggiatoa alba is a colourless, filamentous, gliding bacterium which deposits sulphur when grown in the presence of hydrogen sulphide (Strohl & Larkin, 1978*b*). Ultrastructural features of various *Beggiatoa* strains have been examined by several investigators (Drawert & Metzner-Küster, 1958; Morita & Stave, 1963; Maier & Murray, 1965; Tredway *et al.*, 1977; Strohl & Larkin, 1978*b*; Strohl *et al.*, 1981). However, the unusual cell wall complex of the beggiatoas has not been studied using the freeze-etch technique, so the information on the cell wall and the sulphur inclusions of *Beggiatoa* is not yet complete.

We recently showed by phase, transmission and scanning electron microscopy that the cells of *B. alba* B15LD, as well as 19 other *Beggiatoa* strains studied, divide within the trichome by septation and that the trichomes segment into daughter trichomes through the production of sacrificial cells (Strohl & Larkin, 1978*a*), similar to the mechanism that has been reported for the cyanobacterium *Oscillatoria* (Lamont, 1969; Drews, 1973).

In this paper, we present the results of a study on the nature of the cell envelope and the sulphur inclusions of *B. alba* strain B15LD. We found that the trichome envelope consists of five layers external to the cytoplasmic membrane and that several of the layers show unique morphologies as determined by freeze-etching.

METHODS

Organism and culture conditions. Beggiatoa alba strain B15LD was used throughout this study (Strohl & Larkin, 1978*a*). It was isolated from an enrichment culture from Laccasine, Louisiana, which was kindly supplied to us by Dr M. Joshi, Department of Plant Pathology, Louisiana State University, Baton Rouge, La, U.S.A. Strain

† Present address: Department of Microbiology, The Ohio State University, 484 West 12th Avenue, Columbus, Ohio 43210, U.S.A.

0022-1287/82/0000-9795 $02.00

B15LD had a trichome diameter of about 3·0 μm and was a member of our group E strains in an earlier study (Strohl & Larkin, 1978*b*).

BP (heterotrophic) and MP (mixotrophic) media have been described (Strohl & Larkin, 1978*b*). BPA medium contained 0·05% (w/v) sodium acetate, 0·05% (w/v) asparagine and Pringsheim's basal salts (Strohl & Larkin, 1978*b*).

Electron microscopy. For thin sections, a modified Ryter–Kellenberger (RK) technique was employed as the standard fixation procedure, as previously described (Strohl & Larkin, 1978*b*). The ruthenium red–glutaraldehyde (RRG) fixation was also performed as previously described (Strohl & Larkin, 1978*a*). Thin sections were post-stained with uranyl acetate (Watson, 1958) and then with lead citrate (Reynolds, 1963).

Freeze-etching was done on trichomes which had been grown on BP or MP agar media. The trichomes were scraped off the agar with applicator sticks and were then either placed directly into complementary specimen holders (Steere, 1973) or transferred to a 30% glycerol cryoprotectant solution for 2 h prior to placement in the specimen holders. They were etched for 2 min at −98 °C, shadowed at a 45° angle with platinum/carbon and were then carbon coated. A modified Denton DFE-3 freeze-etch module was used and the procedures as outlined by Steere (1973) were followed.

All micrographs were taken at a magnification of 11 500 × using an RCA EMU-3G electron microscope operating at 50 kV.

RESULTS

Sulphur inclusion morphology

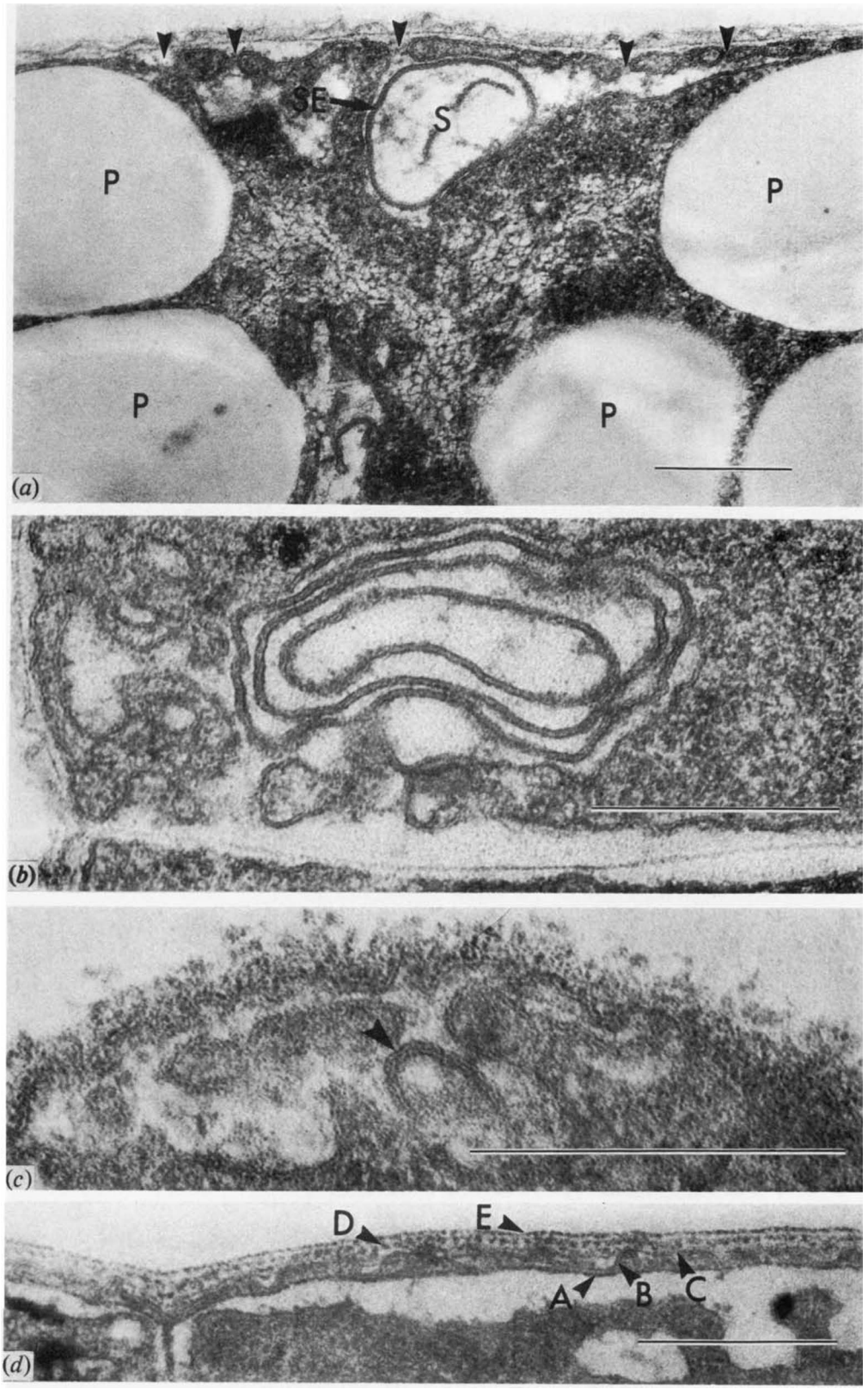
The sulphur inclusions of *Beggiatoa alba* strain B15LD were enclosed within cytoplasmic membrane invaginations and were thus outside the cytoplasm, but interior to the complex cell wall (Fig. 1 *a, b*). The cytoplasmic membrane invaginations containing the sulphur inclusions arose from either the septal regions or the periphery of the cells. Figure 1 (*a*) shows the unique pentalaminar envelope of the sulphur inclusion. The outer two electron-dense layers were 3·5 nm thick and the central dense layer was approximately 2·1 nm thick. The two electron-translucent layers which separated the dense layers were 1·8–2·0 nm thick. The total width of the pentalaminar sulphur inclusion envelopes averaged 12–14 nm. This can be compared with the cytoplasmic membrane which was 6–8 nm in width. Occasionally, sulphur inclusion envelopes were observed in multiple layers (Fig. 1 *b*).

Cells which had been grown on BPA medium (in the complete absence of reduced sulphur compounds) often contained small membraneous structures with the same morphological appearance as a sulphur inclusion envelope (Fig. 1 *c*).

The cell envelope

Figures 1 (*a–c*) show the ultrastructure of *B. alba* B15LD as obtained by the modified RK thin section technique. Although the RK technique proved best for the preservation of the sulphur inclusion envelopes, it was inadequate as a fixation procedure for the *Beggiatoa* cell wall. The RRG technique was the best for the resolution of the complex *Beggiatoa* cell wall (Fig. 1 *d*). Other thin section techniques such as phosphate-buffered glutaraldehyde with osmium post-fixation, cacodylate-buffered glutaraldehyde with osmium post-fixation, and cacodylate-buffered glutaraldehyde–acrolein with osmium and uranyl acetate post-fixation were also inadequate for showing the layers of the *B. alba* B15LD cell wall or the fine structure of its sulphur inclusion envelopes (unpublished observations).

Fig. 1. The structure of *in situ* sulphur inclusions and the cell wall of *B. alba* B15LD. The bar markers represent 250 nm. (*a*) An RK-fixed cell which had been grown on BP medium and then changed to MP medium, showing poly-β-hydroxybutyrate (P) and a sulphur inclusion envelope (SE) surrounding the washed out space of a sulphur inclusion (S): the arrows indicate several cytoplasmic invagination sites which probably correspond to the membrane-enclosed circles in Fig. 3 (*a*). (*b*) A sulphur inclusion with several layers of envelopes. (*c*) An RK-fixed, heterotrophically grown (BPA medium) trichome showing the rudimentary sulphur inclusion envelope (arrow). (*d*) An RRG-fixed trichome showing the peptidoglycan layer (A) and the outer four layers (B, C, D and E, interior to exterior, respectively).


SE
S
P
P
P
P
(a)
(b)
(c)
D
E
A
B
C
(d)

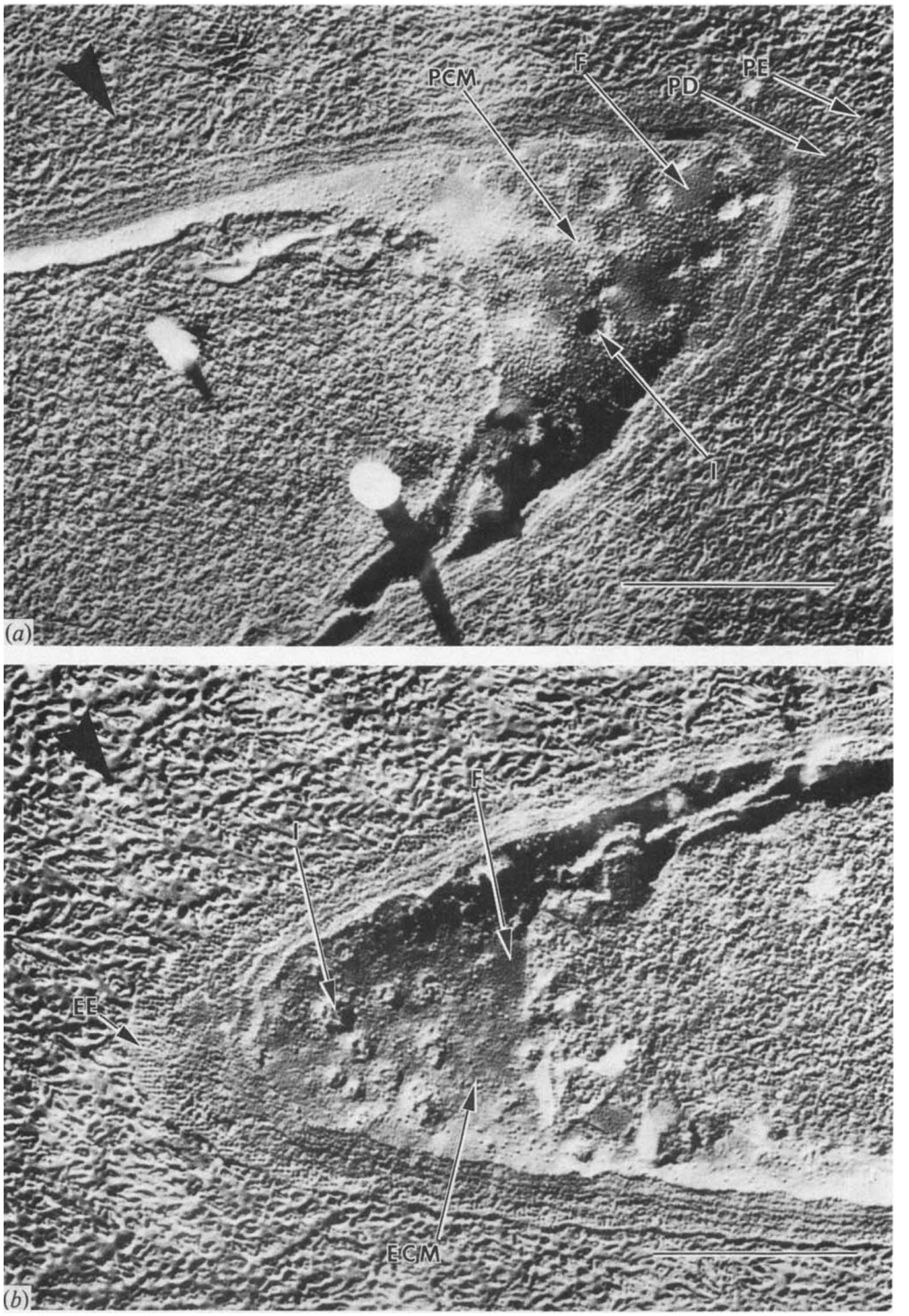
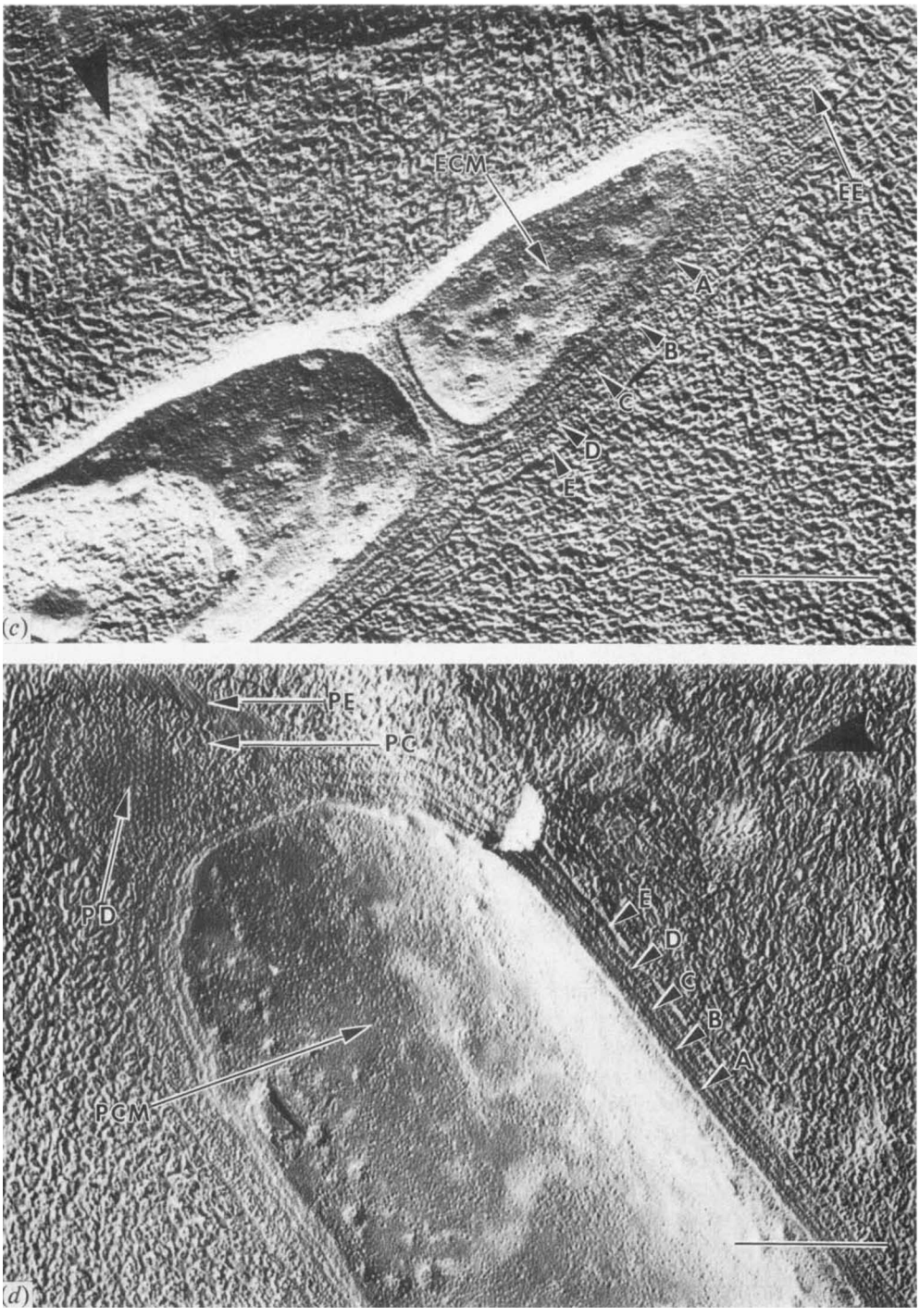


Fig. 2. Glycerol-cryoprotected freeze-etched trichomes of *B. alba* B15LD showing cross fractures of the cell envelopes. Designations for the freeze-etch micrographs are ECM, EA, EB, EC, ED and EE for the exoplasmic fracture faces of the cytoplasmic membrane and the cell wall layers A–E, respectively. Similarly, the protoplasmic fracture faces are labelled PCM, PA, PB, PC, PD and PE for the same respective layers as above. In cross-fractures, the cell wall layers A–E are labelled. The invaginations are designated I and the finely particulate areas, F. The direction of the shadows (black shadows) is



shown in all freeze-etch micrographs by large arrows. The bar markers represent 250 nm. (*a*) A tangential fracture through a trichome exposing the protoplasmic fracture planes. (*b*) A tangential fracture through the same trichome as in (*a*) but in different location, this one exposing the exoplasmic fracture planes. (*c*) A tangential and cross-fracture of a trichome showing a clear delineation of the cell wall layers and the exoplasmic faces of the cytoplasmic membrane and E layer. (*d*) A tangential and cross-fracture of a trichome showing the protoplasmic faces of the C, D and E layers.

Table 1. *Analysis of the cell envelope of Beggiatoa alba B15LD*

		Fixation procedure* and layer thickness (nm)		
		Freeze-etch	Thin section	
Layer	Layer description	CP	RK	RRG
Cytoplasmic membrane	Trilaminar	8·5–10·0	6·3–7·8	5·8
Cell wall, total	Five layers	94·0–105·0	46·0–78·0†	70·0–86·0
Cell wall, A	Single dense	4·0	3·9	6·4
Cell wall, B	Trilaminar, wavy	10·0	7·8–8·0	10·4–12·0
Cell wall, C	Single dense	8·5	6·3–9·5	6·5–12·0
Cell wall, D	Trilaminar, wavy	10·3	NV	10·4–11·0
Cell wall, E	Single dense, regular	17·0–21·0‡	NV	9·3–9·7

* CP, Cryoprotected; RK, modified Ryter–Kellenberger; RRG, ruthenium red–glutaraldehyde.
† Two of the layers (D and E) are not visible by this technique (indicated by NV).
‡ Includes space between the D and E layers.

Five layers were observed in the cell wall complex external to the cytoplasmic membrane (Figs 1*d*, 2*a–d*), and in order from interior to exterior, they were designated A, B, C, D and E. Measurements and descriptions of the cell envelope layers are summarized in Table 1.

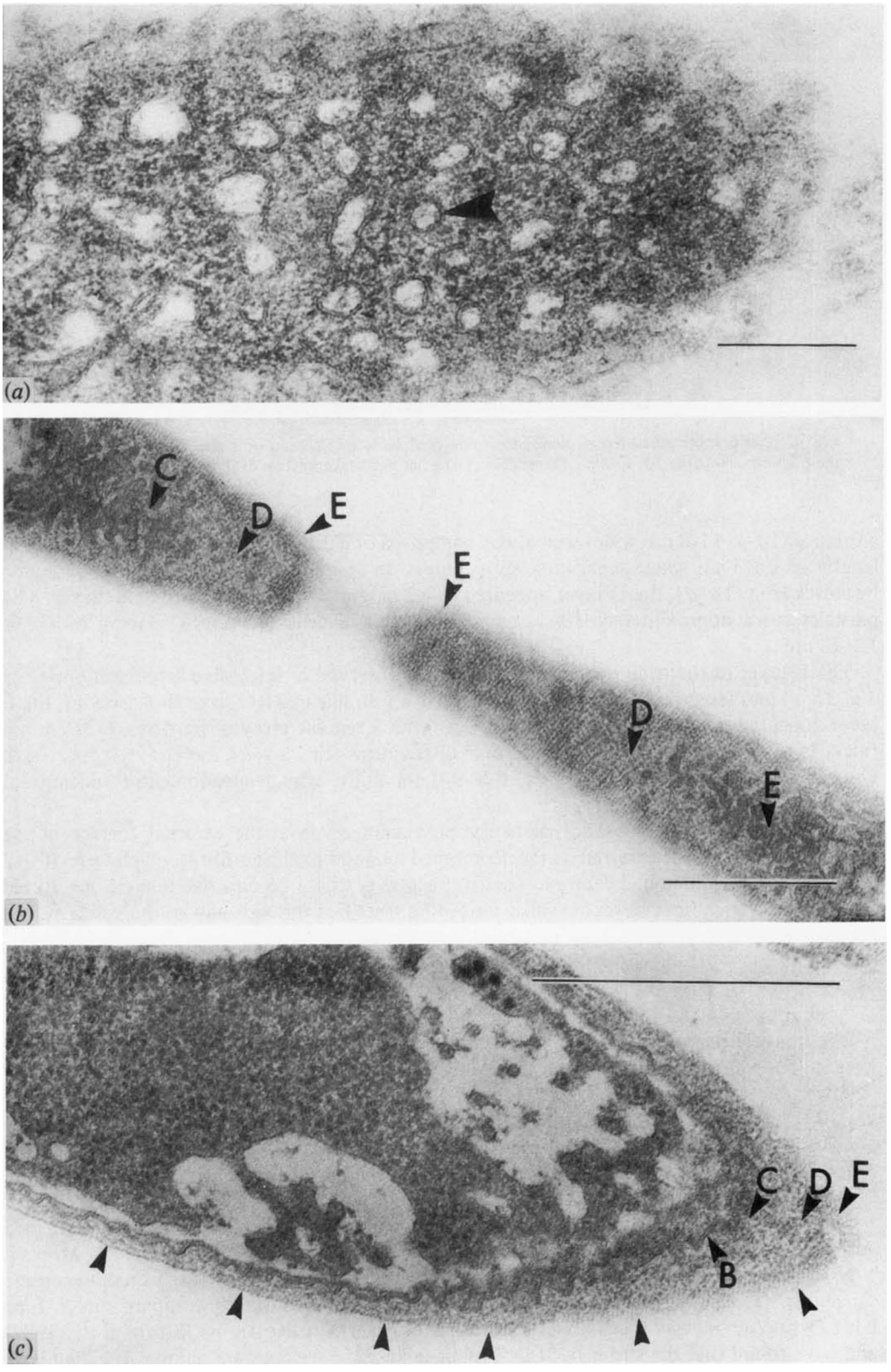
Glycerol-cryoprotected freeze-etch techniques resulted in tangential fractures through both the exoplasmic and the protoplasmic halves of the cytoplasmic membrane. The protoplasmic fracture face of the cytoplasmic membrane (PCM; Fig. 2*a*) appeared highly granulated, whereas the exoplasmic fracture face (ECM) was much less granulated (Fig. 2*b*). The particles on both fracture faces measured 8–9 nm in diameter. Discrete patches of very fine particulation were observed on both fracture faces of the cytoplasmic membrane. The patches were generally circular and measured 90–130 nm in diameter.

Pore-like invaginations in the cytoplasmic membrane were concave on the PCM fracture face (Fig. 2*a*) and were convex on the ECM fracture face (Fig. 2*b*). RK-fixed thin sections of tangentially sliced trichomes appeared to cut through the invaginations (Fig. 3*a*). The invaginations were usually circular or oblong and were approximately 48–72 nm in diameter in both the thin section and the freeze-etch preparations. The membrane surrounding the invaginations was 7·5 nm thick in thin sections (Fig. 3*a*), similar to the thickness of the cytoplasmic membrane.

The A cell wall layer was a single electron-dense layer, approximately 4–6 nm in width (Fig. 1*a*, *d*). The B cell wall layer appeared as an intensely staining electron-dense–light–dense layer (Fig. 1*d*) and was wavy in RK-fixed (Fig. 1*a*) and the RRG-fixed (Fig. 1*d*) thin sections, but straight in freeze-etched trichomes (Fig. 2*c*, *d*). The C layer of the cell wall appeared as a single electron-dense layer and was also visible as a single layer in freeze-etched trichomes (Fig. 2*c*, *d*). Neither the A nor the B cell wall layers were tangentially fractured in freeze-etched trichomes.

The D layer of the trichome envelope was not readily observed in RK-fixed trichomes (Fig. 1*a*), but was observed in the RRG-fixed thin sections (Fig. 1*d*, 3*b*, *c*) and in freeze-etched trichomes (Fig. 2*a–d*). In linear RRG-fixed thin sections the D layer appeared as a trilaminar

Fig. 3. Tangential thin sections of *B. alba* B15LD. The bar markers represent 250 nm in (*a*), and 500 nm in (*b*) and (*c*). (*a*) An RK-fixed trichome showing the cytoplasmic membrane-bound circles which correspond to the invaginations shown in Fig. 1 (*a*), and to the invaginations in the freeze-etch micrographs shown in Fig. 2 (*a–d*): the arrow points to a membrane of the size and density of the cytoplasmic membrane. (*b*) An RRG-fixed trichome showing the patterns of the cell wall layers C, D, and E: these correspond to the freeze-etch patterns shown in Fig. 2 (*d*). (*c*) An RRG-fixed trichome showing the same layers as in (*b*), but here they can be followed to each of the corresponding linearly sectioned layers (arrows).


(a)
C
D
E
E
D
E
(b)
C
D
E
B
(c)

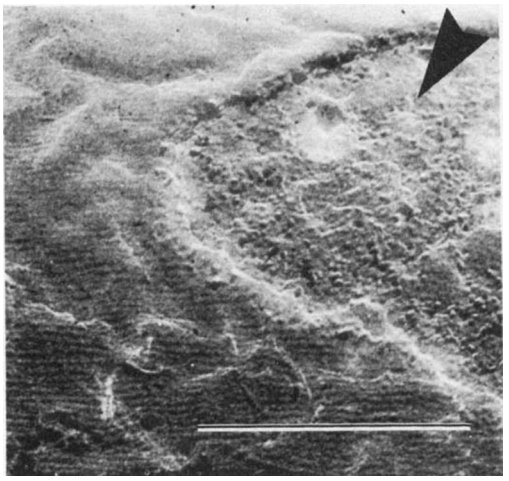


Fig. 4. Non-cryoprotected freeze-etched trichome of *B. alba* B15LD showing the external surface of the trichome. Note the 10–12 nm wide striations. The bar marker represents 500 nm.

structure (10·4–11·0 nm wide) which was composed of paired periodic subunits 12–14 nm in length with a 3 nm space separating the subunits. In tangential thin sections (Fig. 3*b, c*) or fractures (Fig. 2*a–d*), the D layer appeared as a hexagonally arranged particulate layer. The particles were approximately 10–12 nm in size with a centre-to-centre spacing of about 12–18 nm.

The E layer of the trichome envelope was also observed in RRG-fixed thin sections (Fig. 1*d*, 3*b, c*) and freeze-etch preparations (Fig. 2*a–d*). In linear RRG-fixed thin sections, the E layer appeared as a single electron-dense layer with a regular array of particles, 6·5–7·0 nm thick. In tangential thin sections (Fig. 3*b, c*) or fractures (Fig. 2*a–d*), the E layer appeared to consist of linear arrays of particles, 6·8–9·0 nm thick, with centre-to-centre spacings of approximately 12 nm.

Freeze-etching in the absence of a cryoprotectant revealed the external surface of the trichomes (Fig. 4). The external surface contained longitudinal linear fibrils which were 10–12 nm in width and which had centre-to-centre spacings of about 15 nm. The non-cryoprotected freeze-etched trichomes never revealed tangential fractures through any of the cell envelope layers (Fig. 4).

DISCUSSION

The unusual pentalaminar sulphur inclusion envelopes observed in *B. alba* strain B15LD were different from sulphur inclusions observed in other beggiatoas thus far described (Drawert & Metzner-Küster, 1958; Maier & Murray, 1965; Morita & Stave, 1963; Strohl *et al.*, 1981). The *B alba* B15LD sulphur inclusions also differed from the sulphur inclusions observed in filamentous sulphur bacteria such as *Thiothrix* (Bland & Staley, 1978) and *Thioploca* (Maier & Murray, 1965), and the single-celled sulphur bacteria such as *Thiovulum* (Wirsen & Jannasch, 1978) and *Chromatium* (Nicolson & Schmidt, 1971; Remsen, 1978).

The sulphur inclusions in thin sections of other *Beggiatoa* strains have appeared as washed out areas bound by the cytoplasmic membrane (Morita & Stave, 1963; Maier & Murray, 1965) or as single 4 nm thick electron-dense envelopes, external to the cytoplasmic membrane (Strohl *et al.*, 1981). We have used identical fixation procedures to compare the *B. alba* B15LD sulphur inclusion envelopes with those of other *B. alba* strains (Strohl *et al.*, 1981) and have found that the strain B15LD sulphur inclusion envelopes are unique. The *Thiothrix*

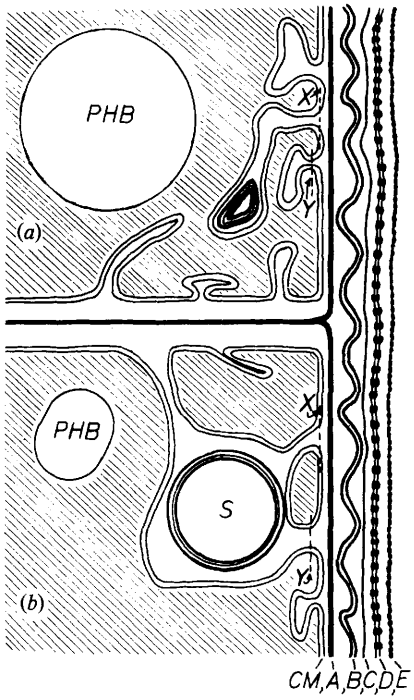


Fig. 5. A two-dimensional model of *B. alba* B15LD showing trichomes grown under heterotrophic conditions (BPA medium, *a*) and under sulphide oxidizing conditions (MP medium, *b*). The rudimentary sulphur granules found in the BPA-grown trichomes expand to contain elemental sulphur (S) while the amount of poly-β-hydroxybutyrate (PHB) in the cells decreases as the cells are switched from the heterotrophic medium to the mixotrophic medium. The dashed line X denotes the probable line of fracture which resulted in the observation of the invaginations (see Fig. 2*a*, *d*); the dashed line Y denotes the probable line of section which resulted in the membrane-enclosed circles observed in Fig. 3(*a*). The cell wall layers are shown as described in the text, in previous figures and in Table 1. This model was drawn as a comparison to the *Beggiatoa* model proposed by Maier & Murray (1965).

sulphur inclusions appeared to have a 'dense interfacial layer' associated with the enclosing cytoplasmic membrane (Bland & Staley, 1978) and the *B. leptomitiformis* sulphur inclusions appeared to have a 'dense irregular material within the membrane' (Maier & Murray, 1965). Thus, only the cytoplasmic membrane, an irregular densely-staining material, or a thin single electron-dense layered envelope appeared to act as the delineating envelope of the sulphur inclusions in those *Beggiatoa* and *Thiothrix* strains.

Strohl *et al.* (1978) reported the preliminary analysis of the isolated sulphur inclusion envelopes from *B. alba* B15LD. They found that the envelopes were destroyed by proteases, phospholipase C, *Pseudomonas aeruginosa* haemolysin and lysozyme. The envelopes were resistant to treatment with trypsin, EDTA, Triton X-100 or sodium dodecyl sulphate (Strohl *et al.*, 1978). Thus, it appears that the sulphur inclusion envelopes of *B. alba* B15LD are unique not only in their morphology but also in their chemical make-up (Schmidt *et al.*, 1971).

We have attempted to freeze-fracture the unusual sulphur inclusions and their envelopes in *B. alba* B15LD, but have been unsuccessful in obtaining clean fractures associated with the sulphur inclusion envelopes. Freeze-fracture of trichomes of *B. alba* B18LD, a strain containing the single electron-dense sulphur inclusion envelopes (Strohl *et al.*, 1981), resulted in fracture planes through the invaginated cytoplasmic membrane but not with the sulphur inclusion envelope (Strohl *et al.*, 1981).

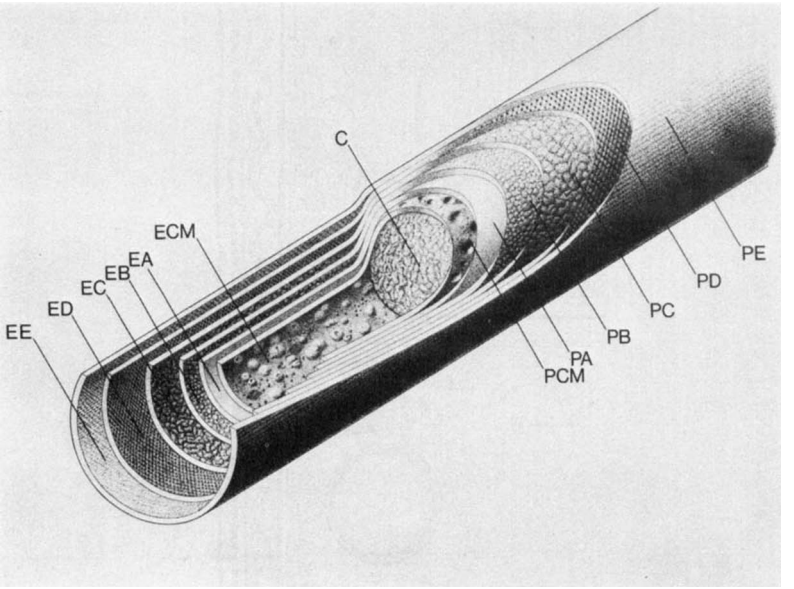


Fig. 6. A three-dimensional model derived from freeze-etch observations of *B. alba* B15LD which shows the exoplasmic (E) and the protoplasmic (P) faces of the cytoplasmic membrane (ECM and PCM, respectively) and of the cell wall layers A–E (as noted in the legend for Fig. 2). The distinctive patterns which were observed in the freeze-etch preparations and which are described in the text are shown. (The A cell wall layer, which corresponds to the peptidoglycan layer, was not observed in tangential fractures, but since it must be included in the model, it is represented without any finite infrastructure.)

The sulphur inclusion envelopes observed in *B. alba* B15LD trichomes grown in the strict absence of reduced sulphur compounds were much smaller than the envelopes found in trichomes grown on MP medium (which had added sulphide). The sulphur inclusion envelopes in the trichomes grown on BPA medium also appeared convoluted and empty, so it is probable that they represent the rudimentary precursors of sulphur inclusions and that they would enlarge to enclose deposited sulphur when sulphide becomes available (Fig. 5). Moreover, in cells exposed for a short time to sulphide, both expanded and rudimentary sulphur inclusion envelopes were observed. It is probable that the rudimentary envelopes in those cells had not yet undergone further synthesis for the enclosure of sulphur. The presence of the sulphur inclusion envelopes in heterotrophic cells (grown in the absence of sulphide) may explain the rapidity with which sulphur inclusions appear microscopically upon exposure to sulphide (Burton & Morita, 1964). This would be an ecologically efficient mechanism (Strohl & Larkin, 1978*b*) by which the beggiatoas could quickly utilize any available sulphide for energy (Güde *et al.*, 1981).

The invaginations in the freeze-etched cytoplasmic membrane and in tangential thin sections were approximately the same size. Because of the location, the size similarity, and the size of the bilayer membrane surrounding them, the invaginations probably correspond to the invaginations of the cytoplasmic membrane which surrounds the sulphur inclusions (Fig. 5).

The 8–9 nm particles on the ECM and the PCM fracture faces of the cytoplasmic membrane are commonly observed in bacterial membranes (Strohl, 1979) and are similar to those which have been described as globular protein molecules or enzyme groups (Moor, 1966).

The *Beggiatoa* cell wall complex or envelope is among the thickest and most complex observed in bacteria (Maier & Murray, 1965). The A cell wall layer is in the same location and is morphologically similar to the L2 layer of the cyanobacteria (Drews, 1973) or to the

peptidoglycan of typical Gram-negative bacteria (Bayer & Remsen, 1970). The lack of a fracture plane through, or associated with, the A (peptidoglycan) layer in *B. alba* is consistent with the observations of most other Gram-negative bacteria (Bayer & Remsen, 1970; Strohl, 1979).

The B cell wall layer of *B. alba* B15LD is morphologically similar to the lipopolysaccharide layer of Gram-negative bacteria and it occupies the same relative space. The B layer is very wavy in thin sections of *B. alba* B15LD, as it was with *Thioploca* and *B. leptomitiformis* (Maier & Murray, 1965). However, in freeze-etched trichromes of *B. alba* B15LD, the B layer was unwrinkled. The corresponding outer layer of the cyanobacteria always appears unwrinkled in either thin sections or in freeze-etch preparations (Drews, 1973), and so it is apparently not identical to the B layer of *B. alba*.

The C, D and E layers of *B. alba* B15LD appear to be unique to only a few organisms. The overall cell wall structure of *B. alba* B15LD is similar to the structure of *Thioploca* (Maier & Murray, 1965). The *B. leptomitiformis* studied by Maier & Murray (1965) lacked the C layer that we observed in our strain, and the two beggiatoas also differed somewhat in the fine structure of the outer two layers.

Drawert & Metzner-Küster (1958) observed a mesh of 6–13 nm diameter fibrils which showed a longitudinal orientation on the surface of their *B. alba*. Longitudinal fibrils, such as we observed on the surface of *B. alba* B15LD, have also been observed on the surface of the cyanobacterium *Spirulina* (van Eykelenberg, 1977). Other Gram-negative bacteria such as *Acinetobacter* (Thornley *et al.*, 1973) and *Spirillum* (Buckmire & Murray, 1970) have patterned surfaces containing tetragonal and/or hexagonal subunits.

By combining the freeze-etch and the thin section data, we have constructed a three-dimensional model of the *B. alba* B15LD cell showing the protoplasmic and the exoplasmic faces of the exposed freeze-etched layers and their respective substructures (Fig. 6).

We thank Ms Ingeborg Geffers, Abteilung Mikrobiologie, Gesellschaft für Biotechnologische Forschung mbH, Braunschweig-Stöckheim, F.R.G., for help with the electron microscopy of Fig. 1(*c*). We also thank Dr L. A. Hook for his kind technical assistance. This work is a result of research sponsored by NOAA Office of Sea Grant, Department of Commerce, under grant no. 04-7-158-44125.

REFERENCES

BAYER, M. E. & REMSEN, C. C. (1970). Structure of *Escherichia coli* after freeze-etching. *Journal of Bacteriology* **101**, 304–313.

BLAND, J. A. & STALEY, J. T. (1978). Observations on the biology of *Thiothrix*. *Archives of Microbiology* **117**, 79–87.

BUCKMIRE, F. A. A. & MURRAY, R. G. E. (1970). Studies on the cell wall of *Spirillum serpens*. I. Isolation and partial purification of the outermost cell wall layer. *Canadian Journal of Microbiology* **16**, 1011–1022.

BURTON, S. D. & MORITA, R. Y. (1964). Effect of catalase and culture conditions on growth of *Beggiatoa*. *Journal of Bacteriology* **88**, 1755–1761.

DRAWERT, H. & METZNER-KÜSTER, I. (1958). Fluorescenz und elektronmikroskopische Untersuchung an *Beggiatoa alba* und *Thiothrix nivea*. VI. Mitteilung der Reihe: Zellmorphologische und zellphysiologische Studien an Cyanophyceen. *Archiv für Mikrobiologie* **31**, 422–434.

DREWS, G. (1973). Fine structure and chemical composition of the cell envelope. In *The Biology of the Blue-green Algae*, pp. 99–116. Edited by N. G. Carr & B. A. Whitton. Oxford: Blackwell Scientific Publications.

GÜDE, H., STROHL, W. R. & LARKIN, J. M. (1981). Mixotrophic and heterotrophic growth of *Beggiatoa alba* in continuous culture. *Archives of Microbiology* **129**, 357–360.

LAMONT, H. C. (1969). Sacrificial cell death and trichome breakage in an oscillatorian blue-green alga: the role of murein. *Archiv für Mikrobiologie* **69**, 237–259.

MAIER, S. & MURRAY, R. G. E. (1965). The fine structure of *Thioploca ingrica* and a comparison with *Beggiatoa*. *Canadian Journal of Microbiology* **11**, 645–656.

MOOR, H. (1966). Use of freeze-etching in the study of biological ultrastructure. *International Review of Experimental Pathology* **5**, 179–216.

MORITA, R. Y. & STAVE, P. W. (1963). Electron micrograph of an ultrathin section of *Beggiatoa*. *Journal of Bacteriology* **85**, 940–942.

NICOLSON, G. L. & SCHMIDT, G. L. (1971). Structure

of the *Chromatium* sulfur particle and its protein membrane. *Journal of Bacteriology* **105**, 1142–1148.

REMSEN, C. C. (1978). Comparative subcellular architecture of photosynthetic bacteria. In *The Photosynthetic Bacteria*, pp. 31–60. Edited by R. K. Clayton & W. R. Sistrom. New York: Plenum Press.

REYNOLDS, E. S. (1963). The use of lead citrate at high pH as an opaque stain in electron microscopy. *Journal of Cell Biology* **17**, 208–212.

SCHMIDT, G. L., NICOLSON, G. L. & KAMEN, M. D. (1971). Composition of the sulfur particle of *Chromatium vinosum* strain D. *Journal of Bacteriology* **105**, 1137–1141.

STEERE, R. L. (1973). Preparation of high-resolution freeze-etch, freeze-structure, frozen-surface and freeze-dried replicas in a single freeze-etch module, and the use of stereo electron microscopy to obtain maximum information from them. In *Freeze-etching Techniques and Applications*, pp. 223–255. Edited by E. L. Benedetti & P. Favard. Paris: Société Française de Microscopie Électronique.

STROHL, W. R. (1979). Ultrastructure of *Cytophaga johnsonae* and *C. aquatilis* by freeze-etching. *Journal of General Microbiology* **112**, 261–268.

STROHL, W. R. & LARKIN, J. M. (1978*a*). Cell division and trichome breakage in *Beggiatoa*. *Current Microbiology* **1**, 151–155.

STROHL, W. R. & LARKIN, J. M. (1978*b*). Enumeration, isolation and characterization of *Beggiatoa* from freshwater sediments. *Applied and Environmental Microbiology* **36**, 755–770.

STROHL, W. R., LARKIN, J. M. & HOWARD, K. S. (1978). Characterization and ultrastructure of *Beggiatoa* sulfur inclusions. *Abstracts of the Annual Meeting of the American Society for Microbiology*, J7, p. 77.

STROHL, W. R., GEFFER, I., LARKIN, J. M. (1981). Structure of the sulfur inclusion envelopes from four beggiatoas. *Current Microbiology* **6**, 75–79.

THORNLEY, M. J., GLAUERT, A. M. & SLEYTR, U. B. (1973). Isolation of outer membranes with an ordered array of surface subunits from *Acinetobacter*. *Journal of Bacteriology* **114**, 1294–1308.

TREDWAY, J. V., LEE, J. D. & BURTON, S. D. (1977). Morphological evaluation of the ultrastructure of marine and freshwater *Beggiatoa* species. *Abstracts of the Annual Meeting of the American Society for Microbiology*, N69, p. 240.

VAN EYKELENBERG, C. (1977). On the morphology and ultrastructure of the cell wall of *Spirulina platensis*. *Antonie van Leeuwenhoek* **43**, 89–99.

WATSON, M. L. (1958). Staining of tissue sections for electron microscopy with heavy metals. II. Applications of solutions containing lead and barium. *Journal of Biochemical and Biophysical Cytology* **4**, 475–479.

WIRSEN, C. O. & JANNASCH, H. W. (1978). Physiological and morphological observations on *Thiovulum* sp. *Journal of Bacteriology* **136**, 765–774.