ELECTRON MICROSCOPY OF THE CELL WALLS OF *BACILLUS MEGATERIUM* AND *RHODOSPIRILLUM RUBRUM*

by

M. R. J. SALTON* AND ROBLEY C. WILLIAMS**

Department of Bacteriology and Virus Laboratory, University of California, Berkeley (U.S.A.)

Most of the isolated cell walls of bacteria so far examined in the electron microscope have been prepared for electron microscopy by the conventional method of air-drying droplets of cell wall suspensions on the supporting film of specimen grids. Cell walls examined in this fashion appear as flat, empty structures possessing the general outline of the organisms from which they have been isolated (DAWSON[1], SALTON AND HORNE[2]). WILLIAMS[3] has recently developed a method of freeze-drying for the preparation of biological specimens for electron microscopy in which the three-dimensional structure of the specimens is successfully preserved. It has been of interest to apply this technique to an examination of the isolated cell walls of bacteria.

Cell walls of *B. megaterium* (strain KM) and *Rhodospirillum rubrum* were prepared by disintegration of washed suspensions of cells in the Raytheon 9 kc Magnetostriction Oscillator, the subsequent steps in the separation of the walls from protoplasmic material being those outlined by SALTON AND HORNE[2]. Fig. 1 shows the appearance of *B. megaterium* cell wall prepared by air-drying a suspension of the walls sprayed onto a specimen grid. The appearance of cell walls of *B. megaterium* prepared for electron microscopy by the freeze-drying method is shown in Fig. 2. A comparison of Figs. 1 and 2 clearly demonstrates the rigid, tubular nature of the cell wall of *B. megaterium* when examined by the freeze-drying method. The flat, collapsed appearance of the cell wall shown in Fig. 1 undoubtedly results from the surface-tension forces acting on the wall during the drying process, such effects having been obviated by the freeze-drying technique. One interesting feature shown in Fig. 2 and observed in many other fields of cell wall preparations of this organism, is the rough texture of the outer surface of the walls. The inner surface of the wall visible in the cell wall fragment in Fig. 2 has a much smoother appearance than that of the outer surface. Treatment of the cell walls of *B. megaterium* with egg-white lysozyme resulted in complete dissolution of the walls and no residual structure could be detected in the electron microscope.

The appearance of an air-dried preparation of a partially washed cell-wall fraction from *Rhodospirillum rubrum* is shown in Fig. 3. Cell walls prepared for electron microscopy by the freeze-drying method are shown in Figs. 4 and 5. The cell walls isolated from this organism were considerably shorter than may have been expected and as shown in Fig. 5 many short, almost spherical cell wall elements were found. A close inspection of the original electron micrograph shown in Fig. 3 revealed the presence

* Present address: Department of Biochemistry, Cambridge, England.

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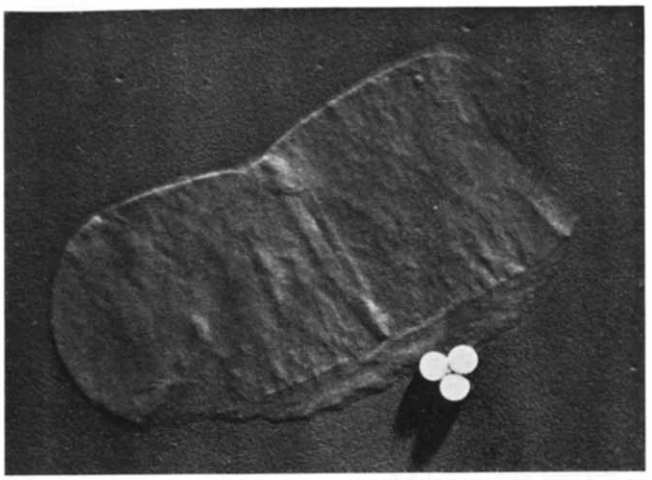

Fig. 1. Air-dried preparation of *B. megaterium* cell wall. × 16,000.

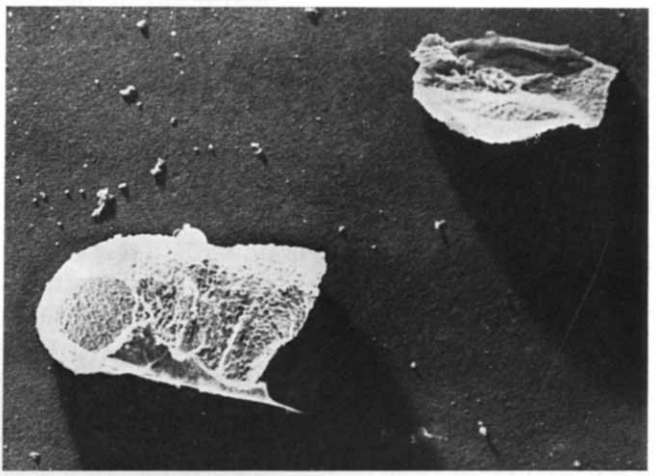

Fig. 2. Cell walls of *B. megaterium* prepared for electron microscopy by the freeze-drying method. × 18,000.

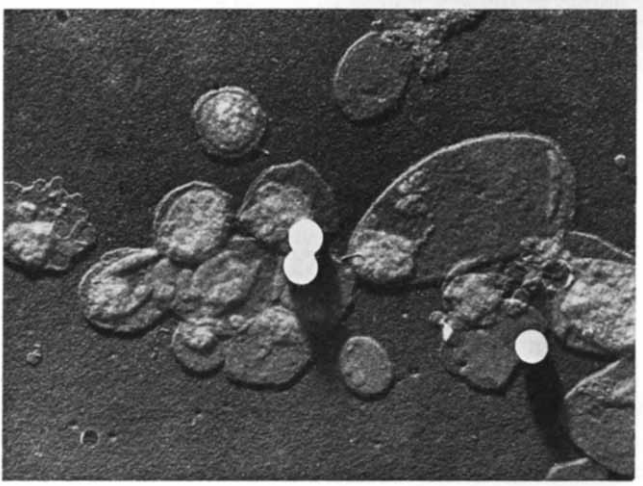

Fig. 3. Partially washed cell wall fraction from *Rhodospirillum rubrum*—air-dried preparation. × 18,000.

All photographs are electron micrographs of preparations shadowed with uranium. The electron-dense spheres are polystyrene latex indicator particles.

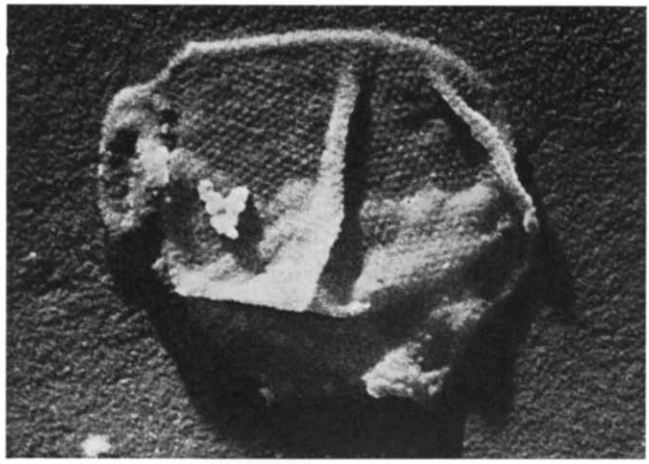

Fig. 4. Cell wall of *Rhodospirillum rubrum* prepared for electron microscopy by the freeze-drying method. Note the presence of macromolecular components. × 73,000.

Fig. 5. *Rhodospirillum rubrum* cell walls—freeze-dried preparation. Macromolecular structure not visible in this preparation. × 38,000.

of macromolecular components. The macromolecular structure in the cell wall of *Rhodospirillum rubrum* was more clearly seen in the material prepared for electron microscopy by the freeze-drying method (Fig. 4). This structure is apparently very similar to that recently shown in the cell wall of a Spirillum species by HOUWINK[4]. Several individual batches of cell walls prepared from light-grown cultures of this *Rhodospirillum rubrum* showed this macromolecular structure in the wall. However, in one batch of cell walls this structure was not apparent (Fig. 5). The cell walls of a dark-grown culture of *Rhodospirillum rubrum* also showed the presence of macromolecular components, similar in every respect to that of the light-grown cells (Fig. 4). It is of interest to note that with dark-grown cells of *Rhodospirillum rubrum*, SCHACHMAN, PARDEE AND STANIER[5] could detect no boundary in the ultra-centrifuge corresponding to that of chromatophores found in extracts from light-grown cells.

Preparations of walls of *Rhodospirillum rubrum* possessing the macromolecular components were examined after treatment with trypsin. There was no detectable change in the appearance of the walls after incubation with trypsin. Some preliminary investigations of the chemical constitution of the cell wall of *Rhodospirillum rubrum* show that it possesses protein (approximately 55%), carbohydrate (23% reducing substances liberated on hydrolysis with 2 *N* HCl) and lipid (21%) components. The high lipid content together with the amino-acid constitution (including aromatic amino acids) of the cell wall of *Rhodospirillum rubrum* is similar to that found for the cell walls

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of other Gram-negative bacteria (SALTON[6]). It seems probable that the macromolecules of the wall of this organism are protein in nature.

The walls of *Pseudomonas fluorescens*, *Rhodopseudomonas spheroides* and *Escherichia coli* were also examined but there was no evidence of macromolecular components. Furthermore, Figs. 1 and 2 show no structure in the cell walls of *B. megaterium*. These results are in complete agreement with HOUWINK's[4] observations. The cell walls of *Pseudomonas fluorescens*, *Rhodopseudomonas spheroides* and *Escherichia coli* prepared for electron microscopy by the freeze-drying method showed a rigidity similar to that shown in Fig. 2 for *B. megaterium*.

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SUMMARY

The isolated cell walls of *B. megaterium* and *Rhodospirillum rubrum* have been examined in the electron microscope after air-drying and freeze-drying the cell wall suspensions on specimen grids. The preservation of the three-dimensional structure of the walls by the freeze-drying method has demonstrated their mechanical rigidity.

The cell wall of *Rhodospirillum rubrum* possesses macromolecular components in its cell wall. There was no evidence of a similar structure in the cell walls of several other bacteria. A preliminary investigation of the composition of the wall of *Rhodospirillum rubrum* has shown the presence of protein, carbohydrate and lipid components.

RÉSUMÉ

Les parois cellulaires isolées de *B. megatherium* et de *Rhodospirillum rubrum* ont été examinées au microscope électronique après avoir été séchées à l'air et à basse température en suspensions. La conservation de la structure spatiale des parois après sèchage à basse température démontre leur rigidité mécanique.

La paroi cellulaire de *Rhodospirillum rubrum* présente des constituants macromoléculaires. Plusieurs autres bactéries ne possèdent probablement pas des structures semblables. Un examen préliminaire de la composition de la paroi de *Rhodospirillum rubrum* montre la présence de protéines, de glucides et de lipides.

ZUSAMMENFASSUNG

Die isolierten Zellwände von *B. megaterium* und *Rhodospirillum rubrum* wurden elektronenmikroskopisch untersucht nach Luft- und Gefriertrocknung der Zellwandsuspensionen auf besonderen Netzen. Die Erhaltung der drei-dimensionalen Struktur der Wände bei der Gefriertrocknung zeigt die mechanische Steifheit dieser Zellwände.

Rhodospirillum rubrum enthält in seinen Zellwänden makromolekulare Komponenten. Es finden sich keine Beweise dafür, dass die Zellwände verschiedener anderer Bakterien ähnliche Strukturen besitzen. Eine vorläufige Untersuchung der Wände von *Rhodospirillum rubrum* zeigte, dass diese Protein-, Kohlehydrate- und Fettkomponenten enthalten.

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