SHORT COMMUNICATION

Ultrastructure of a Gas-vacuolate Square Bacterium

By K. PARKES¹ AND A. E. WALSBY²*

¹Marine Science Laboratories, Menai Bridge, Gwynedd LL59 5EH, Wales, U.K.
²Department of Botany, University of Bristol, Woodland Road, Bristol BS8 1UG, U.K.

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Electron micrographs are shown of the gas-vacuolate square bacterium found at the surface of a brine pool in Sinai. The bacteria have a thickness of 0.1 μm or even less in the central regions. Some are apparently surrounded by sheath material. They contain gas vesicles which are either spindle-shaped or cylindrical with conical ends, and up to 1 μm long. The bacterial surface has 20 nm subunits arranged in a regular array.

INTRODUCTION

Gas-vacuolate bacteria having the form of thin square sheets were recently described occurring in a hypersaline pool near Nabq, on the Sinai Peninsula (Walsby, 1980). The bacteria were collected from the surface of the pool where they had concentrated in large numbers, buoyed up by their gas vacuoles. The identification of these micro-organisms as bacteria was largely based on the presence of gas vacuoles, structures which uniquely disappear on application of a moderate pressure and which occur only in prokaryotic organisms (Walsby, 1972).

An organism with such a square shape is without precedent and it is not surprising, therefore, that the original report of the square bacterium was received with some scepticism (unpublished correspondence to A. E. Walsby). The report mentioned preliminary observations on fine structure but no electron micrographs were published. We present here electron micrographs of the organisms in the original sample described by Walsby (1980), showing details of the shape, wall structure and gas vesicles, which will be useful in further identification of material subsequently collected from hypersaline pools.

METHODS

The brine sample described by Walsby (1980) was left to stand for 3 d so that the buoyant gas-vacuolate bacteria floated to the surface meniscus. A sample of about 50 μl was drawn up from the meniscus in a microsyringe, suspended in 300 μl 4 M-NaCl and centrifuged for 1 h at 700 rev. min⁻¹ in a small centrifuge tube. The pressure generated at the base of the tube during centrifugation was calculated not to exceed 20 kPa (0.2 bar) so that the gas vesicles, which have critical pressures exceeding 100 kPa (Walsby, 1980), would not be collapsed (Walsby & Buckland, 1969). The buoyant square bacteria rose to the surface of the centrifuged suspension and were transferred in small drops to Formvar-coated electron microscope grids. Some of the grids were shadowed with platinum at an angle of 13°, the angle being determined by measurements made on latex beads shadowed at the same time.

RESULTS AND DISCUSSION

Electron microscopy confirmed the observations made by light microscopy: the gas-vacuolate bacteria are square and very thin (Figs 1 and 2). Rectangular forms were also
Fig. 1. Electron micrographs of square bacteria showing the range of shape and gas vesicles: (a–c) rectangular form, (a) with short, spindle-shaped gas vesicles, (b) with peripheral gas vesicles and overlying salt crystals, and (c) packed with over 120 gas vesicles, mostly of the cylindrical form; (d–f) square form, (d) with no sheath, (e) with a thin sheath and (f) with extensive surrounding sheath. The bar markers represent 1 μm.

found. The range of forms described previously suggests that each square grows to a rectangle which then divides into two equal squares (Walsby, 1980).

Figure 1 shows unstained bacteria in which the hollow, electron-transparent gas vesicles
show up against the rather more electron-opaque cytoplasm, as demonstrated previously in other gas-vacuolate prokaryotes (Walsby & Eichelberger, 1968; Konopka et al., 1975). In many other bacteria the gas vesicles form distinct aggregations, which are the gas vacuoles seen by light microscopy. Individual gas vesicles can be seen but not separately resolved under the light microscope. In many of the square bacteria surveyed, the gas vesicles were concentrated at the cell periphery, as in Fig. 1 (b), but in others they appeared to be randomly dispersed (Fig. 1a). Some of the bacteria appeared to be nearly filled with gas vesicles; the one shown in Fig. 1 (c) contains over 120.

The shape of the gas vesicles may be somewhat distorted when viewed in whole mounted cells (see Walsby, 1972; Walsby & Eichelberger, 1968) but it is clear that both spindle-shaped vesicles and cylindrical vesicles with conical ends occur in the square bacteria, as described in species of Halobacterium (Larsen et al., 1967; Stoeckenius & Kunau, 1968). Both forms may occur within the same bacterium, again as in Halobacterium (see Fig. 10a of Walsby, 1972) but a preponderance of one type or the other was often found [cf. Figs 1 (a) and 1 (c)]. The longest cylindrical vesicles exceeded 1 μm in length.

Some of the bacteria appeared to have a sheath around them. It showed as a narrow halo when the bacterium shrank slightly on drying on to the grid (Fig. 1e). The presence and amount of material left surrounding the bacterium was variable; in some cases the material formed a band up to 0.1 μm wide (Fig. 1f).

Metal shadowing revealed a regular array of subunits on the surface of the bacteria, with a periodicity of about 20 nm (erroneously given as 2 nm in Walsby, 1980). The subunit arrangement appeared to be hexagonal but this awaits confirmation by diffractometry. From the minimum length of the shadow cast by the edge of the bacterium normal to the shadowing direction, and the known shadowing angle, the thickness of the bacterium at its edge can be
calculated. The minimum thickness was 0.07 μm. It can be seen in Fig. 2 that where there are gas vesicles along the edge of the cell, the length of the shadow cast is longer, indicating a thickness of 0.15 μm. The difference, 0.08 μm, is roughly equal to the diameter of the gas vesicles which show through the shadowed specimen. The central region of the cell appeared very thin where no gas vesicles were present. A similar indication was also given by other, unshadowed, bacteria. The overall impression gained of the organism is of a highly plastic, flattened envelope, distended in places by granular contents, rather like a rectangular polythene package of frozen peas from which most of the contents have been removed. The flexibility of the bacterium is indicated by the folded square shown in Fig. 2. The folding angle does not seem to correspond with the orientation of the hexagonal lattice, however. The organism is potentially of interest for studying surface subunit structure, as it offers large flat arrays of wall.

Further studies on the square bacterium are required for a full taxonomic description. However, its distinctive morphology suggests that a new taxon should be erected and we would like to propose that, if a new genus is justified, the name *Quadra* (Latin feminine noun, meaning a square) be used. We understand that large quantities of square bacteria have been collected from the original site in Sinai by W. Stoeckenius (University of California) and by M. Kessel and Y. Cohen (Hebrew University, Israel) and they will describe further aspects of these fascinating micro-organisms.

**REFERENCES**


