SHORT COMMUNICATIONS

Extracellular Membranous Structures in a Stable L-form of *Staphylococcus aureus*

By T. EDA, Y. KANDA, C. MORI AND S. KIMURA

*Department of Bacteriology, Teikyo University School of Medicine,
Itabashi-ku, Kaga 2-11-2, Tokyo, Japan*

(Received 26 May 1977; revised 18 July 1977)

INTRODUCTION

Mesosomal vesicles extruded during protoplasting have been demonstrated in many Gram-positive bacteria (see, for example, Cole, 1971; Fitz-James, 1968; Greenawalt & Whiteside, 1975). In L-forms, however, little is known about extracellular membranous structures although cytoplasmic membranous organelles (Cole, 1968, 1971; Corfield & Smith, 1968; Eda, Kanda & Kimura, 1976) or vesicular structures (see, for example, Bibel & Lawson, 1972; Dannis & Marston, 1965; Dienes, 1967) have been reported in various species. Cole (1968) showed tubular, extracellular membranes in group A streptococcal L-forms.

No extracellular tubular membrane structures have so far been described in staphylococcal L-forms. In this report, we describe the ultrastructure of membranous structures extruded into the culture medium by a staphylococcal L-form.

METHODS

Strain. The L-form used (designated 209PL) was isolated from *Staphylococcus aureus* strain 209P by the disc method using aminobenzylpenicillin (Eda, Matsuoka & Tadokoro, 1972). This strain is stable and has been subcultured for several years in the absence of antibiotic without reversion to the parent form.

Medium. The L-form was cultured in L-form broth containing 3.7% (w/v) brain-heart infusion (Difco), 0.5% (w/v) yeast extract (Difco), 4% (w/v) NaCl and 10% (v/v) horse serum (Bio Test).

Electron microscopy. Cultures of the L-form grown in L-form broth at 37 °C were harvested at the stationary phase of growth (48 h) by centrifuging at 10000 g for 30 min (Sorvall RC-2B) and prefixed in chilled 2.5% (v/v) glutaraldehyde in 0.1 M-phosphate buffer (pH 7.2) for 2 h at 4 °C. They were then collected by centrifuging at 10000 g at 4 °C and washed three times with the same buffer in the cold before postfixing with 1% (w/v) OsO₄ in 0.1 M-phosphate buffer (pH 7.4) for 2 h at 4 °C. After dehydration in a graded acetone series, specimens were embedded in Epon 812. Ultrathin sections were cut with a Reichert OMU2 ultra-microtome, and stained with uranyl acetate and lead citrate. Preparations were examined and photographed with a Hitachi HU-12AS electron microscope at 75 kV.

RESULTS AND DISCUSSION

The L-forms varied in diameter from 150 nm to 7 µm, and were bounded by a unit membrane but lacked a cell wall. Each contained ribosomes and fibrillar nucleoid materials. In addition, several small vesicular structures, and tubular or vesiculotubular, sometimes spiral, structures were observed around the larger L-forms (Fig. 1*a*). These vesicular structures, 25 to 100 nm in diameter, were bounded by a unit membrane and had electron-dense particles, probably ribosomes, but did not contain nucleoid materials (Fig. 1*b*). The minimal colony forming units of staphylococcal L-forms are the cells larger than 100 nm in

Bar markers represent 1 μm .

Fig. 1. Thin-section electron micrographs of *S. aureus* strain 209PL. (a) L-forms of various sizes are seen; they lack a cell wall but are bounded by a unit membrane. There are a number of vesicles and tubular structures around the larger L-form. (b) At higher magnification, vesicles and tubular, sometimes vesiculotubular, structures are visible. Tubular structures contain electron-scattering material but lack nucleoid material.

diameter and no changes in ultrastructural appearance are seen in the vesicles after DNAase treatment (Okuda *et al.*, 1977). From these results, it was suggested that these vesicles, smaller than approximately 100 nm, did not contain nucleic acid and so were unable to replicate themselves as stated by Cole (1971). Our present results indicate that these vesicles are the membranous structures extruded outside the cells, but are not the reproductive units, although several investigators (see, for example, Bibel & Lawson, 1972; Dannis & Marston, 1965; Dienes, 1967) have regarded them as one of the reproductive units in an L-form growth cycle.

Tubular structures as seen in Fig. 1(b) measured 25 to 30 nm by 100 to 700 nm and were bounded by a unit membrane. They contained electron-scattering materials but no nucleoid structures. We suggest that these tubular structures are also extruded into the culture medium. They are morphologically similar to those found in streptococcal L-forms by Cole (1968) and also to the extruded mesosomes shown during protoplast formation.

REFERENCES

- BIBEL, D. J. & LAWSON, J. W. (1972). Development of streptococcal L-form colonies. *Journal of Bacteriology* **112**, 602-610.
- COLE, R. M. (1968). The structure of the group A streptococcal cell and its L-forms. In *Current Research on Group A Streptococcus*, pp. 5-42. Edited by R. Caravano. New York: Excerpta Medica Foundation.
- COLE, R. M. (1971). Some implications of the comparative ultrastructure of bacterial L-forms. In *Mycoplasma and L-forms of Bacteria*, pp. 49-83. Edited by S. Madoff. New York: Gordon & Breach.
- CORFIELD, P. S. & SMITH, D. G. (1968). Microtubular structures in group D streptococcal L-forms. *Archiv für Mikrobiologie* **63**, 356-361.
- DANNIS, D. C. & MARSTON, J. H. (1965). Fine structure of staphylococcus L-forms. *Texas Reports on Biology and Medicine* **23**, 729-736.
- DIENES, L. (1967). Morphology and reproductive processes of the L forms of bacteria. I. Streptococci and staphylococci. *Journal of Bacteriology* **93**, 693-702.
- EDA, T., KANDA, Y. & KIMURA, S. (1976). Membrane structures in stable L-forms of *Escherichia coli*. *Journal of Bacteriology* **127**, 1564-1567.
- EDA, T., MATSUOKA, S. & TADOKORO, I. (1972). Studies on staphylococcal L-forms. I. Induction and morphological characteristics of staphylococcal L-forms. *Japanese Journal of Bacteriology* **27**, 657-664.
- FITZ-JAMES, P. C. (1968). The collection of mesosome vesicles extruded during protoplasting. In *Microbial Protoplasts, Spheroplasts and L-forms*, pp. 124-143. Edited by L. B. Guze. Baltimore: Williams & Wilkins.
- GREENAWALT, J. W. & WHITESIDE, T. L. (1975). Mesosomes: membranous bacterial organelles. *Bacteriological Reviews* **39**, 405-463.
- OKUDA, K., TAKAHASHI, T., TADOKORO, I. & EDA, T. (1977). Studies on the minimum reproducible unit of staphylococcal L-forms. *Microbiology and Immunology* **21**, 1-10.