SHORT COMMUNICATIONS

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

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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 209~p) was isolated from Staphylococcus aureus strain 209~ by the disc method using amnibenzylopenicillin (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 OME2 ultramicrotome, 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. 1a). 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. 1b). The minimal colony forming units of staphylococcal L-forms are the cells larger than 100 nm in
Fig. 1. Thin-section electron micrographs of *S. aureus* strain 209P*α*. (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