SHORT COMMUNICATIONS

Morphological Modifications of Bacteria Induced by Spatial Constraints

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INTRODUCTION

A number of morphological modifications have been observed and studied in bacterial cultures during growth and degeneration. They seem to result principally from the action of one or more factors in the growth medium which modify the metabolism of the cells (Duguid & Wilkinson, 1961).

In the course of our studies on the internal structure of the bacterial colony, we observed that bacteria become deformed when dividing in the limited space of the internal canaliculi of cellulose ester membrane filters. Such a means of changing the shape of bacteria has to our knowledge not been reported previously.

METHODS

Bacteria and growth. *Staphylococcus aureus* (1P52.156), *Escherichia coli* and *Pseudomonas aeruginosa* (both from our laboratory collection) were grown on the synthetic medium S of Ryter & Kellenberger (1958) supplemented with Difco yeast extract (3 mg ml⁻¹), and on a semi-synthetic medium (medium PV) containing: Difco peptone, 1 g; Difco beef extract, 0.5 g; NaCl, 0.5 g; agar, 1.5 g; water, 100 ml; adjusted to pH 7.2 with NaOH. For inoculation, a culture was grown in liquid medium for 16 h at 37 °C and then either 1 µl was applied to a Millipore membrane (HAW P 047) which had previously been placed on agar medium or 1 ml was diluted in 20 ml physiological salt solution and filtered through a similar Millipore membrane. This membrane was rinsed twice with 40 ml physiological salt solution and then transferred to agar medium.

The membranes seeded by filtration were incubated at 37 °C for 5 to 6 h; those which had received a surface inoculum were incubated for 24 h.

Electron microscopy. After incubation, filters were gently covered with 2 % (v/v) glutaraldehyde in 0.1 M-sodium cacodylate for 30 min at 4 °C. Membranes seeded by filtration were cut into 2 or 3 mm squares with a razor blade; membranes which had received a surface inoculum were cut as near to the edge of each colony as possible. Both were fixed at 4 °C with 2 % glutaraldehyde in 0.1 M-sodium cacodylate for 2 h, postfixed with 1 % (w/v) OsO₄ and then embedded in Spurr's epoxy resin. After uranyl acetate and lead citrate staining (Reynolds, 1963), sections were cut on a Sorvall Porter Blum microtome no. 2 and examined in a Siemens Elmiskop 1A electron microscope.

Bar markers represent 1 μm .

Fig. 1. Perpendicular sections through membrane filters.

- (a) *Staphylococcus aureus* incubated for 24 h on medium S, showing clusters of bacteria.
- (b) *Pseudomonas aeruginosa* incubated for 24 h on medium PV; arrows indicate the limits of the channels.
- (c) *Escherichia coli* seeded by filtration and incubated for 5 h on medium S.

RESULTS AND DISCUSSION

Sections through colonies of *S. aureus* which were at least 24 h old sometimes showed small groups of very deformed cocci occluded in the filter (Fig. 1a). The cells occupied all the available space. Similar results were obtained with *P. aeruginosa* and *E. coli* (Fig. 1b, c). For *E. coli*, seeding by filtration favoured the penetration of bacteria into the filter.

Nutritional deficiency (Salton, 1960) seems an unlikely cause of these deformations as they were apparent when bacteria had been incubated for only 5 h. Deformation still occurred when a membrane seeded by filtration was transferred to fresh medium every hour during the 5 h incubation. However, deformation did not occur when a cell was in a large cavity of the membrane and was able to develop without reaching the sides of the cavity (A, Fig. 1a). By the same argument, the accumulation of toxic substances resulting from the metabolism of the bacteria seems an improbable cause of deformation.

The observations are probably explained by the physical confinement of the bacteria to

a limited space. The walls of cells inside the filter fit perfectly the configuration imposed by the synthetic fibres, implying that the morphological modification of the cells is induced by spatial constraints and that cells possess parietal plasticity during growth. A cell which develops in a narrow canaliculus (B, Fig. 1*a*) loses its cocciform character and stretches along the axis of the canaliculus. These deformations do not seem to prevent multiplication and successive divisions give a closely arranged mosaic of cells, (C, Fig. 1*a*).

In view of these results, the classic notion of the cell wall as a rigid structure may have to be revised.

These morphological changes, which were observed *in vitro*, may occur in natural environments under certain conditions, for example in soils and sediments with a low granularity. Our observations show a new aspect of bacterial adaptation to the environment and it would be of interest to study the consequences of these changes on cellular metabolism.

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