SHORT COMMUNICATION

The Growth and Form of Bacterial Colonies

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A simple method is described for measuring the profile of bacterial colonies. Profiles were determined for colonies of *Bacillus cereus*, *Escherichia coli* and *Staphylococcus albus* of different ages. In spite of differences in cell morphology, the colony profiles had a common basic structure consisting of a steeply rising leading edge connected by a ridge to an interior region where height also rose, though less steeply, to a flat or domed centre. The colony mass increased exponentially through part of the growth phase. It is suggested that net colony growth consists of a combination of leading edge growth, which is unrestricted and approaches the maximum specific growth rate of the organism, and diffusion-limited growth in the colony interior. Common elements of profiles from each species may be a consequence of such differences in growth rate.

INTRODUCTION

Bacterial colonies, though familiar objects to microbiologists, are structures that are not well-understood. Their shape and profile have been described in subjective terms mainly for taxonomic or diagnostic purposes and there is no evidence that either have been determined accurately. A few workers have described simplified model profiles for particular bacterial species. Pirt (1967, 1975) suggested that colonies of *Escherichia coli* are hemispherical when young but become substantially flat cylinders or discs with convex edges as they age. Palumbo *et al.* (1971) and Wimpenny & Lewis (1977) have assumed that colonies of a *Pseudomonas* species and of four non-motile organisms, respectively, are shaped like sections of a sphere.

This communication describes a simple method for measuring colony profiles and its use with different aged colonies of three morphologically distinct bacterial species. The growth and common elements of the structure of these colonies are discussed.

METHODS

Growth and maintenance of organisms. *Escherichia coli* K12, *Staphylococcus albus* NCIB 8558 and *Bacillus cereus* (from the culture collection of the Microbiology Department, University College, Cardiff) were maintained at room temperature in the dark on Dorset egg slopes (Oxoid). Petri dishes (9 cm diam.) containing 25 ml Tryptone Soya Broth (Oxoid) solidified with Bacto-agar (Difco; 12 g l⁻¹) were dried at 37 °C for 1.5 h before use. The plates were inoculated as follows: a freshly drawn fine glass needle (approximately 20 µm diam.) made from a Pasteur pipette was inserted into the surface growth of a 24 h confluent lawn of organisms and then allowed to touch the surface of the sterile agar without penetrating it. One to five colonies were initiated in this way on each plate. After inoculation, plates were incubated at 37 °C for various times.

Measurement of the colony profile. Each colony on an agar disc cut from the plate with a cork borer was transferred to a clean glass slide. The surface of the colony was exposed to a fine puff of talcum powder and the slide was placed on the stage of an MBR-IE-PH microscope (Leningrad United Optical-Mechanical Enterprises; UK agents, Technical and Optical Equipment, London). The microscope stage had two vernier

Fig. 1. Colony growth. The dry weight of each colony was estimated turbidimetrically as described in Methods and the results were plotted on exponential (a) or arithmetic (b) scales: $\square$, *Bacillus cereus*; $\bullet$, *Escherichia coli*; $\circ$, *Staphylococcus albus*.

controls at right angles with which horizontal measurements could be made to an accuracy of about 0.05 mm. Colony height at any point was determined by focussing on small talcum powder particles using the calibrated fine focus adjustment on the microscope. Fifty scale divisions of the latter correspond to 100 μm and the height of any particle can be determined to an accuracy of about 2 μm . The profile of each colony was determined by height measurements made across the centre of the colony in two directions at right angles. Standard half-colony profiles were determined graphically from the actual full profiles measured at any one time by the following method. It was assumed that all colonies were radially symmetrical so that measurements from each half-profile could be superimposed. Plots of each half-profile were divided into ten equally spaced sections and the height was measured at these positions. Heights at each tenth-interval across the eight half-profiles obtained from two colonies were averaged, as were the radii of each profile. The standard profile was then plotted as the average height at each interval against the average radius at each interval.

Colony growth. The growth of each colony was determined (after its profile had been measured) by transferring the agar disc plus colony to a test tube containing 3, 6 or 9 ml of 20 mM- $\text{KH}_2\text{PO}_4/\text{KOH}$ buffer (pH 7.0), mixing for about 15 s and then reading the $A_{550}^{1\text{cm}}$ value against a blank derived from uninoculated discs treated in the same way. The presence of a small quantity of talcum powder particles made a difference of only 0.002 to 0.004 in the absorbance measurements of the resuspended colony and was ignored. Absorbance values were converted to dry weight using calibration curves prepared from colonial growth of each species.

RESULTS

Colony mass increase

Colony mass was estimated turbidimetrically at various time intervals and the results were plotted on exponential and arithmetic scales (Fig. 1). Colonies of all three species examined grew exponentially for part of the growth period. Specific growth rates of 0.19, 0.12 and 0.13 h^{-1} were obtained for *B. cereus*, *E. coli* and *S. albus*, respectively.

Fig. 2. Standard colony profiles. Measurements from eight actual half-profiles at each colony age (in h) were used to generate a standard profile as described in Methods: (a) *Escherichia coli*; (b) *Staphylococcus albus*; (c) *Bacillus cereus*.

Colony profiles

Profiles of colonies at different times after inoculation were determined microscopically as described in Methods. Averaged profiles calculated from a number of actual profiles are shown in Fig. 2, in which a vertical scale of five times the horizontal scale has been used to emphasize the structure of these rather flat colonies. Colonies of *B. cereus* were wider but flatter than those of the other two species, whilst *S. albus* colonies were the most nearly hemispherical of the three. In spite of the obvious differences in colony height and diameter, and also in the morphology of the individual cells of each species, there was an underlying similarity among all the profiles. Thus each had a 'leading edge' whose height rose, often increasingly steeply, to a ridge. Beyond the point of inflexion marking this ridge, the interior region of the colony also increased in height sometimes with an upward curve but always at a shallower angle than the leading edge slope. Near the centre of the colony the profile became flat or dome-shaped.

It is also clear from the profiles in Fig. 2 that the height rose to a value of about 200 μm in the case of *E. coli* and *S. albus* colonies, whilst those of *B. cereus* remained slightly flatter at approximately 160 μm . Colony radial growth rates for all three species were linear (results not shown), as observed for *E. coli* by Pirt (1967) and for other species by Palumbo *et al.* (1971) and Cooper *et al.* (1968).

DISCUSSION

The three organisms used in this study have quite different cell morphologies, consisting as they do of Gram-positive cocci and Gram-positive and Gram-negative rods of different sizes. There are significant differences, too, in colonial morphology which are easily recognized by eye. Careful measurements of actual colony profiles throughout the normal growth period, however, revealed striking similarities in the fundamental structure of the colonies. Thus, each colony seems to be made up of two different zones: an area at the periphery or leading edge whose height rises relatively steeply, often with an upward curve, and an area in the middle in which height rises much less steeply, though still sometimes with an upward curve, to a flattish or domed centre.

It seems likely that growth is exponential and unrestricted by nutrient diffusion in young colonies and in the leading edge region of older colonies. At some point, indicated by the change in slope of the profile near the ridge between the two areas, nutrient availability becomes restricted possibly due to depletion of a narrow layer adjacent to the colony base. The growth rate in this area falls to a value determined by the rate of diffusion of one or more growth-limiting nutrients through the agar. The model for bacterial colonial growth outlined here conflicts with that proposed for colonies of *E. coli* by Pirt (1967). Pirt suggested that the bacterial colony is a flat structure having a convex leading edge in which all colonial growth takes place. Work reported here suggests (i) that the leading edge is normally concave, (ii) that the colony height continues to rise towards its centre and (iii) that the overall colony growth rate is a combination of unrestricted plus diffusion-limited growth.

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