A Probable Growth Cycle in *Bacillus megaterium*

BY F. J. BERGERSEN

*Department of Bacteriology, University of Otago Medical School, Dunedin, New Zealand*

SUMMARY: The sequence of events which occurs as *Bacillus megaterium* grows and divides is shown to involve cells with fusion nuclei and cells which undergo an apparently sexual process. An account is also given of the part played in cell division by granules associated with the cell membrane. These resemble mitochondria in that they are centres of intense oxidative activity, and they are shown to be related to growing points which have been reported in other organisms.

Life cycles have been described in many micro-organisms, and much work has been done in interpreting observed processes; this has been well summarized and reviewed (Bisset, 1950, 1951a). There have been recent papers by Mudd, Winterscheid, DeLamater & Henderson (1951) and Mudd, Brodie, Winterscheid, Hartman, Beutner & McLean (1951), which describe cytochemical and electron microscope investigations into the existence of mitochondria in bacteria. These have revealed granules of high oxidative activity in certain aerobic organisms. Bisset (1951b), using ordinary stains, has shown that some bacteria have regions of more active growth, which he termed growing points.

I have found that growing points can be demonstrated in HCl-Giemsa preparations of many species of bacteria (Bergersen, 1952). Bisset (1958) has shown that the appearances described by Mudd et al. (1951a, b) are merely growing points and often entire distorted cells. It was with the object of further investigation of the two views that the present work was undertaken.

MATERIALS AND METHODS

The organism used was a strain of *Bacillus megaterium*, isolated and identified in this laboratory. It was maintained on nutrient agar. Cultures for investigation were made in a meat infusion broth, incubated at 37° and examined after various time intervals. HCl-Giemsa preparations were made on coverslips, smears being fixed in osmic acid vapours and hydrolysed at 60° in n-HCl. Living cultures growing on a 0.5% agar medium contained under coverslips in hollow slides were examined by phase microscopy. Such preparations were observed at intervals, the microscope, with the slide in position, being placed in a 37° incubator.

Cytochemical reagents used were Janus Green B, and Nadi reagent which was prepared before use by mixing 1 vol. of 1% α-naphthol in 95% ethanol with 1 vol. of 1% aqueous dimethyl-p-phenylenediamine, and filtering the mixture.
Growth cycle of Bacillus megaterium

OBSERVATIONS

The HCl+Giemsa preparations revealed an orderly series of events. The inocula were taken from actively growing broth cultures which consisted of chains of bacilli with paired nuclear structures and growing points at the poles of each cell (Pl. 1, fig. 1).

After 3 hr. the culture contained elongated cells with bars of chromatinic material (fusion nuclei) (Pl. 1, fig. 2). The lateral cell membranes were studded with three or four growing points, and where the cells were dividing these were often large. Also present were a number of cells with two isolated chromatinic bodies, a number of apparently anucleate cells, and in some microscope fields cells were found which were fusing and apparently transferring nuclear material. The last three types of cells did not contain growing points.

Eight hours after inoculation the bacilli were elongated and in chains of three or four (Pl. 1, fig. 3). The nuclear bodies were still rods and growing points were present in lateral cell membranes and at polar positions.

After 10 hr. these long bacilli began to break up into cells of the same size as those of the inoculum, and the nuclei appeared as zig-zags of short rods in each cell (Pl. 1, fig. 4). Growing points were mainly at transverse septa but some were still present in lateral cell membranes.

Twelve to fifteen hours after inoculation the culture consisted of typical anthracoid filaments, whose cells were of the same type as those of the inoculum (Pl. 1, fig. 5).

These observations suggested that the growing points associated with the cell membrane were concerned in cell division. If this be so they appeared before division actually began.

Phase-contrast studies showed granules in the cell periphery in both lag and logarithmic phases; during active multiplication they were obscured. These granules seemed to occupy positions at the cell membrane corresponding to the growing points (Pl. 2, fig. 6). Dividing organisms were seen to have the granules at the site of division, and in fact division was not observed in their absence provided that they were unobscured by the processes of rapid growth. By limiting the oxygen supply in slide cultures by sealing with wax, growth was halted in the late lag phase; the organisms then showed the granules typical of that stage of growth. When oxygen was again allowed to diffuse into the culture division occurred in the regions of the granules.

A late lag phase slide culture showing these bodies was photographed with the phase microscope, and then Nadi reagent was flooded under the coverslip. After 2-5 hr. incubation with this solution, the same field was photographed with ordinary bright field illumination (Pl. 2, fig. 7). The granules appeared in identical positions and stained a deep blue, indicating that they contained relatively large amounts of cytochrome oxidase. Similar work, using Janus Green B, confirmed the oxidation-reduction activity of the granules which appear in phase-contrast preparations. It seems likely that the growing points seen in stained specimens correspond in some way with these granules.
DISCUSSION

The growing points of *B. megaterium* have some of the properties of mitochondria in that they are centres of oxidation-reduction processes involving cytochrome oxidase. They appear at the site of cell division before this takes place. It is possible that the bodies appearing in HCl+Giemsa preparations, and referred to as growing points, are not the granules themselves, but either densely staining material around them or their remains after partial destruction by hydrolysis. It is not surprising that the vigorous processes of bacterial cell division involve organelles which supply the requisite energy for the chemical syntheses involved. Watson (1952) has shown with rat testis cells that mitochondria are associated with the cell membrane of spermatids. These cells are also in a state of rapid development and division.

The behaviour of the nuclei during the life cycle of *B. megaterium* does not differ from that observed with many other species of Bacillus. There is a fusion of chromatinic material before vegetative division and a probable sexual stage involving morphologically distinct gamete-like cells (Fig. 1).

I wish to thank Dr M. J. Marples for advice and for correcting the manuscript.
F. J. BERGERSEN—GROWTH CYCLE OF *Bacillus megaterium*. PLATE 1
F. J. Bergeresen—Growth cycle of *Bacillus megaterium*. Plate 2
Growth cycle of Bacillus megaterium

REFERENCES


EXPLANATION OF PLATES

PLATE 1

Fig. 1. Cells of the inoculum showing paired chromatinic bodies and polar growing points. HCl+Giemsa. ×1500.

Fig. 2. Bacilli from a 3 hr. culture, showing elongated cells with fusion nuclei and lateral and terminal growing points. Note also anucleate cells and others of the sexual cycle. (See Fig. 1.) HCl+Giemsa. ×1500.

Fig. 3. Organisms from an 8 hr. culture, still containing fusion nuclei and with cell membranes studded with granules. HCl+Giemsa. ×1500.

Fig. 4. A 10 hr. culture. Fragmentation of the elongated cells has commenced and the nuclear material is in a zig-zag of rods in each cell. HCl+Giemsa. ×1500.

Fig. 5. A mature 15 hr. culture. HCl+Giemsa. ×1500.

PLATE 2

Fig. 6. Phase-contrast photomicrograph of late lag phase cells of Bacillus megaterium, showing the peripheral granules. ×1500.

Fig. 7. The same cells as Fig. 6 after treatment with Nadi reagent for 2½ hr. ×1500 (ordinary bright field illumination).

(Received 10 November 1952)