

NOTES

Reversion of *Bacillus Megaterium* Protoplasts to the Bacillary Form

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Photomicrographic evidence of reversion of *Bacillus megaterium* protoplasts to the bacillary form on soft agar plates hypertonic medium is demonstrated.

Protoplasts of *Bacillus megaterium* are able to grow and divide in appropriate media (1-4, 6). Nevertheless, reversion to the bacillary form has not yet been reported. We have not been able to revert *B. megaterium* protoplasts using the procedures developed by Landman et al. (5, 7) for *Bacillus subtilis* protoplasts. In this communication, we present other procedures

and photomicrographic evidence for the reversion to the bacillary form of *B. megaterium* protoplasts.

The strain *B. megaterium* KM Leu⁻ used in these studies is an L-leucine-dependent auxotrophic derivative of strain *B. megaterium* KM. It was isolated in our laboratory.

Two media were used throughout our studies.

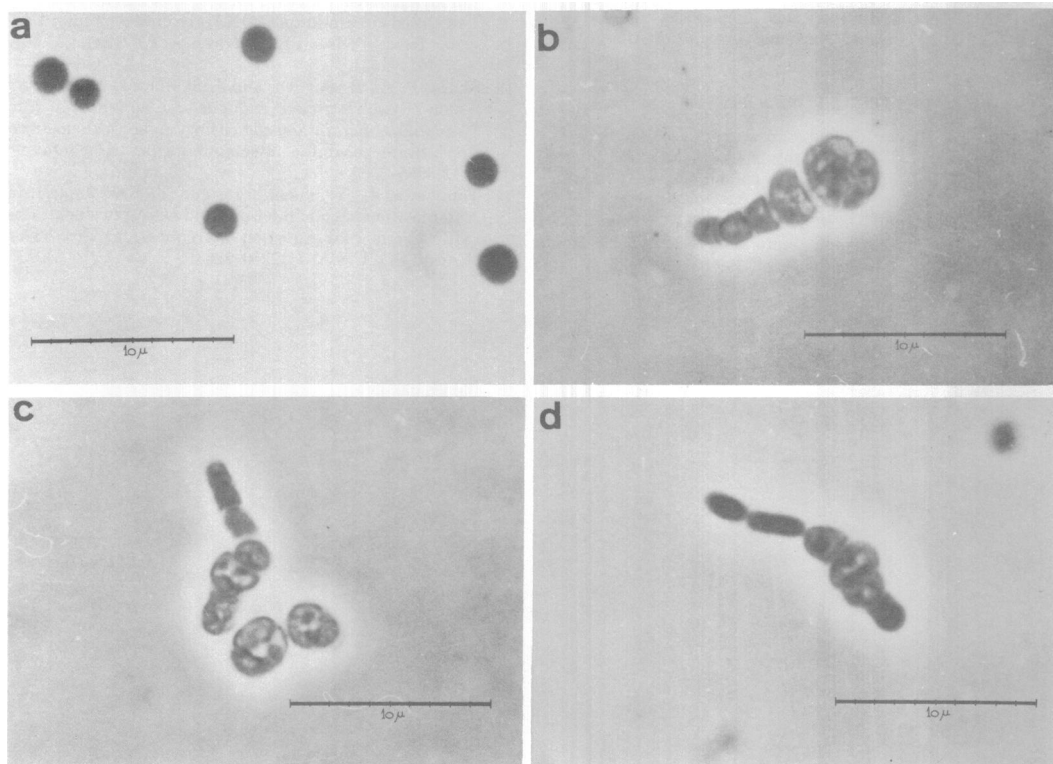


FIG. 1. Phase contrast illuminated photomicrography of (a) protoplasts of *B. megaterium* and (b, c, and d) regenerating bacillary forms in samples of a 48-h protoplast culture.

A hypertonic medium (NH₄Cl, 1.0 g; tris(hydroxymethyl)aminomethane, 12.0 g; KCl, 0.035 g; NaCl, 0.058 g; Na₂SO₄·10H₂O, 0.3 g; KH₂PO₄, 0.14 g; MgCl₂·5H₂O, 4.26 g; glucose, 2.0 g; sucrose, 68.46 g; L-leucine, 0.05 g; and distilled water to 1,000 ml, pH 7.5) was used for culturing the bacteria and protoplasts, whereas the protoplasting medium (the same as the hypertonic medium but lacking KH₂PO₄, glucose, and L-leucine) was used for the lysozyme treatment of bacteria.

Actively dividing 5- to 6-h liquid cultures of the strain grown at 30 C in hypertonic medium were centrifuged and resuspended in 0.1 volume of protoplasting medium. The cell wall was dissolved with lysozyme (50 µg/ml) at 30 C. The protoplast suspension was then centrifuged and suspended in hypertonic medium. (Such protoplast suspensions, if osmotically shocked, do not form any colonies on ordinary media from 10⁻⁷ protoplasts.) To 1.0 ml of this protoplast suspension, 1.0 ml of hypertonic medium containing 0.4% agar (Difco) was added and the mixture was layered onto the surface of plates prepared from hypertonic medium containing 1.0% agar (Difco). The plates were then incubated in a moist chamber at 30 C.

Samples were taken from the top soft agar layer at intervals for microscope examination. Phase contrast photomicrographs were made (Fig. 1).

Growth and division of the protoplasts in the first 24 to 48 h was observed. Multiseptate protoplasts were found similar to those reported by Kusaka (4). After 48 h of incubation, regenerating bacillary forms appeared as demonstrated in Fig. 1.

Regenerated bacilli were then isolated on minimal media without sucrose and these reisolates proved to be leucine auxotrophs like the parent *B. megaterium* KM Leu⁻ strain.

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