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NOTES

Double Mutants of *Bacillus subtilis* Growing as Helices

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Double mutants of *Bacillus subtilis,* deficient in autolysin and *rod* in genotype, grow as helical filaments of unseparated cells when changed from the cocci to rods.

Helical growth and the arrangement of polymers such as DNA and cellulose as helices in cells are common characteristics of living forms. Species as widely separated as some mycoplasmas (1) and *Convolvulus* grow as helices, but in the common straight rod-shaped procaryotes, such a growth form has only very recently been recognized (4, 7). Two types of helical growth of *Bacillus subtilis* have been described. One of these (4) was in a strain carrying a mutation (*div*IV B1) that suppresses division, which was presumed also to carry "additional mutation(s) concerned with the helical growth property" (4). This paper will show that similar growth can be obtained by introducing two known mutations into wild-type strains of *B. subtilis,* neither of which alone causes helical growth.

Three factors were suggested by Mendelson (4) as necessary to give helices from rod-shaped bacteria: (i) suppression of cell division; (ii) a helical orientation of surface expansion; and (iii) fixture of the poles of the first cell so that they cannot rotate, thus creating a torque during elongation of the chain of cells. In the present work properties (i) and (iii) have been given to a wild-type strain of *B. subtilis* by introducing into it two genes *lyt* (2) and *rod* (5) by transformation and transduction (P. J. Piggot, C. Taylor, and H. J. Rogers, unpublished data). The former lesion leads to failure to form active autolysins with the result that the strain grows as long chains of unseparated cells (2). The latter lesion is a conditional morphological mutation allowing the cells to be changed reversibly from normal rod shapes to cocci. Two double-mutant strains having the genetic constitution of *lyt rodA* and *lyt rodB* were made. The former was constructed by crossing FJ3 (2) and 172 *rodA*200B (7) by transformation, and the latter was constructed by crossing FJ4 (2) and 172 *rodB1*-104 (3) by transduction. The genetic and physiological phenotypes of *rodA* and *rodB* are different (3, 6), and different growth conditions are required to cause the morphological change.

When grown under the conditions necessary to form cocci, the double mutant strains grew as long strings of unseparated cells, which gradually formed an apparently disorganized mass of organisms. If the growth temperatures were

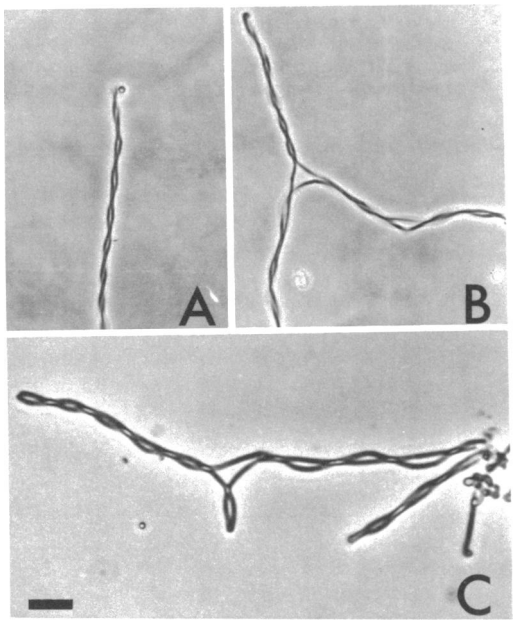

FIG. 1. *B. subtilis mutant strains growing as helical filaments. (A) rodB lyt. Grown in a buffer-salts-glucose-medium (7) containing 10 mM Mg^{2+} and 15 mM Cl^- first at 42°C, inoculated (5% vol/vol inoculum) into a broth medium, and incubated at 35°C. Helices are seen emerging from a small group of round forms. (B) Strain rodB lyt; formation of a branched helix. (C) Strain rodA lyt. Grown in a liquid casein hydrolysate-yeast medium first at 42°C to give round cells and changed to 30°C. The bar represents approximately 10 μm.*

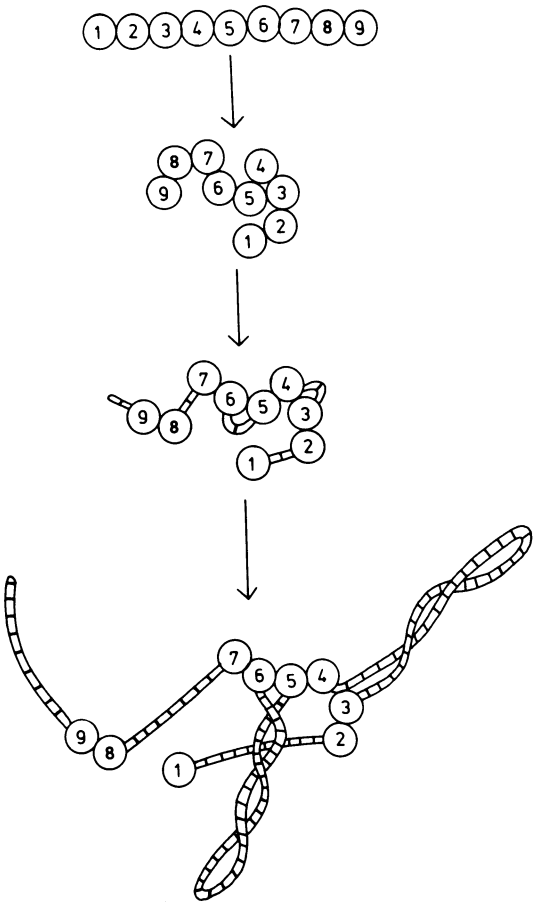

FIG. 2. *Representation of a group of round cells changing to helical chains of rods. The cells inside the group give rise first to short loops with the poles of the original cell attached to cocci held fast to the others. As the cells grow and divide torque is set up by rotation of the poles of the cells, and a helix results. At the edge of the groups, growth of the chains of cells push out some of the cells so that chains of rods are formed interspersed with residual round forms.*

changed (6, 7) so that rods emerged and a high growth rate was ensured by using rich media, long helices of unseparated rods resulted (Fig. 1) from a proportion of the groups of round forms. A few chains of unseparated rods not in helices also occurred, interspersed at intervals with round coccal shapes. From the *lyt rodB* mutant 60 to 70% of the filaments were organized as helices (Fig. 1A and B). The formation of these helices was less common and more variable with the *lyt rodA* strain. In this mutant much tighter helices (8) were also sometimes seen. Neither *lyt* strains nor *rod* strains themselves grow helically. Examination by time-lapse cine photography and by eye showed that the rods emerged as loops between pairs of round-shaped cells, and the ends of the growing chains of unseparated rod-shaped cells were fixed to the mass of unseparated round forms. A possible mechanism by which this happens is shown in Fig. 2. Thus, conditions (i) and (iii) above, suggested by Mendelson (4), are provided, and it seems reasonable to suggest that the helical orientation of surface expansion (ii) may be due to the normal growth of the surface of the wild-type strain as suggested by Tilby (8). This may also be an important clue to the mechanism of growth and possible arrangement of the polymers in the envelope of some rod-shaped procaryotes.

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