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NOTES

Clockwise and Counterclockwise Pinwheel Colony Morphologies of *Bacillus subtilis* Are Correlated with the Helix Hand of the Strain

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Helical macrofiber-producing strains of *Bacillus subtilis* grown on fresh complex medium semisolid surfaces formed "pinwheel"-shaped colonies. Clockwise pinwheel projections arose from colonies of strains that produce right-handed helical macrofibers in fluid cultures. Most strains able to make left-handed helical macrofibers in fluid grew as disorganized wavy colonies without directed projections. A phage-resistant left-handed mutant was found that produces very tight colonies with pinwheel projections that lie counterclockwise relative to the colony. The pinwheel colony morphology is interpreted therefore in terms of the cell surface organization and helical growth.

Helical growth of *Bacillus subtilis* and the production of helical macrofibers in fluid cultures have been interpreted as a reflection of an underlying helical organization of the cell surface (10, 11, 17). Models in which the assembly of the cell surface conforms to the helical architecture have been explored, and evidence has been obtained suggesting that the biomechanical properties of cell surfaces in response to forces generated during growth are largely responsible for cell morphology (10–12). Recently, we have found that the addition of lysozyme or autolysins to macrofibers results in relaxation motions, indicating that helical structures are indeed under strain and suggesting that the peptidoglycan is the structure responsible for maintaining the helical shape deformation (N. H. Mendelson, M. M. Briehl, and D. Favre, manuscript in preparation). We believe, therefore, that the physical properties of cell surfaces, particularly the dynamics of surface structure during growth, must be explored in detail if further insight is to be gained concerning the mechanism and regulation of growth.

Helical macrofibers grown in fluid culture may exist as either right- or left-handed structures (11). A series of mutants were obtained in which the helix hand properties were shown to be under genetic regulation (11). Three classes of strains were found: (i) those able to produce only right-handed structures; (ii) those able to produce only left-handed structures; and (iii) those able to produce either right- or left-handed structures depending upon environmental conditions. Further investigation revealed that this third category is probably the "wild-type" condition found in most helix-producing strains (13). The particular helix hand produced is now known to be regulated by at least four factors: (i) the genetic constitution of the strain; (ii) the nutritional environment in which the cells are cultured; (iii) the temperature at which the cells are cultured; and (iv) the concentration of certain ions in the medium (13). Conditions normally used to culture *B. subtilis* fall within this matrix in the region where right-handed structures are produced.

The initial helix-producing strain of *B. subtilis* B1S, studied in this laboratory, was selected on the basis of colony morphology. In contrast to most other strains, B1S colonies are very tight and grow with a very smooth edge. B1S colonies grown on the complex medium TB previously described (10) contain helical macrofibers similar to those produced in fluid cultures. As in the case of fluid growth, these are always right-handed helical structures. Strain 63SB was derived from B1S on the basis of its greater resistance to autolysis. 63SB retains the helical properties of B1S but generates somewhat

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tighter helical structures than does B1S. When plated on fresh semisolid TB medium, which retains some moisture on the agar surface, 63SB colonies grow in a characteristic pinwheel shape. The colony edge is generally very sharp. Projections arise from the periphery of the colonies at several places and grow always in a clockwise direction relative to the center of the colony. Examples are shown in Fig. 1C.

The pinwheel nature of helical outgrowths from the periphery of 63SB colonies is morphologically similar to the clockwise outgrowths of neurites from retinal explants reported by Heacock and Agranoff (5). These authors reasoned that clockwise curvature would arise if the neurites grow as right-handed helical structures which interact with the surface upon which they are grown. 63SB helical structures are known to be right-handed; hence, we conclude that in the case of these pinwheel colonies the very same physical principles are responsible for the clockwise curvature as in the case of the neurites. To test this hypothesis, we sought pinwheel colonies from left-handed helical strains. Unfortunately, most strains that produce very regular left-handed helical structures in fluid culture do not generate helical structures when grown on semisolid surfaces. Typical colonies are similar to those shown in Fig. 1A. In such colonies the long division-suppressed cell filaments lie parallel to one another, creating the wavy undulations on the colony surface and periphery, and consequently no helix hand may be determined.

In the course of conducting experiments with left-handed helix-producing strains for other purposes, we noted that some phage-resistant isolates grew with very tight colonies reminiscent of the 63SB colony morphology. Strain C6ϕR4 is an SPO1-resistant mutant obtained from strain C6D and, like C6D, is genetically restricted to production of only left-handed helical structures under the growth conditions used in these experiments. The properties of C6ϕR4 have been described by Saxe (Ph.D. thesis, University of Arizona, Tucson, 1979). When plated on fresh, moist semisolid TB medium, C6ϕR4 colonies grow as pinwheels very much like 63SB, with the important exception that the projections from C6ϕR4 colonies curve counterclockwise with respect to the colony center (Fig. 1B). These findings suggest that the pinwheel morphology is indeed a reflection of the helix hand of the cells in the colony and that a new factor must be considered in the interpretation of colony morphology.

A number of factors are currently thought to be responsible for bacterial colony morphology. The interaction of cell surfaces with the substrates upon which they are grown has been recognized long ago as a contributing factor (1). The properties of cell surfaces affect colony morphology, therefore, insofar as they influence the kinds of interactions that take place between the cell surface and the environment. Smooth and rough colonies, for example, are correlated with the chemical composition of the lipopoly-

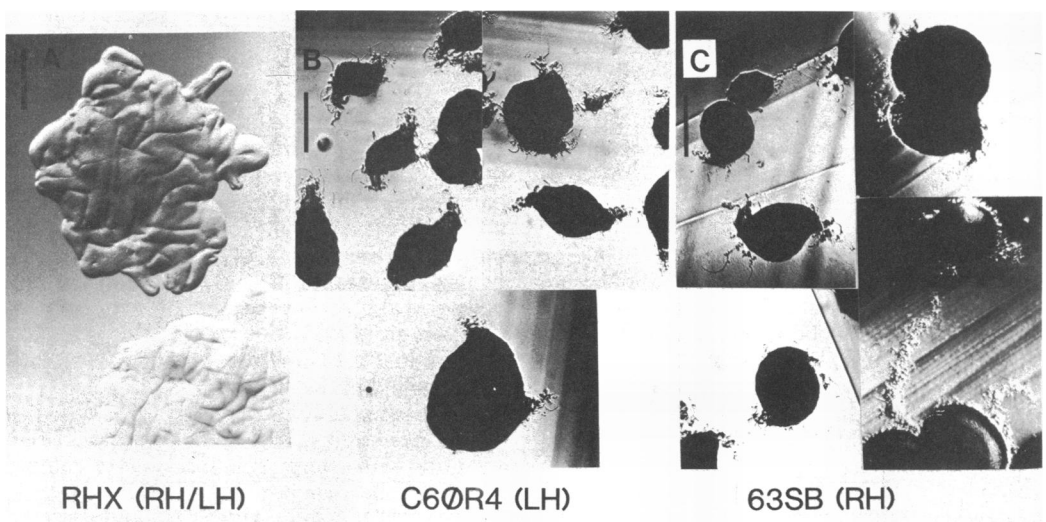

FIG. 1. Colony morphology of helical *B. subtilis* mutants. Cultures were streaked on the surface of fresh semisolid TB medium and incubated at 20°C for 18 to 24 h. Colonies were photographed at low magnification, using a stereoscopic microscope. (A) Colonies of strain RHX, a strain able to produce either right- or left-handed macrofibers in fluid culture depending upon the medium in which the cells are cultured. (B) Colonies of strain C6ϕR4, a SPO1-resistant strain able to grow only as left-handed macrofibers in fluid culture. (C) Colonies of strain 63SB, a strain able to grow only as right-handed macrofibers in fluid culture. Bars = 1 mm.

saccharides located on the outer membrane of gram-negative cells (2, 7). In gram-positive cells such as pneumococci, smooth and rough colonies are related to capsule production which is also correlated with virulence (2, 7, 8, 16). Mucoid colonies of *Escherichia coli* reflect overproduction of capsular materials (8). Cell surface appendages such as pili also appear to influence colony morphology. Recently, a genetic instability has been described that involves pili and causes intraclonal variation in colony morphology (3). The genetic regulation of cell surface properties is of central importance, therefore, in the determination of colony morphology.

There are two other major factors that influence colony morphology: cell growth and motility. Henrichsen has attempted to systematically characterize bacterial movements on semisolid surfaces and to relate these processes to colony morphology (6). Six kinds of movements, swarming, swimming, gliding, twiching, sliding, and darting, were described (6). Of these, the latter four are thought to be driven by mechanisms other than flagella action. Our findings suggest that cellular growth patterns play an important role in the determination of colony morphology. The suppression of cell division and the ability of cells to grow in either helix hand is likely to be the basis for the generation of forces with growth that contribute to colony morphology just as they do in the case of helical macrofiber production in fluid. A similar conclusion was drawn by Gause concerning dextral and sinistral colony forms of *Bacillus mycoides* (4). Murray and Elder noted the similarities between such colonies and colony rotation during swarming of related *Bacillus* species (14). In the latter case motility of the individual cells within the colony apparently provides the force needed for colony rotation. Whether the organization of the cell surface is involved in determining the direction of colony rotation remains to be determined. If so, then some of the properties concerning the helix hand inversion that we have studied in *B. subtilis* macrofibers (13) may provide information that bears upon colony movements as well as colony morphology.

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