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Gliding Motility Mutants of *Myxococcus xanthus*

ROBERT P. BURCHARD

Division of Biological Sciences, University of Maryland Baltimore County, Catonsville, Maryland 21228

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Two gliding motility mutants of *Myxococcus xanthus* are described. The semimotile mutant (SM) originated by high-frequency segregation from the motile FB_t strain. Segregation was enhanced by acridine dye treatment. SM cells glide only when apposed to other cells in a swarm. The nonmotile strain (NM) originated by mutation from SM. NM cells neither glide individually nor cooperatively. FB_t, SM, and NM are indistinguishable with respect to fine structure, vegetative growth rate, glycerol-induced microcyst formation, spheroplasting, bacteriophage sensitivity, and responses to light. The motility mutants are more resistant to penicillin and more sensitive to actinomycin D than is the gliding wild type. The NM mutant is also a morphogenetic mutant; it is unable to form fruiting bodies.

The myxobacteria are capable of gliding movement when in contact with a surface. The mechanism underlying this form of motility is not understood, although a number of hypotheses have evolved (3).

Recently, this laboratory's stock *Myxococcus xanthus* FB_t strain (1) demonstrated short-term high-frequency segregation of a clonal morphology, semimotile mutant (SM) characterized by cells which glide only when in apposition in swarms. A second, nonmotile strain (NM) isolated as a clonal morphology mutant among SM colonies, demonstrated no gliding movement as single cells nor as swarms. A preliminary characterization of these gliding motility mutants is presented.

MATERIALS AND METHODS

Cultivation of vegetative cells. Vegetative-cell suspensions of *M. xanthus* FB_t, SM, and NM were cultured as previously described (1). Growth of colonies was observed on CT-1 agar (1) and on P-T agar. P-T contains 0.5% Phytone peptone (BBL), 0.5% tryptone (Fisher), 0.01 M K_2HPO_4-KH_2PO_4 (*p*H 7.6), and 0.004 M $MgSO_4$.

Fruiting body formation. Drops (0.01 ml) of washed, CT-1-grown, log-phase, vegetative cells were spotted on either of two fruiting media: 2% agar and the same salts as those in P-T plus either 0.025% tryptone or 10^8 to 3×10^8 washed, autoclaved *Escherichia coli* B/ml. Fruiting body formation by *M. xanthus* FB_t usually occurs within 2 days.

Electron microscopy. Cells were fixed in cold 1% glutaraldehyde in Kellenberger buffer at *p*H 5.9 for 30 min. They were then washed three times in Kellenberger buffer and fixed in osmium tetroxide by the method of Ryter et al. (15). After embedding in Epon 812, the samples were sectioned on an MT-2 ultramicrotome (Porter-Blum) with a DuPont diamond knife and observed with a model EMU-3G electron microscope (RCA).

RESULTS

Description of the organism. The normal clonal morphology of *M. xanthus* FB_t is slightly raised with an ill-defined and spreading periphery (4). Both individual cells and small swarms can be observed gliding from the center of the colony (Fig. 1a). The paths of these migrating cells are marked by phase-bright trails, representing slime deposited by the gliders or furrows etched in the agar (18).

On CT-1 agar, *M. xanthus* SM and NM are distinguishable from the FB_t parental strain and from one another. SM colonies are raised and convex; the peripheries are smooth with the exception of some projections of cells. Neither free cells nor swarms move away from the colony edge (Fig. 1b). NM colonies are similar to those produced by SM cells. However, protrusions of cells are absent (Fig. 1c).

The distinctions among the colony types are also illustrated by microcolonies on CT-1 agar (Fig. 2). FB_t microcolonies are loosely organized with cells moving away from the centers. SM microcolonies are somewhat extended; NM microcolonies are compact.

On P-T agar FB_t clonal morphology is similar to that on CT-1 (Fig. 3a). SM colonies are relatively flat with spreading peripheries (Fig. 3b). They are distinguishable from FB_t since there are no free cells at the leading edges. Clusters of cells demonstrate the ability to move from the clone center. NM colonies on P-T are like those

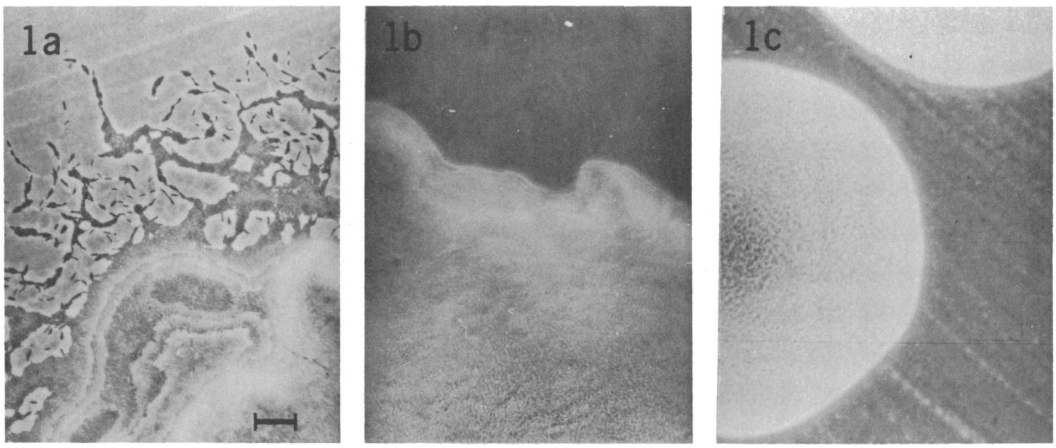


FIG. 1. *M. xanthus* FB_t, *SM, and NM (a, b, and c, respectively) 4-day, CT-1 agar colony peripheries. Bar equals 50 μm. Phase-contrast microscope used for all figures but no. 6.*

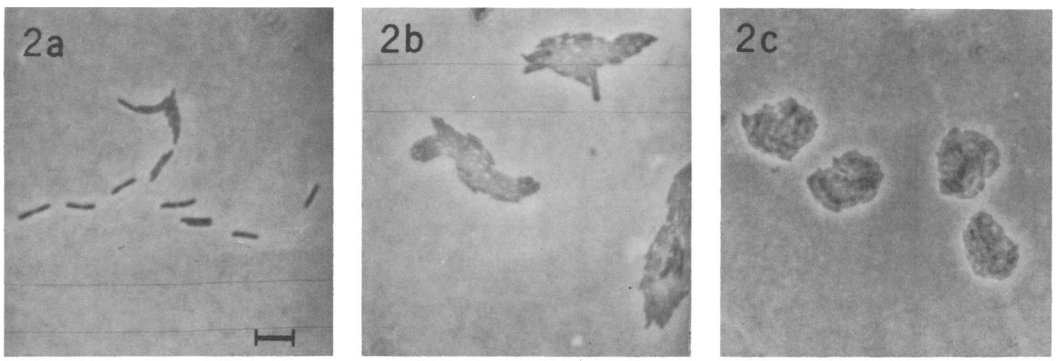


FIG. 2. *(a) M. xanthus* FB_t *microcolony; (b and c) 3 SM and 4 NM microcolonies, respectively. All 1-day colonies on CT-1 agar. Bar equals 10 μm.*

on CT-1: raised, convex, and round with smooth peripheries (Fig. 3c).

Origin of SM and NM. SM arose recently from FB_t during standard viable assays. Over a period of several days, 4 to 20% of the colonies counted demonstrated the characteristic morphology described above. This segregation could not be attributed to any obvious changes in the culture conditions. Since this time FB_t has stabilized.

Subsequently, during a search for nutritional mutants among SM cells treated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine and penicillin, a colony with NM characteristics was observed among numerous SM colonies. SM and NM appear to be stable at this time.

To test the possibility that the wild-type, spreading colony form (Fig. 1a) and single-cell gliding are episomally controlled, a series of experiments was carried out to determine whether acridine dyes promote the $FB_t \rightarrow$ SM segregation (9, 17). Exponentially growing FB_t cells from a culture which had thrown off SM cells were treated for 5 to 16 hr with 20 to 25 μg of acridine orange/ml in CT-1 suspension at 30 C. In one of the two successful preliminary experiments, the segregation frequency increased from an SM-FB_t colony ratio of 5:87 to 50:180 after treatment with acridine orange (20 μg/ml) for 8 hr. This dye does not preferentially inhibit the growth of the parental strain. Acriflavine (0.5 μg/ml) enhanced the segregation frequency also. Subsequent attempts to induce segregation of SM cells from a stabilized FB_t culture have proven unsuccessful.

Nature of the SM and NM alterations. Both clonal morphology and the appearance of stab cultures in 0.7% CT-1 agar suggest a limitation in the mutants' ability to glide. This might be due to an adhesive characteristic preventing otherwise motile cells from moving singly, or there may be some alteration in the gliding mechanism per se.

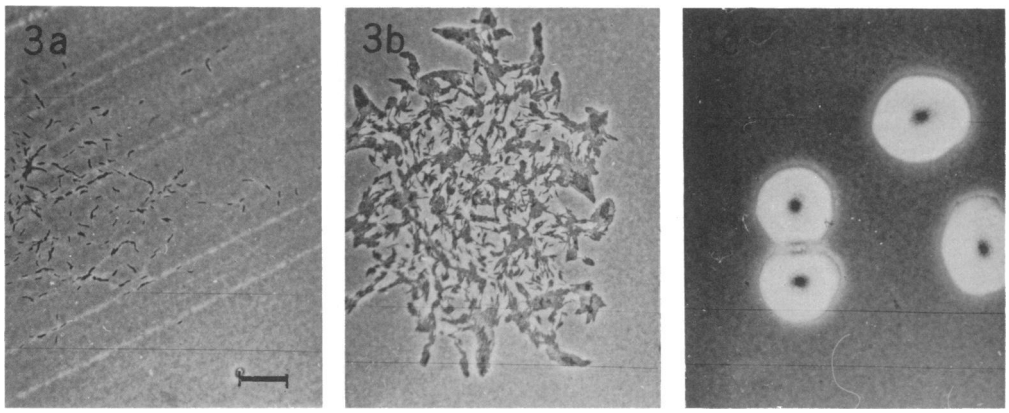


FIG. 3. *M. xanthus* FB_t, *SM, and NM (a, b, and c, respectively) 2-day microcolonies on P-T agar. Bar equals 50 μm.*

To test the first possibility, FB_t and NM cells were mixed in varying proportions and spotted on CT-1 agar (10^7 cells/0.5 cm^2 spot). After incubation for 48 hr, peripheries of the spots were examined for free, gliding cells. At an FB_t-NM ratio of 10^{-4}, cells moved away from the vegetative cell mass, suggesting that if adhesiveness prevents movement of NM cells, it must be a strain-specific phenomenon.

The second hypothesis was tested by determining whether individual SM and NM cells glide under conditions that promote gliding by FB_t cells. Suspensions were spotted on fruiting medium (tryptone) at a density of 10^5 cells/cm^2. The low rate of vegetative growth on fruiting medium permits study of motility independent of cell division. Under these conditions, FB_t cells were observed to move across the agar surface (Fig. 4a). Neither SM nor NM cells (Fig. 4b) moved during observation periods of up to 6 hr.

On spotting high-concentration drops of cells (2×10^7/0.5 cm^2) on fruiting medium, each strain demonstrates distinct behavior (Fig. 5). Single FB_t cells migrate from the periphery of the original spot. Swarms of two or more SM cells move from the periphery. The paths of both single FB_t cells and swarms of SM cells are indicated by phase-bright trails. Single cells in these swarms move relative to one another when they are apposed. This could account for the observed separation of the swarms from the main body of SM cells. Individual SM cells are never seen at the leading edge of the outward-moving swarms. NM spot peripheries remain smooth. No detectable movement of single cells nor swarm formation occurs (Fig. 5c).

Further characterization of the mutants. FB_t, SM, and NM demonstrate generation times of 4.3 hr in CT-1 suspension culture. The chronology of glycerol-induced microcyst formation in strains FB_t, SM, and NM parallels that described for *M. xanthus* FB (7). More than 99% of the cells of all three strains convert to microcysts. In an aqueous solution of 0.03 M NaCl at 45 C (12), all vegetative cells of the three strains convert to spheroplasts. Dark-grown, stationary-phase cells of the three strains undergo photo-induced lysis. Light-grown cells synthesize photo-protective carotenoids (1). The virulent bacteriophage MX-1 (2) infects both motile and motility-limited strains.

Studies of the relationship of these alterations in gliding motility to changes in the cell-wall structure have been carried out in collaboration with Herbert G. Voelz. No wall and membrane-fine structure differences are indicated (Fig. 6a, b, c).

Peptidoglycan is present in the cell walls of *M. xanthus* (19). Vegetative cell growth is inhibited by penicillin G and D-cycloserine. The sensitivity of strains FB_t, SM, and NM to these antibiotics was compared by measuring zones of inhibition of vegetative growth around antibiotic-saturated discs (12.7 mm diameter) on CT-1 agar. In one such experiment, discs were saturated with a 1 μg/ml solution of penicillin G. The diameter of the zone of inhibition of FB_t growth was 26 mm; zones of inhibition for SM and NM each measured 16 mm. The test tube dilution method for determining antibiotic sensitivity yields similar results (Table 1). The strains are equally susceptible to D-cycloserine.

Dworkin (6) demonstrated that growth of most strains of gliding bacteria (all gram-negative) is inhibited by a relatively low concentration of actinomycin D (1 μg/ml), as are the gram-positive bacteria. Nongliding, gram-negative species

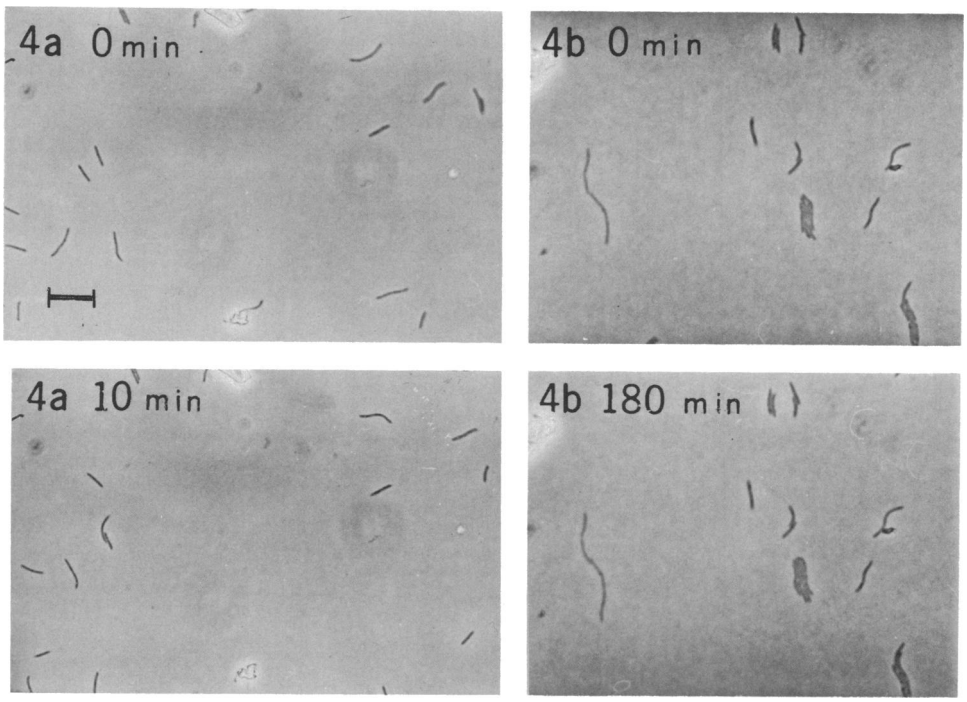


FIG. 4. *(a) M. xanthus FB_t cells before and after 10 min of gliding motility. (b) SM cells before and after 180 min. Cells were plated on fruiting agar. Bar equals 10 μm.*

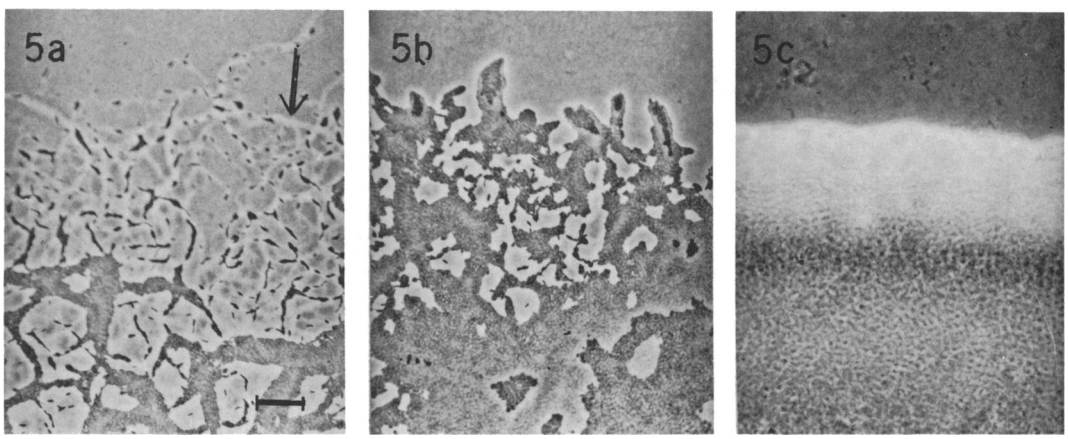


FIG. 5. *Peripheries of spots of M. xanthus FB_t, SM, and NM cells (a, b, and c, respectively) on fruiting agar (2×10^7 cells/0.5 cm^2) after 8 days incubation. Bar equals 50 μm. A trail left by gliding FB_t cells is indicated (arrow).*

are not inhibited by such low levels of this antibiotic. A comparison of actinomycin D sensitivity of NM and the wild-type by zones of inhibition and by the tube dilution method (Table 1) indicates that the nongliders are more susceptible to this antibiotic than are *M. xanthus* gliders.

Fruiting body formation. FB_t cells aggregate in response to a chemotactic stimulus and form macroscopically visible fruiting bodies (Fig. 7a) in which individual cells differentiate into microcysts (5). The cells need not be within the confines of this multicellular stage in the life cycle of the population to differentiate (Fig. 8).

If the aggregative phase of fruiting body formation involves active, gliding motility, one would predict that *M. xanthus* SM and NM are limited

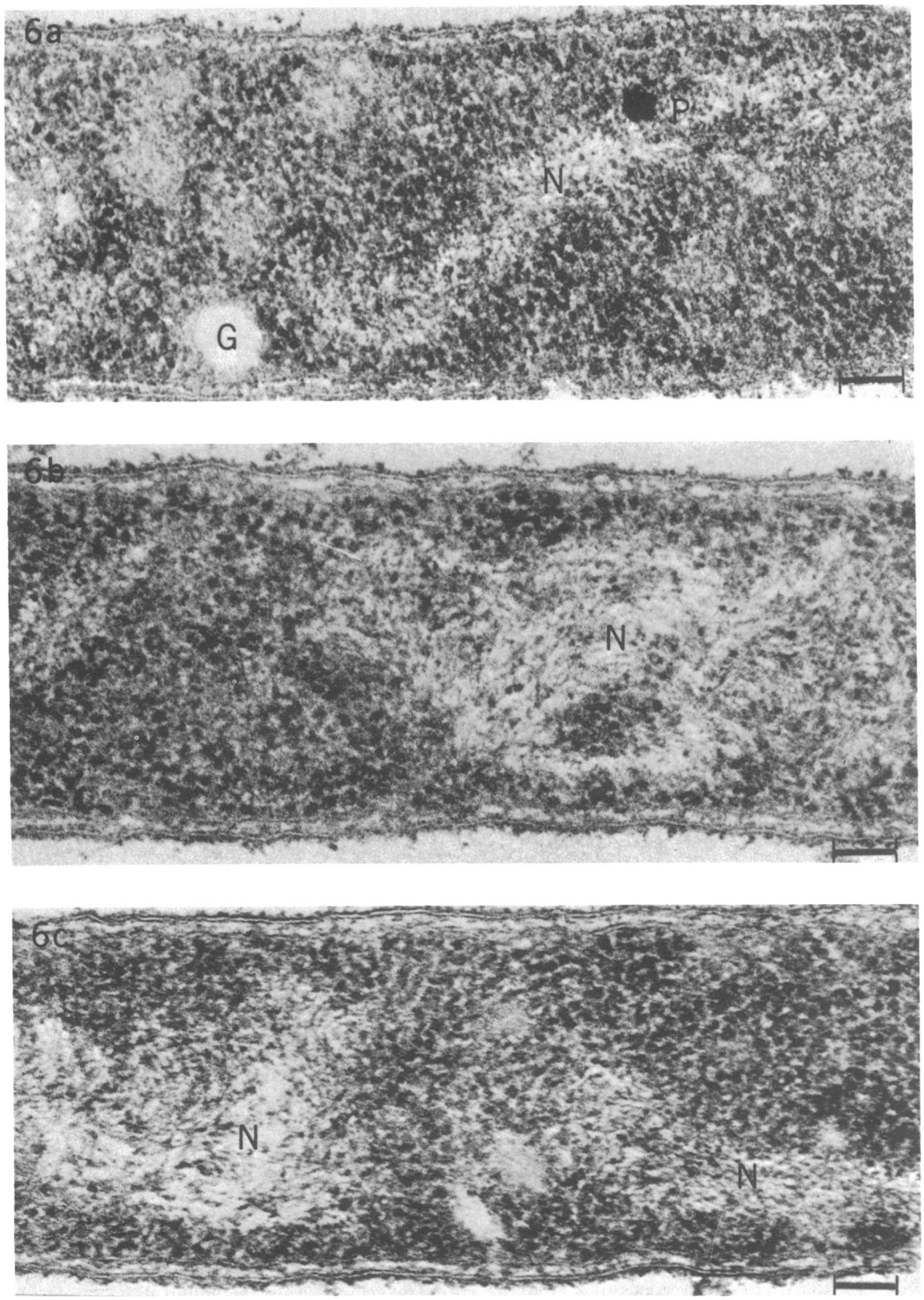


FIG. 6. *Thin sections of M. xanthus* FB_t, *SM, and NM (a, b, and c, respectively). The wall-membrane complex demonstrates typical double-track features. The apparent fuzziness of the wall is characteristic of chemically fixed myxobacteria. N, nucleoid; P and G are presumed to be polyphosphate and glycogen, respectively. Bar equals 0.1 μm.*

TABLE 1. *Antibiotic sensitivity of M. xanthus FB_t and NM by test tube dilution method*

Antibiotic	Strain	Concn (μg/ml)					
		0	3.8	7.5	15	30	60
Penicillin *G*	FB_t	+++[a]	+	±	−	−	−
	NM	+++	+++	++	+	−	−
		0	25	50	100	300	
D-Cycloserine	FB_t	+++	+++	+++	++	−	
	NM	+++	+++	+++	+++	−	
		0	0.3	0.6	1.25	2.5	5
Actinomycin	FB_t	+++	+++	+++	++	+	−
	NM	+++	+++	+	±	−	−

[a] Growth (+); no growth (−).

or unable to form such structures. SM might be expected to fruit as long as the cell density on the fruiting medium is such that cells are apposed to one another. Figure 7b demonstrates that this strain can form fruiting bodies. However, these structures form more slowly, and the rate of cellular differentiation is decreased. The distribution of SM fruiting bodies also differs from that of FB_t; the density of the former is greater. Also, the interfruiting body space is generally covered with a lawn of cells, whereas aggregating FB_t cells leave cell-free spaces on the agar.

The NM strain does not form fruiting bodies under these conditions (Fig. 7c). Some of the NM cells appear to be in the process of conversion to microcysts after extended periods of incubation (10 to 14 days).

DISCUSSION

The observations indicate that *M. xanthus* NM cells are unable to glide individually or as swarms. SM cells are unable to glide as individuals. SM swarms, however, are motile because closely apposed SM cells can move along one another in a form of cooperative gliding. The origin of this SM strain is enigmatic, in light of its sudden, high-frequency segregation from the parental motile FB_t. No such clonal morphology mutant was previously observed by me. No obvious changes in culture conditions could account for this phenomenon. Two preliminary experiments indicated that acridine dyes can increase the segregation frequency, suggesting the possibility that gliding of individual cells may be an episomally controlled phenomenon. Analogous to the characteristics of the F factor in *Escherichia coli* (9), when the gliding factor is in an episomal state, individual cell motility might be eliminated spontaneously or by treatment with acridine dyes. When the factor is integrated into the cell's chromosome, individual cell gliding is stabilized. I have not been able to test this hypothesis because the FB_t strain has stabilized.

Segregation of clonal variants was reported for *M. xanthus* (1). Strain FB throws off cells which give rise to yellow- or tan-pigmented colonies. FB_t was a stabilized form of the tan-pigmented variety. Recently, however, it began again to dissociate cells which produce yellow-pigmented colonies.

Little is known of the mechanism of gliding motility, although a number of hypotheses have been proposed (3, 11, 18). Comparison of semimotile and nonmotile strains of a myxobacterium with the motile parental strain might facilitate the elucidation of the mechanism of gliding.

One approach toward gliding motility is a morphological one. Are there any structures characteristic of gliding organisms which might

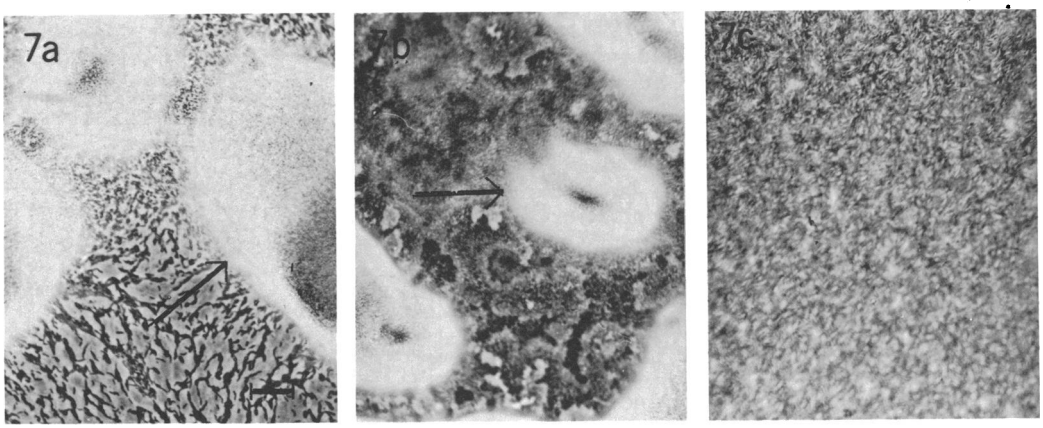
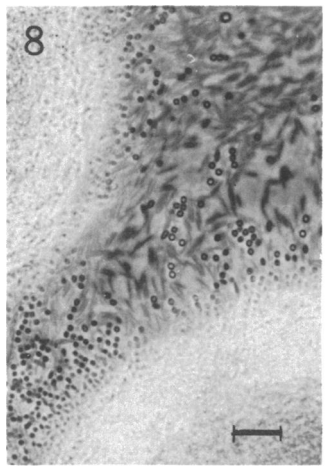


FIG. 7. *Center of spots of M. xanthus FB_t, SM and NM (a, b, c, respectively) on fruiting agar (2×10^7 cells/0.5 cm²) after 8 days incubation. Fruiting bodies are indicated (arrows). Bar equals 50 μm.*



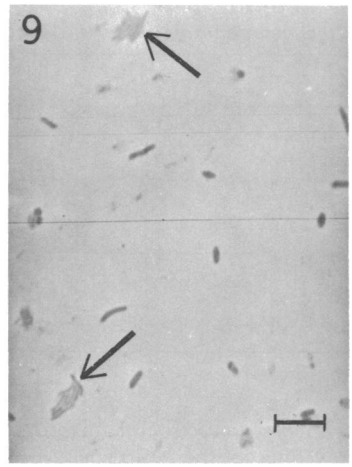


FIG. 8. *M. xanthus* FB_t *fruiting bodies. Microcysts and aggregating vegetative cells are located in interfruiting body spaces. Bar equals 10 μm.*

FIG. 9. *Viable assay of a bacteriophage-infected culture of M. xanthus NM (1-day incubation). Microcolonies are indicated (arrows). Individual cells are nonviable. Bar equals 10 μm.*

account for their motility? Gräf (8) has suggested that a sheath of contractile fibrils observed in *Sporocytophaga* and *Sphaerocytophaga* species is responsible for motility; rhapidosomes arise by degradation of these fibrils. Reichenbach, in examining *Archangium violaceum*, equated the rhapidosomes of that species with the tail component of a defective phage (14). *Chondrococcus columnaris*, another myxobacterium, has fibrils intimately associated with the inner layer of the cells' outer unit membrane (13). Recently, other investigators examined the fine structure of *Cytophaga* and *Sporocytophaga* species; there was no indication of any unusual surface structures in these cells (10, 16).

Fine structure studies in collaboration with Herbert G. Voelz indicate no structural differences among *M. xanthus* FB_t, SM, and NM (Fig. 6). No structures which would provide a morphological basis for gliding have been observed.

Preliminary comparisons of the three strains with respect to some properties of the cell surface suggest that, with the exception of a difference in sensitivity to penicillin which may be significant, they have a number of common properties. The slime produced by motile and nonmotile strains is under study.

Based on most morphological, physiological, and developmental criteria, SM and NM are members of the species *M. xanthus*.

Beside the basic biological interest of SM and NM, these strains proved to be of practical value. First, *M. xanthus*-viable assays via macroscopic colony counts normally require 5 days of incubation. Using SM or NM, I am able to obtain the same data in 16 to 24 hr via microcolony formation (Fig. 9). The motility of FB_t cells prevents accurate resolution of individual microcolonies (Fig. 2a).

Secondly, the compact nature of SM and NM colonies facilitates replica plating; easily resolvable colonies on master and replica plates expedite the isolation of other types of mutants of *M. xanthus*.

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