JOURNAL OF BACTERIOLOGY, Mar. 1979, p. 1417-1420
0021-9193/79/03-1417/04$02.00/0

Vol. 137, No. 3

NOTES

Association of Flexing and Gliding in *Flexibacter*

BETSY DAYRELL-HART† AND ROBERT P. BURCHARD*

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

Received for publication 27 November 1978

Nongliding mutants of *Flexibacter* FS-1 are unable to flex; revertants regain both forms of movement. A variety of conditions and treatments reversibly inhibit both gliding and flexing.

Members of the genus *Flexibacter*, whose basic biology has been described previously (4, 5, 8, 12, 13), demonstrate three apparently different types of movement. On a surface *Flexibacter* filaments demonstrate relatively rapid gliding motility. In suspension they undergo active flexing and straightening. In a wet mount short filaments adhering at one end to the slide can be observed to gyrate around their point of attachment. The mechanism(s) of gliding, flexing, and gyration in these and other gliding bacteria is not known. Other than the goblets observed on the surface of the marine *Flexibacter polymorphus*, for which some gliding-related function has been proposed (10, 11), no morphological basis for any of these movements has been reported.

In this report we present genetic and physiological evidence which suggests that gliding motility and active flexing of *Flexibacter* FS-1 are manifestations of the same mechanism.

Flexibacter FS-1 was isolated from soil by Simon and White (12) and subsequently further characterized (2, 8, 12). Bacteria were grown at 30°C in YE/2 broth, containing one-half the yeast extract of YE (12). In this medium, the bacteria have a generation time of 90 to 100 min. Log-phase cells at an optical density of <0.3 (Bausch and Lomb Spectronic 20, 540 nm), grown on a rotary shaker (60 rpm) were generally long filaments (up to 200 μm).

Flexing was visualized in cell suspensions in a Hawksley counting chamber. The chamber was mounted in a hot stage on a Wild phase-contrast microscope. To observe gliding, cell suspensions or pellets of centrifuged bacteria were spotted on YE/10 medium (2) with 1.5 or 2.5% agar (Difco). After incubation at 30°C for ≤5 h, the plates were examined by phase-contrast microscopy for single cells and groups of cells (swarms) which had glided from the spot peripheries. On 2.5% agar we were able to see phase-bright trails marking the paths over which cells had moved.

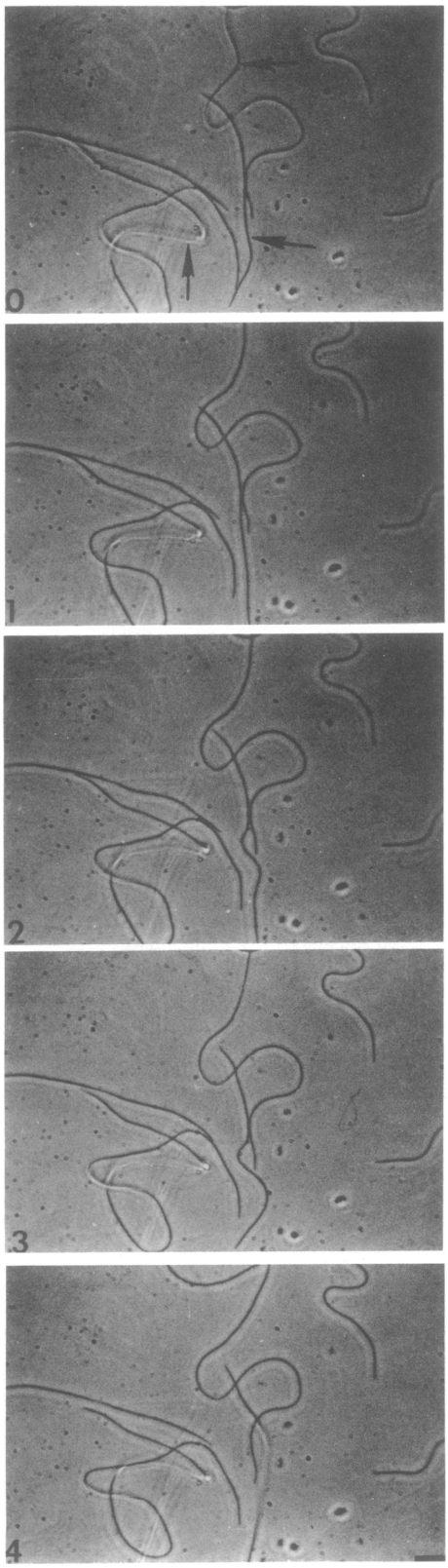
To isolate nongliding mutants, bacteria grown in YE/2 were centrifuged and suspended in 5×10^{-3} M KPO_4 buffer (pH 7.0). A 10-ml volume of suspension was irradiated, with continuous stirring in a 9-cm-diameter petri dish, 35 cm under a 30-W GE germicidal lamp for 60 s. After addition of 2% yeast extract to a final concentration of 0.5%, the bacteria were incubated for 24 to 48 h. Subsequently, they were plated on YE/2 medium containing 2% agar (Difco). This agar concentration limits colony spreading. After 48 h of incubation at 30°C, the plates were examined for raised colonies with smooth edges. Such colonies were subcultured. Daughter colonies were examined for nongliding morphology, and single cells were observed for movement.

In several procedures, *Flexibacter* filaments had to be removed from growth medium and suspended in another solution. To do this without undue shear-induced fragmentation of filaments, bacterial suspensions were centrifuged at $10^3 \times g$ for 10 min or collected on a membrane filter (Millipore Corp., 0.45 μm pore size). Pelleted bacteria or those on filters were then suspended by gentle agitation.

Flexing of *Flexibacter* FS-1 filaments, able to glide on agar (2), was observed in wet mounts (Fig. 1). Flexing was particularly active and frequent in clumps of filaments. When suspensions of filaments were spotted on agar, flexing was observed until the drop had dried, after which gliding motility commenced. Gliding cells were observed to flex infrequently. In contrast, cells of the genus *Cytophaga*, classified within the same family as *Flexibacter*, flex rapidly particularly while gliding on agar (14).

Our observations indicate that only relatively

† Present address: 719 Park Ave., Baltimore, MD 21207.



long filaments are able to glide (2) and flex. Lewin and Lounsberry (5) have also reported that active flexing occurs in more elongate forms of various *Flexibacter* stains.

Short *Flexibacter* filaments, generated late in logarithmic growth and by growth in glucose salts medium (12), are unable to glide (2). We were unable to observe flexing in such cells (Table 1), although such observations are made less reliable because of the inherent difficulty in observing flexing of short filaments.

We isolated six, independent, UV-induced, nongliding mutants (Ng 1 to 6) (Fig. 2). All produced filaments during logarithmic growth, although they were shorter than those typical of the wild type in several of the mutants. However, even in these mutants filament length was $\gtrsim$90 μm. None of these mutants was observed to flex under our experimental conditions.

Spontaneous revertants of Ng 2 and Ng 4 to the wild-type gliding phenotype also demonstrated the ability to flex.

Motility of *Myxococcus xanthus*, another gliding bacterium, can be reversibly inhibited by treatment of these bacteria with certain proteolytic enzymes (1). *Flexibacter* filaments were suspended in phosphate buffer (5×10^{-3} M, pH 7.0) containing 1 mg of subtilisin per ml (Sigma Protease VIII) and incubated for 1 h at 30°C. Flexing and gliding motility were inhibited in these cells; both forms of movement reappeared after 2 to 3 h of incubation in growth medium. Control cells incubated in bovine serum albumin (1 mg/ml) glided and flexed normally.

Gliding of *M. xanthus* is also reversibly inhibited by osmotic shock (1). Using the shock

TABLE 1. *Flexing and gliding of Flexibacter: mutants and effect of various conditions*

Strain, morphological state, or treatment[a]	Movement	
	Gliding	Flexing
Wild-type filaments	+	+
Wild-type short cells	−	−
Nongliding (Ng) mutant filaments	−	−
Revertants of Ng 2 and 4	+	+
Protease treatment	−	−
EDTA	NT[b]	−
Hypertonic sucrose	−	−
47°C Incubation	−	−
KCN (5×10^{-3} M)	−	−
Dinitrophenol (2×10^{-4} M)	−	−
Sodium arsenate (2×10^{-2} M)	+	+

[a] See text for details.
[b] Not tested.

FIG. 1. *Flexing of Flexibacter FS-1. Arrows indicate points at which flexing and straightening are evident. Cells are depicted at 1-s intervals. Bar represents 10 μm.*

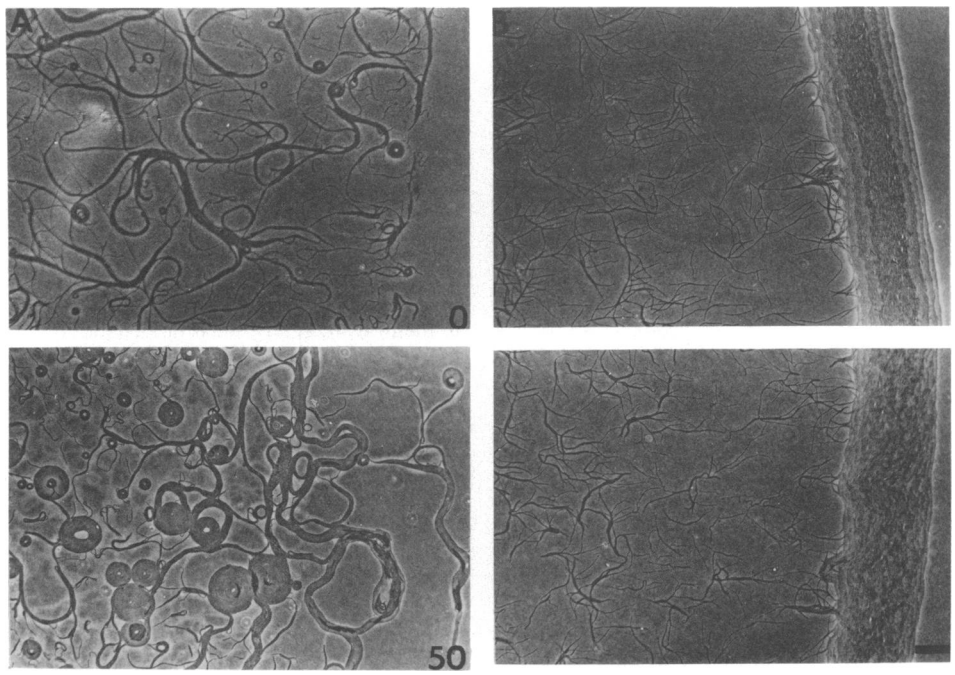


FIG. 2. *Gliding of Flexibacter FS-1 (A) on YE/10 agar at t = 0 and after 50 min. Some movement had already occurred prior to drying of the spot of cells. (B) Nongliding mutant Ng 4 under the same conditions. Bar represents 50 μm.*

procedure of Nossal and Heppel (6), we found that both gliding and flexing of *Flexibacter* are inhibited. However, it may be that one or more of the steps of the osmotic shock procedure are themselves inhibiting *Flexibacter* movement. Filaments washed with and suspended in Tris-hydrochloride buffer (pH 7, 0.01 M) flexed normally. They also glided when plated on YE/10 agar.

Plasmolysis is the next step in the osmotic shock procedure. Exposure of flagellated bacteria to hypertonic sucrose temporarily inhibits motility (7). Incubation of *Flexibacter* FS-1 filaments in YE/2 growth medium containing 20% sucrose (20 min at 30°C) caused inhibition of both flexing and gliding. Filaments exhibited both forms of movement after a 1- to 2-h recovery period in growth medium.

Addition of EDTA to washed filaments suspended in Tris buffer, the next step in the osmotic shock procedure, inhibited flexing. Subsequent addition of $CaCl_2$ (10^{-3} M) and $MgCl_2$ (10^{-3} M) to EDTA-treated cells restored flexing immediately. We were unable to determine the effect of EDTA on motility since we have thus far been unable to develop a defined medium on which *Flexibacter* can glide.

We determined the maximum temperature at which flexing and gliding will occur. A suspension of *Flexibacter* filaments was spotted on YE/10 agar plates prewarmed to the test temperature. After 4 h of incubation at that temperature, gliding was checked. Cells were able to glide at 45°C. At 47 to 48°C, trace or no gliding was observed. Active flexing occurred up to 47°C in cell suspensions gradually heated to that temperature. However, if 30°C-grown cells were shifted to 37°C, flexing was inhibited immediately. This inhibition may be related to the initiation of filament fragmentation induced by temperature shift (8).

Ridgway (9) reported that KCN and uncouplers of oxidative phosphorylation inhibit gliding motility of *F. polymorphus*. We have incorporated inhibitors in the flexing and gliding media to determine their effects on movement in *Flexibacter* FS-1. KCN (5×10^{-3} M) inhibited gliding absolutely and stopped flexing within 0.5 h; at 10^{-2} M, flexing ceased almost immediately. The uncoupler dinitrophenol had the same effect; at 2×10^{-4} M, flexing was inhibited within 0.5 h and no gliding was observed. Flexing was inhibited immediately at 10^{-3} M. Arsenate, which reduces the ATP and phosphoenolpyruvate concentration in bacteria (3), has little influence on gliding of *F. polymorphus* (9). Similarly, we have found that neither flexing nor gliding of *Flexibacter* FS-1 was inhibited by sodium arsenate (2×10^{-2} M).

The facts that UV-induced nongliding mutants were also unable to flex, and that when such mutants revert, they simultaneously re-

gained both types of movement, suggest that flexing and gliding have at least one gene in common. Furthermore, treatments and conditions which inhibited gliding also inhibited flexing. These observations suggest that expression of the flexing phenotype is under the same control as that of gliding motility.

We thank Jeremy Klein and David Lee for their technical assistance, Richard Chisolm and Michael O'Neill for their aid with microcinematography, and David White for his valuable suggestions.

This work was supported by National Science Foundation grant PCM77-24461.

LITERATURE CITED

1. **Burchard, R. P.** 1974. Studies on gliding motility *Myxococcus xanthus*. Arch. Microbiol. **99**:271-280.
2. **Costenbader, C. J., and R. P. Burchard.** 1978. Effect of cell length on gliding motility of *Flexibacter*. J. Bacteriol. **133**:1517-1519.
3. **Klein, W. L., and P. D. Boyer.** 1972. Energization of active transport by *Escherichia coli*. J. Biol. Chem. **217**:7257-7265.
4. **Lewin, R. A.** 1969. A classification of flexibacteria. J. Gen. Microbiol. **58**:189-206.
5. **Lewin, R. A., and D. M. Lounsberry.** 1969. Isolation, cultivation and characterization of flexibacteria. J. Gen. Microbiol. **58**:145-170.
6. **Nossal, N. G., and L. A. Heppel.** 1966. The release of enzymes by osmotic shock from *Escherichia coli* in exponential phase. J. Biol. Chem. **241**:3055-3062.
7. **Okrend, A. G., and R. N. Doetsch.** 1969. Plasmolysis and bacterial motility: a method for the study of membrane function. Arch. Microbiol. **69**:69-78.
8. **Poos, J. C., F. R. Turner, D. White, G. D. Simon, K. Bacon, and C. T. Russell.** 1972. Growth, cell division, and fragmentation in a species of *Flexibacter*. J. Bacteriol. **112**:1387-1395.
9. **Ridgway, H. F.** 1977. Source of energy for gliding motility in *Flexibacter polymorphus*: effects of metabolic and respiratory inhibitors on gliding movement. J. Bacteriol. **131**:544-556.
10. **Ridgway, H. F.** 1977. Ultrastructural characterization of goblet-shaped particles from the cell wall of *Flexibacter polymorphus*. Can. J. Microbiol. **23**:1201-1213.
11. **Ridgway, H. F., R. M. Wagner, W. T. Dawsey, and R. A. Lewin.** 1975. Fine structure of the cell envelope layers of *Flexibacter polymorphus*. Can. J. Microbiol. **21**:1733-1750.
12. **Simon, G. D., and D. White.** 1971. Growth and morphological characteristics of a species of *Flexibacter*. Arch. Mikrobiol. **78**:1-16.
13. **Soriano, S.** 1973. Flexibacteria. Annu. Rev. Microbiol. **27**:155-170.
14. **Stanier, R. Y.** 1940. Studies on the cytophagas. J. Bacteriol. **40**:619-635.