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Effect of Cell Length on Gliding Motility of *Flexibacter*

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Short *Flexibacter* FS-1 cells, generated during logarithmic growth in glucose-salts medium or by filament fragmentation during the transition from log to stationary phase in rich medium, are unable to glide. Motility returns when cells elongate. This strain also dissociates stable, short, nongliding variants.

The genus *Flexibacter* consists of gram-negative, flexible rods or filaments that demonstrate gliding motility (1, 4). Simon and White (3) reported that an isolate of this genus, designated FS-1, showed a significant variation in cell length ranging from 10 to 400 μm, depending on temperature of incubation and composition of the growth medium. They also observed that

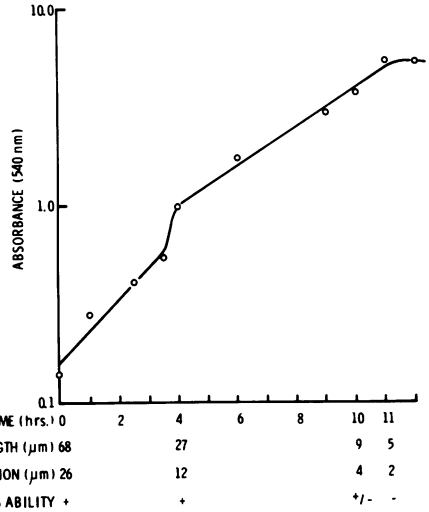

FIG. 1. *Growth curve of Flexibacter FS-1 in YE medium at 30°C. During the log → stationary phase transition, cell lengths and gliding ability were determined.*

filaments entering stationary phase undergo an increased rate of cell division, resulting in fragmentation. Poos et al. (2) proposed that this fragmentation, also induced by metabolic inhibitors, "could have obvious survival advantages if short cells are able to seek out [by gliding] more favorable habitats." Because of our interest in gliding motility, we set out to test this hypothesis.

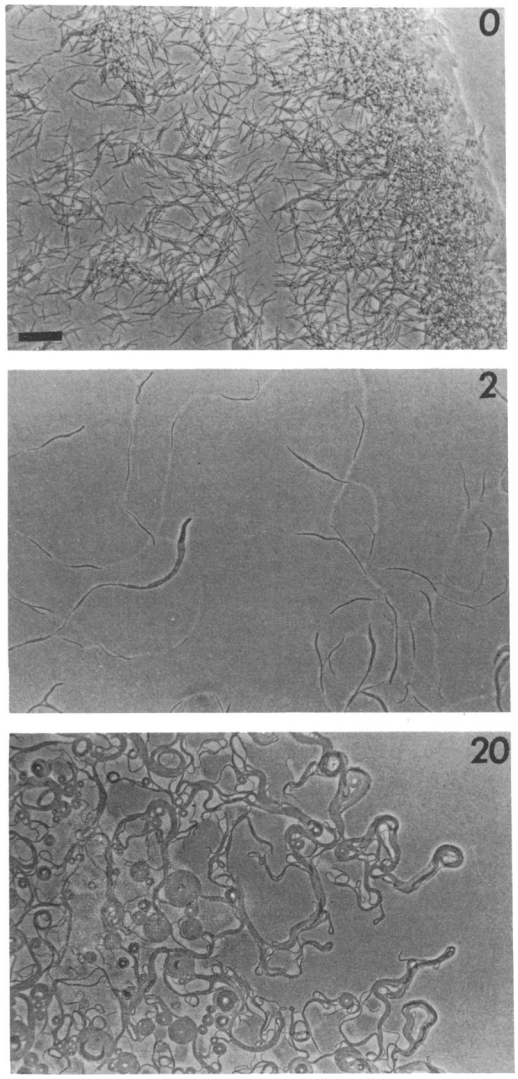

FIG. 2. *Log-phase Flexibacter FS-1 grown in YE, 0, 2, and 20 h after spotting on YE/10 agar. Bar represents 50 μm.*

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Flexibacter FS-1 was cultivated in yeast extract broth (YE) or glucose plus salts (NO_3^-) at 30°C(3). Growth curves were obtained by following the optical density of cultures at 540 nm with a Bausch and Lomb Spectronic 20. Gliding studies were performed on YE/10 medium, which contained 1/10 the yeast extract of YE plus 1.5% agar. The gliding assay was facilitated by the lower nutrient concentration. Chloramphenicol (25 to 250 μg/ml; Sigma Chemical Co.) was incorporated into this medium in some assays. Filaments were able to glide, although not extensively, on agar containing 250 μg of chloramphenicol per ml, a level that inhibits amino acid incorporation (2) and filament elongation.

Gliding was assayed by direct observation of movement of single cells and swarms under Teflon cover slips on YE/10-coated slides and by spotting on agar 10-μl samples of cell suspension, generally diluted to an absorbance of ≤0.05. After incubation at 30°C, the dried spots were examined hourly by phase-contrast microscopy for projections of cells extending from the spot periphery, for swarming, and for the presence of phase-bright trails left behind gliding cells. Cell length measurements were done with a Numonics Graphics Calculator on phase-contrast photomicrographs. At least 20 cells were measured for each point. Mean cell lengths and standard deviations are presented.

Exponentially growing *Flexibacter* filaments were able to glide on YE/10 (Fig. 1, 2). Fragmenting filaments had the ability to glide until shortly after entry into stationary phase, at

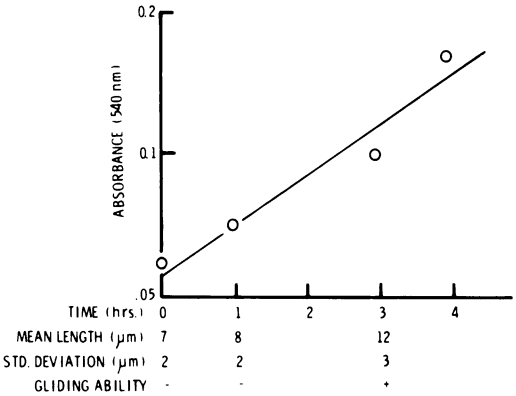

FIG. 3. *Growth curve of Flexibacter FS-1 early stationary-phase cells after transfer to fresh YE medium. Cell lengths and gliding ability were determined.*

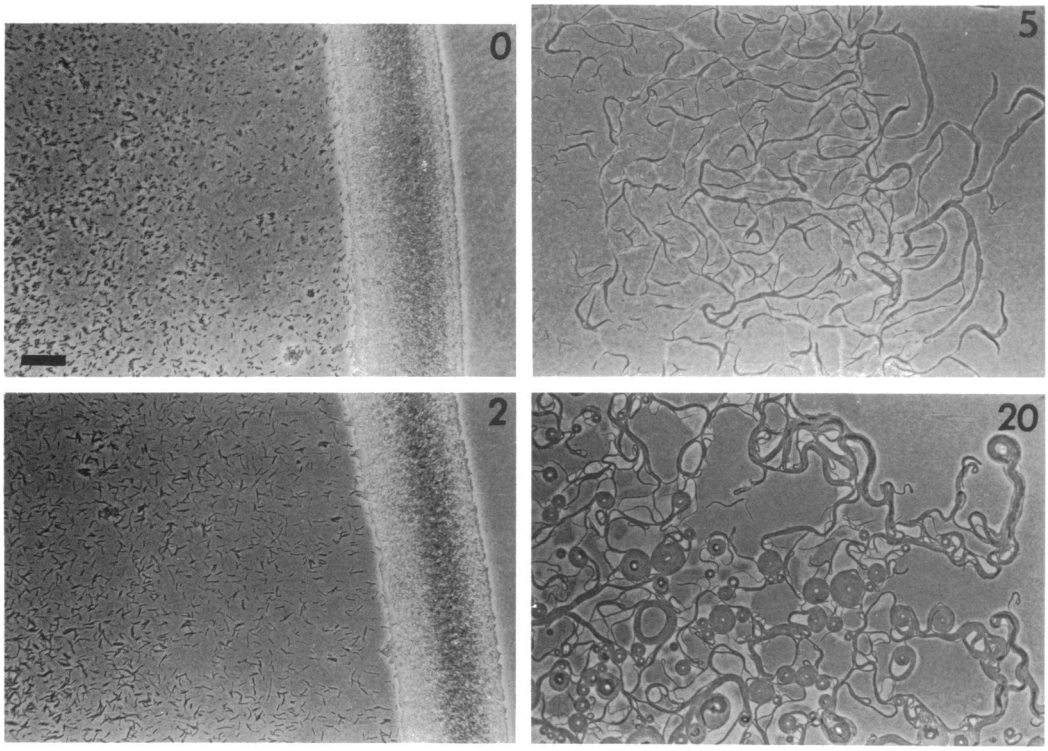

FIG. 4. *Log-phase Flexibacter FS-1 grown in glucose plus salts (NO_3^-), 0, 2, 5, and 20 h after spotting on YE/10. Bar represents 50 μm.*

which time the mean cell length was 5 ± 2 μm. When stationary-phase cells were inoculated into fresh YE broth, gliding motility was regained after cells had elongated to 12 ± 3 μm (Fig. 3).

Is the inability of short stationary-phase cells to glide a function of length or physiology? Simon and White (3) reported that cells growing exponentially at 25°C in glucose plus salts, in which NO_3^- is the nitrogen source, have a mean length of 10 μm. We have observed that cells grown on the same medium at 30°C have a mean length of 3 ± 1 μm. They are not able to glide on YE/10 or on glucose-salts agar. Only after outgrowth in YE to a cell length of 6 ± 2 μm did gliding ability return (Fig. 4). Filaments can glide on glucose-salts agar.

On subculture, FS-1 sometimes disssociates colony variants, designated FS_{ng}. The colonies are raised and demonstrate smooth peripheries, neither showing the single cells nor projecting swarms typical of the parental strain. Observations of these cells on YE/10 indicated that they are unable to glide, whether they be the relatively short filaments from log-phase cultures (20 ± 7 μm) or the fragmented filaments (6 ± 2 μm) of stationary-phase cultures. Whether it is cell length or some other factor that accounts for the inability of these variants to glide has not been determined.

In conclusion, our data demonstrate that short cells of *Flexibacter* FS-1, whether generated during the transition to stationary phase or by growth in glucose plus salts (NO_3^-), or possibly in the dissociation of the FS_{ng} variant, are unable to glide. As short cells elongate into filaments, they recover ability to glide. This may provide a good system for study of the mechanism of gliding motility.

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