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Effect of Temperature Shifts on Gliding Motility, Adhesion, and Fatty Acid Composition of *Cytophaga* sp. Strain U67

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Gliding motility and flipping of 25°C-adapted *Cytophaga* sp. strain U67 were inhibited when the bacteria were shifted to a ≤12°C environment; motility was not blocked by a shift to 13°C. Bacteria adapted to 4°C were motile over the entire 4 to 25°C temperature range tested. U67 adhesion to the substratum appeared to be unaffected by temperature shifts. Bacteria adapted to 4°C had higher proportions of unsaturated and branched-chain fatty acids than did those grown at 25°C. When 25°C-adapted bacteria were subjected to a gradual temperature decline, the time of reappearance of gliding competence at 4 to 5°C was correlated with these changes in fatty acid composition.

The gliding bacteria are a taxonomically heterogeneous assemblage (19) that translocates on surfaces by a mechanism(s) that is still not understood. Several mechanistic models for motility invoke actively moving components or longitudinally or helically propagated waves of compression or deformation in the cell envelope (reviewed in references 1, 3, and 17). Two of these models (in addition to gliding) account for the nontranslocational motile behaviors of members of the family *Cytophagaceae*. These behaviors include flipping on one pole, pivotting, and active propulsion of microscopic particles that adhere to the bacterial surface (2, 10, 18, 20).

One of these comprehensive models was developed from observations of *Cytophaga* sp. strain U67. The model involves longitudinal movements of bacterial surface adsorption sites in a track system fixed to the underlying peptidoglycan layer of the cell envelope (10). Observations of the sinistral revolution of *Flexibacter polymorphus* during gliding and its helical propulsion of microspheres led Ridgway and Lewin to propose that the translocational force is produced at many independent but functionally coordinated adsorption sites rather than by linearly arrayed tracks (20). In fact, rotation around the cellular long axis may be a general characteristic of gliding in members of the family *Cytophagaceae* (7).

The hypothesized active movement of components within the outer membrane or of the outer membrane itself implies some degree of fluidity. In fact, the gliding bacteria are considered to be flexible cells with a relatively fluid cell envelope (8). Changes in cell envelope fluidity, such as that predicted to result from a sharp decline in temperature to one below the cell envelope lipid crystalline-gel phase transition, might inhibit the function of the putative mobile elements. Only after homeoviscous adaptation of cell envelope lipids (21) would motility predictably be restored. Thermal regulation of bacterial membrane lipid fluidity has been reviewed, but there have been no reports of such regulation occurring in the gliding bacteria (4, 12, 13).

We report here that shifting 25°C-grown *Cytophaga* sp. strain U67 cells to 4 to 5°C growth conditions resulted in the inhibition of motility until the bacteria adapted to the lower temperature. Changes in motility correlated with shifts in cellular fatty acid composition suggestive of homeoviscous adaptation.

MATERIALS AND METHODS

Bacteria and culture conditions. *Cytophaga* sp. strain U67 was grown in C62 liquid medium (0.05% tryptone plus 0.05% yeast extract, pH adjusted to 7.0 [9]) at 25°C. In most temperature shift experiments, 1 liter of logarithmic-phase cells in a 2-liter baffled culture flask was shifted to a 200-rpm rotary shaker in a cold room (4 to 5°C).

Adhesion assay. The bacterial suspension was loaded into a Petroff-Hausser counting chamber and incubated for 5 min at the test temperature. Bacteria in three 8×10^5-μm^3 volumes were counted. The 5 μl of cell suspension within the chamber was displaced by delivering an equal volume of sterile growth medium at one end of the cover slip and drawing it through the chamber with a 1-cm-wide piece of bibulous paper placed at the other end. This process flushed out loosely adherent as well as suspended cells. The flushing was repeated twice. The bacteria remaining in the chamber were enumerated after flushing.

Motility assays. With phase-contrast optics (magnification, ×100), motility on C62 agar was detectable, at the peripheries of drops of a bacterial suspension that had been spotted on the gel, as radially advancing projections and rafts of cells.

Single-cell motility was observed in wet mounts with phase-contrast optics (×400). After attachment to the slide surface, motility-competent bacteria demonstrated short glides interspersed by flips during which they lifted off the surface and remained attached on the posterior pole. The entire length of the cell reestablished contact with the slide and set off in a new direction.

The temperature of wet mounts was monitored with an electronic probe applied to the slide itself. The wet mounts were observed in a brass slide holder with internal channels through which was circulated an ethylene glycol solution from a refrigerated reservoir.

Rapid temperature shifts were achieved in a slide holder with both a proportionally regulated heating element and channels through which ice water was rapidly circulated.

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TABLE 1. *Cytophaga* sp. strain U67 adhesion at 5 and 24°C

Temp (°C):		No. of bacteria[a]		% Adherent bacteria
For bacterial growth	In the adhesion chamber	Preflush	Postflush[b]	
25	24	73 ± 5.5	67 ± 5.9	92
25	5	61 ± 8.1	62 ±10.7	100
4	24	56 ± 9.3	46 ± 2.6	82
4	5	56 ± 6.1	45 ± 3.1	80

[a] Bacteria were incubated in a Petroff-Hausser counting chamber for 10 min before the three 8 × 10^5-μm^3 volume equivalents were counted. The chamber depth was 20 μm. Results are reported as means ± standard deviations.
[b] Bacteria in the same volume were counted again after three flushes with 5 μl of C62 medium to displace the fluid in the chamber.

This method enabled lowering of the wet mount temperature from 25 to 12°C in approximately 3 min. The temperature could be shifted between 12 and 13°C in a few seconds.

Fatty acid analysis. Logarithmic-phase bacteria were centrifuged at 20,000 × *g* for 10 min at 4°C. Cell pellets were saponified and processed for total cellular fatty acid analysis as described previously (23). The resulting fatty acid methyl esters were analyzed by capillary gas-liquid chromatography and by combined gas-liquid chromatography–mass spectrometry as described previously (15).

RESULTS

Motility. *Cytophaga* sp. strain U67 cells grown at 25°C (growth rate constant [*k*] = 0.52) and observed in wet mounts at ambient temperatures (22 to 24°C) demonstrated rapid adhesion to the glass, followed by short glides interspersed with flips (2, 7, 10). On agar, the predominant movement was that of rafts of closely associated gliding cells that could be detected within a few minutes; isolated bacteria demonstrated little translocation.

Bacteria adapted to 25°C were unable to glide or flip in 4 to 5°C wet mounts. This paralysis was not the result of an inability to adhere to cold glass surfaces, since the bacteria demonstrated comparable adhesion at 25 and 5°C (Table 1). Bacteria adapted to 25°C, spotted on 4°C agar, and incubated at 4°C recovered the ability to glide after 2 to 3 days, as demonstrated by the appearance of projections and rafts of cells.

The motility of 25°C-adapted *Cytophaga* sp. strain U67 cells at intermediate temperatures was also tested. Since translocation in wet mounts was intermittent, motility was quantitated by recording the behavior on videotape and subsequently counting the number of flips per cell per minute. The data from these experiments are reported in Table 2 and Fig. 1. Shifting the bacteria to a 12°C wet mount resulted in complete inhibition of flipping motility. Gliding was also inhibited when 25°C-adapted cells were spotted onto 12°C agar and only became detectable after 7 h. Gliding was evident at gradually shorter times as the temperature of the agar onto which 25°C-adapted cells were spotted was increased above 12°C; no sharp transition was observed. *Cytophaga* sp. strain U67 cells adapted to growth at 4°C (*k* = 0.035) were motile over the 4 to 25°C range tested (Fig. 1 and Table 2).

In another experiment, 25°C-adapted U67 cells were placed in wet mounts on a temperature-controlled stage and observed while the temperature was rapidly lowered. Flipping ceased abruptly when the slide reached 12°C. Motility resumed when the slide temperature was raised above 13°C. This entire process of raising and lowering the slide temperature was repeated many times, with the motility transition always occurring at the 12 to 13°C point.

TABLE 2. Flipping frequencies at various temperatures for *Cytophaga* sp. strain U67 grown at 25 and 4°C[a]

Temp (°C):		No. of microscopic fields examined	Total no. of cells	Avg flipping frequency	SD for flipping frequency
For growth	Of the slide				
25	25	4	115	2.18	1.39
	21	4	92	1.04	0.78
	17	4	91	1.09	0.72
	13	4	93	0.93	0.52
	12	NM		0.00	
	5	NM		0.00	
4	25	5	130	0.93	1.10
	20	5	114	1.19	1.16
	15	5	174	2.02	1.33
	10	5	187	1.63	0.99
	5	5	209	1.72	0.67
	0	NM		0.00	

[a] Flipping frequencies were determined for U67 grown at either 25 or 4°C on microscope slides at different temperatures. The number of microscopic fields was used in the statistical analyses because the flipping frequency was determined one field at a time, as opposed to one cell at a time. A one-way analysis of variance was also performed on the flipping frequency data. The 25°C-adapted cells had an *F* value of 4.94, with a critical value at $\alpha = 0.05$ of 3.49; the 4°C-adapted cells had an *F* value of 7.75, with a critical value at $\alpha = 0.05$ of 2.87. Both of these cases indicated that the flipping frequencies of the adapted cells at the different slide temperatures were significantly different from each other. NM, No motility.

Fatty acid composition. The fatty acid compositions of *Cytophaga* sp. strain U67 cells adapted to growth at 25 and 4°C were compared (Table 3). Some variations in the proportions of individual fatty acids among parallel runs were found. A comparison of classes of fatty acids demonstrated that 4°C-adapted bacteria had a higher ratio of unsaturated to saturated fatty acids than did 25°C-adapted bacteria. The relative amount of branched-chain fatty acids was higher in 4°C cells, while 25°C cells contained a higher proportion of hydroxy fatty acids.

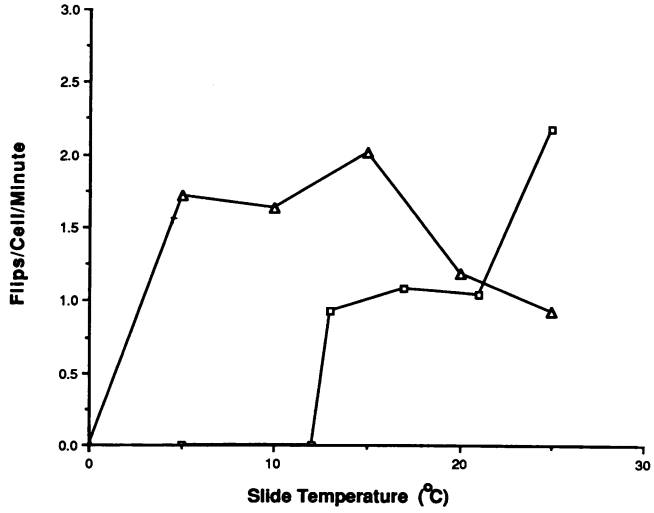

FIG. 1. Motility of *Cytophaga* sp. strain U67 adapted to 4°C (△) and 25°C (□). Flipping frequencies were determined at various temperatures as described in Materials and Methods. Statistical analyses are presented in Table 2.

TABLE 3. Fatty acid compositions of *Cytophaga* sp. strain U67 grown at 4 and 25°C[a]

Fatty acid	% of total fatty acids in expt:					
	1		2		3	
	25°C	4°C	25°C	4°C	25°C	4°C
i-$C_{14:0}$	0	0	0	0	0	0
$C_{14:0}$	0	0	0	0	0	0
i-$C_{15:1}$	0	8	0	9	4	9
a-$C_{15:1}$	0	2	0	3	0	3
i-$C_{15:0}$	25	19	26	21	29	19
a-$C_{15:0}$	0	6	0	5	2	8
$C_{15:1}$	0	2	0	1	3	3
$C_{15:0}$	6	4	6	3	9	4
3-OH $C_{14:0}$	0	0	0	0	0	2
i-$C_{16:0}$	0	3	0	3	0	2
$C_{16:1}$	23	23	22	23	25	23
$C_{16:0}$	9	3	8	2	7	3
i-3-OH $C_{15:0}$	11	8	12	8	6	6
i-$C_{17:1}$	6	4	7	4	4	4
a-$C_{17:1}$	0	0	0	0	0	0
$C_{17:1}$	0	0	0	0	0	1
i-3-OH $C_{16:0}$	0	4	0	3	0	3
3-OH $C_{16:0}$	7	3	6	2	5	2
$C_{18:1}$	0	0	0	0	0	0
$C_{18:0}$	0	0	0	0	0	1
i-3-OH $C_{17:0}$	13	7	12	8	6	4
Total	100	96	99	95	100	97
SFA	71	57	70	55	64	54
UFA	29	39	29	40	36	43
HFA	31	22	30	21	17	17
BFA	55	61	57	64	51	58

[a] Three separate gas-liquid chromatography fatty acid analyses for whole cells adapted to either 25 or 4°C for 48 h were done. For fatty acids, the number before the colon indicates the carbon chain length, and the number after the colon indicates the number of double bonds in the chain; a and i designate anteiso or iso branchings, respectively, at the end of the chain; hydroxyl groups, if present, are preceded by their location on the acyl chain. SFA, Saturated fatty acids; UFA, unsaturated fatty acids; HFA, hydroxy fatty acids; BFA, branched-chain fatty acids. These four categories are not mutually exclusive. Numbers were rounded to the nearest percent. Thus, totals did not always add up to 100%.

TABLE 4. Fatty acid compositions of 25°C-adapted *Cytophaga* sp. strain U67 during a shift to 5°C[a]

Fatty acid	% of total fatty acids at time (h):										
	0	2	4	6	8	12	12*	16*	20*	24*	36*
$C_{14:0}$										1	
i-$C_{15:1}$	4	6	7	9	8	10	9	9	10	10	12
a-$C_{15:1}$								1	1	1	
i-$C_{15:0}$	28	26	27	30	27	26	26	23	25	24	27
a-$C_{15:0}$						1	1	2	2	2	3
$C_{15:1}$	1	3	4	5	4	4	5	3	4	3	1
$C_{15:0}$	9	8	8	9	8	6	8	7	6	6	6
3-OH $C_{14:0}$										1	
i-$C_{16:0}$								1	2	1	
$C_{16:1}$	24	23	27	26	27	24	23	22	22	20	23
$C_{16:0}$	8	5	5	4	5	3	4	3	3	3	1
i-3-OH $C_{15:0}$	6	6	6	7	7	8	6	7	5	5	5
i-$C_{17:1}$	4	7	7	7	7	7	6	6	6	6	7
a-$C_{17:1}$										1	
$C_{17:1}$		1				2	3	2	3	2	3
i-3-OH $C_{16:0}$		3				2	1	3	2	2	3
3-OH $C_{16:0}$	5	4	3			2	2	2	2	2	
$C_{18:1}$											
$C_{18:0}$	1	1					1	1	1	1	
i-3-OH $C_{17:0}$	6	6	5	3	7	5	5	6	4	5	5
Total	96	99	99	100	100	100	100	98	98	96	96
SFA	63	59	54	53	54	53	54	55	52	53	50
UFA	33	40	45	47	46	47	46	43	46	43	46
HFA	17	19	14	10	14	17	14	18	13	15	13
BFA	48	54	52	56	56	59	54	58	57	57	62

[a] See the legend to Fig. 2. Fatty acid nomenclature and abbreviations are as described in Table 3, footnote *a*. Samples were taken from one culture from 0 to 12 h. A second culture was sampled from 12 to 36 h after the temperature shift (12* to 36*).

To determine whether the development of the fatty acid profile characteristic of 4 to 5°C-adapted bacteria was a prerequisite for motility at 4 to 5°C, we examined the characteristics of 25°C-adapted *Cytophaga* sp. strain U67 cells during a gradual decline in the culture temperature. As measured by culture absorbance, the bacteria continued to grow during and after the temperature decline to 5°C (Fig. 2). The motility of single cells in 5°C wet mounts as well as bacteria on 4°C agar recovered between 8 and 12 h. In other experiments, the time to the recovery of motility was as short as 4 to 5 h. The ability to glide in the cold developed after the major changes in the classes of fatty acids characteristic of 4°C-adapted cells had occurred (Fig. 2 and Table 4).

DISCUSSION

We have confirmed the previous report (10) that *Cytophaga* sp. strain U67 cells grown at 25°C and shifted rapidly to a 4 to 5°C substratum (agar or glass) were unable to glide. The bacteria did, however, survive this temperature downshift and regained the ability to glide at 4 to 5°C after 2 days at that temperature. If the temperature shift was more gradual, the bacteria continued to grow and were motility competent in the cold soon after the culture temperature had reached 5°C. Similarly, 25°C-grown bacteria demonstrated no motility when shifted rapidly to 12°C. However, when shifted to 13°C wet mounts, the bacteria continued to flip and glide.

One explanation for these observations may be that a crystalline-gel phase transition occurred in the cell envelope lipids between 12 and 13°C. One or both of the membranes of the cell envelope of these 25°C-adapted bacteria may have become relatively rigid at temperatures below the phase transition, prohibiting the functioning of the hypothesized machinery of gliding. We did not observe any obvious change in adhesion or in the rigidity of the bacteria that were rapidly chilled. Unlike some other gliding bacteria (8), 25°C-adapted *Cytophaga* sp. strain U67 appears to be relatively rigid (7, 10).

Motility inhibition might also have been effected by a cold temperature-induced decrease in the fluidity of sulfonolipids, which have been demonstrated to be involved in the gliding of these bacteria (6). It is also conceivable that temperatures below the lipid phase transition blocked motility by inhibiting proton motive force-derived energy transduction from the cytoplasmic membrane to the gliding machinery itself.

The fatty acid profile of *Cytophaga* sp. strain U67 is similar to that of other members of the family *Cytophagaceae* in its diversity of molecular species (11, 16, 22–24). In 25°C-grown cells, seven or eight fatty acids each represented at least 5% of the total fatty acid composition, clearly distinguishing *Cytophaga* sp. strain U67 from *Escherichia coli*, a prototypical gram-negative bacterium which has only three major fatty acids: palmitic, palmitoleic, and *cis*-vac-

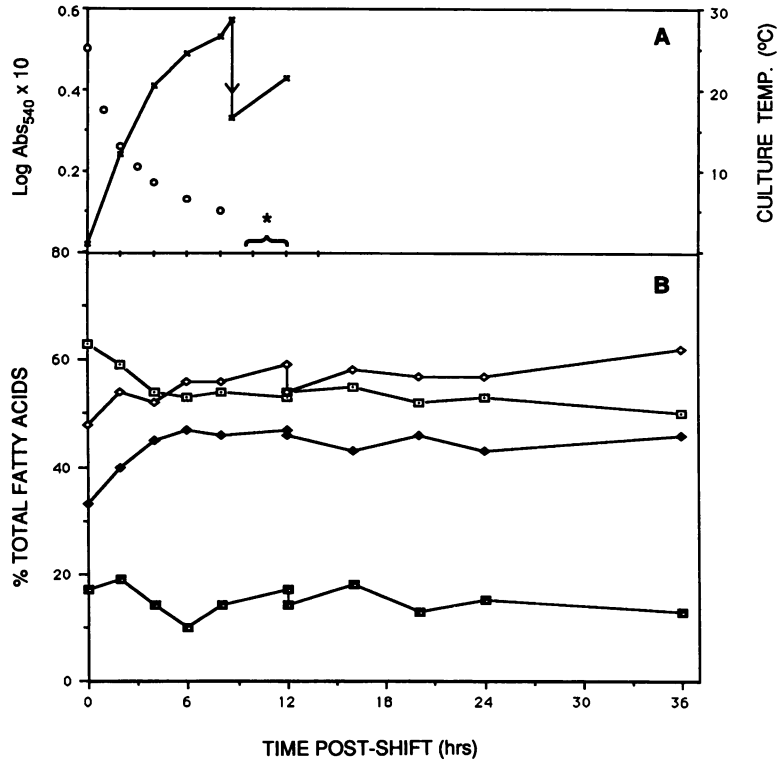

FIG. 2. *Cytophaga* sp. strain U67 fatty acid composition during a culture shift from 25 to 4°C. (A) Culture absorbance (Abs) (×) and culture temperature (○) were determined after shifting a 1-liter culture of logarithmic-phase, 25°C-grown cells to a cold room (5°C) at time zero. The bacteria were subcultured into fresh 5°C medium at 8.7 h (arrow). Gliding competence at 4 to 5°C developed during the interval indicated by the asterisk. (B) Culture samples were taken for fatty acid analyses from one culture between 0 and 12 h and from a parallel culture (which had been shifted from 25 to 5°C at −12 h) between 12 and 36 h. The categories of fatty acids are described in Table 3, footnote *a*; they are not mutually exclusive. Symbols (fatty acids): ⊡, saturated; ◆, unsaturated; ■, hydroxy; ◇, branched chain.

cenic (13). Unlike *Cytophaga* cells, *E. coli* cells grown at their optimal temperature (37°C) continue to swim, albeit more slowly, following a temperature shift to 4°C (14).

During more gradual temperature shifts, the overall pattern of changes in the classes of fatty acids was predictable. Cells adapted to 4°C had a higher ratio of unsaturated to saturated fatty acids than did 25°C-adapted cells. The monounsaturated 18-carbon fatty acid (*cis*-vaccenic) that undergoes the greatest change in response to temperature shifts in *E. coli* (5) was absent in *Cytophaga* sp. strain U67. In addition to the unsaturated fatty acids, those with branched chains also have been reported to increase membrane fluidity (8). Branched-chain fatty acids represented over half of the total fatty acid composition of 25°C-grown *Cytophaga* sp. strain U67 cells, and the relative amount of this category increased further during adaptation to the cold.

Harwood and Russell (8) also suggested that the relatively high level of hydroxy branched fatty acids (e.g., i-3-OH $C_{15:0}$) typical of some gliding bacteria may also contribute to membrane fluidity. This category represented about 30% of the total in 25°C-adapted U67 cells. However, the relative proportion of these acids was lower in 4°C-adapted U67 cells, suggesting that they play no role in homeoviscous adaptation. Shortening of the average fatty acid chain length may be another adaptation to cold temperature (8). The average fatty acid chain length of 25°C-adapted U67 cells was almost identical to that of 4°C-adapted U67 cells.

Our data do not establish a cause and effect relationship between homeoviscous adaptation and the development of gliding competence at 4 to 5°C. However, in examining the time course of acclimatization during a temperature shift, we found correlations between the return of motility and the rise in the proportions of unsaturated and branched-chain fatty acids, suggesting that increases in the proportions of these fatty acids are important for homeovisious adaptation and the reappearance of gliding motility in the cold.

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