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Motility and Thermotactic Responses of *Thermotoga maritima*

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***Thermotoga maritima*, a thermophilic eubacterium, is motile at temperatures ranging from 50 to 105°C. The cells are propelled by a single flagellum which most of the time spins clockwise. Changes in the swimming direction ("tumbles") are achieved by short reversals of the direction of filament rotation. The average speed of swimming cells depends on the temperature, reaching a maximum value of about 60 μm/s at 85°C. The cells show a thermotactic response to temporal temperature changes. When the temperature is raised, the rate of tumbles is increased, while decreasing temperature decreases the tumbling rate.**

Bacteria respond to changes in ambient conditions by sensing temporal changes of environmental parameters and adjusting their motility accordingly. This enables them to move towards favorable places and to avoid lethal environments. A variety of stimuli can serve as sensory input: chemicals, gases, light, temperature, pH, etc. (for a review, see reference 1). In the presence of a constant stimulus, intervals of straight swimming ("runs") are interrupted by short intervals in which the cell body changes its orientation ("tumbles"), thus resulting in a random change of the direction of swimming. The resulting motion of the cell is a three-dimensional random walk. Local variations of the environmental parameters are sensed by temporal comparisons during motion. When a cell swims in a direction where an attractive stimulus increases, the amount of directional change is reduced, while a decrease in the stimulus has the effect that the tumbling rate increases. This biases the random walk along the gradient of the stimulus. Besides attractive stimuli there exist also repellent stimuli (11), which are sensed in the same way but cause an opposite response: the cell moves away from the source.

We have investigated the motility of the thermophilic eubacterium *Thermotoga maritima*, which has been previously described by Huber et al. (5). The rod-shaped cells, about 4 μm in length and 0.5 μm in diameter (Fig. 1), thrive on marine seafloors which are geothermally heated. Besides *Thermus thermophilus* (17) and *Aquifex pyrophilus* (6), members of the order *Thermotogales* are the only known hyperthermophilic representatives of the eubacteria. *T. maritima* grows anaerobically at an optimum temperature of 80°C by fermentation of carbohydrates. The motile cells each possess a single flagellum that drives them with a speed of up to 60 μm/s. Nothing is known so far about the tactic behavior of this organism. A motile bacterium, however, is expected to have a sensory system. In the natural habitats of *T. maritima*, extreme differences in ambient temperature are present on small spatial scales, ranging from about 10°C up to over 100°C (19). It is, therefore, important for the cells to avoid lethal temperatures. We wanted to find out whether organisms, thriving under such environmental conditions, are capable of sensing thermal stimuli, thus enabling them to move towards more favorable conditions. Former studies on thermotaxis were carried out with mesophilic organisms (13, 14). It was found that high temperature acts as an attractive stimulus for the cells: the tumbling rate is low during an increase in ambient temperature and high during a decrease in temperature. In contrast to these findings, *T. maritima* cells show a phobic response to rising temperature over the whole temperature range in which they are motile.

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MATERIALS AND METHODS

Growth media and conditions. Samples of *T. maritima* MSB8 cells were kindly provided by K. O. Stetter, University of Regensburg, Regensburg, Germany.

The growth medium was a modified MMS medium (5) (6.93 g of NaCl, 1.75 g of $MgSO_4 \cdot 7H_2O$, 1.38 g of $MgCl_2 \cdot 6H_2O$, 0.16 g of KCl, 25 mg of NaBr, 7.5 mg of H_2BO_3, 3.75 mg of $SrCl_2$, 0.025 mg of KJ, 1.5 g of $CaCl_2$, 0.5 g of KH_2PO_4, 15 ml of trace minerals [see below], 1 ml of resazurin [0.1%], 1 g of starch [p.a.], 0.1 g of yeast extract in 1 liter of double-distilled water at pH 6.5). Oxygen was removed by the addition of 20 ml of Na_2S solution (2.5% from 35% stock) after 30 min of N_2 treatment.

The trace minerals stock solution (10× concentration) used was as follows: 30 g of $MgSO_4 \cdot 7H_2O$, 5 g of $MnSO_4 \cdot 2H_2O$, 10 g of NaCl, 1 g of $FeSO_4 \cdot 7H_2O$, 1 g of $CoSO_4$, 1 g of $CaCl_2 \cdot 2H_2O$, 1 g of $ZnSO_4$, 0.1 g of $CuSO_4 \cdot 5H_2O$, 0.1 g of $KAl(SO_4)_2$, 0.1 g of H_3BO_3, 0.1 g of $Na_2MgO_4 \cdot 2H_2O$, 2 g of $Ni(NH_4)_2(SO_4)_2$ in 1 liter of double-distilled water, pH adjusted to 1 with H_2SO_4.

The cells were grown in stoppered 28-ml serum tubes (Bender & Hobein GmbH, Munich, Germany). Cultures (1 ml) were transferred into 20 ml of fresh medium and incubated at 80°C up to a final density of 10^7 cells per ml. The grown cells were stored at 4°C.

Electron microscopy. Electron microscopy was performed with a Philips CM12 microscope. Specimens of *T. maritima* cells were prepared on copper grids covered with a thin carbon film and negatively stained with 2% uranyl acetate.

Light microscopy. For light microscopical investigations we used an inverted microscope (Zeiss Axiovert 10; Zeiss, Oberkochen, Germany), equipped with a 100-W halogen lamp, a long-working-distance (LD) condenser (numerical aperture, 0.5), an LD Achroplan objective lens (32×, Ph2, numerical aperture, 0.5), and additional equipment for differential interference contrast.

Observation of motile cells. Figure 2 gives a schematic overview of the experimental setup. The temperature-controlled observation chamber has been described previously (4). Heating and cooling is achieved by two peltier elements. The cells were drawn into a flat capillary with an inner diameter of 0.2 mm (Vogel GmbH, Gießen, Germany) which was sealed with vacuum grease on both ends and placed between the peltier elements. A personal computer (Macintosh IIfx) was used to control the temperature, to place time markers on the video recording, and for the later analysis of the motion sequences. In the experiments, either constant temperature or periodic temperature profiles were applied by software control. After a time interval t_i at a constant temperature T_1, the sample was heated to a higher temperature T_2 with a rate R and then kept constant for the same time interval t_i. After that time it was cooled down again with rate $-R$ back to T_1, and the cycle started again. The parameters t_i, T_1, T_2, and R could be chosen with the program. The accuracy of the temperature control was to ±0.25°C. Cell motions under the microscope were recorded with a charge-coupled device camera (COHU4722; IPPI GmbH, Munich, Germany) on a video recorder (Panasonic AG7330). A video interface allowed us to superimpose a text overlay with comments at the beginning of each sequence. This helped to find a single motion sequence on the tape during the later analysis. The start and end of a temperature change interval during the experiment were marked with a tone signal on the sound track of the video tape. These markers served as reference points during the motion analysis of the sequences.

Motion analysis. For the motion analysis of the recorded video sequences, we used the public domain program IMAGE, version 1.43 (W. Rasband, National

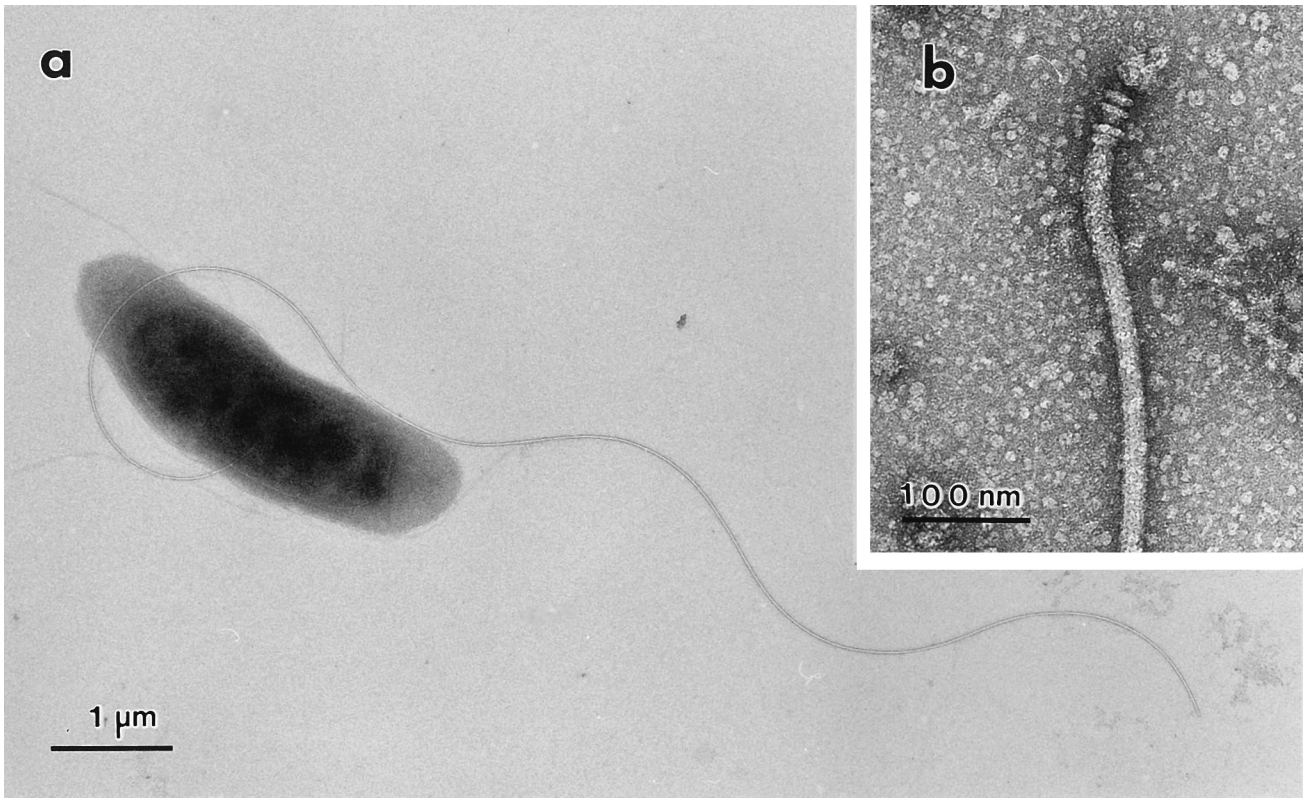

FIG. 1. Electron micrographs of a *T. maritima* cell (a) and its flagellum (b). Specimens were negatively stained with 2% uranyl acetate.

Institutes of Health). The source code in Pascal is available via anonymous ftp. For analysis and display of bacterial tracks, the program was modified by additional routines. Images were digitized from the video recording with a frame grabber board (Scion Video 1000; Scion Corp. Frederick, Md.) at a rate of 6.25 frames per s, i.e., every fourth video frame was digitized. The analysis of the images was performed in a manner analogous to that described by Sager et al. (18). In the digitized motion sequences, the outlines of the cells were separated from the bright background by choosing a proper gray-value threshold. The center-of-mass coordinates of each cell were calculated, giving a set of cell coordinates for each frame. From this set of points, the tracks of the moving cells were determined by searching the nearest neighbor of each center of mass within a given search radius in the next frame. The calculation of a track was aborted if no nearest neighbor was found within that radius or if two tracks came so close together that they became indistinguishable. Cells that did not move for more than half of the time being traced and tracks shorter than 1 s were also excluded from the analysis. To compare the tracks generated by the trace-finding algorithm with the digitized motion sequences, both were superimposed on the display and replayed step by step. Erroneous tracks could be corrected interactively with the mouse by an editing program. The final data set contained the x and y coordinates of the cell tracks, the velocity (proportional to the length of a segment connecting two points), the change of the angular direction (i.e., the angle in the forward direction between two successive velocity vectors), and the elapsed time from the start of the analysis.

In order to analyze the duration of swimming and tumbling intervals at different temperatures, we distinguished runs from tumbles in a way similar to that used by Berg (2): if the directional change between two steps of a track was greater than 35 degrees, the step was considered a tumble, whereas directional changes below that threshold value were classified as smooth swimming.

The motility during temperature changes was analyzed as follows. The video records of five temperature changes up and down were digitized. Digitization was started 5 s before the onset of the temperature change, marked by the tone signal on the record. Therefore, each digitized frame corresponded to a defined instant of time with respect to the start of the temperature change. The time course of the temperature was determined in an independent measurement by using a thermo-resistor, mounted between two coverslips and placed at the point of observation. This arrangement provided a close approximation to the temperature inside the capillary.

RESULTS

Motion of cells. Only 5 to 10% of the observed cells in a grown culture were motile. Labelling of the cells with an antibody against xylanase, which bound unspecifically to the flagellins, revealed that this small fraction resulted from the fact that ca. 90% of the cells had no flagellum at all. Electron microscopy showed that the flagellum inserts laterally in the cell at a position where the outer membrane is in contact with the cytoplasmic membrane. An example is shown in Fig. 1a. The flagellum (Fig. 1b) has the characteristic features of eubacterial flagella: a basal body with four rings is joined by a curved hook with the flagellar filament; the diameter of the filament is 18 nm.

The direction of flagellar rotation was determined from cells which accidentally adhered with their flagellum to the surface of the coverslip. Observed under the microscope, these tethered cells rotated counterclockwise most of the time. According to the convention for judging the sense of flagellar rotation, looking from outside along the flagellar filament (10), the flagellum rotates clockwise. This clockwise rotation was interrupted by short reversals to counterclockwise rotation. With swimming cells these reversals could be recognized as short backing-up movements followed by a move in a new direction (Fig. 3). In peritrichous bacteria the flagella form a bundle during smooth swimming. Reversal of the sense of flagellar rotation makes the single filaments fly apart and act in different directions, thus reorienting the cell body (12). The resulting motion is usually called a tumble. For the sake of simplicity, we adopt the term tumble to describe the changes in swimming direction of *T. maritima.*

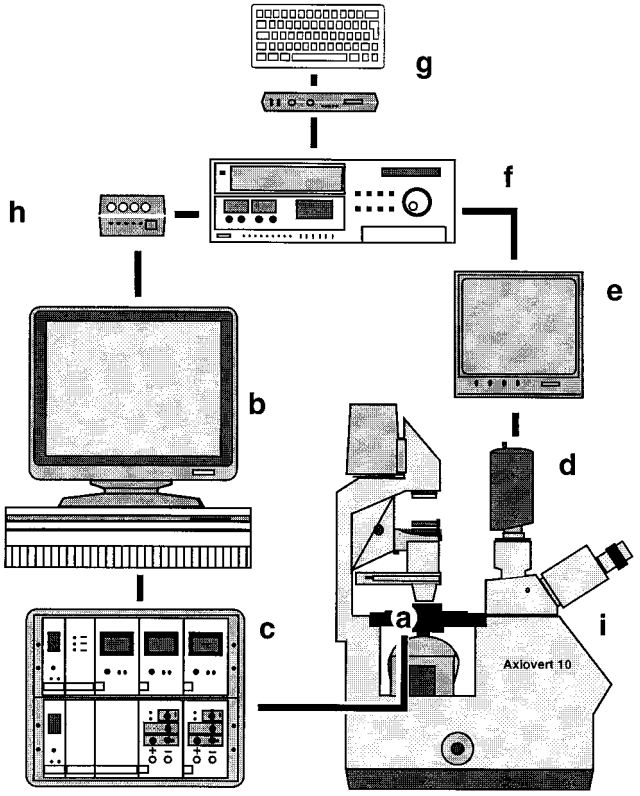

FIG. 2. The experimental setup. (a) Observation chamber; (b) computer; (c) control unit containing the power supply for the peltier elements and the electronics of the temperature sensors; (d) charge-coupled device camera; (e) video monitor; (f) video recorder; (g) text overlay interface; (h) tone signal generator. The temperature of the observation chamber is controlled by the computer. It receives the actual temperature from the control unit via an analog/digital converter and sends back signals on an 8-bit digital port enabling either heating or cooling of the peltier elements. Motion sequences are recorded with the charge-coupled device camera on a video recorder. Temperature steps are marked on the audio channel of the video record as tone pulses, triggered by the computer. Text comments can be added by means of a video text overlay with an alphanumeric keyboard.

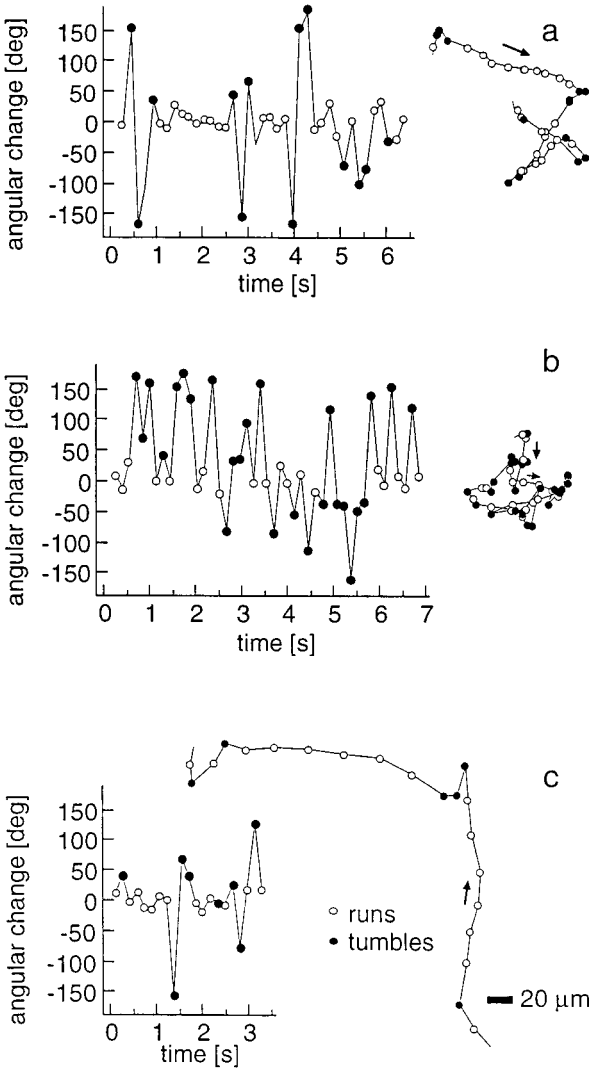

FIG. 4. Typical motion tracks of *T. maritima* cells and corresponding changes of direction. (a) Track at constant temperature, 65°C; (b) during an increase in temperature from 65 to 75°C with 0.3°C/s; (c) during a decrease in temperature from 75 to 65°C with 0.3°C/s. The time interval between successive points is 0.16 s. deg, degree.

No difference in motilities was observed between cells that were taken from the growing culture at 80°C and those taken from a culture which had been stored at 4°C. When a sample was heated, starting from low temperature, the first swimming cells could be observed at 50°C, i.e., slightly below the minimum growth temperature. We could observe swimming cells at up to 105°C, the maximum temperature that we could reach with our experimental design. This is 15°C above the maximum growth temperature. The presence of carbohydrates as an energy source is obligatory for motility. Cells suspended in growth medium free of nutrients did not swim. However, after repeated resuspension in growth medium containing starch, they were found to be motile again.

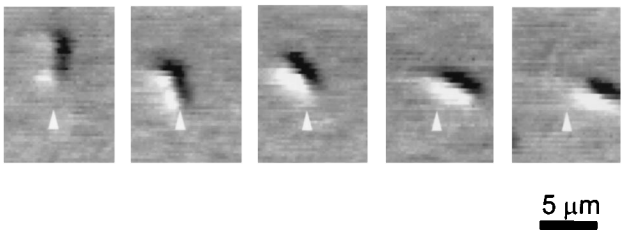

FIG. 3. Single-frame sequence showing a *T. maritima* cell during a tumble, digitized from the recording. The time interval between successive images was 40 ms. Initially the cell is moving from the upper to the lower border. It stops at a point, which is indicated by the white triangle, retracts, and then continues to swim to the right.

Motion analysis. As noted earlier, the motion of the cells consists of runs, i.e., intervals of smooth swimming, frequently interrupted by short tumbles, in which the cells are reoriented randomly. As an example of a motion analysis, Fig. 4 shows, on the right, three characteristic tracks and, on the left, the corresponding graphs of angular changes during swimming, for constant (a), increasing (b), and decreasing (c) temperatures.

The temperatures and numbers of tracks used in a more detailed motion analysis are summarized in Table 1. The differences in the numbers of tracks available at different temperatures reflect the fact that cells move faster at higher temperatures; more tracks had to be discarded, as these cells moved too quickly out of the field of view. Figure 5 presents a

TABLE 1. Motion data for *T. maritima* cells

Temp (°C)	No. of tracks	Avg time interval of recorded tracks (s)	Ratio of tumbles/runs[a]
Constant			
65	155	2.11	0.42
75	144	1.61	0.47
85	48	1.33	0.64
95	119	1.23	0.59
Change (0.3°C/s)			
65–75	65	2.57	1.16
75–65	70	1.46	0.18

[a] For definition, see the text.

visualization of the distribution of step sizes and angles, obtained from these measurements, in polar diagrams; 0° corresponds to the forward direction, and the 35° lines indicate the threshold, separating runs from tumbles according to our definition.

Motion at constant temperatures. It is obvious from Fig. 5a through d that the average velocity at constant temperatures strongly increases between 65 and 95°C. The distributions are peaked in the forward direction: basically they are very similar; for instance, the ratio of runs to tumbles is almost the same for the different temperatures (Table 1).

Figure 6 shows the average duration of runs and tumbling events and the average velocity as functions of the temperature. The average velocity (Fig. 6b) increases by a factor of about 3 between 65 and 85°C and then plateaus.

There are small changes in the average duration of runs and tumbles (Fig. 6a), apart from a slight decrease in the lengths of run intervals at higher temperatures. This tendency could be due to a systematic error in the analysis: the average duration of swimming may be underestimated at high temperatures because faster cells can be monitored only for relatively short time intervals.

Response to temperature changes. Initially we applied temporal temperature changes with various rates and observed the responses of the cells by eye. The minimum rate of temperature change for which a visible response could be detected was 0.1°C/s. The cells responded to temperature changes over the whole temperature range in which they were motile. During a temperature rise, cells tumbled for prolonged intervals; a temperature decrease caused them to speed up and suppress tumbling. This can be seen in Fig. 5e and f, where the distributions of motion steps during temperature changes from 65 to 75°C and 75 to 65°C, respectively, are shown. They differ clearly from the corresponding distributions at the constant temperatures 65 and 75°C (Fig. 5a and b, respectively). Shortly after a constant temperature had been reached, the cells adapted to their normal swimming patterns. For a more quantitative evaluation, we collected data on temperature changes between 65 and 75°C with changing rates of 0.3°C/s. In this temperature range, the cells could be easily tracked because of their moderate velocity. The rate of ±0.3°C/s, which provided a 30-s time interval for the observation of motion in the presence of a gradient and a stimulus of sufficient strength to induce a clear response of the cells, was a compromise.

Figure 7 shows the time course of the temperature (a and c) and the average velocity and average change of the swimming direction (b and d) during a temperature step from 65 to 75°C within 30 s (a and b) and from 75 to 65°C, again within 30 s (c and d). The response to rising temperature (Fig. 7b) is a quasi-continuous tumbling of the cells, leading to a decrease in the speed and an increase in directional changes. During a temperature decrease (Fig. 7d) there was little variation in the directional changes but a clear increase in the velocity. The rates of temperature changes are also plotted (Fig. 7a and c), to indicate that the rate correlates positively with the velocity and negatively with the directional changes. After a constant temperature was reached, the normal tumbling frequency was restored; however, adaptation of the velocity to the value measured at a constant temperature (Table 1) takes more than 30 s.

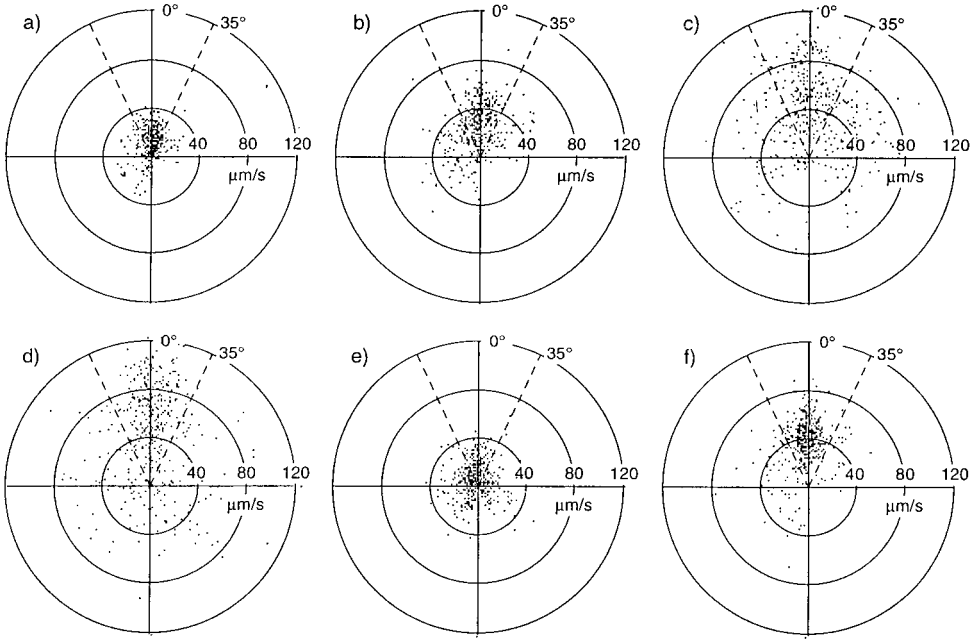

FIG. 5. Distribution of motion steps at various temperatures ([a] 65°C; [b] 75°C; [c] 85°C; [d] 95°C) and during temperature changes ([e] 65 to 75°C with 0.3°C/s; [f] 75 to 65°C with 0.3°C/s). Each polar diagram contains 400 values of the data from Table 1.

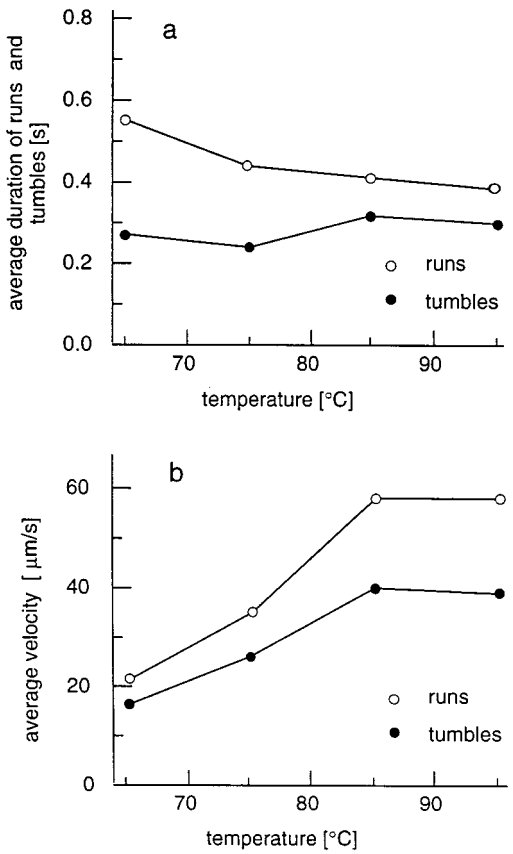

FIG. 6. Average duration of motion intervals (a) and average velocity (b) at constant temperatures (65, 75, 85, and 95°C).

DISCUSSION

The motion of *T. maritima* cells was analyzed by observation of free-swimming and tethered cells. *T. maritima* swims with a single flagellar filament that rotates clockwise. Short reversals to counterclockwise rotation cause a backing-up motion and a reorientation of the cell. The same mechanism has been reported for the motility of *Caulobacter crescentus* (9). We could not determine the handedness of the filament; therefore, it is possible that the cells are either pushed or pulled by their flagellum. It is also not known whether the filament undergoes a polymorphic transition during reversal of the direction of rotation, changing its handedness and helical parameters. Such polymorphic transitions were observed during tumbling in peritrichous bacteria (12). Comparing the distribution of angular changes between successive runs of *T. maritima* with that of the peritrichous *Escherichia coli* (data not shown), we found that the latter has a stronger tendency towards forward angles. The single flagellum of *T. maritima* seems to provide faster propulsion and a more effective reorientation.

The motion of cells, adapted to a constant temperature, is an unbiased three-dimensional random walk. The average swimming speed showed an almost linear increase from 21 to 58 μm/s when the temperature was raised. The machinery which generates propulsive motion is strongly dependent on the absolute temperature. Besides the changes in the activity of the cellular enzymes, there is also a significant change in the physical properties of the medium. The viscosity of water decreases almost linearly by about 25% from 0.46 to 0.35 mPa · s between 60 and 80°C (3). As the frictional drag on a swimming cell increases linearly with the velocity, this means that the reduction of the viscosity contributes only a small fraction to the 2.8-fold increase in velocity between 55 and 85°C. The output power of the motor is maximal at 85°C. At higher temperatures, the decrease in viscosity is obviously compensated for by a reduction of motor power, and therefore, the velocity remains constant.

In contrast to the speed, the frequency of changes in the swimming direction is almost constant in the same temperature range. Cells that are adapted to a constant temperature show a constant rate of tumbling. Their motion can be described as a three-dimensional random walk with a temperature-dependent length of running intervals.

The temperature-controlled observation chamber allowed us to investigate the changes in bacterial motility as a response to temperature changes. Such detailed analysis has not been carried out before with thermophilic organisms. A temporal stimulus with a rate of 0.3°C/s is encountered by a bacterium, which moves along a spatial gradient of 100°C/cm with a speed of 30 μm/s. Temperature variations of this magnitude have been found with hydrothermal vents (20). The tactic response to temperature changes could be observed over the whole temperature range in which the cells are motile. The response is almost instantaneous after the onset of the temporal gradient. The change in motility correlates with the rate of temperature change, i.e., thermoresponse is a gradient-sensitive mechanism. Adaptation appears to be more complex than stimulation; there are at least two mechanisms with different time constants. While the tumbling frequency of the cells returns to its unstimulated value shortly after a constant temperature has been reached, the speed approaches its normal value much more slowly. To gain more detailed insights into the relationships between stimulus, response, and adaptation, the observation of individual, tethered cells over prolonged time intervals appears to be an alternative and possibly better approach (8). It would allow us to study the dynamics of individual cells and, thus, to eventually observe differences between the cells.

It is intriguing to compare the thermosensory behavior of *T. maritima* with the observations obtained with *Escherichia coli*. In the latter case, it has been reported that the methyl-accepting chemotaxis proteins (MCP) Tar and Tsr are involved in the thermoreception. They are chemoreceptors for amino acids such as serine (Tsr) and aspartate (Tar) and for sugars (Trg) and dipeptides (Tap) (11), which serve as nutrients for the cells. In wild-type cells the numbers of Tsr and Tar receptors are very large compared with the numbers of the other two receptors. Rising temperature is sensed as an attractive stimulus, which reduces the frequency of tumbling (7). Thermosensing can be inhibited by the addition of serine, while the simultaneous addition of serine and aspartate results in an inverted response. Mutant cells with an increased level of Trg or Tap are also sensitive to changes in the temperature (16); however, while Trg mutants show the same response as wild-type cells, i.e., a reduction of the tumbling rate with rising temperature, the tumbling rate is increased in Tap mutants. This phobic reaction is also observed with *T. maritima*. The addition of serine had no effect on the thermoresponse of the cells. This is not unexpected, as the bacterium grows exclusively by fermentation of carbohydrates. Sensing of amino acids is of no use for the cell, and therefore, it is very improbable that a receptor exists for these substrates. Attempts to identify MCP-homologous proteins in *T. maritima* by immunoprecipitation with an antibody against Trg gave a negative result (15). If ancestral MCP proteins are present in *T. maritima*, they have only weak homology to the MCPs in *E. coli*. It is possible that

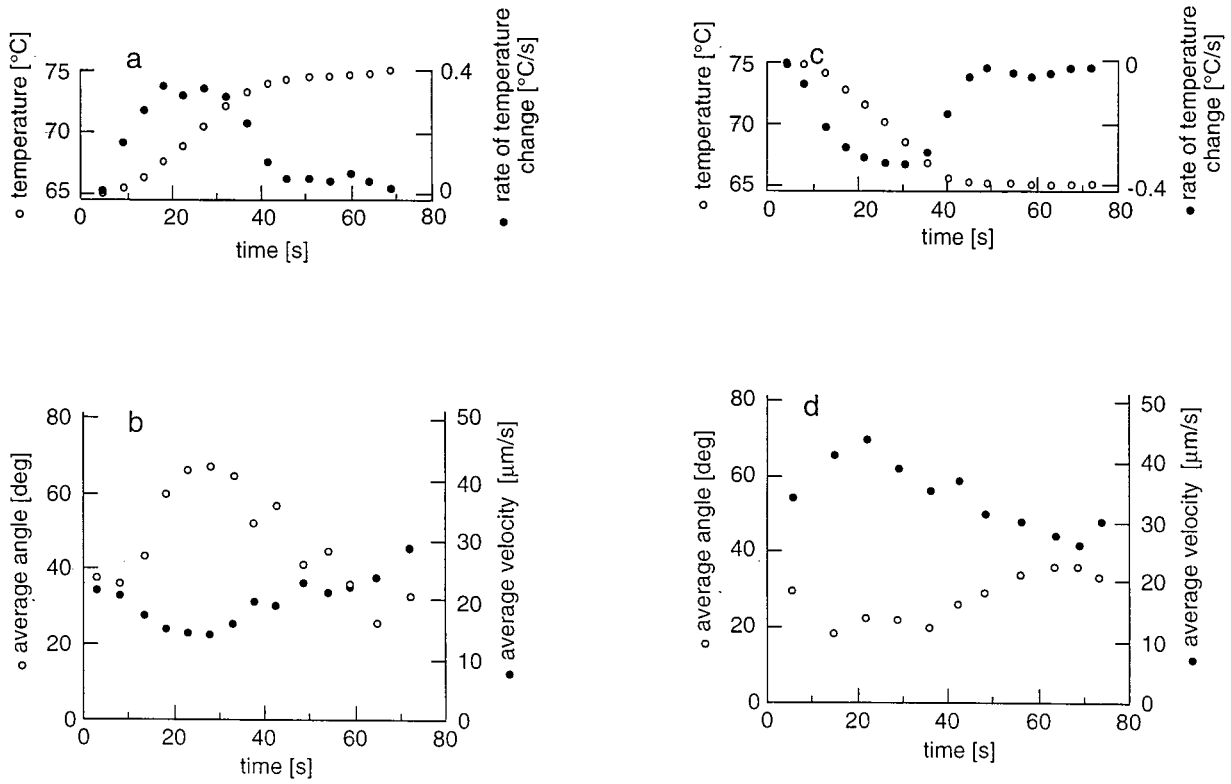

FIG. 7. Motion of *T. maritima* cells during temperature changes. (a and c) Temperature and its time derivative; interval, 10°C; rate, 0.3°C/s and −0.3°C/s, respectively. (b and d) Motion parameters. In order to reduce statistical fluctuations, every point is an average of 200 original datum points (the corresponding time intervals are slightly different).

the mechanism of the thermophobic behavior of *T. maritima* is quite different from the well-known MCP-dependent signal transduction in *E. coli.*

What might the advantage of this phobic response for a thermophilic bacterium in its natural habitat be? It can serve as a survival mechanism, which keeps cells away from high-temperature regions which are lethal for them. However, temperature acts as a repulsive stimulus over the whole temperature range in which the cells are motile. A bacterium is thus biased towards lower temperatures, even those below the optimum growth temperature. This does not seem to be a good strategy for finding optimal growth conditions. In principle, an optimal thermosensor should respond in a dual way to rising temperature, depending on the absolute temperature: above the optimum growth temperature, increasing temperature should be a repulsive stimulus, and below the optimum growth temperature, it should be an attractive stimulus. The solution to this paradox may be that temperature is not the only environmental parameter that is important for the cell. It is very likely that other sensory inputs will, together with the temperature, contribute to a balanced response of the cell. At high temperatures the phobic temperature response may be the predominating component, while at lower temperatures environmental stimuli such as the availability of nutrients might dominate the tactic behavior. There is very little knowledge about the habitats of these cells and the ecological relationships with other organisms in the natural environment. The aim of this work was to give a quantitative phenomenological description of thermotactic behavior. It will be the task of future work to identify the underlying molecular mechanisms and also to elucidate their function in the natural environment.

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