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Unidirectional Motility of *Escherichia coli* in Restrictive Capillaries

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In a 6-μm capillary filled with buffer and in the absence of any chemotactic stimuli, *Escherichia coli* K-12 cells swim persistently in only one direction. This behavior of *E. coli* can be simply explained by means of the length and relative rigidity of their flagella. Single-cell motility parameters—swimming speed, turn angle, and run length time—were measured. Compared with the motility parameters measured in bulk phase, turn angle was influenced because of the effect of the geometrical restriction.

The swimming of motile bacteria as a result of movement of their flagella has long been known. According to the mechanism of bacterial motility proposed by Berg and Anderson (4), movement is generated through rotation of the bacterial helical flagella, which act like a propeller. The rotation of the flagella was further demonstrated by Silverman and Simon (29), who were able to tether *Escherichia coli* mutants to the surface of a microscopic slide via their flagellar hooks, by introducing antipolyhook antibody in the bacterial suspension. The movement of cells was then monitored under a microscope. When a bacterium is tethered, it spins counterclockwise and clockwise alternatively with almost the same angular speed, changing its direction randomly and abruptly (3, 29). It is well established that flagellar movement is driven by a motor system (4, 16, 19, 20), that the counterclockwise and clockwise rotations of bacterial flagella correspond to smooth swimming and tumbling, respectively (17), and that bacteria direct their migration by changing the frequency of tumbling (5, 21, 30).

Bacterial swimming is called chemotaxis when it occurs preferentially in the direction of an increasing concentration of attractants or a decreasing concentration of repellents. In the absence of such gradients microorganisms may still swim, but only randomly, in which case their movement is referred to as random motility. When there is no spatial or temporal concentration gradient of chemotactic stimuli, bacterial swimming resembles a three-dimensional random walk, during which bacteria frequently change their moving direction. When cells are tracked individually, the parameters often used to describe their swimming behavior are swimming speed, tumbling frequency (reciprocal mean run length time), and index of directional persistence, which is equal to the mean of the cosine of the run-to-run angle in three dimensions (23). For *E. coli*, a peritrichous bacterium, the duration of a tumbling is ~0.1 s and the interval between tumblings is ~1 s. After each tumble, the bacterium randomly chooses a new swimming direction (5).

Bacterial motility is exhibited not only in bulk phase but also, and more commonly, in geometrically restrictive environments (12, 15, 25, 28). In the latter, the interaction between bacterial cells and neighboring surfaces may play an important role in bacterial transport. In this article, the term "interaction" between a cell and a surface or between two cells is not confined to physical contact. It includes mutual proximity that allows the fluid flow field around a cell, as well as its physicochemical characteristics, to influence (and be influenced by) its proximate surfaces and cells. In restrictive geometries, due to spatial limitations, motile bacteria may be prevented from reversing their swimming direction. Moreover, they may also reach the solid surface before they finish one run. This migration behavior differs significantly from that in unrestricted bulk. Recently, a number of studies have addressed bacterial motility on or close to solid surfaces (8, 14) and through restrictive passages. Using light scattering, Berg and Turner (6) studied motility and chemotaxis of bacterial populations in an array of fine-glass capillaries. They found the net migration of wild-type *E. coli* cells in 10-μm capillary tubes to be faster than in 50-μm capillary tubes. This was attributed to the straight segments of bacterial runs being aligned with the axis of the smaller microcapillaries. In studying the motility of individual bacterial cells, Biondi and Quinn (7) found that the swimming speeds in unrestricted bulk were comparable to those in a rectangular chamber with one restrictive dimension.

The objective of our work is to study the effect of two-dimensional geometrical restriction on the swimming behavior of motile bacteria. Studying the movement of bacterial cells in small capillaries will help elucidate the mechanism of bacterial transport through contaminated soils and sediments, which is necessary for in situ bioremediation of contaminated sites.

MATERIALS AND METHODS

Microcapillaries. A microcapillary with a desired inside diameter was made as follows. A 30-μl micropipette with a 0.275-mm inside diameter and a length of 78 mm (Drummond Scientific Company) was pulled in the center with a micropipette puller (Narishige). By controlling the pulling times and the pulling length, capillaries with different inside diameters can be made. A 6-μm capillary is made by pulling the micropipette three times, with pulling lengths of 7, 6, and 6 mm, respectively. During the pulling process, nitrogen flows through the capillary to prevent fusion, and the pulling speed is controlled manually. The length of the capillaries can vary from 5 to 10 mm, so that any given capillary segment captured by the microscope has a uniform radius. Another fact about capillary dimensions that should be noted is that the capillary inside diameter is burdened by a 0.4 μm uncertainty. The inside diameter was measured with a video microscope, and the capillary was in the air. Because of the effect of the curvature of the capillary, there is a dark area near the capillary wall and the inside wall edge cannot be determined precisely.

Bacteria. *E. coli* K-12 (American Type Culture Collection) was used in this study. One liter of culture medium contained 11.2 g of K_2HPO_4, 4.8 g of KH_2PO_4, 2.0 g of $(NH_4)_2SO_4$, 0.25 g of $MgSO_4 \cdot 7H_2O$, 0.0005 g of $Fe_2(SO_4)_3$, and 1 g of galactose (autoclaved separately) in deionized pure water. *E. coli* cells were grown to stationary phase at 30°C and 180 rpm in an incubator shaker (New Brunswick Scientific, Inc.) and harvested by centrifugation. The cells were washed three times (1) with a pH 7 motility buffer (2) (11.2 g of K_2HPO_4, 4.8 g of KH_2PO_4, and 0.029 g of EDTA per liter of water). The centrifugation force was 7,000 × *g*, and each of the four centrifugations took 18 min. From the direct observation under the microscope, the motility was still good, in agreement with

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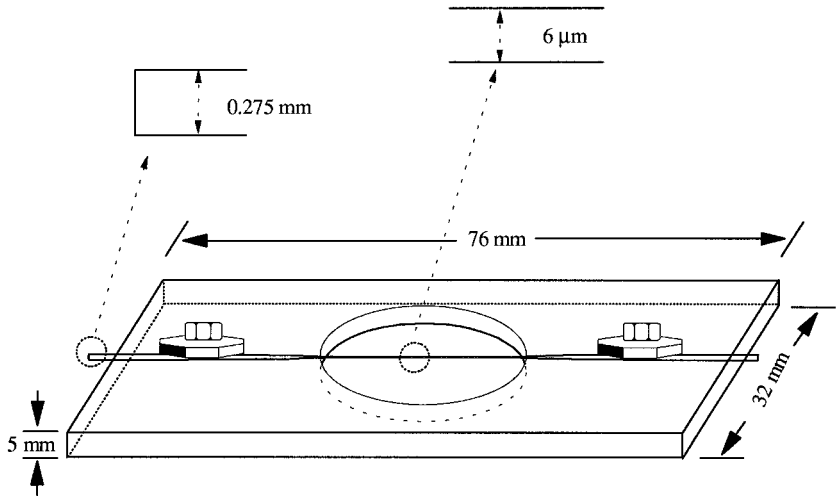

FIG. 1. Schematic of the capillary and capillary holder. The objective (40×, long working distance) is under the capillary, and the light comes from the top of the capillary, passes a green filter and a light condenser, and reaches the objective.

the assertion of Adler (1) that the washing procedure is safe for preserving the motility. Finally, the cells were suspended in the motility buffer with the density of $\sim 10^9$ cells per ml. During the process of making the capillary, the bacterial suspension was stored at 4°C to reduce the metabolism of the bacteria.

Motility assay. A capillary with a desired inside diameter was mounted on a capillary holder (Fig. 1) and then placed under a video microscope with the capillary in the air. With a long working distance objective (40×; Nikon) and a zoom connected between the video camera and microscope, the total magnification was ×600. The bacterial suspension was introduced into the capillary by way of a micropipette and a manual syringe as follows. First, the bacterial suspension was suctioned into a micropipette (outside diameter, ~20 μm); then, the micropipette was held with a micromanipulator (Narishige) and inserted into the capillary. The bacterial suspension was injected into the capillary manually with a syringe. The movement of cells was microscopically followed and captured with a high-resolution black-and-white video camera (Cohu) at room temperature (~22°C) and recorded with a 30 frames per s videocassette recorder (Sony). Because of the minuteness and fragility of the capillary, it was not possible to seal its ends. This led to convective flows, most likely caused by "meniscus pumping" due to the unequal evaporation rates at the menisci of the two ends after injection. Such flows were completely eliminated generally 1 h after the cells were introduced into the capillary. During the period of unperturbed unidirectional motility in the capillary, 1,123 *E. coli* cells were tracked.

The run length time was determined by counting the number of videotape frames between two consecutive tumbles and converting it to real time. Turn angles and run lengths were determined with an image analysis system (Bioscan). Run lengths were determined from the *x-y* pixel positions of the beginning and the end of a run, and swimming speeds were calculated from the run length and run length time. Turn angles were measured when two consecutive runs occurred on the same focus plane. By image analysis, a straight line can be created from the beginning to the end of a run, and the angle between this line and a horizontal reference line can be obtained automatically. Turn angle can thus be calculated from the two angles formed between the run line and the reference line of two consecutive runs.

RESULTS AND DISCUSSION

In the absence of a chemoattractant gradient, motile cells swim randomly in unrestricted bulk. However, in capillaries with inside diameters smaller than the length of bacterial flagella, the pattern of bacterial swimming changes. In this study, experiments were conducted with capillaries with inside diameters of 6, 10, and 50 μm. The swimming behavior of bacteria in the 50-μm capillary was similar to that in unrestricted bulk. In 6- and 10-μm capillaries, however, we found that the bacteria not only swam with reduced randomness along the capillary but were also unable to reverse their swimming direction. While tumbling did occur, the turn angle was rarely larger than 90°. Whereas the swimming patterns of bacteria in the 6- and 10-μm capillaries are very similar, in this article we report only data for the 6-μm capillary, in which the swimming of the cells occurs mostly on the same focal plane. Single-cell motility parameters were compared with previously reported values, but it should be noted that the microenvironment locally inside the capillaries and around individual cells may be different from those reported in the literature. The distribution of turn angles in the capillary was biased from normal (Fig. 2), with a mean angle of 38° and a standard deviation of 25°. The turn angles for *E. coli* measured by Berg and Brown (5) were 68 ± 36°, and the distribution was almost normal.

The run length time was 0.81 ± 0.70 s (mean ± standard deviation), and the distribution is shown in Fig. 3. Run length time measured by Phillips et al. (24) for *E. coli* AW405 was 0.84 ± 0.71 s with an exponential distribution, while for the same strain Berg and Brown (5) reported a run length of 0.86 ± 1.18 s. The tumbling probability can be obtained by plotting the natural logarithm of the fraction of run length times which

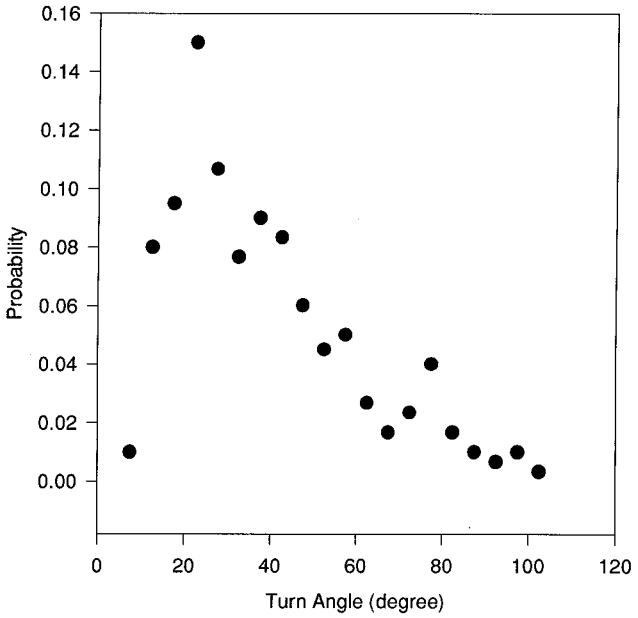

FIG. 2. Turn angle distribution of *E. coli* in a 6-μm capillary. The plot is based on 300 measurements, and turn angles were grouped in 5° clusters.

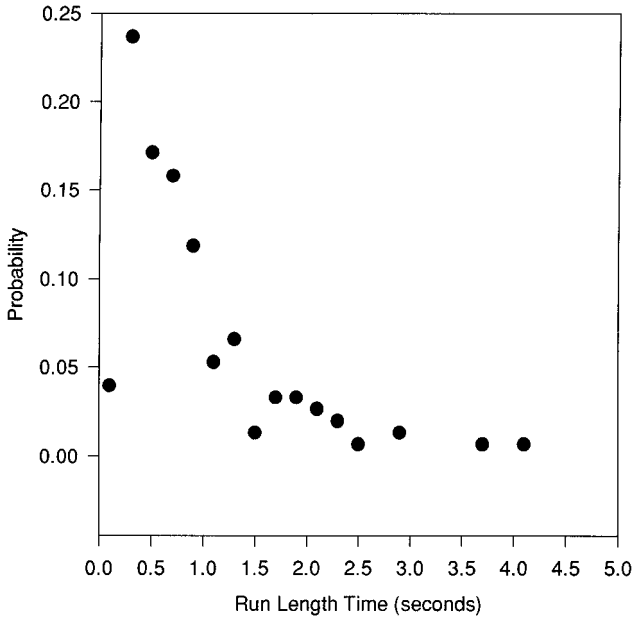

FIG. 3. Run length time distribution of *E. coli* in a 6-μm capillary. This plot represents 300 measurements, while the run length times were grouped in 0.2-s clusters.

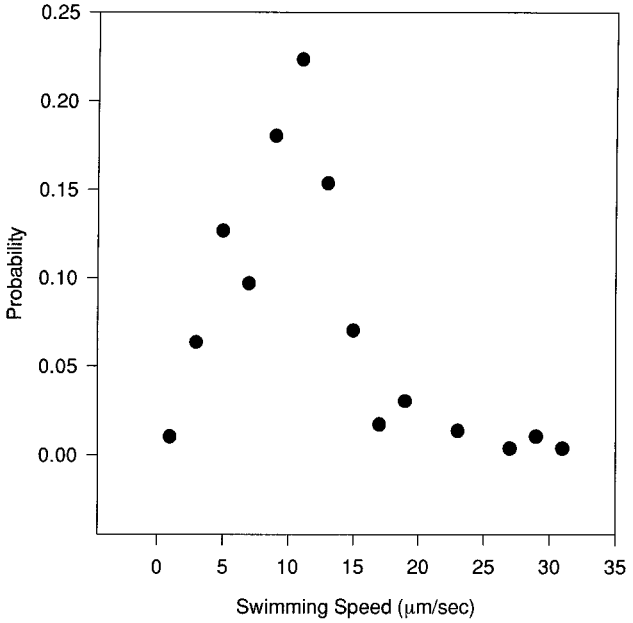

FIG. 5. Swimming speed distribution of *E. coli* cells in a 6-μm capillary. The plot is based on 300 measurements, and the velocities were grouped in 2-μm/s clusters.

are greater than a given time versus time (24) (Fig. 4). The tumbling probability thus obtained is 1.4 s^{-1}.

The mean swimming speed in our experiment was 11.4 ± 3.5 μm/s and is almost normally distributed (Fig. 5). Swimming speed is known to be affected by viscosity (13, 26), temperature, and culture medium (18) and to vary from strain to strain. Besides the influence of experimental conditions and strain type, experimental methods may also affect the results. For *E. coli* AW405, Berg and Brown (5) measured a swimming speed of 14.2 ± 3.4 μm/s at 32°C in a motility medium with a viscosity of 2.7 cP in an unrestricted geometry. However, for the same strain, the swimming speed determined by Phillips et al. (24) in bulk was 24.1 ± 6.8 μm/s, much higher than that determined by Berg and Brown. The swimming speed reported here is low compared with those reported previously. One of the reasons is that our bacteria were starved for about 3.5 h, which included the time used for bacterial washing, capillary preparation, bacterial injection, and about 1 h of waiting for the convection flows to disappear before the recording started. Also, our culture medium was not as rich as that of Berg and Brown (5) and Phillips et al. (24), and as known (18), bacteria grown in a relatively richer medium swim faster. It should be noted, however, that the bacteria remained active and alive during the entire experiment and that motility was preserved.

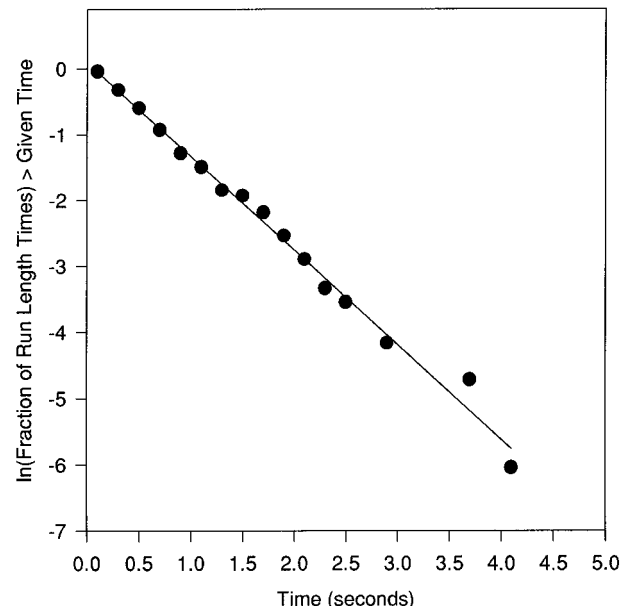

FIG. 4. Distribution of run length times presented in terms of the natural logarithm of the fraction of run length times larger than a given time versus run time in a 6-μm capillary. The absolute value of the slope gives the tumbling probability, 1.4 s^{-1}.

In their relatively unidirectional movement, bacteria swam radially as well but always maintained their unidirectional axial motion, as they failed to turn around. When bacterial cells swam towards the capillary wall, their interaction was rarely a collision. In many instances the cells swam along the wall. Cells swimming in opposite directions inside the same capillary exhibited the same mean swimming speed, thus removing any possibility of forced convection flows.

Even though still photographs cannot fully convey the intricate details of bacterial swimming in very fine pores, a number of important observations may nevertheless be made by looking at a typical sequence of stills. Figure 6 portrays such a sequence of photographic stills, each 1/3 s apart from the next, for a section of a 6-μm capillary over a period of 5.33 s. The swimming of the eight different cells shown is representative of the cells that were tracked. To facilitate the discussion of the cells' movement, the individual bacteria were colored differently. Of particular interest are stills showing bacteria crossing paths. Still 8, for example, shows five bacteria sharing a space of less than 8 μm within a capillary tube, thus being forced to interact. While some bacteria maintain a steady swimming

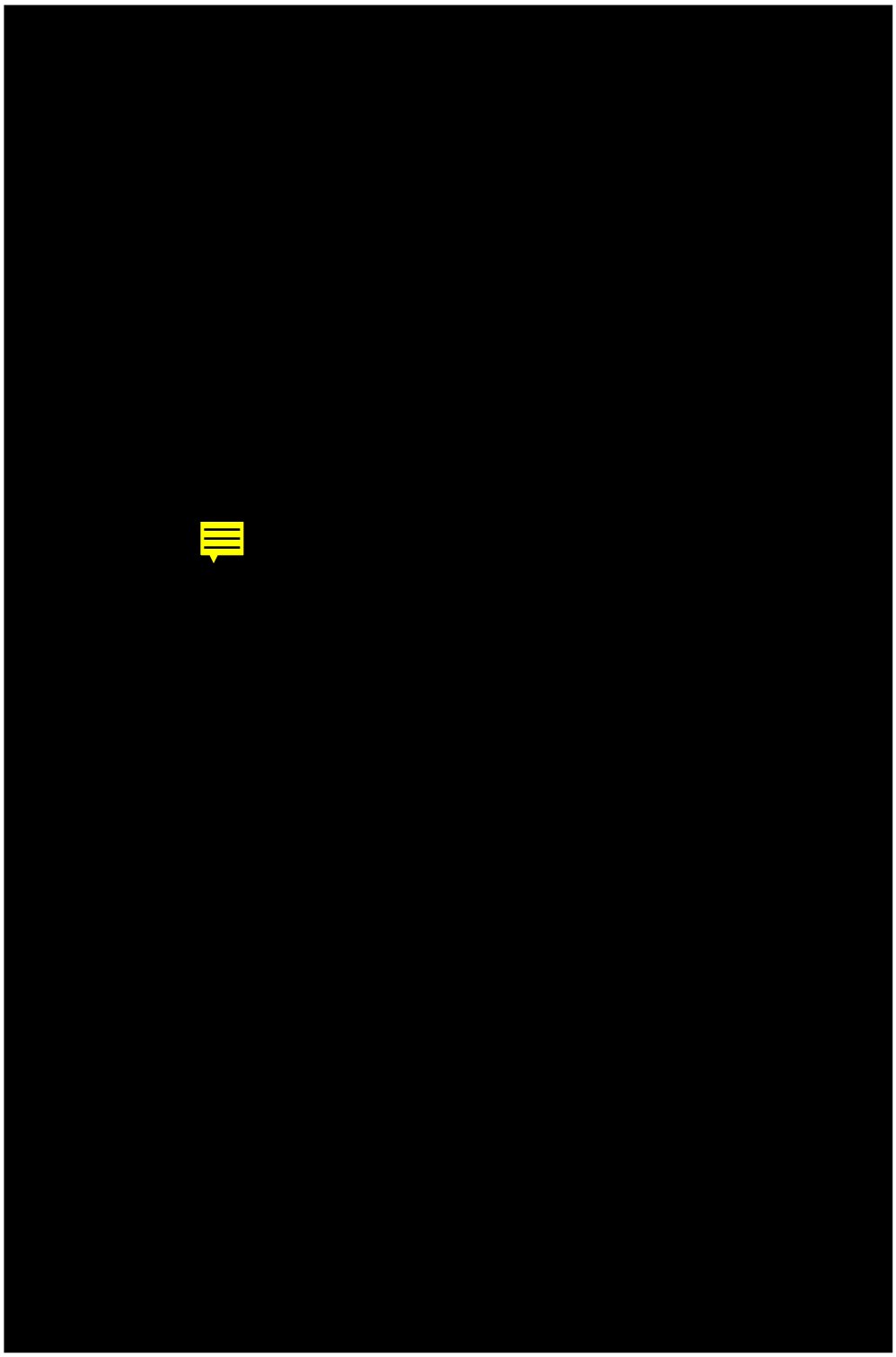

FIG. 6. Sequence of 15 photographic stills, 0.33 s apart, depicting the swimming of eight *E. coli* cells in a 6-μm capillary. By monitoring the real-time swimming of these cells, it was observed that the purple cell close to the right edge in each still appeared to be tethered to the wall. This cannot be seen in the figure.

speed for several seconds, others change it somewhat, especially in proximity of other bacteria. In stills 4 through 10 it is worth noticing the movement of the slowly moving yellow cell, as it is about to be passed by the blue cell (still 4), is about to cross paths with the red and purple cells (stills 5 and 6), is at the same axial position with the purple cell (still 7), has just crossed paths with the red and purple cells and is about to be passed by the green and orange cells (still 8), and is finally

passed by the green cell (still 9) and later by the orange cell (still 10). The series of interactions with other cells causes the yellow cell to have a net backward movement of ~1.6 μm for ~0.6 s. Similar effects have recently been observed in numerical simulations of simultaneous chemotaxis and induced fluid flow around cells (11). It was observed that the convection induced by some cells can cause their neighbors to be displaced opposite to their swimming direction. After these interactions are ended, the cell resumes its prior motion.

Such net backward displacement was observed with 93 cells (or 8.3% of the total number tracked in the 6-μm capillary). In such cases, the cells did not reverse direction of swimming, since our visual observations suggest that the cells did not change their orientation. In one instance, two bacteria experienced a head-on collision, which caused them to pause for 0.2 to 0.3 s before resuming their prior swimming speeds and directions. In the only observed apparent discrepancy from unidirectional movement, the bacterium in question seemed to be tethered for ~1.6 s to the capillary wall while other bacteria swam past it in both directions as it dangled. Then, for a period of ~3.6 s it moved ~12.5 μm opposite to its original direction, before subsequently turning and resuming its original direction. Seventeen cells (1.5% of the total number tracked) in this experiment adhered onto the capillary wall. In no case, however, did we observe aggregation of the cells.

A speculative explanation of the observed phenomena involves the action and the relative dimensions of the flagella. When an *E. coli* cell is swimming, individual rotating flagella form a bundle due to hydrodynamic forces, and when it tumbles the flagella unravel in a disorderly fashion (9, 10, 27). The relationships between peritrichous-bacterium motility, chemotaxis, and changes in the quaternary structure of the flagella were thoroughly investigated by Macnab and Ornston (22). While the dimensions of the cell body are 1 to 2 μm, the flagella are several times longer (5 to 10 μm). Therefore, when a bacterium starts to tumble or turn in a narrow capillary, with the capillary dimension being smaller than flagellar length, the movement not only of the cell's body but of the flagella as well is restricted. The flagella therefore cannot fully reorient themselves 180°, in turn making it impossible for a given bacterium to permanently reverse its swimming direction. In their studies of chemotaxis of bacterial populations in glass capillary arrays, Berg and Turner (6) reported that diffusion coefficients were much higher in 10-μm capillaries than expected in free solution. This increase was attributed to the occurrence of cell displacement mainly along the axial direction. Although their experimental conditions, such as growth conditions, bacterial swimming medium, and structure of capillary tubes, were different, our direct observation of bacterial swimming is in qualitative agreement with Berg and Turner's finding.

Conclusions. In the first reported experiments tracking the movement of individual bacteria inside capillaries with diameters smaller than or comparable to the length of the flagella, we have found that, in the absence of any chemotactic stimuli, individual *E. coli* K-12 wild-type cells can swim in only one direction. Unidirectional movement of an individual cell is defined as movement along the capillary tube towards one of its ends, while reversal of direction means the switch of bacterial movement towards the other end of the capillary. Movement consists of runs and tumbles and does not imply straight-line motion parallel to the capillary's axis. To explain the cells' failure to reverse their swimming direction, it is hypothesized that their flagella are not short or flexible enough. The finding of this phenomenon is important in understanding flagellated bacterial migration through any porous medium with pores or passages in the range of a few micrometers to a few tens of micrometers. In measuring single-cell motility parameters (swimming speed, turn angle, run length time, and tumbling probability) in a 6-μm capillary, we found that only the turn angle was significantly affected by the geometric restriction.

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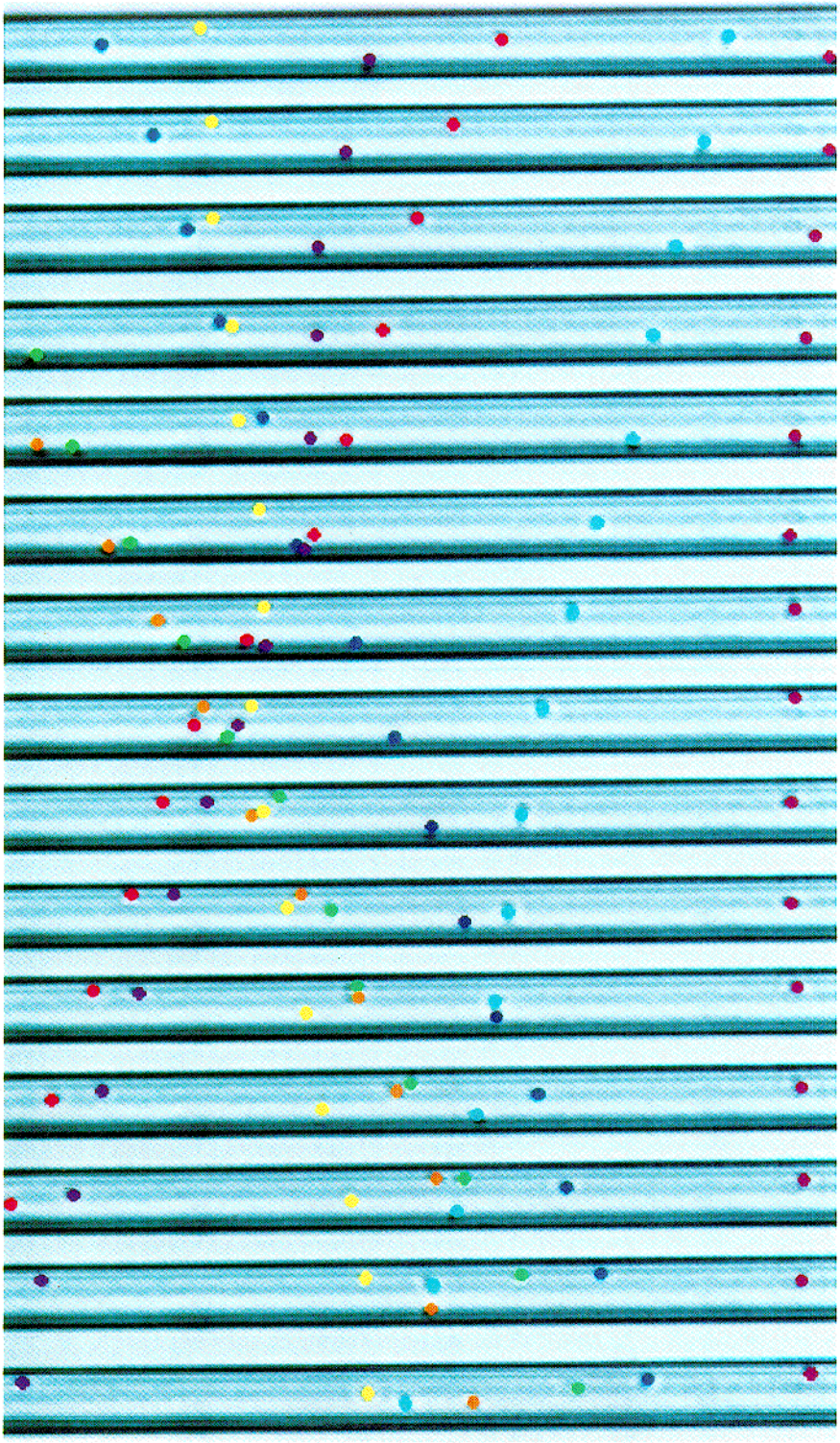
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