Random Motility of Swimming Bacteria in Restricted Geometries

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The effects of restricted geometries on the swimming behavior of Escherichia coli were examined. Videomicroscopy and image analysis techniques were used to measure single-cell motility parameters (cell velocity, tumbling probability, and turn angle) in microfabricated 2-D channels ranging from 2 to 20 μm in height. Macroscopic motility coefficients were calculated from the single-cell data and compared to literature values. These observations indicate that single-cell parameters remain nearly constant in confined spaces in which channel heights approach the size of the bacterium. The nearly constant swimming behavior of individual cells in confined geometries indicates that the macroscopic motility coefficient describing population behavior will be greater than the unrestricted value by a factor proportional to the dimensionality of the system.

Introduction

Designing and interpreting processes such as bioremediation requires a detailed knowledge of biotransport. In order to predict the flux of cells through microporous substrates such as soil, oil-covered beaches, and river-bed sediments (Jenneman et al., 1985; Quenson et al., 1988; Gannon et al., 1991; Jenkins et al., 1994; Sarkar et al., 1994), one must know the factors controlling *bacterial* movement in these environments. (Pores in soil vary depending on the soil type. Fine pores are considered to be approximately 2 to 20 μm in size (Russell, 1973).) While much is known of the swimming behavior of bacteria in unrestricted environments (Adler and Dahl, 1967; Berg and Brown, 1972; Macnab, 1979; Ford et al., 1991; and Phillips et al., 1994), the added complication of propulsion through a microporous environment can affect the cell's ability to rotate or maintain an optimal speed, which in turn will alter the rate at which bacteria penetrate a given substrate.

The bacterium considered in this study is *Escherichia coli*.. Visual observations have revealed several distinctive features of its run-and-tumble behavior. The cell body is approximately 1×2 μm with flagella 20 nm in diameter that extend approximately 8 μm in length (O'Brien and Bennett, 1972). These cells swim in nearly linear trajectories ("runs") propelled by a counterclockwise rotation of their flagella. Each run is interrupted by an erratic rotation ("tumble") of the cell in place. This is caused by a change to a clockwise rotation of the flagella. By continuously alternating between runs and tumbles, the trajectory of the cell closely resembles that of a random walk, similar to that of a Brownian particle (Berg, 1983). For this reason, the motile behavior of a population of cells is described in terms of an equivalent diffusion coefficient, μ, the random motility coefficient.

Experimentally the behavior of individual flagellated bacteria can be described in terms of run lengths, run times, and turn angles (the angle between two successive runs) of a cell. From these one can calculate the tumbling probability, λ_T, the cell speed, s, and the index of directional persistence (the mean cosine of the angle between two consecutive runs), ψ_d. Since these parameters give a complete description of the motion of an individual cell, they can also be related to the net motion of a population of cells. Othmer et al. (1988) derived an expression (Eq. 1) that relates the macroscopic motility coefficient to the microscopic single-cell parameters, where the factor n_d represents the dimensionality of the system:

$$\mu = \frac{s^2}{n_d \lambda_T (1 - \psi_d)} \tag{1}$$

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Several researchers have measured either the macroscopic motility coefficient, μ, or selected single-cell motility parameters. The classic work of Berg and Brown (1972) and others has been recently reviewed (Phillips et al., 1994; Biondi, 1995). Berg and Brown devised a three-dimensional tracking microscope (Berg, 1971) to measure each of the single-cell motility parameters for *E. coli* AW405. They showed that the cell velocities were distributed normally, while the turn angles were skewed from isotropic in favor of angles smaller than 90 deg. Their measurements also verified that given the Poisson nature of the tumbling probability, the run times (the times between tumbles) were exponentially distributed. Phillips et al. (1994) measured the motility coefficient for *E. coli* AW405 in various concentrations of serine, an attractant known to affect *E. coli* motility, using a stopped flow diffusion assay (Staffeld and Quinn, 1989). Cell speed and run-time distributions were also measured at the single-cell level. By comparing these two results, they calculated a value for the index of directional persistence, ψ_d.

The motility experiments and mathematical model discussed previously pertain to cells swimming in an unbounded medium. Few researchers have studied quantitatively the effects of restricting geometries on bacterial motility. Berg and Turner (1990) conducted experiments in which random motility coefficients were measured in restricting environments (uniform capillaries with diameters of 10 and 50 μm) for several cell strains varying in their run and tumble times. They noted that the random motility coefficients measured for wild-type *E. coli* in a 10-μm microcapillary were 2.3 times greater than those observed in 50-μm capillaries, and concluded that the motility behavior of cells in a confined space was enhanced by interactions with the pore wall. More specifically, they hypothesized that the increase in the motility coefficient is due to "guidance." As the size of the cell approaches that of the size of the pore, movement is restricted solely to the axial direction.

Barton and Ford (1995) measured motility coefficients in a more complex environment. They examined the transport of *Pseudomonas putida* as the cells migrated through sand columns. They found that motility coefficients decreased quite dramatically with a decrease in average particle (and hence pore) size and note that this is likely due to the tortuosity of such a complex porous network (see also Duffy et al., 1995, for a related simulation study).

As stated earlier, these experiments were conducted by observing cell-population dynamics. To date few researchers have examined individual cells as they move through restricting environments. Liu and Papadopolous (1995, 1996) observed *E. coli* as they migrated through 6-μm, 10-μm, and 50-μm capillary tubes. They noted that the turn-angle distribution in 6-μm capillaries was nonisotropic, while cell speed and run-time data were similar to that measured in the bulk. Frymier et al. (1995) and Frymier and Ford (1997) examined bacteria as they approached and swam near solid surfaces. They measured cell velocities as a function of distance from the planar surface and approach angle, and compared these measurements to theoretical velocities.

The goal of this study is to measure each of the single-cell parameters for bacteria in a range of restricting channel heights and to use these measurements to predict macroscopic motility coefficients. Single-cell motility parameters were measured for *E. coli* in channels ranging between 2 and 20 μm in height. The channels were prepared using silicon etching techniques to etch polymer films on glass-slide surfaces. Using a microscope connected to a video camera and a time-lapse videocassette recorder, we were able to record cell movement within a channel. Single-cell parameters were measured directly from the video tapes using a computer-aided image-analysis system. Since all of the single-cell parameters were measured, a random motility coefficient could be calculated and compared to the measurements of Berg and Turner (1990), Phillips et al. (1994), and Barton and Ford (1995). A significant advantage of the videomicroscopy technique used here is that it provides direct visual information on the swimming behavior of the bacteria. This helps to better understand the motility data. The single-cell parameters measured in restricting geometries varied little with respect to those measured in the bulk demonstrating that these cells can swim through pores only slightly larger than their own body with little change in their behavior. The calculated motility coefficients compared well with macroscopic motility measurements made by Berg and Turner (1990) and Phillips et al. (1994).

Experimental Studies

Cell culture

The strain used in this study was *Escherichia coli* AW405, which is *K-12, gal-1, gal-2, thr, leu, his-4, lac, xyl, ara, strA, tonA, tsx*, and wild-type for chemotaxis. To make a stock supply of cells (stored at -55°C), samples were taken from the outer edge of spreading colonies. These colonies were grown on swarm plates made with Luria broth and 0.2% agar (Adler, 1966). For experiments, a sample of the stock solution was transferred into a flask of minimal media with an inoculating loop. The medium contained 5 g/L of glucose, which served as an energy source and 0.25 g/L of each of the necessary amino acids for growth of this strain: *L*-threonine, *L*-leucine, and *L*-histidine. The inorganic portion of the medium consisted of 11.2 g/L K_2PO_4; 4.8/g/L KH_2PO_4; 2.0 g/L $(NH_4)_2SO_4$; 0.25 g/L $MgSO_4 \cdot 7H_2O$; and 0.0005 g/L $Fe_2(SO_4)_3 \cdot 5H_2O$ (Adler and Dahl, 1967). The inoculated culture was grown in a rotary shaker at 150 rpm and 30°C and harvested at an absorbence of between 0.45 and 0.65 at 590 nm. (For more details on growth procedures for this strain, see Phillips et al., 1994). All experiments were carried out at room temperature ($24° \pm 2$°C) within 2 h of harvesting the cells.

Channel formation

The channels were micromachined using traditional silicon etching techniques (Peterson, 1982). Silicon, however, could not be used for these experiments since cell visualization is best done with transmitted light (silicon would necessitate the use of reflected light). As an alternative, channels were fabricated by etching a UV-sensitive polymer that had been spin-coated onto a glass slide. A diagram of a channel used in these experiments is illustrated in Figure 1. The polymer used was determined to be nontoxic to bacterial motility during the relevant experimental time by comparing single-cell parameters measured in these fabricated channels with those

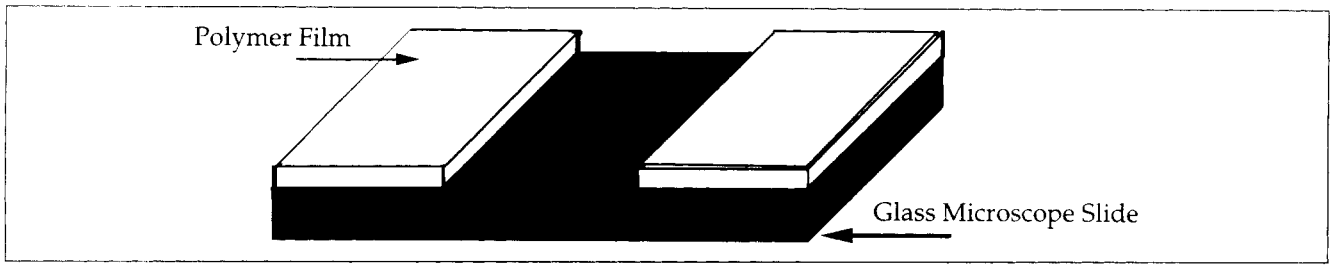

Figure 1. Glass microscope slide with etched polymer (photoresist) film.

Photoresist was spin-coated onto glass microscope slides; the thickness of the film could be controlled to ±5%. For experiments, a coverslip was sealed across the top of the channel.

measured in glass channels of an equivalent size (data not shown).

Before applying the polymer, the glass slides were cleaned in H_2O_2–H_2SO_4 [1:2 v/v] for 9 min at 120°C, rinsed in DI water, and then placed in a mixture of H_2O–NH_4OH–H_2O_2 [5:1:1 v/v] at 75°C for 10 min. After a final rinse in DI water, the slides were spun dry. To avoid recontamination, they were coated immediately.

The polymer, photoresist, was spin-coated onto a glass slide at a thickness equivalent to the desired channel height. (Photoresist is used traditionally in etching lithography and is primarily composed of the following compounds: 2-ethoxy ethyl acetate, xylene, and *n*-butyl acetate.) Two resists were used: Shipley 1813 photoresist (Newton, MA) for channel heights less than 4 μm and Hoechst 4903 ultrathick photoresist (Dallas, TX) for film thicknesses equal to or greater than 10 μm. Spinner speeds were chosen to achieve the desired film thickness. For 3-μm geometries, using the 1813 resist, the spinner speed was approximately 2,500 rpm, while for the thick resist, 10-μm films were made at speeds of 6,000 rpm. After spin coating the polymer onto the glass, the slides were soft baked (3 min. at 85°C for the Shipley resist and 1 min at 65°C for the thick resist) to evaporate the volatile solvents. To make the channels, the slides were first exposed to 405-nm light through a ferrox mask using a Kasper #2001 mask aligner. The exposure time and energy varied with the type of photoresist and the channel thickness. This process imprints a mask of the channel geometry onto the polymer. The slides were then rinsed in a resist-developing solution, where the developer dissolves the polymer within the masked region. Following the developing procedure, the slides were hard baked (2 min, 120°C). At this time a cover slip was sealed on top of the channel. The thickness of the polymer film was measured and tested for uniformity (variations in depth were less than ±5% of the stated height) with an alpha step (Tencor, Mountain View, CA). Since the adhesion of photoresist to glass is weak (compared to silicon), the slides had to be baked and developed with care.

Method and procedure

For experiments, the bacteria were suspended in motility buffer (11.2 g/L K_2 PO_4, 4.8 g/L KH_2PO_4, and 0.029 g/L EDTA in distilled water; Adler and Templeton, 1967) so that the final cell concentration was $\leq 10^7$ cells/ mL. This was sufficiently low to minimize cell–cell interaction. The cells were not washed free of growth medium prior to dilution; however, the final concentration of glucose during these experiments was sufficiently small to assume that cell growth was negligible. (Due to lack of nutrients in the motility medium, the swimming behavior of the cells is expected to become slightly diminished after approximately 2.5 h; hence, experiments were conducted in the first 30 min. after dilution.)

Bacteria were pulled into the rectangular channels by capillary action. Minute amounts of polyvinylpyrrolidone (0.1% final concentration) were added to the motility buffer to prevent the cells from sticking to the glass surfaces (Berg and Turner, 1990). This changed neither the pH nor the viscosity of the medium. The channels were sealed at both ends with vacuum grease to minimize convection. The cells were observed at the midpoint between the glass slide and the coverslip at a total magnification of 400× (40× microscope objective, numerical aperture = 0.65) using a Zeiss phase-contrast microscope, which was connected to a video camera (Dage-MTI, 67M Series) and a time-lapse videocassette recorder (JVC BR-9000U) to record bacteria movement. An image-analysis system (Imaging Technology, Series 151 Image Processor) was programmed to track the run length, run time, and turn angle for individual cells. To ensure that the actual three-dimensional run length, rather than a projection, was measured, data were taken only if the cell remained in focus (depth of field = 1.5 μm; this was achieved by placing a green filter between the light source and the cell suspension, thus limiting the light wavelength to less than 700 nm) for the entire duration of the run. The computer determined the run length of the bacterium by marking the position of two consecutive tumbles (the beginning and end of a run). To determine the run time, the number of videotape frames between tumbles was counted and converted to real time (30 frames/s). The turn angle was calculated as the angle between two consecutive run vectors. Additional information on the data analysis has been published by Phillips et al. (1994).

Results and Discussion

Approximately 250 bacteria taken from three different cultures were tracked in each of the five channel heights (2, 3, 10, 15 and 20 μm) and in bulk fluid. Single-cell motility parameters were measured for each of the channel sizes; however, in both the 20-μm channel and in the bulk, a statistically meaningful turn-angle measurement was not possible (see Turn Angle Measurements) (Table 1). The turn angle presented in Table 1 for unhindered *E. coli* was measured by Berg and Brown (1972).

Table 1. Single-Cell Motility Data for *E. coli* in Rectangular Channels of Six Different Heights*

Channel Hgt. (μm)	Cell Speed (μm/s)	Mean Run Time (s)	Tumbling Probability (1/s)	Mean Turn Angle (deg)
2	20.8 ± 5.8	1.54 ± 1.02	0.7	61 ± 49
3	29.3 ± 8.7	0.95 ± 0.57	1.1	69 ± 50
10	26.4 ± 7.1	0.95 ± 0.56	1.1	64 ± 48
15	25.4 ± 7.7	0.89 ± 0.65	1.1	67 ± 48
20	26.9 ± 6.0	0.89 ± 0.53	1.1	N.A.
∞	26.2 ± 6.7	0.89 ± 0.59	1.1	68 ± 36

*The unrestricted mean turn-angle measurement was reported by Berg and Brown (1972) using their 3-D tracking microscope. All the single-cell motility measurements in this study were made using the image-analysis tracking technique (approximately 250 cells tracked for each). A mean turn angle could not be determined in channels larger than 15μm because the cell tumbled out of view too frequently for a statistically meaningful value to be determined.

Run time and tumbling probability measurements

The mean run time was measured for each channel height. As stated previously, the run-time distribution is exponential if the tumbling results from a Poisson process. Figure 2 shows the run-time distribution measured in the 10-μm channel as well as the ideal exponential curve corresponding to the mean of the distribution. It appears that the data are well represented by the exponential distribution with a mean ($\pm$ standard deviation) of 0.95 ± 0.56 s (Devore, 1982). The slight shift in the distribution is due to the inability to measure run times shorter than approximately 0.3 s. Similar distributions were found for all channel heights. From each run-time distribution, a tumbling probability was calculated as the reciprocal of the mean run time (Eq. 2):

$$\lambda = 1 \neq \tau \qquad (2)$$

A uniform tumbling probability of 1.1 tumbles/s was observed for channel heights of 3 μm and greater. A tumbling probability of 0.7 tumbles/s was calculated from the mean run time in the 2-μm channel. The run time and tumbling probability determined by Phillips et al. (1994) ($\tau = 0.84 \pm 0.71$ s; $\lambda = 1/\tau = 1.2$ tumbles/s) and Berg and Brown (1972) ($\tau = 0.86 \pm 1.18$ s; $\lambda = 1.2$ tumbles/s) agree well with the present measurements.

One difficulty in measuring run times in microchannels was that the flagella occasionally adhered to the wall during a run, causing the cell to stick momentarily. If this single interrupted run were recorded as two distinct runs, the distribution would have become skewed. We found that it was often difficult to detect the difference between a tumble and a transiently adhering cell. The addition of the polyvinylpyrrolidone to the motility medium alleviated this problem. Another difficulty in measuring run times in narrow channels arises from the cell's tendency to swim in large circles instead of linear runs and tumbles. Other researchers have noticed this and have suggested mechanisms for such behavior (Frymier et al., 1995; Berg and Turner, 1990; Ramia et al., 1993). We noted that when the cells were swimming in circles near the glass surface (circle diameter on the order of 50μm), they tumbled much less frequently. Data obtained from these cells were not collected, and this may add a small bias to the reported run-time measurements.

Velocity measurements

From each run time and length measurement, a cell velocity was calculated. In Figure 3, the velocity distribution for the 10-μm channel is compared with the ideal normal curve (determined from the mean and standard deviation of the data set), as well as the velocity distribution for the unhindered data. As can be seen, the velocity distribution determined in the 10-μm channel is normal and similar to the distribution determined for the unrestricted data.

Surprisingly, the mean velocity for each of the channel heights, with the exception of the 2-μm channels, was re-

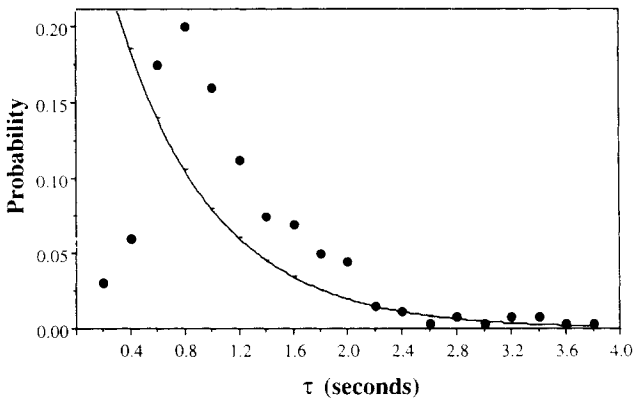

Figure 2. Run time, τ, probability distribution in 10-μm channels.

The curve plotted is the ideal exponential curve given the mean of the 10-μm data ($\tau_{10} = 0.95 \pm 0.56$ s). A total of 250 run times were measured.

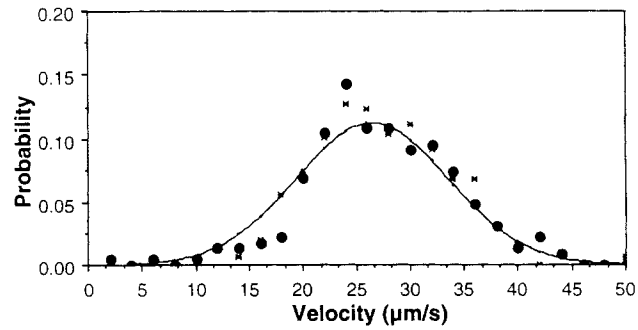

Figure 3. Probability distribution for cell velocity in the 10-μm channels (circles) and for the unrestricted geometry (stars).

The curve plotted is the ideal normal curve given the mean and standard deviation for the 10-μm data ($s_{10} = 26.4 \pm 7.1$ μm/s). A total of 250 velocity measurements were made.

markably similar (Table 1). When one considers the ratio of the cell body size (an ellipsoid of dimensions 1×2 μm) to the channel size, one would expect hydrodynamic drag to reduce the cell velocity to a fraction of that found in the bulk. For comparison, a similar image-based tracking technique was applied to compare the diffusion of a 9-μm-diameter spherical latex particle in a 30-μm channel to that in an unbounded medium (Biondi and Quinn, 1995). The effects of hydrodynamic drag reduced the diffusion coefficient of the particle in the 30-μm channel to almost half that in the unbounded medium, which is consistent with predictions of enhanced drag in restricted channels (Happel and Brenner, 1973). Enhanced drag of this magnitude is not observed directly in the restricted bacteria experiments even though the size ratios are similar. In fact, the measured cell speed in the 3-μm channel is 10% larger than the measured value in the unbounded medium.

Drag effects only became apparent when cells were swimming in the 2-μm channels. Here, the channel size is close to that of the cell. Furthermore, the length of a flagellum is between 6 and 10μm. In 2-μm channels the cell is incapable of continuously executing linear runs, and instead frequently swims in a sporadic zigzag path. Tumbles are executed with difficulty since flagella are unable to extend fully.

Several investigators have noted that swimming bacteria do not respond to hydrodynamic drag, as would be predicted from size and shape alone (Schneider and Doetsch, 1974; Greenberg and Canale-Parola, 1977; Berg and Turner, 1979). To explain such behavior, considerable work has been done to model bacterial hydrodynamics and flagellar propulsion (Hancock, 1953; Higdon, 1979; Shreiner, 1971; Coakley and Holwill, 1972; see Ramia et al. (1993) for a review of this and related literature). The work of Ramia et al. (1993) is most relevant to the experiments described in this article. They modeled the hydrodynamics of flagellated bacteria swimming near a plane wall and concluded that there is a small propulsive advantage to swimming very close and parallel to a planar surface. They predicted that when the cell swims within a critical distance of the wall (approximately 1 μm for *E. coli*) the propulsive advantage will result in a swimming speed slightly larger than that in the bulk ($\sim 5\%$). For the velocity measurements made in this study, in the 3-μm channels, the cell will always find itself within 1 μm of the wall, and accordingly the mean velocity measured was 29 μm/s, which is an increase from the unrestricted velocity of approximately 10%.

Turn-angle measurements

Turn angles were determined for the trajectory of each cell tracked. Two consecutive three-dimensional run vectors, $r_1 = a_1 i + b_1 j + c_1 k$ and $r_2 = a_2 i + b_2 j + c_2 k$, were measured and the turn angle, Φ, was calculated as the angle between them (Eq. 3):

$$\Phi = a\cos\left(\frac{r_1 \cdot r_2}{|r_1||r_2|}\right) \tag{3}$$

Since the bacterium being tracked runs entirely within the plane of focus of the objective (see method and procedure), the vectors r_1 and r_2 have no k component. Thus, Eq. 3 calculates the turn angle exactly. (If a projection of the two runs were measured, the calculated turn angle would be incorrect because of the missing k component.)

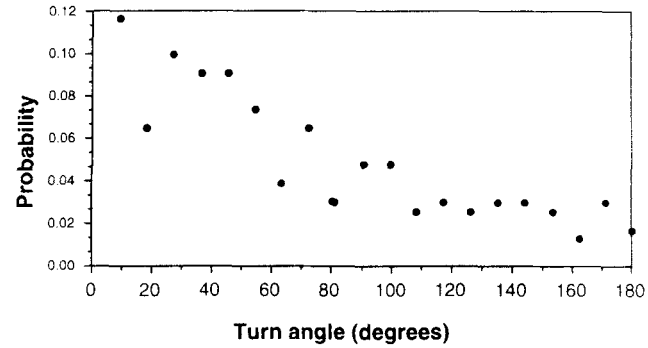

Figure 4. Turn-angle probability distribution, $64 \pm 48°$, in 10-μm channels.

The data include 250 turn-angle measurements. Similar nonisotropic distributions were measured in 2-, 3-, and 15-μm channels.

In previous work using this technique (Phillips et al., 1994), turn-angle measurements were seldom possible because the bacterium tumbled out of the plane of focus before two sequential runs could be measured. In the restricted geometries, the probability of tumbling out of focus is small and therefore a cell could frequently be tracked for three or four consecutive runs.

To our knowledge, there are two previous turn-angle measurements reported for flagellated bacteria. In the first, Berg and Brown (1972), using a three-dimensional tracking microscope, measured a turn-angle distribution in an unrestricted environment ($68° \pm 36°$) for *E. coli* A405 and found the distribution to be skewed from isotropic (sinusoidal) with a bias toward smaller turn angles. Liu and Papadopolous (1995) measured the turn angles of *E. coli* K-12 in a 6-μm capillary ($38° \pm 25°$) and found the distribution in these geometries to be nonisotropic with a strong bias toward smaller turn angles. Figure 4 shows a plot of the turn angle distribution measured in this study in a 10-μm channel.

A nonisotropic turn-angle distribution was also measured in the restricting geometries used in this study (see Table 1) and could be explained by considering the interaction of the cell with the channel wall. Direct observations reveal that the bacteria are frequently unstable at the beginning of a run, and occasionally appear inactive for up to several milliseconds after a tumble as if the cell were lodged between the glass plates. Also, the turn angles of a cell appear to be smaller, on average, than those observed in the bulk. These observations are not surprising considering the size of the geometries through which the bacteria are swimming. In these environments the flagella are unable to extend themselves fully (Figure 5), so the cell can easily become lodged between the two plates. This would also cause the cell to have difficulty reorienting itself, which would result in small turn angles.

Dimensionality and the macroscopic motility coefficient

The single-cell motility parameters measured in this study appear to be nearly constant, regardless of whether the cell is

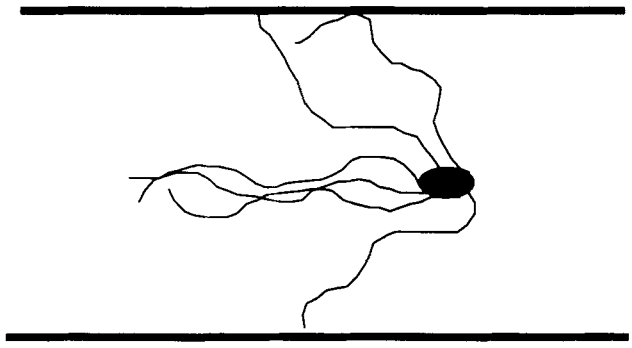

Figure 5. *E. coli* in a 10-μm channel.

The cell body size is $1 \times 2\,\mu$m and the flagella range in length between 6 and 10μm. In a 10-μm pore, the cell is unable to extend its flagella fully while tumbling. This biases the cell's turning such that turn angles of 0° are favored.

swimming within a restricted or unrestricted geometry. In restricted geometries, the number of dimensions in which the cell is free to swim becomes important. Clearly, when the bacterium is swimming in an unrestricted environment, three dimensions of movement are wholly available to the cell. In the smaller channels employed in this study, the similarity in size between the cell and the channel restricts movement to two dimensions. In the capillary assay performed by Berg and Turner (1990), the cells were restricted to movement solely in the axial direction—a one-dimensional situation. Our observations indicate that regardless of the dimensionality of the system, the single-cell motility parameters are nearly constant and independent of the proximity of the bounding walls (The error of this approximation will increase as the restricting geometry dimension decreases below 3 μm, as the cell velocity will be considerably decreased.)

Since each of the single-cell motility parameters has been measured, a macroscopic motility coefficient, μ, can be calculated (Eq. 1). The channels used here restrict the swimming behavior to two dimensions, and therefore the parameter n_d, the dimensionality, in Eq. 1 should be set equal to two. Using the motility data from the 10-μm channel in Table 1, the motility coefficient is calculated to be 4.8×10^{-6} cm^2/s. If the single-cell motility parameters used in this calculation can be assumed to be valid for one- and three-dimensional systems (while there is a difference between the turn-angle distribution measured in restricting geometries and that measured in the bulk, this does not translate into a significant difference in the index of directional persistance (10–20%)), a motility coefficient can be calculated in these dimensions as well, merely by changing the dimensionality parameter, n_d. Accordingly, in three dimensions (unrestricted) $\mu = 3.2 \times 10^{-6}$cm^2/s, while in one dimension (capillary tubes) $\mu = 9.6 \times 10^{-6}$ cm^2/s. The calculated unrestricted value is in approximate agreement with that determined by Phillips et al. (1994), who measured a bulk motility coefficient of 2.1×10^{-6} cm^2/s. Further comparisons can be made to the work of Berg and Turner (1990), who reported motility coefficients of 2.6×10^{-6} cm^2/s in a 50-μm capillary tube and 6.1×10^{-6} cm^2/s in a 10-μm capillary tube. The agreement between the calculated motility coefficients determined in this study and those measured by Berg and Turner (1990) indicates that their enhanced motility coefficients are most likely due to the reduction in dimensionality, and are not due to changes in the single-cell parameters. Interestingly, one can also compare the motility coefficients noted earlier with those measured by Barton and Ford (1995), who found a significant decrease in motility with a decrease in average pore size. Our study suggests that cell velocities and run times in packed beds will not be notably different from those in the bulk. However, the bias in turn-angle distributions toward smaller turns (or simply the difficulty in reversing directions) might affect the net motility of the cell. In the complex pore network of packed sand, the cell could frequently encounter dead ends or pore structures too small to penetrate. If the cell has difficulty reversing directions, the net penetration time through the soil will be extended. Another important consideration is that due to the tortuosity of a packed bed, the cell must travel further to achieve the same net displacement of a cell migrating through linear pores. There are undoubtedly many complex phenomena at play in the migration of bacteria through porous media. We believe that the present study illustrates how such an environment might affect the swimming behavior of the individual cell.

Conclusions

Single-cell motility parameters for *E. coli* (cell velocity, tumbling probability, and turn angle) were measured in micromachined channels using videomicroscopy and image-analysis techniques. We found that run-time (and hence tumbling probability) measurements were nearly constant in all channel sizes. Turn-angle measurements were also similar at the different channel heights; however, the distributions were not isotropic. We attribute this to the interaction of flagella with the pore wall interfering with the turning behavior of the cell. Mean cell velocities remained constant in channels ranging in height between infinity and 10 μm. Cell speeds measured in 3-μm channels were 10% larger than those measured in unhindered environments. These results are in good agreement with the predictions of Ramia et al. (1994) who postulated that there is a propulsive advantage (which could increase the cell velocity by up to 5%) to swimming near a wall. A 25% decrease in cell speed was measured in 2-μm channels, which was attributed to steric hindrance; the cell's swimming mechanism was disrupted significantly by the presence of the wall.

From the single-cell parameter measurements, a two-dimensional cell-population motility coefficient was calculated. These calculations were extended to one- and three-dimensional motility coefficients by assuming that the single-cell parameters remain unchanged in these environments. These results are in reasonable agreement with the literature values for motility coefficients measured in a pore and in the bulk (Berg and Turner (1990) and Phillips et al. (1994)), but notably overestimate the motility coefficients measured by Barton and Ford (1995) in packed beds. In comparing our data with these studies, it appears that the complexity of the porous system through which the bacterium swims is the dominating factor in predicting its motility.

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