JOURNAL OF BACTERIOLOGY, Feb. 1997, p. 1428–1430 Vol. 179, No. 4
0021-9193/97/$04.00+0

Turn Angle and Run Time Distributions Characterize Swimming Behavior for *Pseudomonas putida*

KEVIN J. DUFFY AND ROSEANNE M. FORD*

Department of Chemical Engineering and Biophysics Program, University of Virginia, Charlottesville, Virginia 22903-2442

Received 10 June 1996/Accepted 26 November 1996

The swimming behavior of *Pseudomonas putida* was analyzed with a tracking microscope to quantify its run time and turn angle distributions. Monte Carlo computer simulations illustrated that the bimodal turn angle distribution of *P. putida* reduced collisions with obstacles in porous media in comparison to the unimodal distribution of *Escherichia coli*.

Soil bacteria of the species *Pseudomonas putida* propel themselves through their surrounding medium by rotating flagella that form tufts at one end of their bodies (10). A single cell traces a path that consists of a series of runs interrupted by changes in direction. As with *Escherichia coli*, the changes in direction are initiated by a reversal in the rotational direction of the flagellar motors of the bacteria (4, 6, 10, 12–14). In the absence of a chemical gradient this swimming pattern resembles a three-dimensional random walk similar to Brownian motion in molecular diffusion, except that changes in direction are due to the reversal of flagellar rotation and not molecular collisions.

P. putida PRS2000 cells were originally obtained from Wayne Coco at the University of Illinois in Chicago (2). A small loopful of cells was inoculated into a solution consisting of a buffer, ammonium sulfate, and a mineral base, as described previously (2). Cultures were incubated for 20 to 30 h to a stationary cell density of approximately 10^9 cells/ml. One milliliter of this suspension was diluted 50-fold in motility buffer (1) with a pH of 7.0.

The tracking microscope developed by Berg (3) tracks individual bacteria using a movable stage. The configuration of the microscope and its use are described comprehensively in Frymier et al. (9). The data were analyzed by using the algorithm developed by Berg and Brown and their empirically determined criteria for *E. coli* (6). Several alternatives for the angle criterion were considered for analyzing the behavior of *P. putida*. Angular speeds for flagging a tumble of from 360 to 600°/s were tried. However, the value of 420°/s previously used by Berg and Brown (6) appeared to give results most consistent with visual observations of three-dimensional images of the trajectories. In all, a total of 1,056 turn angles for 80 bacteria were measured.

A three-dimensional visualization of the experimental traces for each of three bacteria is given in Fig. 1. Examples of various turning behavior are illustrated. A range of angles is possible (Fig. 1a). Bacteria sometimes continue with a bias in the forward direction (Fig. 1b) and sometimes reverse their direction (Fig. 1c).

For *P. putida* there is a bimodal distribution for frequency of turn angles θ (Fig. 2). (Note that these results were presented previously in Duffy et al. (7) for a different data set.) If the bacteria chose new angles at random, then the frequency would be maximum at $\theta = 90°$. For *P. putida* there are peaks at approximately $\theta = 40°$ and $\theta = 160°$. Angles which correspond to a bacterium continuing in a direction close to its original direction and angles for which the new direction is approximately opposite to the original direction predominate. An overall bias exists toward the smaller angles. This distribution is qualitatively different from that reported for the wild-type bacterium *E. coli* AW405, which Berg and Brown (5) found to have a unimodal distribution with a bias toward smaller angles (Fig. 2). These bacteria tend to continue in approximately their original direction after a change in direction. Frymier (8) measured the turn angle distribution for another strain of *E. coli* (NR50) with the tracking microscope in our laboratory (Fig. 2). The distribution for NR50 is also unimodal with a bias toward smaller turn angles, although a less significant bias than for the AW405 strain.

The distribution given in Fig. 2 for *P. putida* is consistent with what was seen qualitatively by Harwood et al. (10) with their two-dimensional motion analysis system. It also confirms what we qualitatively observed under the microscope. The bacteria appear to dart in approximately straight-line segments, and then, after stopping intermittently, often continue in the same general direction. Every so often a reversal in direction occurs.

While the distributions of turn angles are not the same for *E. coli* and *P. putida*, they do have the same qualitative distribution of run lengths (Fig. 3) (5). This indicates that the probability of turning is approximately random and that each event is independent of the others. This is also important in understanding the macroscopic migration of *P. putida* because under these circumstances its migration can be modeled with the equations for Fickian diffusion.

The average swimming speed was also calculated for each bacterium. If averages of less than 17 μm/s are excluded (10), then the overall average is 27.5 μm/s and the average value of the maximum for each bacterium is 53.8 μm/s. These values are less than those reported by Harwood et al. (10) (44 and 75 μm/s). This was not surprising because we were pushing the limits of the tracker. It was designed to follow *E. coli* bacteria, which swim about half as fast as *P. putida* bacteria. However, we are confident that the run length and turn angle distributions are at the very least qualitatively accurate (i.e., there is no correlation between swimming speed and the turn angle or run time distributions.)

P. putida is a soil-inhabiting species. Thus, its environment is composed of a bulk liquid phase and a solid impenetrable

* Corresponding author. Mailing address: Department of Chemical Engineering, Thornton Hall, University of Virginia, Charlottesville, VA 22903-2442. Phone: (804) 924-6283. Fax: (804) 982-2658. E-mail: rmf3f@virginia.edu.

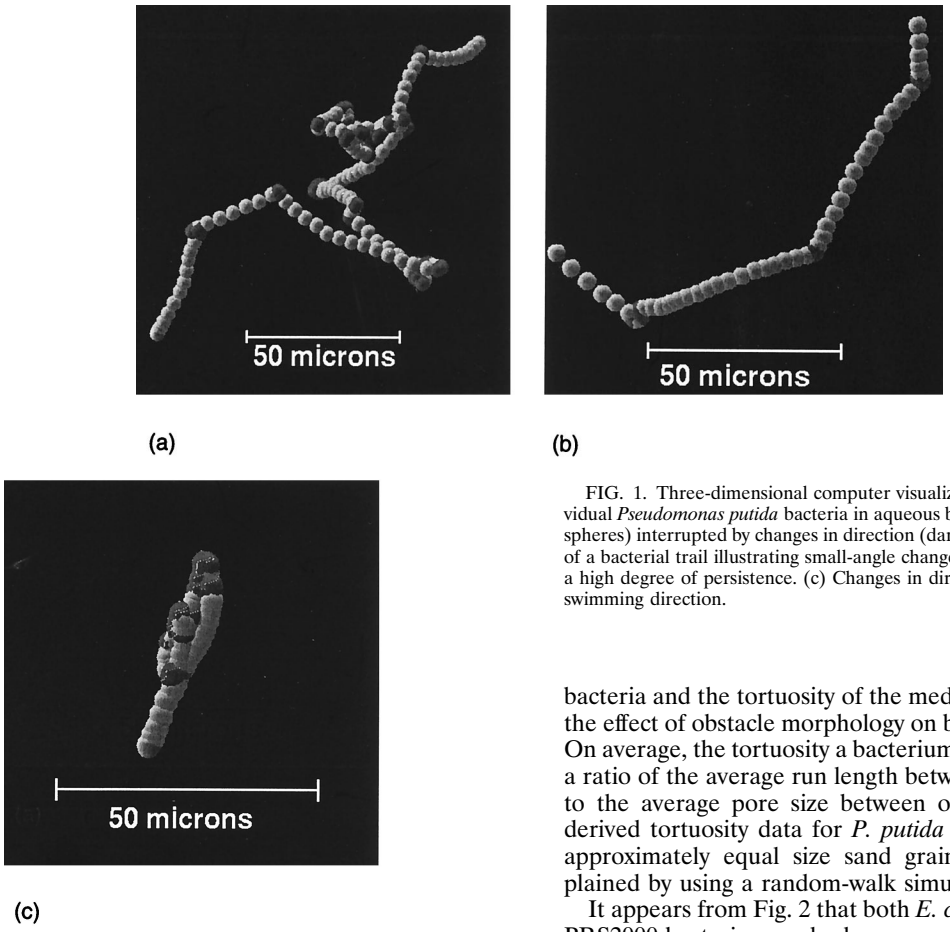

FIG. 1. Three-dimensional computer visualization of the path of three individual *Pseudomonas putida* bacteria in aqueous buffer. (a) A series of runs (light spheres) interrupted by changes in direction (dark spheres). (b) A short segment of a bacterial trail illustrating small-angle changes in direction corresponding to a high degree of persistence. (c) Changes in direction resulting in a reversal of swimming direction.

phase, in other words a porous medium. Bacterial movement in a porous medium can be characterized by multiplying the normal swimming behavior in the bulk liquid solution by a retention factor due to the obstacles. The degree of retention is a combination of the restricted volume available to the bacteria and the tortuosity of the medium. Tortuosity refers to the effect of obstacle morphology on bacterial migration (2, 7). On average, the tortuosity a bacterium experiences depends on a ratio of the average run length between changes in direction to the average pore size between obstacles. Experimentally derived tortuosity data for *P. putida* moving in a medium of approximately equal size sand grains were successfully explained by using a random-walk simulation (7).

It appears from Fig. 2 that both *E. coli* AW405 and *P. putida* PRS2000 bacteria spend a large proportion of their time continuing in approximately the same direction before and after a turn. This increases their bulk random motility and the effective volume that a population of bacteria can cover. Why does *P. putida* not simply use the same distribution as *E. coli*? To help answer this question cellular dynamic simulations were run as described in Duffy et al. (7). Parameter values for the bacterial size (2 μm), the swimming speed (44 μm/s), and the duration (0.025 s) and frequency (0.5 s^{-1}) of turning for a *P. putida* bacterium were estimated from empirical data (7). The porous medium was modeled by using a "box" of equal-size spheres that were randomized by a molecular dynamics simulation. To achieve a realistic porosity (σ = 0.37) the spheres

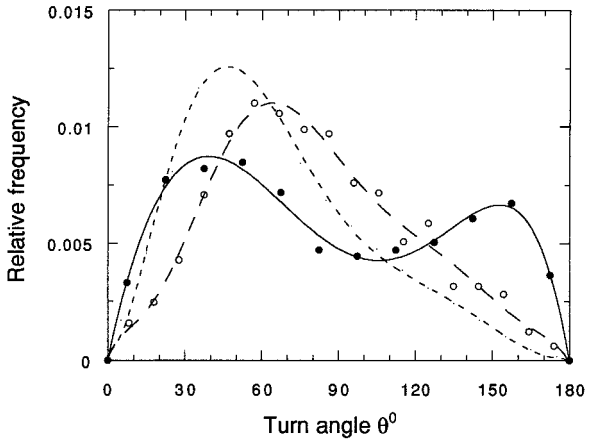

FIG. 2. Distribution of changes in direction (turn angles θ) for *P. putida* measured with the tracking microscope (solid circles and line). Distributions for two strains of *E. coli* are presented for comparison (NR50, open circles and dashed line [8]; AW405, dotted line [5]).

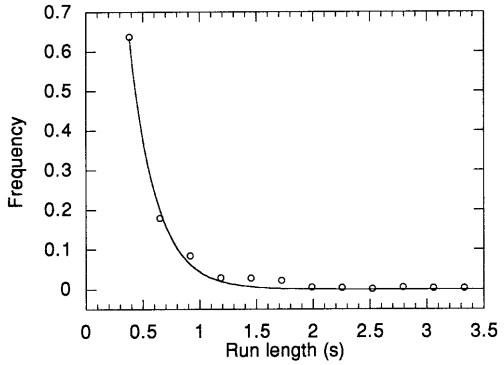

FIG. 3. Distribution of the frequency of run lengths. The solid line is an exponential curve fit to the data.

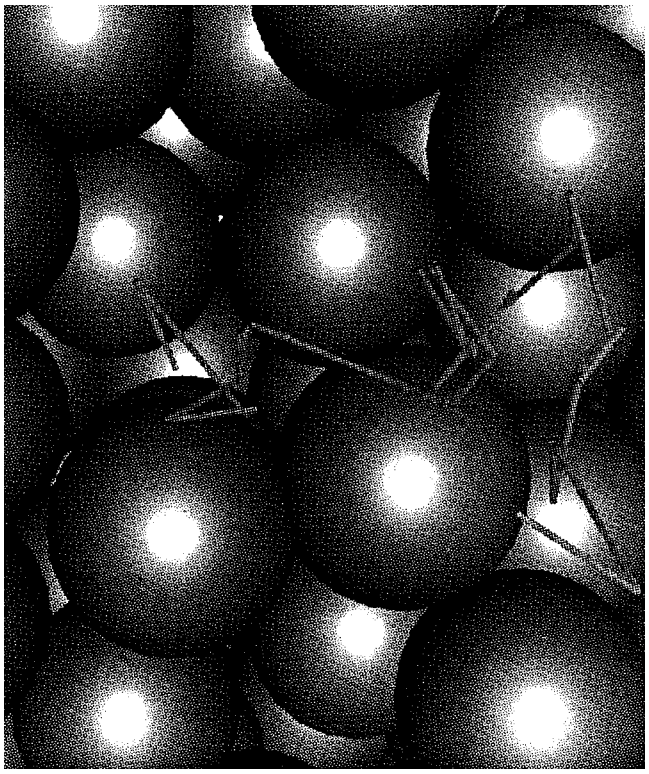

FIG. 4. Visualization of a simulated bacterial trace in a model porous medium.

were allowed to overlap, but this was minimal. A sphere diameter of 100 μm was used. The result is a distribution of pore sizes with an average diameter of 39 μm (11). A visualization of the model porous medium and a simulated bacterial trace are shown in Fig. 4. The trace compares well with experimentally observed traces in bulk liquid media (Fig. 1).

Simulations using the unimodal distribution (*E. coli*) and the bimodal distribution (*P. putida*) were compared, holding all other parameters the same (Table 1). Random motility in bulk medium is larger if one uses the *E. coli* distribution (Table 1). However, in the porous medium there is no appreciable difference in the random motility between the *E. coli* and *P. putida* turn angle distributions. The *E. coli* distribution, which results in a higher random motility in bulk medium, does not result in a higher motility in the porous medium. Calculations of tortuosity (8) using these data indicate that *P. putida* organisms experience less tortuosity if they move in accordance with their own distribution (Table 1). An increase in tortuosity will result in an increase in bacterium-obstacle collisions (8). It appears that *P. putida*, being a soil bacterial species, utilizes a strategy that minimizes collisions with the surface. It remains to be explained why both *E. coli* and *P. putida* have turn angle distributions that are not random. A possibility is that this allows an increase in effective volume covered by the population while reducing the potential contacts between individuals.

TABLE 1. Computer simulation results showing the effects of the turn angle distribution on random motility

Distribution[a]	Bulk motility (B) (cm^2/s)	Porous motility (P) (cm^2/s)	Tortuosity ($= \sigma B/P$)
Unimodal (*E. coli*)	2.0×10^{-5}	5.0×10^{-7}	14.8
Bimodal (*P. putida*)	1.4×10^{-5}	4.8×10^{-7}	10.8

[a] The bacterial species exhibiting the distribution is in parentheses.

This research was supported by a grant from the IBM Environmental Research Program.

We thank Howard Berg for the loan of the tracking microscope and Paul Frymier for help with using it and for permission to use his data. We also thank Peter Cummings for help with the computer visualizations.

REFERENCES

1. **Adler, J., and M. M. Dahl.** 1967. A method for measuring the motility of bacteria and for comparing random and non-random motility. J. Gen. Microbiol. **46:**161–173.
2. **Barton, J. W., and R. M. Ford.** 1995. Determination of effective transport coefficients for bacterial migration in sand columns. Appl. Environ. Microbiol. **61:**3329–3335.
3. **Berg, H. C.** 1971. How to track bacteria. Rev. Sci. Instrum. **42:**868–871.
4. **Berg, H. C., and R. A. Anderson.** 1973. Bacteria swim by rotating their flagella filaments. Nature **239:**380–382.
5. **Berg, H. C., and D. A. Brown.** 1972. Chemotaxis in *Escherichia coli* analysed by three-dimensional tracking. Nature **239:**500–504.
6. **Berg, H. C., and D. A. Brown.** 1974. Chemotaxis in *Escherichia coli* analyzed by three-dimensional tracking. Antibiot. Chemother. (Basel) **19:**55–78.
7. **Duffy, K. J., P. T. Cummings, and R. M. Ford.** 1995. Random walk calculations for bacterial migration in porous media. Biophys. J. **68:**800–806.
8. **Frymier, P. D.** 1995. Bacterial migration and motion in a fluid phase and near a solid surface. Ph.D. thesis, University of Virginia, Charlottesville, Va.
9. **Frymier, P. D., R. M. Ford, H. C. Berg, and P. T. Cummings.** 1995. Three-dimensional tracking of motile bacteria near a solid planar surface. Proc. Natl. Acad. Sci. USA **92:**6195–6199.
10. **Harwood, C. S., K. Fosnaugh, and M. Dispensa.** 1989. Flagellation of *Pseudomonas putida* and analysis of its motile behavior. J. Bacteriol. **171:** 4063–4066.
11. **Huizenga, D. G., and D. M. Smith.** 1986. Knudsen diffusion in random assemblages of uniform spheres. Am. Inst. Chem. Eng. J. **32:**1–6.
12. **Macnab, R. M.** 1977. Bacterial flagella rotating in bundles: a study in helical geometry. Proc. Natl. Acad. Sci. USA **74:**221–225.
13. **Silverman, M., and M. Simon.** 1974. Flagella rotation and the mechanism of bacterial motility. Nature **249:**73–74.
14. **Taylor, B. L., and D. E. Koshland, Jr.** 1974. Reversal of flagella rotation in monotrichous and peritrichous bacteria: generation of changes in direction. J. Bacteriol. **119:**640–642.