APPLIED MICROBIOLOGY, Apr. 1969, p. 584-588
Vol. 17, No. 4

Motility Tracks: Technique for Quantitative Study of Bacterial Movement

Z. VAITUZIS AND R. N. DOETSCH
Department of Microbiology, University of Maryland, College Park, Maryland 20742

Received for publication 30 December 1968

A method for recording movements of bacteria in time and space on a single photograph is described. Quantitative information on the behavior of various motile organisms may easily be obtained for comparative studies. The method possesses certain advantages over cinematography, and illustrations of applications of the technique are presented.

Aside from cinematographic and television techniques, the study of bacterial movement is notorious for its paucity of readily available, simple, reproducible methods (1). As a consequence, results of work on bacterial motile behavior are reported in vague subjective terms such as "fast," "somewhat slow," "erratic," and the like, which convey scant information concerning the events observed. It is probable that lack of interest in this area of bacteriology is due in large measure to the poorly developed state of relevant techniques. Accordingly, the work described here represents an attempt to rectify this situation by dealing with the problem quantitatively.

MATERIALS AND METHODS

Cultures. The following bacteria were used: *Escherichia coli* (ATCC 13070), peritrichous flagella; *Pseudomonas aeruginosa* (University of Maryland collection), single polar monotrichous flagellum; *Bacillus licheniformis* (9945-A), peritrichous flagella, from F. J. Tyeryar; *Sarcina ureae* (ATCC 13881), peritrichous flagella; *Thiospirillum jenense* and *Chromatium okenii*, lophotrichous flagella, both from R. L. Gherna. The latter two organisms were cultured and observed in a synthetic medium specifically developed for large photosynthetic purple sulfur bacteria by Pfennig and Lippert (4). The other bacteria were grown in 5-ml amounts of Trypticase Soy Broth (BBL) in screw-top tubes (18 by 125 mm) for 18 hr at 30 C on an oscillating shaker (Lab-Line Instruments, Melrose Park, Ill.). Quantities of these cultures (0.1 ml) were transferred to 5-ml amounts of fresh broth; after 1 hr of incubation at 30 C, their motility tracks were studied. One drop (ca. 0.05 ml) of culture was placed on a glass slide (0.96 to 1.06 mm thickness) and immediately covered with a cover slip (0.13 to 0.16 mm thickness); 1 min was then allowed for equilibration at 20 C before photography was started. The cultures were not sealed under the cover slip and, over the period of observation (generally less than 5 min), no diminution of motile activity was noted.

Microscopic methods. A Zeiss photomicroscope with the following arrangement was employed. A 10× planachromat (NA 0.22) objective was used in conjunction with the condenser set at "phase 3." The substage auxiliary lens was always kept out, but a green interference filter occasionally was introduced. Photography was done with Kodak Tri-X Pan or Panatomic-X film, and exposures of 1 to 15 sec were made by means of a Time-O-Lite interval timer (Master Model, Industrial Timer Corp., Parsuppany, N.J.) introduced in the line. Illumination was provided by either 12 v, 60 w or 6 v, 15 w lamps. This arrangement provided an extremely bright image on a black background similar to that seen in dark-field microscopy.

Velocity calculation. A planometer (American Map Co., New York, N.Y.) calibrated in millimeters was used to measure the maximum lengths of the photographically produced motility tracks. The velocity, expressed in micrometers/sec, was calculated from the formula: $V = (L \times E)/T$; where V (velocity in micrometers/sec) $= L$ (measured length in millimeters of the photographically produced track) $\times E$ (micrometer equivalent per millimeter as calculated from the enlargement) $\div T$ (time of exposure in seconds). For example, if 1 mm on the photograph is equal to 10 μm (final magnification on film, 100×), and a motility track of 10 mm is measured for a 10-sec exposure time, calculated velocity would be 10 μm/sec. If one sets the final magnification at 10× and the exposure time at 10 sec, the velocity may be read directly from the planometer by multiplying the track distance in millimeters by 10 and expressing this as micrometers/sec. In all cases, the enlarged length (in millimeters) of the organism is subtracted from the total track length prior to calculation of velocity. The mean velocity, as used in this paper, was calculated from the average of the five longest tracks found in a given photograph. It is not, therefore, the mean of all velocities observed, but the mean of the maximum velocities observed.

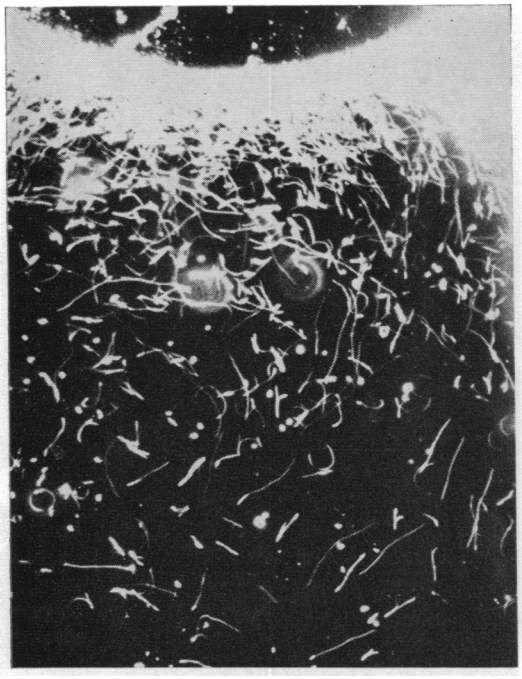

FIG. 1. *P. aeruginosa oriented around an air bubble.* × *193. Exposure time 1 sec.*

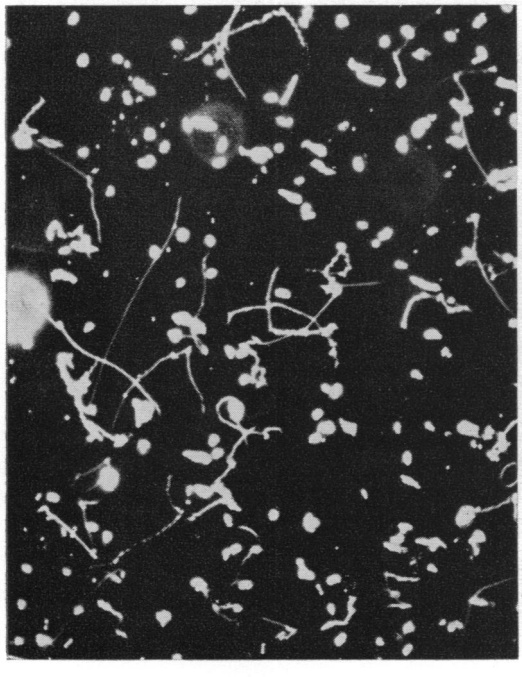

FIG. 2. *E. coli.* ×*244. Exposure time 5 sec.*

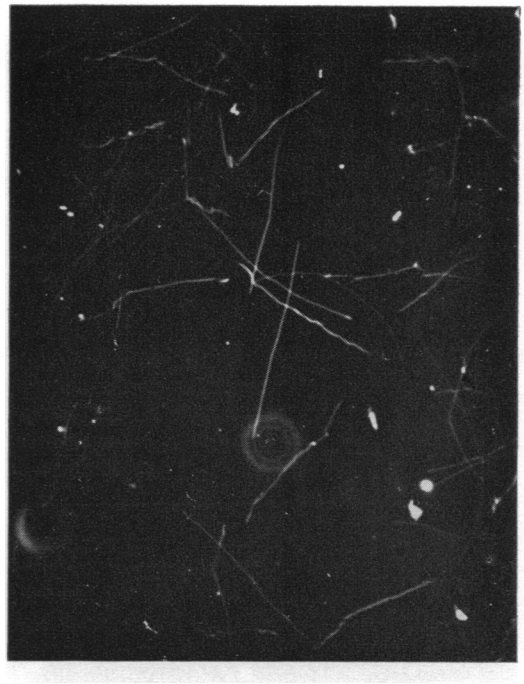

FIG. 3. *B. licheniformis.* ×*244. Exposure time 5 sec.*

RESULTS AND DISCUSSION

Motility tracks result from photographic overexposure of moving bacteria, whereby characteristic movements of the *individual* bacteria are depicted and permanently preserved without recourse to subjective or descriptive terminology. Motility tracks of the bacteria studied are shown in Fig. 1–8. The calculated velocities and other data are presented in Table 1.

The measurement of bacterial velocities began with the work of Gabricevskij (2) and, thereafter, reports appeared in the literature at irregular intervals. It is apparent from published results that variations in cultural conditions, i.e., temperature, *p*H, osmotic pressure, and nutritional level, may affect motility both qualitatively and quantitatively. Our results show graphically that *individual* organisms of a given culture vary in speed of locomotion. This, of course, has been known to be the case, but only in a qualitative way. We believe that the motility track technique represents a significant advance in that a precise quantitative statement may be made concerning observed variations. Elimination of hand-operated stop-watches, as used by Ogiuti (3) and others, as well as such devices as ruled stage and ocular micrometers, is of obvious advantage in minimizing observational errors. Furthermore, subjective estimates and fatigue factors have been obviated.

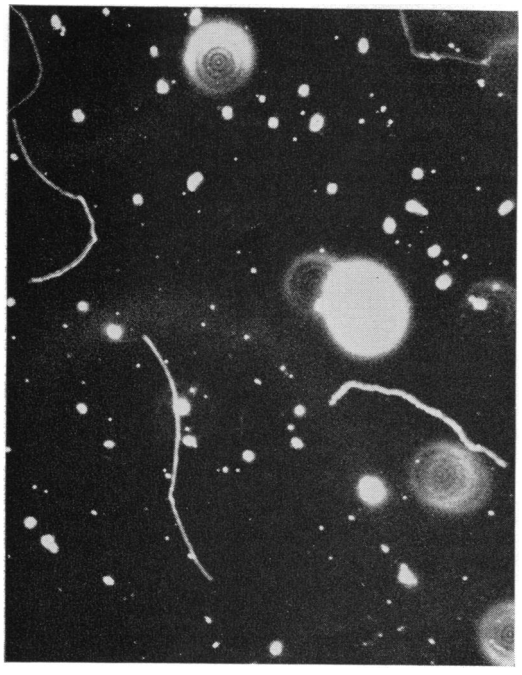

FIG. 4. *S. ureae.* ×*244. Exposure time 5 sec.*

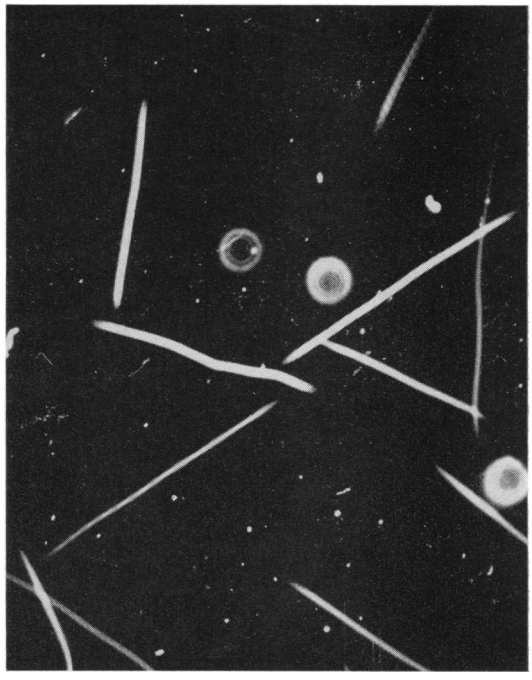

FIG. 6. *T. jenense.* ×*152. Exposure time 2.8 sec.*

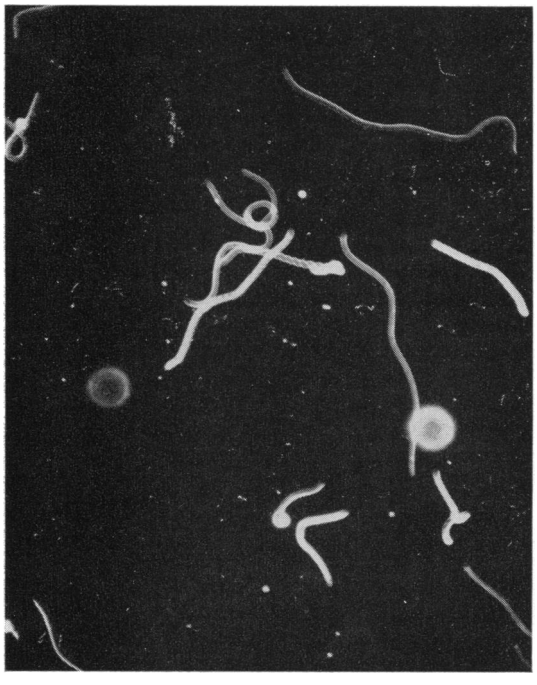

FIG. 5. *C. okenii.* ×*152. Exposure time 3.8 sec.*

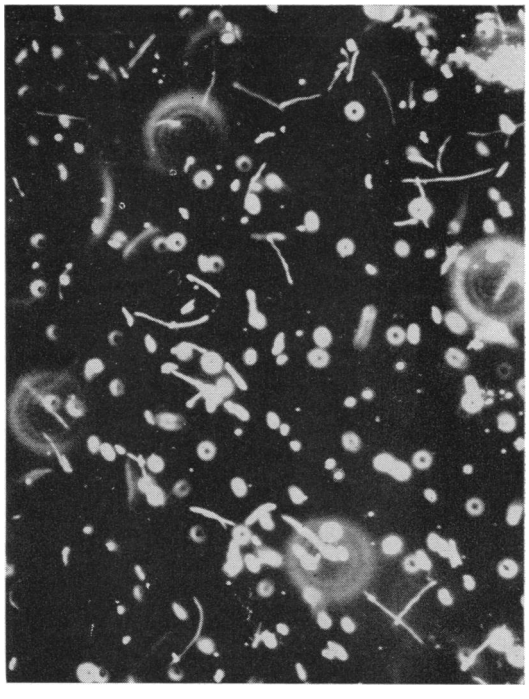

FIG. 7. *P. aeruginosa after 30 min in 97.9% D_2O.* ×*152. Exposure time 2 sec.*

The results show that among the bacteria observed here, those polarly flagellated, whether monotrichous or lophotrichous, move more rapidly than do the peritrichous forms, although definitive studies have yet to be made on this interesting point. A superficial examination of the photographs might lead one to conclude that mean free paths of the larger forms were greater than those of the smaller ones. However, bacteria such as the pseduomonads move over many more cell lengths per unit distance than do larger bacteria. For example, *P. aeruginosa* (1.5 μm in length) would move approximately 666 cell lengths per mm, whereas *T. jenense* (35 μm in length) would move through only 28 cell lengths per mm.

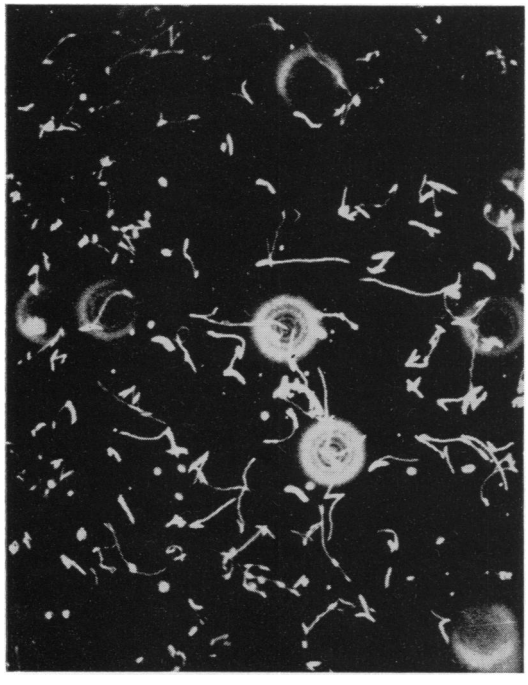

FIG. 8. *P. aeruginosa in 5% polyvinylpyrrolidone. ×244. Exposure time 5 sec.*

TABLE 1. *Velocities of various bacteria as calculated from motility tracks*

Organism	Mean cell length	Mean[a] velocity	Cell lengths moved[b]
	μm	μm/sec	no./sec
P. aeruginosa	1.5	55.8	37
P. aeruginosa in 5% PVP[c]		9.8	6
P. aeruginosa in 97.7% D_2O		5.9	4
E. coli	2	16.5	8
B. licheniformis	3	21.4	7
S. ureae	4[d]	28.1	7
C. okenii	10	45.9	5
T. jenense	35	86.5	2

[a] Based on the five longest track measurements observed.

[b] Calculated on the basis of mean velocity divided by mean cell length and rounded off to nearest whole number.

[c] Polyvinylpyrrolidone.

[d] Calculated from the width along the diagonal of an octet of cells.

Quantitative information derived from motility track studies will be useful in experiments on chemical and physical interference with the motility process (5). Figure 8 shows motility tracks of *P. aeruginosa* suspended in 5% (w/v) polyvinylpyrrolidone (type NP-K90, mol wt 300,000, Antara Chemical Co., New York, N.Y.). Comparison of the mean velocity of organisms in this preparation shows that it has been reduced to 10% of the control. In an experiment in which *P. aeruginosa* was suspended in 97.9% D_2O (New England Nuclear Corp., Boston, Mass.), the mean velocity after 30 min was only 17% of the control (Fig. 7 and Table 1).

The motility track technique may also be of value in studies of chemotaxis in bacteria. Responses of individual organisms in time and space may be shown on a *single* photograph (Fig. 1). This is an advantage not shared by cinematographic techniques. In addition, the motility track technique is valuable in delineating with precision the changes of direction, reversals, and rotations observed in motile behavior heretofore not amenable to quantitation by any method. On the other hand, details of flagellar motion cannot be seen except by frame analysis of cinematograph film.

Some factors involved in interpreting motility tracks ought to be kept clearly in mind. It is assumed that the track is made by an organism moving parallel to the cover slip and at a uniform velocity. One cannot easily determine whether an organism might be swimming at some angle to this plane; if so, the velocity would be calculated as less than its actual value. There should be no field drifting due to thermal or other causes. This may be noted, however, by observing whether the nonmotile organisms and particles of debris form discrete haloes due to random Brownian agitation. If not, and if parallel tracks of equal lengths are observed throughout the field, one may assume drifting has occurred. The organisms must not be so obligately aerobic that they cease moving almost immediately when placed under the cover slip. In any event, the motility tracks may be photographed after a 1-min equilibration period has elapsed, so oxygenation will not often be a

critical factor. A thermal filter should be used when illumination and heat production is great, as with a 250-w lamp, or if a long period of observation of a single preparation is contemplated.

The motility track method is probably amenable to further refinements, for example, by use of stroboscopic or laser illumination, and work on these techniques is in progress.

ACKNOWLEDGMENT

This research was supported by Public Health Service grant AI 07835-02 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

1. Doetsch, R. N., and G. J. Hageage. 1968. Motility in procaryotic organisms: problems, points of view, and perspectives. Biol. Rev. 43:317–362.
2. Gabricevskij, G. N. 1900. Über aktiv Beweglichkeit der Bakterien. Z. Hyg. 35:104–122.
3. Ogiuti, K. 1936. Untersuchungen über die Geschwindigkeit der Eigenbewegung von Bakterien. Japan. J. Exptl. Med. 14:19–28.
4. Pfennig, N., and K. D. Lippert. 1966. Über das Vitamin B_{12}-Bedürfnis phototropher Schwefelbakterien. Arch. Mikrobiol. 55:245–256.
5. Smith, J. L., and R. N. Doetsch. 1968. Motility in *Pseudomonas fluorescens* with special reference to survival advantage and negative chemotaxis. Life Sci. 7:875–886.