LIGHT-SCATTERING STUDY OF THE TEMPERATURE DEPENDENCE OF *ESCHERICHIA COLI* MOTILITY

GORDON BANKS, DALE W. SCHAEFER, *and* S. S. ALPERT

From Sandia Laboratories, Albuquerque, New Mexico 87115, and the Department of Physics and Astronomy, University of New Mexico, Albuquerque, New Mexico 87131

ABSTRACT Two light-scattering techniques are used to study the temperature dependence of translational and rotational motility in *Escherichia coli*. The method of number fluctuation spectroscopy is developed theoretically and experimentally to measure the translational swimming speed of a smooth swimming strain of *E. coli*. Interference fluctuation techniques are used to study the rotational component of the motion. The results demonstrate that the thrust remains proportional to the torque generated by the flagella throughout the range studied and also show that relative changes in translational swimming speed may be inferred from the dynamics of rotational motion.

INTRODUCTION

The behavior of bacteria when subjected to environmental changes provides an ideal arena for the study of biological responses at the cellular level (1, 2). There is considerable interest, therefore, not only in the tactic responses of bacteria but also in the mechanisms by which motile microorganisms organize and drive their flagella (3). In this work we develop both theoretically and experimentally a new laser light-scattering method to measure the root mean square (rms) swimming speed of *Escherichia coli* cheC497. This method is based on the analysis of the dynamics of fluctuations in the total number of bacteria in the focused waist of a laser beam and is applicable to bacteria which swim smoothly for distances long compared with the diameter of the waist. We find that the rms translational swimming speed is a linear function of temperature. In addition, we use the previously developed method of interference fluctuation spectroscopy (IFS) (4) to study the center of mass motion of the bacteria. The results of the two classes of experiments demonstrate that the translational and rotational components of the swimming motion directly scale. Our results also demonstrate that IFS is sensitive to relative changes in translational swimming speed even if the bacteria in the cultures display significant rotational modes of motility (5).

In this work we measure the rms translational swimming speed by the correlation analysis of the light scattered from solutions which are so dilute that fluctuations are observed in the number of bacteria in the scattering region. This method has at least two advantages over classical motility measurements based on microscopic observa-

tion. First, classical methods based on timing the transit of a bacterium across a grid have the disadvantage of a relatively long "yardstick." The number fluctuation (NF) method requires only that the organism swims straight for a distance long compared with the diameter of a focused laser beam (here 25 μm). Second, the NF method eliminates prejudice due to selection of specific individuals.

In addition to measurement of the rms swimming speed by NF we also study rotational motion by IFS (sometimes called homodyne light scattering). This method, which was first applied to bacterial motion by Nossal and co-workers (4), relies on the analysis of fluctuations which arise because the motion of the particles produces a constantly changing *interference* condition at the detector. In contrast to NF these interference fluctuations are sensitive to motion over distances of the order of the wavelength of light ($\sim$0.5 μm). If care is taken to ensure that rotational motion is not present, Nossal et al. (4) have shown that the rms swimming speed can be measured by IFS. In the case of wobbling cultures of the type used here, however, experimental results indicate that IFS is primarily sensitive to the rotational component of the motion (5). Therefore, we are able to study the coupling between rotational and translational motion by exploiting both number and interference methods.

The potential of these methods is demonstrated through a study of the thermal response of *E. coli*. Classical experiments have left confusion in this area because most methods do not distinguish changes in swimming speed from other factors such as growth rate and loss of flagella.

THEORY

If a focused beam of laser light is used to illuminate a volume containing swimming bacteria, a detector placed in the field of the scattered light will detect a fluctuating intensity. The far-field intensity as a function of time will be related to both the number of scatterers occupying the scattering volume at any given time and the position of the fluctuating diffraction pattern generated by the scatterers. It is necessary to characterize the dynamics of these intensity fluctuations. Such dynamic phenomena are conveniently described either through time correlation functions or power spectra. Because of the very long characteristic time of number fluctuations it is convenient to perform experiments in the time domain. An expression is needed, therefore, for $\langle I(0)\, I(t)\rangle$, the intensity correlation function for light scattered from a system in which there are on average $\langle N\rangle$ scatterers in the scattering region. Schaefer and Berne (6) have demonstrated that if $\delta N(t)$ is the fluctuation of the number of scatters in the scattering volume away from $\langle N\rangle$, then

$$\langle I(0)\, I(t)\rangle \propto 1 + \frac{\langle \delta N(0)\, \delta N(t)\rangle}{\langle N\rangle^2} + |\, R_E(K,t)\,|^2, \tag{1}$$

where $R_E(K,t)$ is the self-intermediate scattering function (4, 6) and K is the magnitude of the scattering vector (4). In the experiments reported here, we measured the

photocurrent correlation function, $R_i(K,t)$, which in the time domain of interest is directly proportional to Eq. 1. The second and third terms of Eq. 1 represent the number and interference fluctuations. Depending on $\langle N \rangle$ either of these terms can be made to dominate the intensity correlation function.

The second, or NF term of Eq. 1 is important only at low population densities ($\langle N \rangle \sim 1$). The characteristic decay time of $\langle \delta N(0)\, \delta N(t) \rangle$ is roughly the residence time of a typical particle in the scattering volume and this decay time depends on the details of the swimming motion. In the case of strains of bacteria which are likely to change directions several times while in a typical scattering region (e.g., wild type *E. coli*), $\langle \delta N(0)\, \delta N(t) \rangle$ is sensitive to V_T, the translational swimming speed, and $\langle L \rangle$, the mean distance between direction changes (7). Although theoretical analysis of $\langle \delta N(0)\, \delta N(t) \rangle$ is quite difficult for this case, number fluctuation experiments have been used to confirm that chemotaxis in *E. coli* is a gradient-sensitive effect (8).

Since the amplitude of wobble motion is very small compared with the scattering volume, $\langle \delta N(0)\, \delta N(t) \rangle$ depends only on the translational component of the motion. We have

$$\langle \delta N(0)\, \delta N(t) \rangle = \rho \iint d\mathbf{r}_1 d\mathbf{r}_2 I(\mathbf{r}_1) I(\mathbf{r}_2) p(\mathbf{r}_2 - \mathbf{r}_1; t), \tag{2}$$

where $I(\mathbf{r})$ is the intensity of light scattered by a particle when at position $\mathbf{r}$ and $p(\mathbf{r}_2 - \mathbf{r}_1; t)$ is the probability of observing a particle at position $\mathbf{r}_2$ at time t if that particle was at position $\mathbf{r}_1$ at time 0. ρ is the number density.

The scattering volume will generally be fixed by the focused laser beam in two dimensions and by a narrow slit in the third. In this case

$$I(\mathbf{r}) = \exp\{-2(x^2 + y^2)/\sigma_1^2 - 2z^2/\sigma_2^2\}, \tag{3}$$

where σ_1 is the $1/e^2$ radius of the focused laser beam σ_2 is the $1/e^2$ point of the intensity distribution in the third dimension.

For strains of bacteria which swim in relatively straight lines for distances large compared with the average dimension of the scattering volume,

$$p(\mathbf{r}_2 - \mathbf{r}_1; t) = \iint d\mathbf{V} dV_T \delta(\mathbf{r}_2 - \mathbf{r}_1 - \mathbf{V}_T t)\, \delta(|V| - V_T)[P(V_T)/4\pi V_T^2], \tag{4}$$

where $P(V_T)$ is the distribution of translational swimming speeds.

Inserting Eqs. 3 and 4 into Eq. 2, we have

$$\frac{\langle \delta N(0)\, \delta N(t) \rangle}{\langle N \rangle} = \int_0^\infty dV_T \frac{P(V_T)}{2V_T} \int_{-V_T}^{V_T} dV_Z \cdot \exp - \left\{ \frac{V_Z^2 t^2}{\sigma_2^2} + \frac{(V_T^2 - V_Z^2) t^2}{\sigma_1^2} \right\}. \tag{5}$$

This equation indicates that the entire speed distribution $P(V_T)$ could be extracted

from NF data. We find, however, that because of signal-to-noise considerations, it is reasonable to measure only the second moment of the distribution through an expansion of the logarithm of Eq. 5 about $t = 0$:

$$\ln \frac{\langle \delta N(0)\, \delta N(t) \rangle}{\langle N \rangle} = - \langle V_T^2 \rangle t^2 \left(\frac{2}{3\sigma_1^2} + \frac{1}{3\sigma_2^2} \right) + \cdots. \tag{6}$$

Thus, analysis of the initial decay of $\ln \langle \delta N(0)\ \delta N(t) \rangle$ yields the rms swimming speed. The beauty of the NF method is that Eq. 6 is independent of the details of rotational motion. Moreover, the interpretation of the data are not obscured by the presence of nonmotile individuals (see below).

The third term of Eq. 1 represents fluctuations due to the changing diffraction pattern generated by interference conditions within the scattering volume. This term dominates in the high density regime and in this case the method is referred to as IFS. Since R_E is sensitive to motion over distances of the order of the laser wavelength it is reasonable to believe that this term is sensitive to the rotational as well as the translational motion. Indeed, experiments indicate that rotational motion dominates this term (5). Boon et al. (9) have developed an approximate theory for $R_E(K,t)$ for rotating (or wobbling) rods. Their theory leads to an expression for the correlation function which can be expanded for small t into

$$\ln | R_E(K,t) |^2 \simeq - (K^2 t^2/3) \langle V_C^2 \rangle + \cdots, \tag{7}$$

where $\langle V_C^2 \rangle$ is a parameter proportional to the mean square speed of the center of mass of the particle. In the case of the wobbling rod

$$\langle V_C^2 \rangle \propto \langle \langle \Omega^2 b^2 \sin^2 \alpha \rangle \rangle + \langle V_T^2 \rangle, \tag{8}$$

where the rods of length b are rotating with frequency Ω about one end and translating with velocity V_T about the axis of rotation, α being the angle between the axis of the rod and the translation axis. The data presented here were analyzed through the initial decay of $\ln | R_E(K,t) |^2$. Previous experiments (5) indicate that the first term of Eq. 8 dominates the second.

RESULTS AND DISCUSSION

The experimental work consisted of the measurement of the photocurrent autocorrelation function of light scattered from low and high density populations of *E. coli* cheC497 (10, 11). This strain has been characterized as swimming at constant speed for long distances without abrupt changes in direction (10). The samples were grown in closed tubes containing Hutner's medium (12) + 0.5% dextrose at 30°C (5) which had been passed through a 0.22 μm filter. Coherent light from a Spectra-Physics 125 HeNe laser (6,328 Å) (Spectra-Physics, Inc., Mountainview, Calif.) was focused into the sample which was immersed in a temperature-controlled bath (±0.01°C). It was

found that when raising or lowering the temperature of the cultures, the characteristic decay times of the correlation function would fall or rise, respectively, for several minutes but would eventually stabilize at a value which was repeatable upon subsequent measurements. Therefore, about 1/2 h between measurements was necessary.

In those samples studied at high densities, $R_i(K,t)$ was measured at a scattering angle of 50° during the stationary phase of the growth cycle at 30°C. The bacteria were studied directly in the growth medium rather than resuspended in a motility medium since it has been found that the centrifugation necessary to separate the bacteria damages the motility, presumably by destroying some of the flagella. The temperature was then raised to 35°C and after stabilization the correlation function was measured at the higher temperature. The temperature was then returned to 30°C and the measurement repeated to verify reproducibility within 5%. The temperature was then lowered for experiments at 25°C. Another culture was grown at 25°C and studied in the same manner at 25° and 20°C.

Since the density of the bacteria was high ($\sim 10^8$ cm^{-3}) some multiple scattering occurs. We do not believe multiple scattering significantly effects our conclusions because: (*a*) four-fold dilution does not alter the correlation time (5), and (*b*) theoretical considerations indicate that multiple scattering does not effect the correlation time to second order in the density (13).

In the NF experiments, a few drops of a culture grown at 30°C to a high density

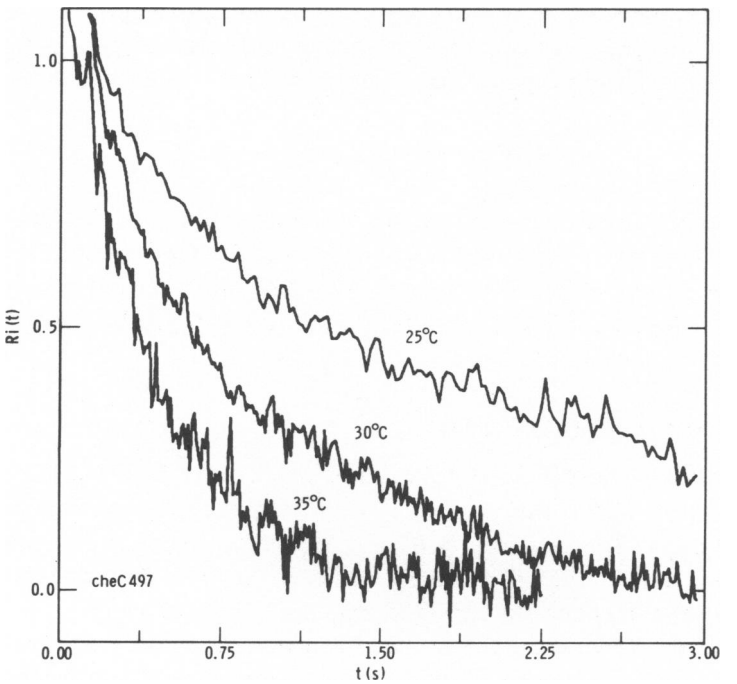

FIGURE 1 Photocurrent correlation function at low population densities (number fluctuations).

were diluted into a cell containing "motility" medium which sustains motility but not growth (14), consisting of 0.01 M phosphate buffer (pH 7.0), 10^{-4} M EDTA, and 0.5% dextrose. The final density was $\sim 10^6$ cm^{-3}. The motility medium was used since the NF measurements required a long period of data collection during which the culture must remain vital but at a constant low density. $R_i(t)$ was measured for three separate cultures at 25°, 30°, and 35°C at a scattering angle of 90°. Whereas the motility of the samples approached 100% in Hutner's medium it was difficult to prepare samples in motility medium in which the fraction of nonswimming bacteria was less than 20%. Fortunately, in the NF experiment the effect of nonmotile bacteria appears as a time independent background and can be easily subtracted. The scattering volume parameters σ were found by using a moving sample holder to translate a solution of polystyrene spheres perpendicular and parallel to the beam (15); σ_1 was determined to be 12.6 μm and σ_2 to be 1×10^2 μm.

The data for NF experiments are shown in Fig. 1. A background due to nonmotile bacteria has been subtracted from these data. As explained above, these data were analyzed through Eq. 6 to yield $\langle V_T^2 \rangle^{1/2}$, which is plotted in Fig. 2. This figure indicates that the rms swimming speed is an approximately linear function of temperature between 25° and 35°C.

The swimming speeds in Fig. 2 can be compared with data of other workers. The swimming speed obtained at 30°C is about 15% higher than that obtained by Berg and Brown (10) by tracking. This discrepancy is consistent with the fact that their apparatus is unable to track the most rapid individuals. Adler and Templeton (14) found that motility of *E. coli* B275 is approximately constant between 25° and 35°C. Although it is possible that the discrepancy arises due to the strain differences, or lack of preci-

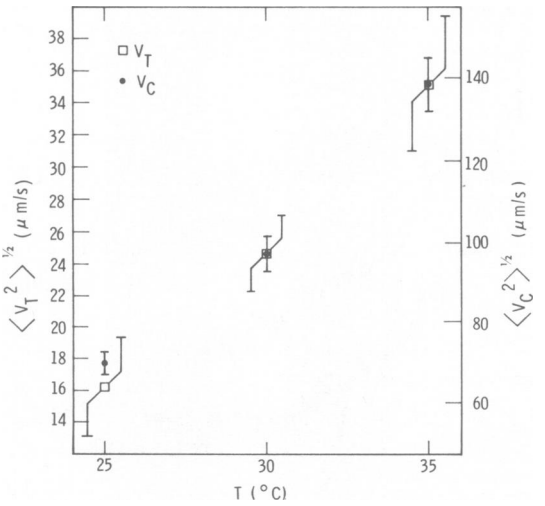

FIGURE 2 Temperature dependence of $\langle V_T^2 \rangle^{1/2}$ and $\langle V_C^2 \rangle^{1/2}$. The error bars represent our estimate of confidence limits based on the repeatability of measurements at each temperature.

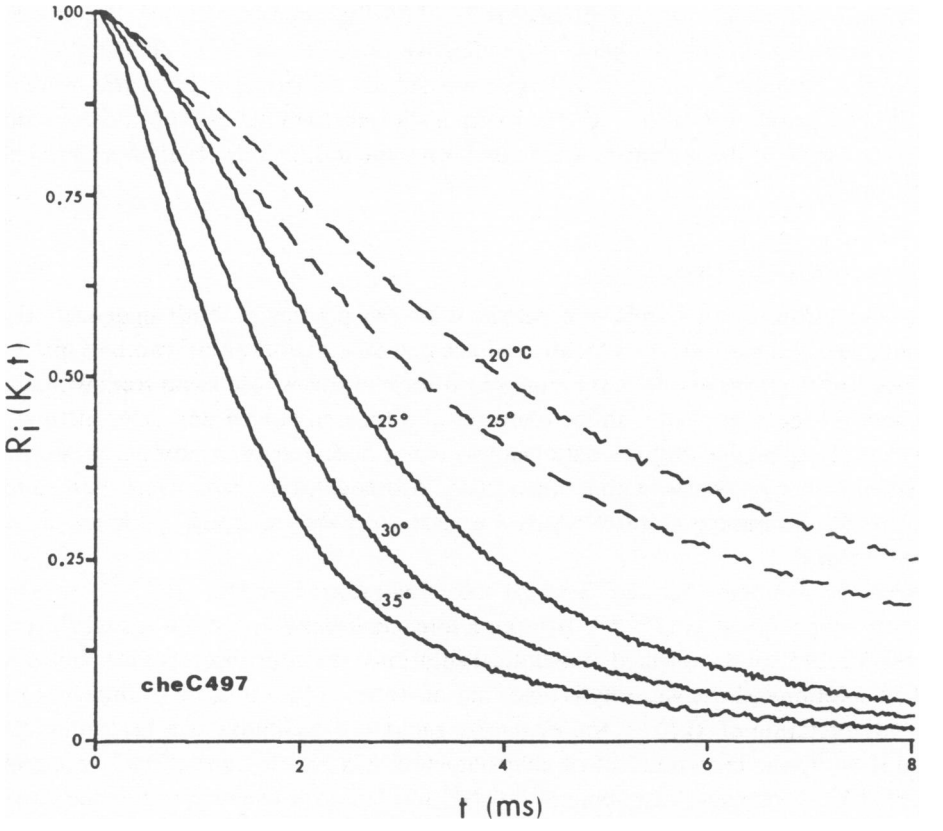

FIGURE 3 Photocurrent correlation function at high population densities (interference fluctuations) for bacteria grown at 25°C (broken lines) and 30°C (solid lines). Measurement temperatures are indicated above the curves.

sion, it is more likely that motility, as defined by Adler and Templeton, is sensitive to factors other than the swimming speed. Finally, our results show the trend found by Ogiuti (16) for *Salmonella.*

Scattering data for high density cultures grown at 25° and 30°C are shown in Fig. 3. The center-of-mass velocity parameter $\langle V_C^2 \rangle^{1/2}$ extracted using Eq. 7 from the data for cultures grown at 30°C is plotted as the right ordinate of Fig. 2. This parameter is also an approximately linear function of temperature. The presence of a slowly decaying background for samples *grown* at 25°C is probably due to the presence of nonswimming individuals which precludes extraction of $\langle V_C^2 \rangle^{1/2}$.

These results do not contradict those of other investigators with regard to the presence of nonmotile bacteria in cultures grown or incubated at high temperatures (17). The present data were collected from samples grown at a temperature (30°C) chosen for the production of optimal numbers of motile specimens, and studied after relatively short incubation times at the higher and lower temperatures. Indeed, in an experiment

done on a culture grown and studied at 37°C (at high densities), $R_t(K,t)$ showed a slowly decaying exponential behavior, indicative of scattering from diffusing bodies. When the exponential which fit at large t was subtracted from the data, the residuals exhibited a profile similar to Fig. 3 but with a shorter decay time. This indicates that although some of the specimens were nonmotile, the motile individuals were simming very rapidly.

CONCLUSIONS

The conclusions of this work are pertinent to the physics of both light-scattering phenomena and motility. First of all, we have demonstrated that NF can be exploited to measure the translational swimming speed for cultures whose mean free swimming distance is long compared with the diameter of a focused laser beam. This method is particularly appealing because data analysis is not obscured by rotational motion or by the presence of nonswimming individuals. Moreover, NF methods are best suited to very fast swimming cultures which are least amenable to study by direct microscopic means.

We have also demonstrated that rms speeds extracted from IFS ($\langle V_C^2 \rangle^{1/2}$) data are directly proportional to $\langle V_T^2 \rangle^{1/2}$. Although some ambiguity is cast on this result since the data could not be collected on identical solutions, the conclusion is probably valid for all solutions whose viscosity is near that of water. Thus, in spite of difficulties in the interpretation of $\langle V_C^2 \rangle^{1/2}$, the extremely rapid IFS technique can be legitimately used to study the relative effect of environmental factors on translational swimming speeds (18). Moreover, a combination of NF and IFS experiments provide the means to study the coupling of rotational and translational motion, which is important in the understanding of flagellar action.

The results found here are consistent with either the helical wave (19) or rotating flagella (3) model of bacterial motility. In either case, if the flagellar bundle does not form directly along the axis of the bacteria, rotation of the head is required for the generation of thrust. (The fact that the wobble motion disappears when the bacteria are made to swim in high viscosity media can be accounted for by a model based on hydrodynamic and elastic considerations [20].) Either model of bacterial motility leads to the conclusion that the rotational (wobble) modes of motion will be coupled to the translational modes for a constant viscosity medium.

In our experiments the scaling of $\langle V_C^2 \rangle^{1/2}$ with $\langle V_T^2 \rangle^{1/2}$ indicates that the thrust generated by the flagella remains proportional to the torque generated by the flagella throughout the range of temperature and viscosity studied. This result can be contrasted with experiments at viscosities twice that of water, in which case rotational motion is suppressed and *E. coli* swims smoothly with very little wobble (5, 20).

The data presented above suggests that this strain would be repelled by heat. That is, since the speed increases with increasing temperature, a bacterium executing otherwise random behavior would spend a lesser fraction of time in hotter regions. Since

this strain lacks the control systems necessary for chemotaxis (10), it is reasonable to suggest it would not exhibit any other thermal response than that suggested above.

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