Estimation of chemotaxis intensity of *Salmonella* cells by the capillary assay and biased random walk simulation

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Abstract—Bacterial cells exhibit chemotaxis by repeating a straight swimming (run) and an abrupt change of the swimming direction (tumble). Cells detect the change in the concentration of a chemical attractant during the run and decrease the frequency of the tumbles if the cells have swum toward the favorable direction. As for the chemotaxis mechanism, a mathematical model has been proposed where the frequency of the tumble is correlated with the chemotaxis intensity. In this study, we observe the chemotactic behaviors of bacterial cells and compare the measurement with the mathematical model to quantitate the chemotaxis intensity.

Index Terms—Bacterium, Flagellum, Chemotaxis, Run and tumble, Biased random walk.

I. INTRODUCTION

Bacterial cells such as *Escherichia coli* and *Salmonella typhimurium* are peritrichous that have multiple flagella. The cells swim by rotating their helical flagella with flagellar motors at the proximal ends of the flagella. When the motors rotate counterclockwise (CCW) viewing from the flagellum to the motor, flagella form a bundle and the cell propels itself straight (run). When the rotational direction of the motor switches to clockwise (CW), the corresponding flagellum is loosened from the bundle and the swimming direction of the cell changes (tumble) [1, 2]. For *E. coli* cells, the mean run and tumble lengths (the authors used the term “lengths” as the time duration) are about 1 and 0.1 s, respectively [3].

Many of peritrichous bacteria exhibit chemotaxis; the cells accumulate around the favorable substance (attractant) or recede from unfavorable one (repellent). The cell senses the difference in concentration of the attractant between two adjacent time points. If the cell senses that the concentration of an attractant has increased, the cell decreases frequency of the tumbles. Thus the cells accumulate around an attractant though they cannot choose the directions after tumbles. For the chemotactic behaviors of *E. coli* cells toward L-serine, mean run lengths were reported as 2.19 s and 1.40 s for up and down the gradient, respectively [3].

Some mathematical models have been proposed for the bacterial chemotaxis. The continuum model based on telegraph equations was developed by Schnitzer [4], where the tumble frequency and swimming velocity are described as functions of the position and swimming direction. In contrast, to associate the degree of chemotaxis with characteristics of cells’ behavior easily observed, Goto and Nakai have proposed another mathematical model based on the biased random walk [5]. In this model, the cell changes frequency of tumbles depending on the difference in the concentration of an attractant at the starting and ending points of one leg. They defined chemotaxis intensity $\alpha$ as the probability that the cell suppresses a tumble and continues a run. According to this model, the distribution of cell number density becomes steady and exponential function of distance from the attractant source, where the exponent largely depends on the chemotaxis intensity. In this study, we measured the bacterial cells’ behavior around an attractant to estimate the chemotaxis intensity $\alpha$. A conventional method, capillary assay, was employed for the measurement of the chemotactic behaviors. In the presence of the concentration gradient of an attractant, we measured the tumble frequency of cells to directly determine the chemotaxis intensity $\alpha$. Then, the chemotaxis intensity was applied to the biased random walk simulation [5] and the calculated cell accumulation was compared with the observed one.

It is known that chemotactic behavior widely varies with the chemicals even in the same bacterial strain. For example, difference in accumulation of *Bacillus subtilis* cells toward many kinds of sugars has been reported [6]. Our study aims to estimate and predict the accumulation of cells by quantifying the chemotaxis intensity from the behavior of a single cell toward an attractant.

II. METHODS

A. Observation of accumulating cells around an attractant

A strain of peritrichous bacteria, *Salmonella typhimurium* SJW1103 was used. The size of the cells is 1–2 $\mu$m in length and 0.6–0.8 $\mu$m in width. Cells were cultured in 3 mL of LB medium (1% polypeptone, 0.5% yeast extract, 0.5% sodium chloride) overnight at 30°C. Then, 0.1 mL of the bacterial suspension was put into 3 mL of the motility buffer (pH 7.0, 10 mM KH$_2$PO$_4$, 0.1 mM EDTA, 10 mM sodium lactate) and incubated for 3–4 hours at 30°C. The solution was centrifuged for 1 minute at 10000 rpm (CHIBITAN-II, Merck, Japan). The supernatant liquid of 0.9 mL was removed and the buffer of the same volume was added to remove the culture medium for the detection of an attractant.

The capillary assay was employed, where cells accumulate around the tip of a capillary filled with an
attractant [7]. A capillary was made by pulling a glass tube with a filament (GD-1, Narishige, Japan) by a puller (PC-10, Narishige). The inside diameter of the tip was 3–4 micrometers. L-serine (MW = 105, Wako, Japan) was used as an attractant. Since the surface tension is strong inside the capillary due to the filament, the flow would be induced around the capillary tip if liquid were filled inside the capillary. To prevent the flow, the attractant solution was jellified with agar. One molar L-serine with 0.3% agar (Agarose L, Wako, Japan) dissolved into the motility buffer was heated with a hot plate at 80°C and then aspirated into the capillary. Then the outside surface of the capillary was washed with distilled water. As shown in Fig. 1, the capillary and the bacterial suspension were inserted between slide glass (S7224, 76 mm × 26 mm, thickness 1.2–1.5 mm, Matsunami, Japan) and cover glass (18 mm × 18 mm, thickness 0.13–0.17 mm, Matsunami, Japan). To prevent convection due to the evaporation, the edge of the cover glass was sealed with petroleum jelly. The thickness of the suspension was so large (~300 μm) that the cells’ swimming was almost three dimensional.

Cells accumulating around an attractant were observed by an inverted microscope (IX71, Olympus, Japan) with an objective lens (LUCPLFN40XPH, NA = 0.60, Olympus). The capillary tip was set at the center of the view. Images were recorded with a digital camera (DP27, Olympus) at 30 fps with resolution 1216 × 960 pixels (corresponding to the field of view 280 μm × 220 μm). Images were analyzed by the tracking software (DippMotionPro, Ditect, Japan) to measure the frequency of tumbles, and by the freeware (ImageJ, NIH, USA) to measure the cell distribution.

Although the concentration field of L-serine cannot be seen directly, the characteristic time of the diffusion can be roughly estimated from the diffusion constant $D$. In aqueous solution, the diffusion constant $D$ is on the order of $10^{-9}$ m²/s at room temperature. Consequently, the time $\tau$ that it takes for the L-serine molecules at the capillary tip to diffuse outside of the view is approximately estimated as

$$\tau \approx \frac{(100 \, \mu m)^2}{D} = 10 \, s.$$  \hspace{1cm} (1)

From this estimation, concentration field of serine is thought to be formed in several tens of seconds.

To check the stable and concentric concentration field in the capillary assay above, a fluorescent dye was used instead of L-serine. The capillary filled with 10 mM fluorescein (MW = 332, Wako, Japan) was observed by the laser scanning confocal microscope (FV10i, Olympus, Japan) with a 10X objective lens. Figure 2 shows the snapshot (a) and the fluorescence intensity (b). The fluorescence distribution around the tip seems approximately concentric, which continued at
least for 20 minutes. The light intensity monotonically decreases along the line OP (~ 500 \( \mu \)m). The diffusion constant is inversely proportional to the size of the particle. The size of L-serine is roughly estimated to be 0.7 times as large as that of fluorescein if both molecules are spherical (cubic root of the ratio of the molecular weight: \((105/332)^{1/3} \approx 0.681\) ). Thus, similar concentration gradient of an attractant is also assumed in the observation of the chemotactic behavior of cells (within 15 minutes, see IIIA and IIIC.).

**B. Simulation based on the biased random walk**

The chemotactic behavior of cells was numerically simulated by the biased random walk model [5], where the tumble frequency changes depending on moving direction of cells, as schematized in Fig. 3. An attractant source is at the origin and the concentric concentration field due to diffusion is assumed. The rules for modeled cells are as follows:

1. Cells migrate a constant distance \( \Delta r \) during a unit time step \( \Delta t \).
2. A cell moves toward the same direction as the previous time step with the probability \( \alpha \) when the cell has moved toward the direction where the concentration of an attractant increases, i.e., toward the source of an attractant. Or the cell changes its direction with the probability \( 1 - \alpha \), where the direction in the next step is determined randomly. Note that the cells only judge whether they swim up or down the gradient, and that they do not sense how large the concentration gradient is. The parameter \( \alpha \) ( \( 0 \leq \alpha \leq 1 \) ) denotes the intensity of the chemotaxis; \( \alpha = 1 \) is the maximum intensity and \( \alpha = 0 \) is minimum, that is, the random walk without biases.
3. A cell changes its direction to the random direction when the cell has moved toward the direction where the concentration decreases.

Initially, \( 10^5 \) cells were arranged randomly and uniformly in the three dimensional region \( r < 30 \Delta r \), where \( r \) is the cell’s distance from the origin (attractant source). Enough time steps later, the distribution of cells was analyzed in the region \( |z| < \Delta r \) for the comparison with the quasi-2-dimensional microscopic observation.
III. RESULTS

A. Run duration

To confirm that the actual cells obey the rules of the biased random walk model, the frequency of tumbles was investigated in the capillary assay. Figure 4 shows the example of observed trajectory of a swimming cell (a) and the distribution of run duration (b). From the trajectory, run duration, the time between two successive tumbles, was measured, where each run was categorized into two groups depending on its direction in the concentration gradient of an attractant. Since the concentric concentration field is assumed, the cell approaching the tip of a capillary senses that the concentration of an attractant increases (up the gradient), and vice versa (down the gradient). From images several minutes after the start of observation, we analyzed 146 trajectories of cells and obtained data of 150 runs for “up”, and 277 runs for “down”. Both distributions have a peak around 1–2 s but longer runs are seen in “up”. The mean run duration is 2.0 ± 0.9 s for “up” and 1.3 ± 0.5 s for “down”. The run duration is larger for “up”. Note that the run duration “down” is not constant but has a significant fluctuation, which is different from the simulation model above.

B. Estimation of the intensity of chemotaxis $\alpha$ from the run duration

The above observed run duration can be compared with the biased random walk model to estimate the chemotaxis intensity $\alpha$. Let us use the average value of the run duration although there are some fluctuations as seen in Fig. 4 (b). If all the cells swimming toward “down” have the same run duration $t_d$, the probability $p(n t_d)$ that the run duration “up” becomes $n t_d$ is calculated as a directional change after $n - 1$ straight runs;

$$p(n t_d) = \alpha^{n-1}(1 - \alpha). \quad (2)$$

The average run duration for “up” direction $t_u$ is calculated as

$$t_u = \sum_{n=1}^{\infty} n t_d p(n t_d) = \frac{t_d}{1 - \alpha}. \quad (3)$$

Thus, the chemotaxis intensity $\alpha$ is described with the ratio of the run durations as

$$\alpha = 1 - \frac{t_d}{t_u}. \quad (4)$$

Since $t_d = 1.3$ s, $t_u = 2.0$ s for the observed run duration, we obtain $\alpha \approx 0.35$.

The distribution “up” in Fig. 4 (b) has second peak around 2.5–2.75 s. This appears to be twice the first peak (1.5–1.75 s) corresponding to $n = 2$ in eq. (2). By applying eq. (2) to observed run duration “down”, we can guess the distribution of run duration “up”. However, actual distribution of run duration “down” is too broad to produce the second peak in the estimated distribution “up”.

C. Steady distribution of the cell number density around an attractant

Previous numerical study using the biased random walk model reported that the steady distribution of cells largely depends on the chemotaxis intensity $\alpha$ [5]. In this subsection, the cell distribution around the capillary is compared with the numerical simulation to estimate $\alpha$. Figure 5 shows the cell distribution around a capillary filled with 1 M L-serine. The profile of cell number density (b) was deduced from the bright-field microscopic image (a), which had been processed with...
subtracting background for the visualization of cells. Note that the blurred cells out of focus were also counted. In Fig. 5 (a), the range where the visible cells exist is estimated to be within 10 μm from the focal plane. In Fig. 5 (b), data are not shown in the region where distance is less than 50 μm because uncertainty of z-position affects the 3D distance from the capillary tip. As time elapses, the number of cells increases and the distribution becomes almost steady after 8 minutes. The number density is larger at the position near the capillary tip. The number density is well fit to the exponential function of the distance from the capillary tip, which is similar tendency with the simulation [5].

To compare the observed result (Fig. 5) with the simulation, let us estimate the actual size of the step length \( \Delta r \). Considering that \( \Delta r \) corresponds to the run duration “down” (Fig. 4) and swimming speed of the cells is \( 23 \pm 5 \) μm/s (mean ± S.D.), the average value of \( \Delta r \) is \( 1.3 \) s × \( 23 \) μm/s = 30 μm. Thus, the slope \(-1/(100 \mu m)\) (decrease in an order of one magnitude per 100 μm) in the exponential distribution in Fig. 5 corresponds to \(-1/(3.3\Delta r)\).

D. Estimation of the intensity of chemotaxis \( \alpha \) from the distribution of the cell number density

Numerical simulation based on the biased random walk was conducted in a 3-dimensional region to estimate the chemotaxis intensity \( \alpha \) from the comparison with the observation. Figure 6 shows the steady distribution of cells with various values of \( \alpha \). For the comparison with the 2-dimensional microscopic observation, cells within the region \(|z| < \Delta r\) were counted and the distance (horizontal axis) was taken as in the 2-dimensional plane. The distribution became stable in 200, 700, 2000, 3000 time steps \((\Delta t)\) for \( \alpha = 1, 0.4, 0.2, 0.1 \), respectively. Considering that \( \Delta t \sim t \sim 1\) s (run duration), the distributions in Fig. 6 are in the state after several tens of minutes. Each distribution has exponential decay and its slope varies widely depending on \( \alpha \) value. Similar tendency was reported in our previous work with one dimensional simulation [5]. The exponent of the distribution \( \alpha = 0.4 \) or \( \alpha = 0.6 \) is similar to the observed one, \(-1/(3.3\Delta r)\). These \( \alpha \) values are close to \( \alpha = 0.35 \), deduced from the change in the run duration in IIIB.

As seen in Fig. 4 (b), the run duration does not have a constant value but has a fluctuation around the average value; the distribution seems Gaussian for “down” and Poisson for “up”, reflecting eq. (2). Consequently, a step length in the simulation that corresponds to the run length “down” is actually not a constant value \( \Delta r \), whose influence on the steady distribution of cells has to be elucidated. According to the distribution “down” Fig. 4 (b), the distribution of the step length is roughly approximated with a triangular distribution ranged from 0 to 2\( \Delta r \), as shown in the inset of Fig. 7 (the step length is different among cells but constant in the same cell). Simulation with this triangular velocity distribution \((\alpha = 0.35)\) is compared with that with constant velocity in Fig. 7. Although cell distributions are slightly different at the region \( \Delta r < 3 \) and \( \Delta r > 15 \), the slope is almost the same at the intermediate region. The estimation of \( \alpha \) value can be done by the average value of the swimming velocity or the run duration of cells and almost independent of their fluctuations.
IV. CONCLUSION

Bacterial cells’ behaviors toward an attractant are measured by the capillary assay and compared with the mathematical model to quantify the intensity of chemotaxis. From the observation in the capillary assay, the intensity of chemotaxis is estimated by two methods; one is from the change in the run duration (time between successive two tumbles) and the other is from the spacial distribution of the cell number density. For both methods, we confirm the qualitative correspondence with the mathematical model based on biased random walk; the run duration increases when the cells swim toward the attractant source and the distribution of cell number density becomes steady and exponential decay with distance. Estimated chemotaxis intensity values from both methods are quite similar, which shows the chemotactic behavior of individual cells directly causes the accumulation of cells. This estimation is robust against fluctuations; fluctuations in the swimming velocity or the run duration do not affect the steady distribution of cells around an attractant.

Although this study deals with the chemotaxis of only one bacterial strain toward one chemical, it sheds light on the quantitative estimation of cells’ accumulation. In the future, our study may be applied for the chemotaxis of many kinds of bacteria towards many kind of chemo-attractant.

Acknowledgment

This work was supported by JSPS KAKENHI Grant Number JP19K04193 and JP18K03950.

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