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Simulation Study of Bacterial Colony with Multiplying Rods

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Abstract. A microscopic model for a bacterial individual is proposed, and applied investigation of the bacterial colony. *B. Subtilis* is considered as a sample, which is regarded as a rod molecule moving and rotating on the substrate including the diffusive nutrients. The model is analyzed by Monte Carlo simulations, and several patterns observed in experiments have been generally reproduced.

1. Introduction

Bacteria exhibit various colony patterns according to the substrate softness and nutrient concentration though they are simple unicellular organisms (SINGLETON and SAINSBURY, 1981). Particularly colony pattern of bacteria species *B. Subtilis* has been vigorously studied from both experimental and theoretical viewpoints (FUJIWARA *et al*., 1989; OHGIWARI *et al*., 1992; WAKITA *et al*., 1994, 2001; KAWASAKI *et al*., 1997; MATSUSHITA *et al*., 1998). From the experimental studies, a morphological phase diagram of colonies of *B. Subtilis* is determined by varying both the concentration of nutrient and the substrate softness. The phase diagram is composed of five patterns, DLA-like, Eden, DBM-like, concentric ring and homogeneous disk-like (OHGIWARI *et al*., 1992). Though boundaries between these phases are somewhat ambiguous, their physical and geometrical characters are clearly defined by parameters such as the roughness exponent.

In order to investigate these biological patterns and compare them with peculiar patterns caused by physical factors, numerical simulations of the model equations based on the reaction-diffusion equations have been studied recently (WAKITA *et al*., 1994; KAWASAKI *et al*., 1997; MATSUSHITA *et al*., 1998; KOZLOVSKY *et al*., 1999). Result of these theoretical studies revealed that similar colony patterns were reproduced and parameters in the model equations were discussed. These models, however, are constructed from a macroscopic viewpoint and thus detailed movement of each individual is disregarded.

In this paper we propose a microscopic model treating bacterial individual and apply it to formation of the bacterial colony. *B. Subtilis* is regarded as a rod molecule which moves and rotates on the substrate including diffusive nutrients. Though the rods basically

move and rotate randomly, they also choose one of two modes, “run” or “tumble”, depending on the amount of the surrounding nutrient. The rod molecule ingests the nutrient and grows until the rod divides into two new individuals.

We study this multiplying rod model by means of Monte Carlo simulation. By varying the substrate softness and nutrient concentration, growth of the colony in various environment is investigated. In the result of the simulation, several patterns observed in experiments have been globally reproduced. From investigating a diffusion coefficient of the bacteria, their activity in DBM-like and disk-like colonies are microscopically determined.

2. Model

We consider a hard-rod system in two dimensions as is shown in Fig. 1. A length of the rod l_b grows from 6a to 14a where a is a unit length corresponding to the radius of the rod. The rod which represents *B. Subtilis* moves ahead and rotates continuously on the surface of agar plates (BERG, 1992). The mimic bacteria ingests the nutrient and grows until

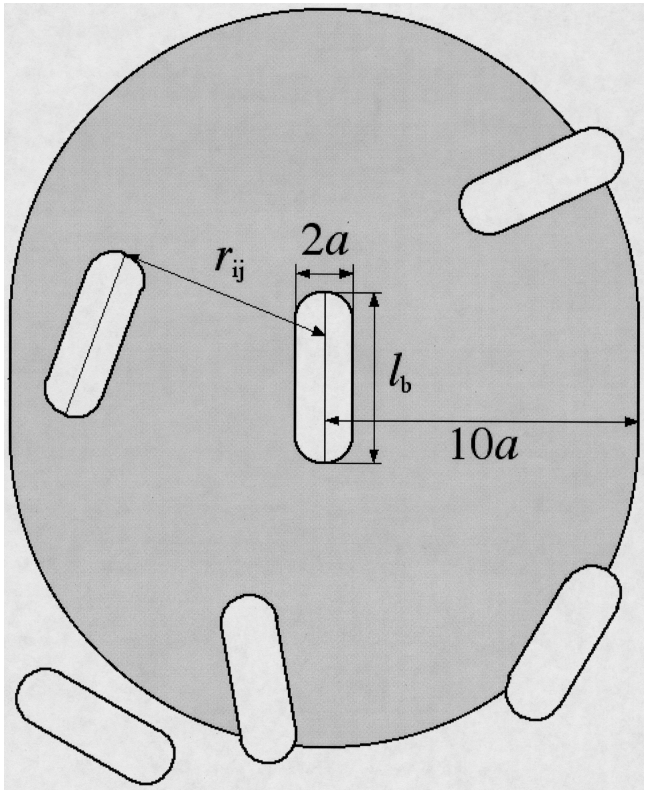

Fig. 1. Bacteria regarded as hard rod diffusing on an agar plate. The diffusion coefficient of the rod depends on the number of surrounding rods within the gray area.

the bacteria divides into two new individuals at $l_b = 14a$. Therefore the number of rods increases with the simulation step and will form a bacterial colony. In the colony, there is a direct repulsive interaction between rods. The potential energy between the rods i and j is represented as

$$u(r_{ij}) = \begin{cases} (2a / r_{ij})^{12} & (r_{ij} > 2a) \\ \infty & (r_{ij} \le 2a) \end{cases} \tag{1}$$

where r_{ij} is the distance between central segments of rods i and j. Since $u(r_{ij})$ includes a softcore term $(2a/r_{ij})^{12}$, the rods can push and shove each other (WAKITA *et al*., 2001). As a result, more active rods push other rods. This biological repulsion corresponds to a "short-range repulsive chemotaxis" (KOZLOVSKY *et al*., 1999).

The movement of the rod is classified as passive or active (BERG, 1992). A passive movement is caused by a fluctuation of surrounding mediums and thus it depends on the temperature T and the viscosity of the mediums. From the Stokes' law, the mean displacement $\sqrt{\langle \delta_P^2 \rangle} = \sqrt{2\Delta\tau k_B T / f_{xy}}$ and the mean rotational angle $\sqrt{\langle \theta_P^2 \rangle} = \sqrt{2\Delta\tau k_B T / f_r}$ of the rod for a unit time $\Delta\tau$ are adopted (BERG, 1992). Here $f_{xy} = 3\pi\eta l_b/\ln(l_b/a)$ is the viscous drag coefficient of the rod moving at random and $f_r = \pi\eta l_b{}^3/3(\ln(l_b/a) - 1/2)$ is the rotational frictional drag coefficient of the minor axis. k_B is Boltzmann's constant. η is the coefficient of viscosity of the surrounding mediums, which depends on not only the concentration of agar but also the lubricant such a surfactant secreted by the bacteria. An increase in the amount of lubricant decreases the friction between the bacteria and the agar surface. From a reaction-diffusion model for a bacterial colony including a time-evolution equation of a lubricant, Kozlovsky *et al*. suggested that the coupling of the bacterial motion to the lubricant should be replaced by a density-dependent diffusion coefficient for the bacteria (KOZLOVSKY *et al*., 1999). We adopt the suggestion into our model and define the coefficient of viscosity $\eta = \eta_0/s(n)$ for each rod i, where n is the number of surrounding rods which are less than $10a$ from the rod i. Since the function $s(n)$ is regarded as an increasing function, we assume $s(n) = 2^n \times 0.01 + 0.99$.

On the other hand, the active movement of a bacteria is driven by rotation of several flagellar filaments. When these flagella turn counterclockwise, they form a synchronous bundle that pushes the body steadily forward; this mode is said to "run." When they turn clockwise, the bundle comes apart and the flagella turn independently. As a result, the bacteria moves in a highly erratic manner; this mode is said to "tumble." In our model, a mimic bacteria also moves due to either "run" or "tumble" mode. In the "run" mode, a bacteria goes ahead with the mean displacement $\sqrt{\langle \delta_A^2 \rangle} = \sqrt{2\Delta\tau A_b / f_a}$, where the value A_b represents an "activity" of the bacteria, which equals the amount of nutrients ingested by the individual. f_a is a viscous drag coefficient moving lengthwise written as $f_a = 2\pi\eta l_b/(\ln(l_b/a) - 1/2)$. In the other mode, "tumble", a bacteria randomly turns clockwise and counterclockwise by the mean rotational angle $\sqrt{\langle \theta_A^2 \rangle} = \sqrt{2\Delta\tau A_b / f_r}$.

The alternative modes are determined by a parameter μ which relates to the recent

memory for an amount of nutrients near a bacteria (MACNAB and KOSHLAND, 1972). The parameter μ is defined as $\mu = (1/n_\mu)\sum_{i=1}^{n_\mu}\varphi_{n-i} - \varphi_n$, where φ_n is the amount of nutrients near the bacteria at the simulation time step n. A period of keeping the memory n_μ is used as $n_\mu = 5$ in the simulation. If $\mu > 0$, the bacteria will switch its movement from "tumble" to "run" and vice versa. In the result, the bacteria automatically moves towards a nutrient-rich area. Therefore a coupling of these two modes depending on a change in the concentration of nutrients causes "chemotaxis towards nutrient" which is just a biological feature (ADLER, 1966; SINGLETON and SAINSBURY, 1981).

A quantity of the nutrient in the thin agar plate is represented by a mesoscopic value $\phi_{i,j}$ at the intersecting point on a triangular lattice. Each $\phi_{i,j}$ varies due to the diffusion of the nutrient under the constraint of a Ginzburg-Landau expanded free energy V,

$$V = \alpha\sum_{i,j}\phi_{i,j}^2 + \frac{\beta}{2}\sum_{i,j}\Big\{\left(\phi_{i,j}-\phi_{i+1,j}\right)^2 + \left(\phi_{i,j}-\phi_{i-1,j}\right)^2 + \left(\phi_{i,j}-\phi_{i-1,j-1}\right)^2 + \left(\phi_{i,j}-\phi_{i,j-1}\right)^2 + \left(\phi_{i,j}-\phi_{i-1,j+1}\right)^2 + \left(\phi_{i,j}-\phi_{i,j+1}\right)^2\Big\}. \quad (2)$$

The first term in Eq. (2) is a diffusion term and the parameter α corresponds to the diffusion coefficient. The second term acts on suppressing a variation of $\phi_{i,j}$ when the parameter $\beta > 0$. In addition to the potential energy V, the ingestion by bacteria also affects the diffusion of the nutrient.

3. Simulation and Results

We prepare a two-dimensional square space $2048a \times 2048a$ which roughly corresponds to a 1 mm × 1 mm dimensional agar plate and initially place a few rods on the center of the space. We also set a value of the nutrient homogeneously in the agar plate as $\phi_{i,j} = \phi_0$. In a Monte Carlo trial, a rod is chosen randomly. For a passive movement of the rod, its center of mass and orientation are shifted randomly subject to $\sqrt{\langle\delta_P^2\rangle}$ and $\sqrt{\langle\theta_P^2\rangle}$. These trials are accepted or rejected according to the Boltzmann weight $\exp(-\Delta U/k_BT)$, which is calculated from a change of the total potential energy $U = \sum_{i\neq j}^{N} u_{i,j}$ and the temperature of the system T.

Likewise, an active movement of the rod randomly goes ahead and rotates subject to $\sqrt{\langle\delta_A^2\rangle}$ and $\sqrt{\langle\theta_A^2\rangle}$. The acceptance of these trials are judged by the Boltzmann weight $\exp(-\Delta U/A_b)$, which does not depend on the temperature of the system but the activity of the bacteria A_b.

While the rods move on the off-lattice space, the nutrients diffuse on a triangular lattice whose unit length is a. In a Monte Carlo trial, an intersecting point $\phi_{i,j}$ is chosen randomly and a part of the $\phi_{i,j}$ moves to one of the six nearest neighbor points in the triangular lattice. The trial is accepted or rejected according to the Boltzmann weight $\exp(-\Delta V/k_BT)$ where ΔV is a change of the potential energy V during the trial. In addition, the nutrient is ingested by bacteria and decreases.

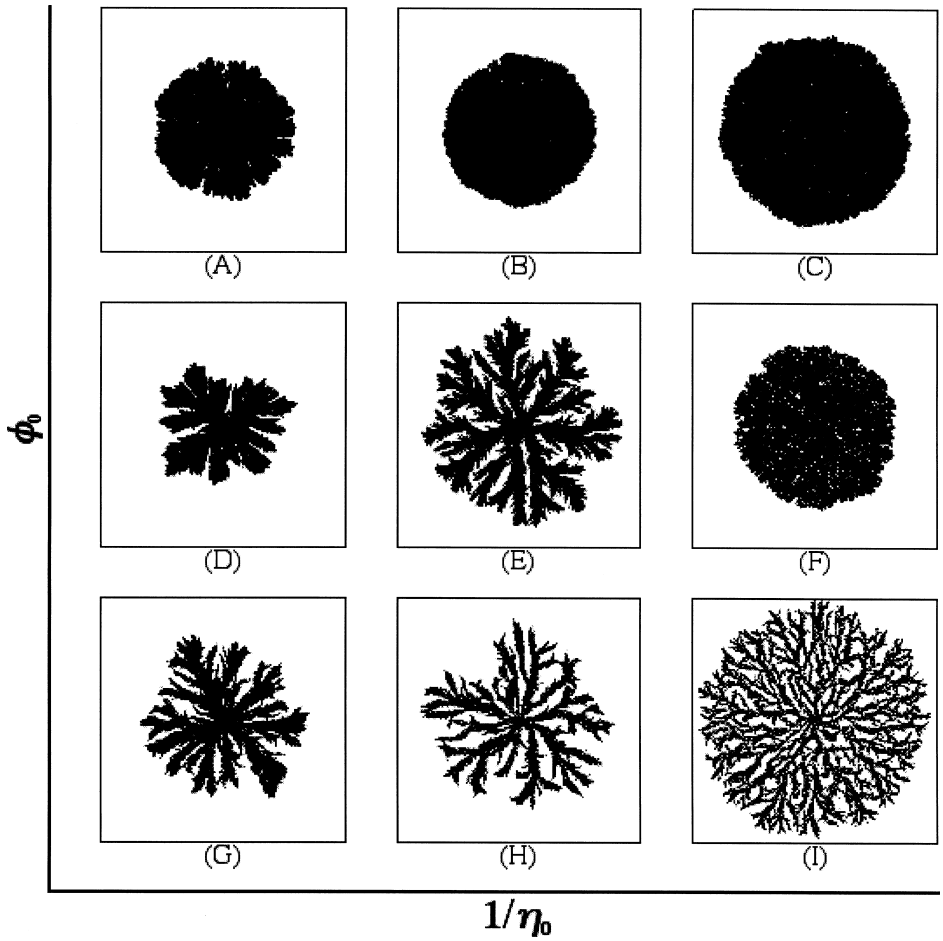

Fig. 2. Patterns of bacterial colonies for various values of initial quantities of the nutrient ϕ_0 and coefficients of viscosity η_0; (η_0, ϕ_0) = (A) (500, 40), (B) (50, 40), (C) (5, 40), (D) (500, 10), (E) (50, 10), (F) (5, 10), (G) (500,4), (H) (50, 4), (I) (5, 4). The number of bacteria in each colony is 10000–20000.

Simulations are run up to $1\sim3 \times 10^4$ Monte Carlo steps (MCS) at various coefficients of viscosity $\eta_0 = 5\sim500$ and initial quantities of the nutrient $\phi_0 = 4\sim40$. Parameters chosen are $k_BT = 1$, $\Delta\tau = 1$, $\alpha = 1$ and $\beta = 1$. In the result of the simulation, we obtain typical colony patterns shown in Fig. 2. At nutrient-poor and solid agar medium, the bacterial colony shows the DLA-like pattern (Fig. 2(G)). Using a box count method, the fractal dimension of 1.763 is obtained in a good linear regression line, which almost corresponds to the experimental result 1.716 (FUJIKAWA and MATSUSHITA, 1989). Since its size corresponds to 1 mm × 1 mm, the result shows a pattern in a small region at the center of the DLA-like pattern observed by experiment. Therefore our result of the fractal dimension is slightly higher than the experimental one. Eden-like pattern appears in the region of nutrient-rich

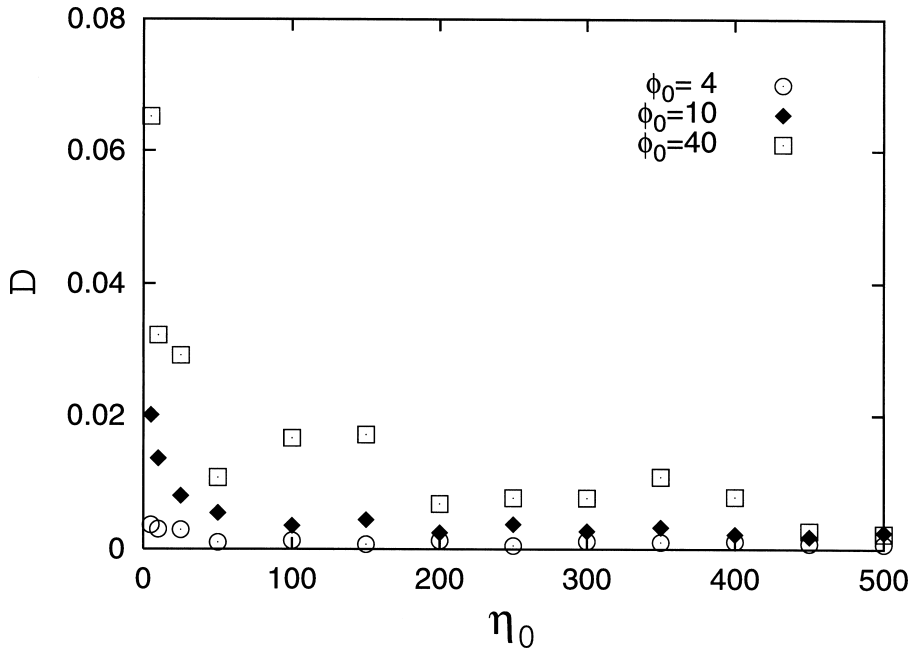

Fig. 3. The diffusion coefficient of bacteria D versus coefficient of viscosity η_0 without any lubricant for ϕ_0 = 4, 10, 40.

and solid agar medium shown in Fig. 2(A). In the region of solid agar medium, movement of rods are restricted and growth of the colony is controlled by an extension and a division of the rods. On the other hand, rods actively move at soft agar medium and form DBM-like (Fig. 2(I)) or homogeneous disk-like colony (Fig. 2(C)). To investigate the activity of the rods at each environment, we calculate a diffusion coefficient of the rods $D = \langle \delta^2 \rangle / 4\tau$ shown in Fig. 3. Here $\langle \delta^2 \rangle$ is a mean-square displacement of the rods during the time τ. Though the diffusion coefficient D is almost unchanged for $1/\eta_0 > 50$, it rapidly increases at $1/\eta_0 \leq 50$. This result qualitatively corresponds with the experimental observation (OHGIWARI *et al.*, 1992).

4. Summary

In order to study the morphology of bacterial colonies microscopically, we performed Monte Carlo simulations of multiplying rods model for various values of the viscosity of the surrounding mediums and the nutrient concentration. We obtained the same colony patterns that are found in the experimental studies except for a concentric ring-like pattern (OHGIWARI *et al.*, 1992). Though this microscopic model is proposed with respect to *B. Subtilis*, it is possible to apply our model to another bacteria species such as *E. coli* by changing microscopic specifications of the model (BUDRENE and BERG, 1991). Hence our rod model is useful for studying how the microscopic specifications of bacteria affects

unique behavior of the bacterial colony. BEN-JACOB *et al*. (1994) propose a mesoscopic model for the formation of bacterial colonies, called "Communicating Walker model", which incorporates random walkers representing aggregates of bacteria (BEN-JACOB *et al*., 1994). Though this model seems to resemble our model, it is actually different in a sense that the movement of bacteria in our model is considered individually.

There is a report on the concentric ring-like pattern based on the reaction-diffusion type model in the two-dimensional space (MATSUSHITA *et al*., 1998). As a matter of fact, the concentric ring is a three-dimensional structure which is constructed by accumulating disks of different sizes (WAKITA *et al*., 2001). Therefore, it is hard to realize the concentric ring-like pattern by means of the two-dimensional model. Although our model is also studied in two dimensions, it is more easily extended for studying three dimensional behavior than the reaction-diffusion type model.

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