Self-Organized Pattern Formation of a Bacteria Colony Modeled by a Reaction Diffusion System and Nucleation Theory

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Self-organized pattern formation is observed in bacterial colony growth. The recently reported knotted-branching pattern of the *Bacillus circulans* colony consists of the trajectories of aggregates which grow, move, and reproduce simultaneously. We modeled these processes by combining a reaction diffusion system of nutrient dynamics, nucleation theory for aggregate generation, and individual based dynamics of motion and growth of aggregates. The branching pattern produced by computer simulation shows great similarity with experiments. Response to the initial nutrient concentration is also consistent with the experiments.

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Self-organized pattern formation is observed in bacterial colony growth [1–6]. Such cooperative behavior can be considered as an adaptive response under unfavorable environments [1]. Reaction diffusion equations have been applied to study the branching pattern formation [7–10]. However, in most studies, bifurcation of a branch is reproduced just as a result of numerical calculation and the mechanism is still of great interest. It is recently reported that *Bacillus circulans* forms a knotted-branching pattern (KBP) which consists of the trajectories of aggregates which grow, move, and reproduce simultaneously [2]. By observing growth, movement, and generation processes of an aggregate, the underlying mechanism of the branching pattern formation might be understood. In this paper, we briefly introduce recent experimental results and their implications. Then, our hypothesis for the aggregate dynamics is explained, followed by mathematical formulation. Results of the computer simulation are posed and discussed.

When a droplet containing bacteria is inoculated at the center of a Petri dish filled with agarose gel medium, *B. circulans* forms various colony patterns [Figs. 1(a)–1(d)]. Hardness of the medium (agar concentration) and nutrient concentration are major factors affecting the pattern. Some patterns are characterized by aggregates of bacteria. The observed pattern is considered as trajectories of moving aggregates [11]. When hardness of the medium is so low that bacteria can move inside individually, only a simple and typical disklike pattern is produced [Fig. 1(d)]. Experimental study shows that formation of an aggregate caused by a high density of bacteria is a key which triggers the formation of KBP [2]. Once aggregates are formed, the colony expanding rate increases drastically. A radius of the aggregate linearly increases with time while its velocity increases sharply and then decreases [11]. The generation process, which is essential to the bifurcation of a branch, had been left untouched. From a microscopic view, the generation process is a creation of a new aggregate. Bacteria with the size of a few micrometers form an aggregate with the size of a few millimeters. We focus upon the similarity between this self-organized phenomenon and nucleation. Nucleation theory developed in physical chemistry is applied to model a generation process.

Aggregate formation of bacterial systems has been considered as a consequence of attractive chemotaxis [12–14]. Such long-distance force plays an important role in the first step for globally distributed bacteria to aggregate. When the average distance between cells becomes as small as the cell size ($\approx 10\ \mu$m), the short-distance force becomes dominant. Surprisingly, the existence of

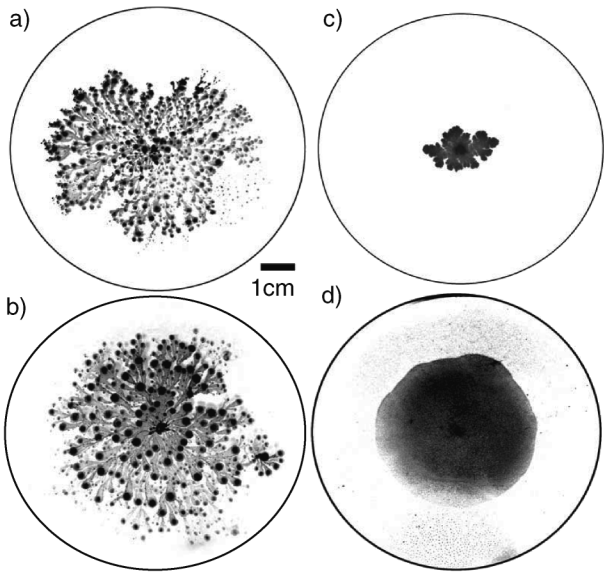

FIG. 1. Colony patterns of *B. circulance*. (a) A typical knotted-branching pattern (KBP). (b) When nutrient concentration is denser. The pattern is more isotropic with more branches and less gaps. (c) When agar medium is harder. This branching pattern is called an Eden-like pattern in which knots are not clearly distinguished. (d) When agar medium is softer. No spatial structure is formed.

van der Waals attractive force between cells had been experimentally verified [15]. There is also an experimental study showing that the bacterial surface is negatively charged and that electrostatic repulsive force works between cells [16]. These studies suggest that a critical step in aggregation of bacteria should be considered with colloid theory [17]. The Derjaguin-Landau-Verwey-Overbeek theory in physical chemistry predicts nucleation in such a system [18–20]. Actually, many bacteria coagulate above the critical coagulation concentration [21]. An aggregate of *B. circulans* is also very closely packed with uniform direction [11] and might be regarded as a nucleus. Therefore, in the present study, we regard aggregate formation as the nucleation process.

Individual bacterium can hardly move in hard agar. Aggregation is considered to be an adaptive response as it reduces viscous drag, allowing bacteria to move toward nutrient concentration. Moreover, some bacterial species are known to secrete lubricant fluid around the aggregates so that they can move on the hard and dry agar surface [Fig. 2(a)]. Ben-Jacob *et al.* called the lubricant an envelope [1]. As an aggregate moves, the envelope is formed along with the trajectory. Bacteria can disperse in the envelope, i.e., the envelope is considered as a solvent of bacteria. Dispersed bacteria keep reproducing in the envelope after an aggregate goes away and the branch is formed [11]. As the thickness of the envelope is limited, reproduction of bacteria easily leads to supersaturation followed by nucleation. The initial nuclei size (i.e., critical nuclei size expected from classical nucleation theory) is much smaller than the observed aggregate size. We assume that viscous drag force, which is a major obstacle for minute particles to move quickly, prevents the initial nuclei from escaping the parent envelope. As the nuclei grow, viscous drag becomes smaller than propulsion generated by the flagella of all active bacteria. When the size reaches the critical escape size, it escapes the parent envelope to move toward nutrient concentration (nutrient chemotaxis). At the beginning of a movement, nutrient concentration is locally small but it gradually increases as it leaves the parent nucleus. High nutrient concentration results in a high reproduction rate. The model of reproduction is illustrated in Fig. 2(b). Experimental study shows that a nucleus grows larger even after it stops, which suggests that the stop is not due to the lack of nutrient but due to the existence of frictional force [11]. As a radius becomes very large, friction between the nucleus and the agar surface becomes dominant and the nucleus stops. All these dynamics are globally governed by spatiotemporal dynamics of nutrient concentration.

For simplicity, we assume a nucleus's shape remains similar as it grows characterized by the radius r. The surface area S and the volume V are written as $S = \lambda_2 r^2$ and $V = \lambda_3 r^3$. A unit of weight is rescaled so that the weight of a nucleus $m = V$. A nucleus's position is represented by the coordinates in two-dimensional space. Bacteria on the surface of the nucleus are active but bacteria at the inner part are inactive spores [22] which do not reproduce nor propel a nucleus because they are densely packed and nutrient concentration is low [11]. Thus, the number of active bacteria generating propulsion is proportional to the surface area. The equation of motion of a nucleus is formulated as

$$m\frac{d\vec{v}}{dt} = p\frac{\vec{\nabla}n}{|\vec{\nabla}n|}S - \mu m g\frac{\vec{v}}{|\vec{v}|}. \tag{1}$$

The first term on the right-hand side represents the nutrient chemotaxis. The second term represents friction between a nucleus and an agar surface. Apparently, as the radius increases, a propulsion term and a friction term become dominant in that order.

Each nucleus is surrounded by the lubricant envelope with constant thickness E_d. In the envelope, dispersed bacteria are active, the number of which being b. The supersaturation level is denoted by $\theta = \alpha(b/SE_d)$ ($\theta = 1$ when saturated). The reproduction process is denoted by the following differential equations:

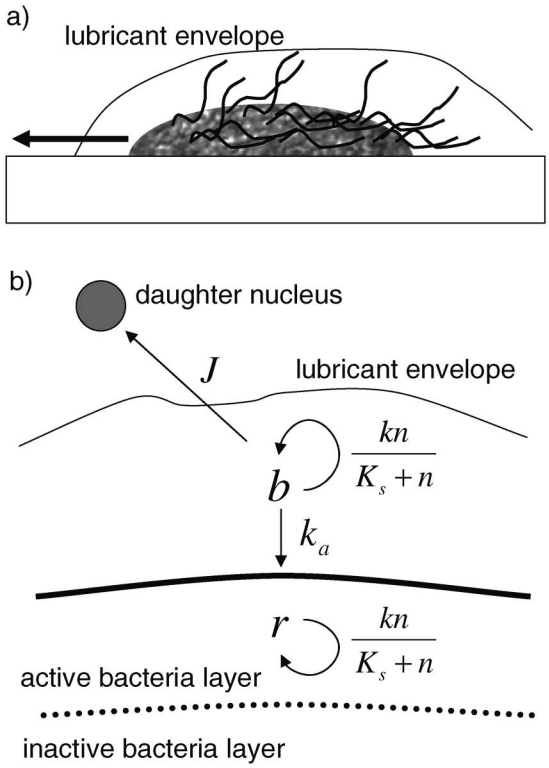

FIG. 2. Schematic illustration of the model. (a) An aggregate (nucleus) swims in the surrounding lubricant, which stands still due to friction. The lubricant secreted by an aggregate also plays the role of a solvent of dispersed bacteria. (b) Bacteria reproduce in both the envelope and the active layer of a nucleus. As the envelope is supersaturated and adsorption (nucleus growth) is dominant, net adsorption rate is considered in the model.

$$\frac{db}{dt} = \frac{kn}{K_s + n} b - k_a(\theta - 1)S,$$
$$\frac{d}{dt}(\rho V) = \frac{kn}{K_s + n} \rho a S + k_a(\theta - 1)S, \quad (2)$$

where ρV is the number of bacteria in a nucleus with volume V. We adopt a Mechaelis-Menten–type equation for bacterial reproduction. aS represents the volume of the active layer; k_a represents the net adsorption rate. If the nutrient concentration is very large and the supersaturation level is almost 1 at the earlier stage of nuclei growth, a radius becomes a linear function of time. By putting this result into Eq. (1) and integrating it, we get $\nu = X \ln r - Yr + Z$. Both results agree with the experimental observation [11].

For the nucleation process, we adopt a classical nucleation theory. The difference in free energy between N monomers and a *N-mer* nucleus is $\Delta G_N = -\phi N + S\varphi$, where ϕ is the difference of the chemical potentials of a monomer at supersaturation and that at equilibrium with a nucleus. φ represents the surface free energy of a *N-mer* per unit area. The nucleation rate is denoted by $J = \omega \exp[-(\Delta G^*)/(kT)]$ where ΔG^* is a maximum of ΔG_N. The generated nucleus stays in the parent envelope until it reaches critical escape size η. So we assume a nucleus with size η is generated at a rate J. Each nucleus is generated with the envelope saturated with the dispersed bacteria, i.e., $\theta = 1$. Hence, the number of bacteria in the parent envelope is decreased by $\eta + (1/\alpha)E_d\lambda_2(\eta/\lambda_3)^{2/3}$ to keep the mass balance. The daughter nucleus is put randomly near the parent.

Spatiotemporal dynamics of nutrient concentration is represented by

$$\frac{\partial n}{\partial t} = D\nabla^2 n - \sum_i \delta(\vec{x} - \vec{x}_i) \frac{kn}{K_s + n}(b_i + \rho a S_i),$$

where the subscript i represents the ith nucleus. A nucleus loses activeness (sporulates) when experiencing nutrient concentration that is less than Ω. Once it sporulates, it does not reproduce, move, nor recover activeness.

In computer simulation, nutrient dynamics in a two-dimensional agar surface is calculated. Growth, motion, and generation are also simulated for all nuclei at various points on the surface. The history of the resulting colony growth dynamics is stored in a computer memory and the trajectory of all nuclei including spores is considered as a colony pattern.

Computer simulations proved that the model can reproduce essential characteristics of KBP (Figs. 3 and 4). Our scenario of the formation of KBP is as follows. A nucleus size is monotonically increasing while its velocity increases and then decreases. Thus, thickness of a branch increases as a nucleus moves outward. Finally, a nucleus stops but reproduction does not stop until it exhausts the nutrient, resulting in a large nucleus at the end of the branch. This is a knot. In a rough approximation, the number of bacteria in the envelope grows exponentially while the radius increases linearly. This will result in an explosive increase in the supersaturation level in the latter period of growth, which causes the generation of a new nucleus through nucleation. Nucleation decreases at a super saturation level but it may recover the nucleation threshold as long as nutrient concentration is high. Therefore, a few branches may be produced by the parent nucleus. Note that nutrient concentration is expected to be high when the parent is isolated or located at a projection.

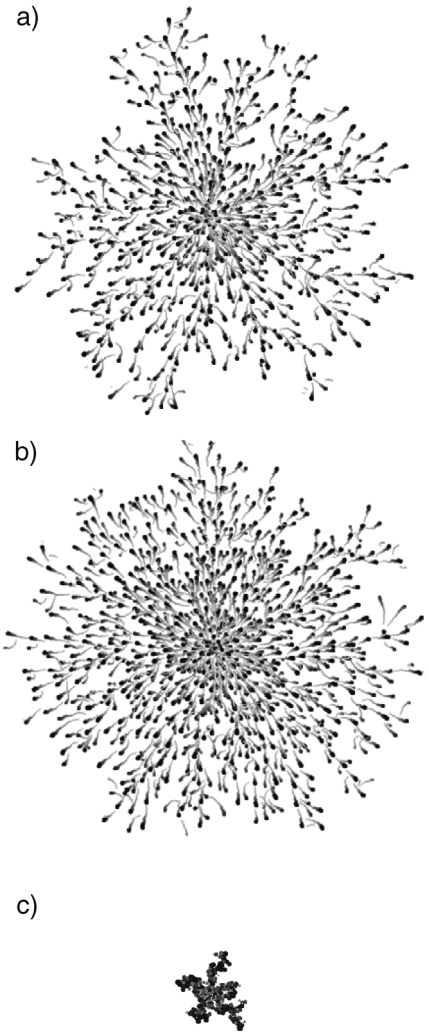

FIG. 3. Simulation results. The final patterns when all nuclei become inactive are shown. The shaded area represents the size of the nucleus when it passes the corresponding point of the branch. For appropriate parameters, KBP is reproduced (a). When the initial nutrient concentration is increased, the number of branches increases and the pattern becomes more isotropic (b). When a friction coefficient is large, which corresponds to a hard agar condition, a nucleus can hardly move and thus nutrient concentration is expected to be almost the same among nuclei. However, simulation reproduced a branching pattern (c). All these results are consistent with corresponding experimental results (Fig. 1).

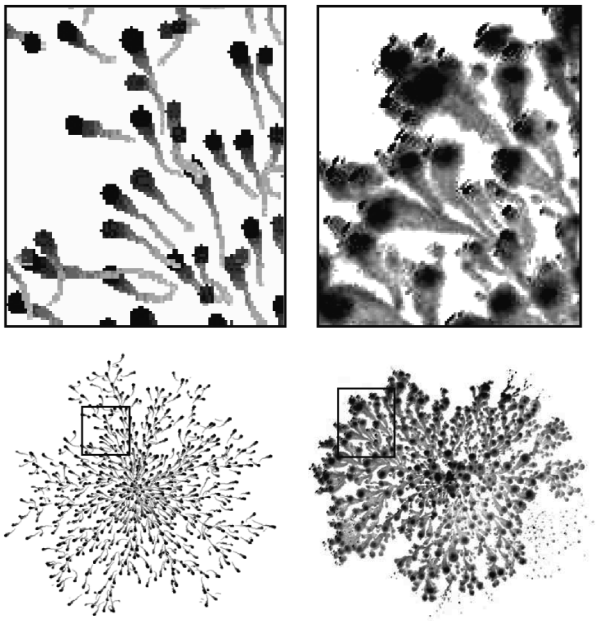

FIG. 4. Enlarged pictures of simulation (left) and experimental (right) results. The great similarity is observed.

As a chain of the branches grows concentrically outward, an aggregate at the head of the chain becomes more isolated. Then, multiple generations will occur to fill gaps, which will result in a beautifully branching colony pattern. Despite many complex factors in both the model and the experiment, the diffusion of nutrient governs the pattern formation. The branching mechanism is similar to a diffusion limited aggregation formation [23].

The colony pattern of *B. circulans* is similar to that of *Paenibacillus vortex* in a point that rotating aggregates (vortex) migrate to draw patterns. For the latter system, a combined model of vortex formation and migration of the vortex has been proposed [14]. In their model, the vortex is formed by short range attractive chemotaxis while the global pattern of the vortex trajectory is governed by long range repulsive chemotaxis. On the other side, in our model, aggregate formation is due to fundamental (or physicochemical) forces between cells, which are experimentally verified and global dynamics of aggregates is due to nutrient chemotaxis, which is also experimentally well known. Our model does not explain rotational motion of the aggregate while assumptions of our model are more strongly related to experimental facts. The introduction of the mechanism of vortex formation into the present study would be the next important step. Hereafter, the fruits achieved by the physical chemistry of microorganisms will provide new hypotheses and new possibilities to the study of pattern formation of microorganisms.

The major inconsistency with the experiment is the tendency for an aggregate size to increase as the initial nutrient concentration increases [Figs. 1(b) and 3(b)]. We suspect that the tendency is due to vertical diffusion in agar. If a super saturation level exceeds the nucleation threshold, rich nutrient is consumed to produce daughter nuclei. In order for a nucleus to grow large without too frequent nucleation, nutrient concentration must stay at a proper level. A continuous supply of nutrient from the bottom half of agar may enable this. As our model considers only two-dimensional diffusion, the tendency is not reproduced. However, preliminary experiments (data not shown) show the dependence of the colony pattern on agar thickness. The effect of three-dimensional diffusion of nutrient is left for future work.

The knotted-branching pattern has a distinct structure offering insight into underlying biological and physical mechanisms. Based on this feature, our model reproduces the pattern. The present study shows that a complex colony pattern can be explained by the combination of well-known physical laws and biological observation.

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