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Modeling Spatio-Temporal Patterns Generated by *Bacillus subtilis*

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Colonies of bacteria, *Bacillus subtilis*, that grow on the surface of thin agar plates show various morphological patterns in response to environmental conditions, such as the nutrient concentration, the solidity of an agar medium and temperature. For instance, the colony pattern shows a dense-branching morphology with a smooth circular envelope (DBM-like) in a nutrient-poor semi-solid agar medium, and it turns to a simple disk-like colony as both the nutrient concentration and the agar's softness increase. These patterns have been shown to involve cell movement inside colonies. In a DBM-like colony, individual cells actively move, particularly in the expanding periphery of the colony, while they become immotile at the inner region of the colony where nutrient is very low. In a disk-like colony, cells are highly active in the whole region of the colony. Based on such experimental observations, we develop a diffusion-reaction model, in which density dependent cell movements are incorporated in a nonlinear diffusion term and the local growth of the cell population is assumed to be regulated by the level of nutrient concentration available for the cell. Numerical simulations of the model under different environmental conditions closely reproduce various colony patterns ranging from DBM-like pattern to the homogeneous disk-like one in a unifying manner. The analysis also predicts the growth velocity of a colony as a function of the nutrient concentration.

1. Introduction

Bacteria grown on the surface of thin agar plates develop colonies of various spatial patterns, depending on both bacterial species and environmental conditions (Budrene & Berg, 1991, 1995). Recently, Fujikawa & Matsushita (1989) observed that the bacterium *Bacillus subtilis*, inoculated on a nutrient-poor solid agar, exhibits fractal morphogenesis similar to diffusion-limited aggregation (DLA) (see also, Matsushita & Fujikawa, 1990; Ohgiwari *et al*., 1992; Ben-Jacob *et al*., 1992, 1994a, b). When the agar medium becomes softer (nutrient-poor semi-solid medium), however, bacterial colonies turn to show a dense-branching morphology (DBM) with a smooth circular envelope. When both the nutrient concentration and the agar's softness further increase, simple circular colonies grow almost homogeneously in space (Wakita *et al*., 1994). Similar morphological changes have also been found for various other bacterial species, for example, *Serratia*, *Salmonella*, *Escherichia*, *Porteus* and their mutant strains (Matsuyama *et al*., 1989; Matsuyama & Matsushita, 1992, 1993; Shimada *et al*., 1995). Various models have been developed for explaining each characteristic colony pattern. Examples include a diffusion limited aggregation model for DLA-like colonies (Matsushita & Fujikawa, 1990; Fujikawa & Matsushita, 1991), a communicating walkers model for the DLA- and DBM-like morphology (Ben-Jacob *et al*. 1994a, b), and a simple diffusion-reaction model

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for homogeneous circular colonies (Wakita *et al*., 1994).

In this paper we present a new diffusion-reaction model that captures the qualitative features of the growth pattern from DBM to homogeneous circular colonies. In the next section, we first introduce experimental results reported so far on morphological changes in *B. subtilis* with the variation of environmental conditions. Then we develop a mathematical model that incorporates cell movement in the nonlinear diffusion term and cell proliferation in the reaction term. The nutrient concentration is also subject to a diffusion-reaction equation, in which it changes due to diffusion and consumption by the cells. Upon numerical simulation, the model reproduces various colony patterns as observed under different environmental conditions in a unifying manner. Finally we discuss implications of the model and how the various pertinent parameters influence the growth of branching and its elongation velocity.

2. Experimental Results

We briefly summarize the experimental studies done by Ohgiwari *et al*. (1992). They used a bacterial strain of *B. subtilis*, which is rod-shaped with flagellae (about 0.7 μm in diameter and 2 μm in length), and motile in water by collectively rotating them. The bacteria was point-inoculated at the center of an agar plate containing peptone as a nutrient in a plastic petri dish with a diameter of 88 mm. Under the condition they used, the average pore size of the agar-gel network was smaller than the size of the bacteria so that bacterial colonies grew two-dimensionally on the agar surface. Wakita *et al*. (1994) examined the morphology of colonies as a function of the concentration of agar C_a and the concentration of nutrient C_n (peptone) in the incubation medium, while other parameters including temperature (35°C) were kept constant. The observed colony patterns changed drastically with the variations in C_a and C_n as shown in Fig. 1, where the abscissa indicates the inverse of the concentration of agar, $1/C_a$, representing the softness of the agar medium. When C_a was higher than 8 g l^{-1} (hard agar plates), colony patterns were DLA-like at low C_n (region A) and "Eden-like" at high C_n (region B). Here "Eden-like" refers to a round and compact colony, the growing interface of which shows a self-affine fractal (Vicsek *et al*., 1990). The DLA-like colony patterns had self-similarity with a fractal dimension of about 1.72 (Matsushita & Fujikawa, 1990). As C_n increased, the DLA-like branches thickened gradually, and finally fused together to form a compact pattern (in region B). In the region E where the nutrient was poor and the agar softness was intermediate, the colony grew radially with a dense-branching morphology (DBM), and its advancing envelope was smoothly rounded in contrast to DLA-like colonies. A typical DBM-like colony is shown in Fig. 2. As seen in this figure, internal branches stopped growing by screening effects from surrounding main branches. In the region D where the agar was soft, dense branches fused together to form a homogeneous circular colony. In the region C, the colony grew by producing many thin branches characteristic of the DBM structure. However, a peculiar pattern like a concentric ring was

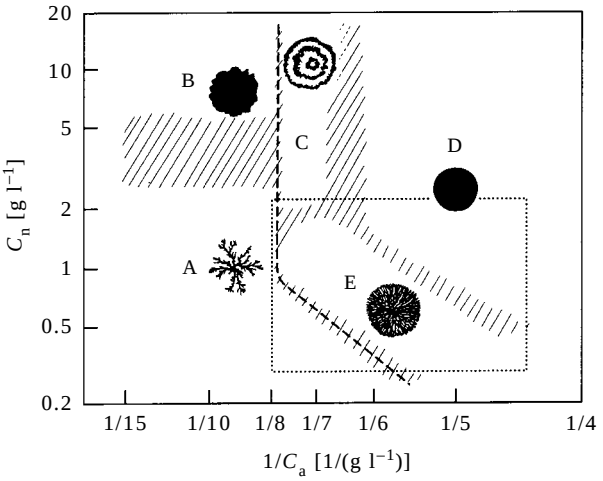

FIG. 1. Phase diagram of pattern changes in the colony of *B. subtilis* as a function of the concentration of nutrient C_n and the solidity of agar medium expressed as $1/C_a$, where C_a is the agar concentration. Colony patterns are classified into five types: DLA-like (region A), Eden-like (region B), concentric ring-like (region C), homogeneous disk (region D), and DBM-like (region E). The shaded area stands for the zone where colonies take intermediate ambiguous patterns. The present analysis focuses on the region enclosed by the dotted rectangle (redrawn from Wakita *et al*., 1994).

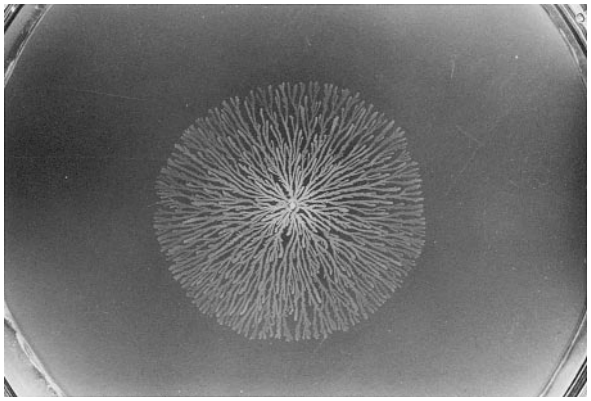

FIG. 2. A typical DBM-like colony pattern. The agar plate contains 0.5 g l^{-1} of peptone and 5 g l^{-1} of agar. The colony is photographed 2 days after inoculation.

found in the upper part of this region (Fujikawa, 1992).

The growth rates were also different among these five morphologies. Growth to the size of about 5 cm required about a month in A and a week in B, while a few days in C and E and half a day in D. In fact, by microscope observation of growing zones of colonies, Ohgiwari *et al*. (1992) recognized two distinct types of growing processes. In regions A and B, no active movement of individual cells was found. Only cells located in the outermost part of a colony were accessible to nutrient and proliferated by cell division, while cells in the inner part changed to spores and entered into the resting phase. On the other hand, in the regions C, D and E, where agar plates were relatively soft, the bacteria exhibited a random walk-like movement, which seems to drive the growth of colonies. For instance, in the region E, the outermost growing front tip was enveloped by a thin layer (thickness—5 μm) of bacterial cells whose movement was sluggish. But cells located inside the layer moved around actively. Sometimes they collided with the layer, broke through it and rushed out. But the cells which rushed out became immediately sluggish. Deep inside the growth front, where the nutrient was almost depleted, the cell movement was again inactive. Thus, it seems that the morphological changes of colonies crucially depend on the capability of cell movement. In fact, Ohgiwari *et al*. (1992) found that a non-motile mutant with no flagella showed only DLA- and Eden-like patterns in all ranges of agar concentration examined. In other words, regions A and B in Fig. 1 expanded into the entire region, and regions C, D and E disappeared. In the following, we focus on regions D and E (particularly the region enclosed by a dotted rectangle), where cells are potentially movable in the two-dimensional space.

3. Mathematical Model

We consider a system consisting of bacterial cells and nutrient in two dimensions. Both cells and nutrient undergo diffusion while cells proliferate by consuming the nutrient. Let us denote the population density of the cells at time t and spatial position $\boldsymbol{x} = (x, y)$ by $b(\boldsymbol{x}, t)$, and the concentration of the nutrient by $n(\boldsymbol{x}, t)$. Then b and n are in general governed by the following equations:

$$\frac{\partial n}{\partial t} = D_n \nabla^2 n - f(n, b), \tag{1}$$

$$\frac{\partial b}{\partial t} = \nabla \cdot \{D_b \nabla b\} + \theta f(n, b), \tag{2}$$

where D_b and D_n are the diffusion coefficients of the bacterial cells and nutrient, respectively. Here we assume that the diffusion coefficient of the nutrient D_n is constant, while the diffusion coefficient D_b of the bacterial cells depends on both the bacterial density and the nutrient concentration. As described in the previous section, the movement of bacterial cells is inactive in the inner region where the cells are exposed to a low level of nutrient, while cells move around actively at the periphery of the colony where the nutrient is relatively high. However, cells become inactive again at the outermost front of bacterial colonies, where cell density is apparently low. This presumably suggests that bacteria are immotile when either n or b is low and become active as n or b increases. Furthermore, microscopic observations have revealed that although each bacterial cell undergoes random diffusion essentially in the same manner, some deviate from the standard due to stochastic fluctuations. Since the functional form of D_b has not been empirically determined, here we propose the simplest one as,

$$D_b = \sigma n b, \tag{3}$$

where $\sigma = \sigma_0(1 + \Delta)$. The value of σ_0 generally increases as the agar concentration decreases. Δ indicates the stochastic fluctuation of random movement.

The term $f(n, b)$ represents the consumption rate of nutrient by the cells and $\theta f(n, b)$ is the growth rate of bacteria, where θ is the conversion rate (growth yield) of consumed nutrient to bacterial growth. Here we assume that the rate of consumption is governed by Michaelis–Menten kinetics:

$$f(n, b) = knb/(1 + \gamma n) \tag{4}$$

where k is the intrinsic consumption rate. This function is approximately linear in n when n is small and becomes saturated as n increases: k/γ is the maximum consumption rate of the nutrient by a cell. For the region of DBM, where the nutrient concentration is relatively low, function (4) could be reduced to:

$$f(n, b) = knb. \tag{5}$$

Although we adopt mostly the functional form (5) in the present analysis, we will discuss later the effect of the saturation term γn on the colony morphology.

As the initial condition, we set

$$\begin{aligned} n(\boldsymbol{x}, 0) &= n_0, \\ b(\boldsymbol{x}, 0) &= b_0(\boldsymbol{x}), \end{aligned} \tag{6}$$

where n_0 is the initial concentration of the nutrient, which is uniformly distributed, and $b_0(\boldsymbol{x})$ is the density of the initial inoculum, which is localized at the center of the two-dimensional space.

Thus, we have a set of eqns (1) and (2) supplemented by (3), (5) and (6), which involves several parameters. To reduce the number of parameters, let us introduce the following variable transformations,

$$n' = \sqrt{\frac{\theta}{D_n}}\, n, \quad b' = \sqrt{\frac{1}{\theta D_n}}\, b, \quad x' = \sqrt[4]{\frac{\theta k^2}{D_n}}\, x, \tag{7}$$

$$y' = \sqrt[4]{\frac{\theta k^2}{D_n}}\, y, \quad t' = k\sqrt{\theta D_n}\, t.$$

Substituting (7) into (1) and (2) and omitting the prime for notational simplicity, we have the following equations:

$$\frac{\partial n}{\partial t} = \nabla^2 n - bn \tag{8}$$

$$\frac{\partial b}{\partial t} = \nabla\cdot\{\sigma n b \nabla b\} + nb \tag{9}$$

with initial conditions,

$$n(\boldsymbol{x}, 0) = \sqrt{\frac{\theta}{D_n}}\, n_0 \equiv v_0, \quad b(\boldsymbol{x}, 0) = \sqrt{\frac{1}{\theta D_n}}\, b_0(\boldsymbol{x}) \equiv \beta_0(\boldsymbol{x}). \tag{10}$$

Thus the model includes essentially three variable parameters, σ, v_0 and $\beta_0(\boldsymbol{x})$, where v_0 and $\beta_0(\boldsymbol{x})$ measure the relative magnitudes of the initial nutrient concentration and the bacterial density, respectively. In the following, we will examine how each variable regulates the spatial patterns of colonies.

4. Results of Simulations

We performed computer simulations of (8) and (9) on a two-dimensional rectangular grid (1600×1600) by using an alternating-direction implicit method. The initial distribution of cells is chosen as a normal distribution, $\beta_0(\boldsymbol{x}) = \beta_M \exp\{-(x^2 + y^2)/6.25\}$, where β_M is the maximum density at the center of the plane. Nutrient is uniformly distributed at a level v_0 initially. The time step and the grid width used in the simulations are 0.1414 and 0.4205, respectively. In each grid unit at every time step, diffusion coefficient of cells, σ, is perturbed from the mean σ_0 with random variable Δ, which is taken from a triangular distribution supported by $[-\rho, \rho]$ $(0 \leqslant \rho \leqslant 1)$. Since the experiments are carried out in a petri dish, we impose zero flux boundary conditions.

We first examine the effects of v_0 and σ_0 for a given choice of $\beta_M = 0.71$ and $\rho = 1$. The typical morphological features are shown in Fig. 3. With a fixed value of σ_0 (i.e. the agar concentration is fixed), the colony pattern becomes more ramified with decreasing nutrient levels. For example, given $\sigma_0 = 4$, homogeneous disk pattern occur at sufficiently high levels of v_0. As v_0 decreases, tip-splitting patterns with dense smooth fingers appear. At still lower values of v_0, the colony pattern shows thin and dense branching morphology with round envelope. For a given nutrient level, on the other hand, the colony patterns becomes more ramified with thinner branches as σ_0 decreases. Particularly, when $v_0 = 0.35$ and $\sigma_0 = 1$ [Fig 3(c)], the envelope of the colony appears somewhat irregular, showing a pattern intermediate between DBM-like and DLA-like ones. These predicted morphological changes with the variation of v_0 and σ_0 conform well qualitatively to the observed patterns as shown in the phase diagram of Fig. 1.

Figure 4 shows the time evolution of the DBM patterning for $v_0 = 0.71$, $\sigma_0 = 1$, $\rho = 1$ and $\beta_M = 0.71$. The initial inoculum splits radially in the beginning, followed by a cascade of tip-splitting to develop a DBM-like structure. Meanwhile interior branches stop growing, as indicated by an arrow in Fig. 4. This suggests that protruding main branches exert screening effects against interior ones, whose growth is limited by the lowered nutrient concentration. In fact, as shown in Fig. 4, the nutrient is completely depleted within the colony, while there remains enough nutrient outside the colony so that only the cells located on the periphery of the colony can proliferate. Since every leading tip is exposed to similar environmental conditions, its advancing speed is almost the same, resulting in a colony with a round envelope. When v_0 and σ_0 are extremely low [see Fig. 3(c)], however, local anisotropy caused by stochastic fluctuations accelerates the growth of branches that happen to protrude among others.

We examined the time development of colony growth and calculated the average velocity of tip elongation for varying sets of v_0 and σ_0. After the initial transient stage, the velocity approaches a constant value in each case. Figure 5 shows the ultimate velocity as a function of v_0 for $\sigma_0 = 1$ and 4. The velocity increases acceleratingly as v_0 increases.

The effect of stochasticity of cell movement is examined by changing the parameter of the triangular distribution, ρ, from 0 to 1. Even when ρ is zero, there appear branched patterns with low degrees of ramification because of anisotropy originating from

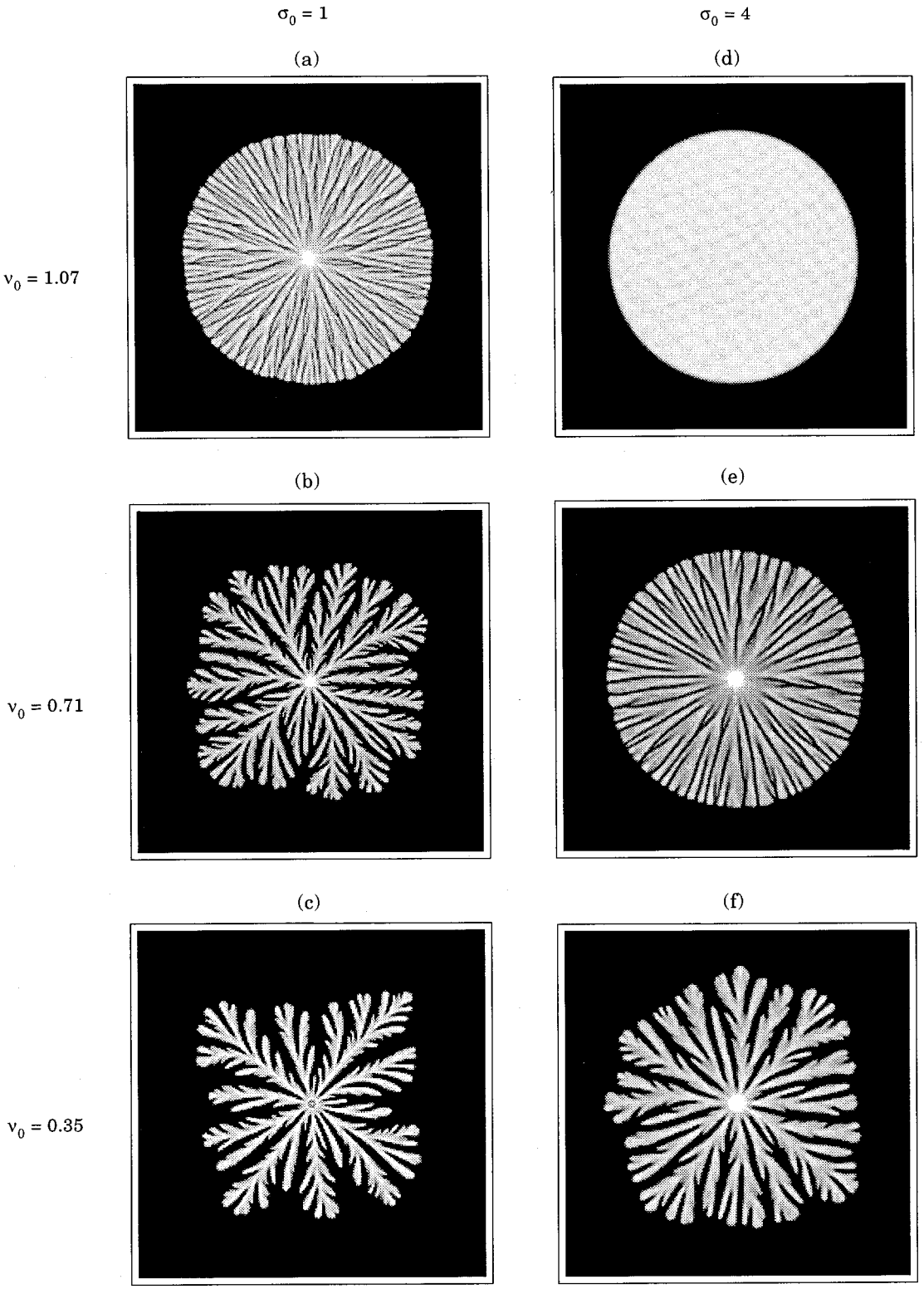

FIG. 3. Morphological changes of colonies predicted from the diffusion-reaction model (8) and (9) with varying nutrient concentration, v_0, and bacterial diffusivity, σ_0. $\beta_M = 0.71$, $\rho = 1$. The time for the colony to reach the size as in figure is (a) $t = 396$, (b) $t = 2828$, (c) $t = 19233$, (d) $t = 127$, (e) $t = 566$ and (f) $t = 4525$.

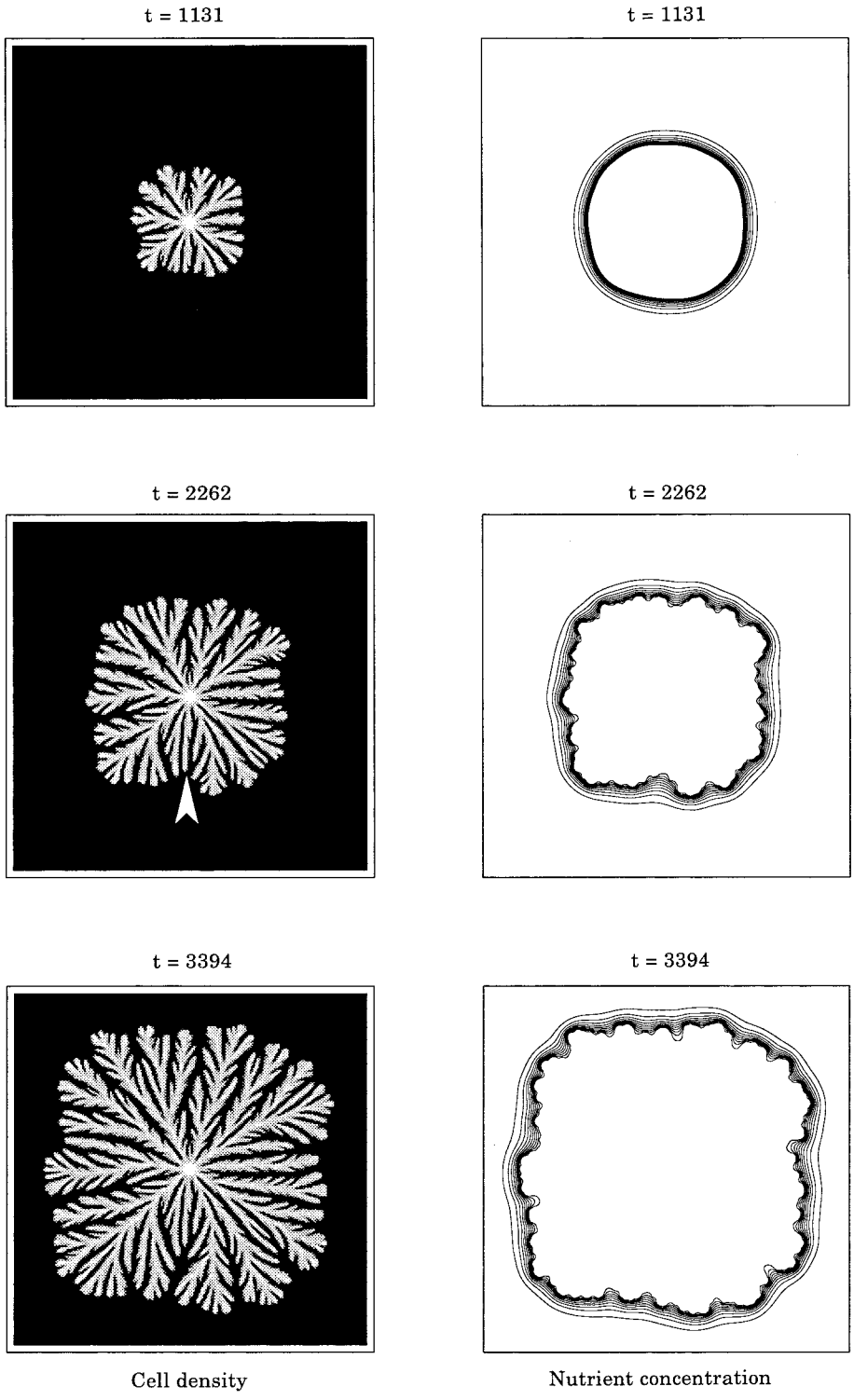

FIG. 4. Time-evolution of the cell density and the nutrient concentration with the DBM-like pattern shown in Fig. 3(b). The internal branches stop growing, an example of which is indicated by an arrow. In the r.h.s. panels are shown the contours of the nutrient concentration: the outermost line represents the initial level v_0, and the innermost one represents level 0. The number on the top of each panel indicates the time lapsed.

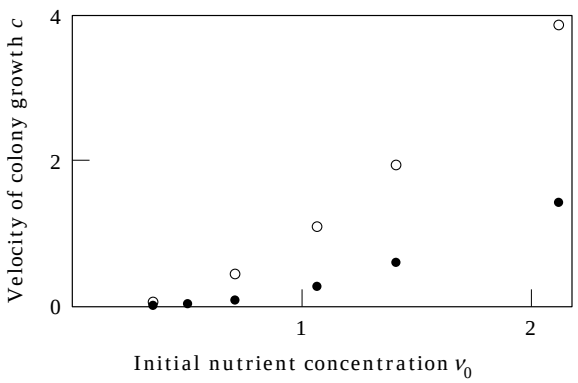

FIG. 5. The velocity of colony growth c as a function of the initial nutrient concentration v_0 for $\sigma_0 = 1$ (●) and 4 (○). $\beta_M = 0.71$, $\rho = 1$.

numerical discretization on a square lattice (see Fig. 6). As ρ increases,the frequency of tip splitting increases, though it becomes saturated as ρ approaches 1. On the other hand, the growth rate of the colony increases only slightly as ρ becomes larger.

Finally the effect of the cell density at the initial inoculum is examined by changing β_M. We found that the value of β_M does not essentially affect the colony morphology, though the growth rate of the inoculum is initially accelerated as the value of β_M is increased.

5. Discussion

The present diffusion-reaction model consisting of two variables, n and b, has been shown to reproduce various colony patterns ranging from DBM-like pattern to the homogeneous disk-like one. To our knowledge, this is the first demonstration of branching pattern formation of bacterial colonies in the framework of the diffusion-reaction system. One of the key elements of the model is the adoption of a nonlinear diffusion term (3) such that bacterial movement becomes immotile when either n or b tends to zero. Although the simplest functional form is used here, other functions might suffice as long as that condition is satisfied. In fact, we examined a case where $D_b = \sigma bn/\{(1 + \mu n)(1 + \mu' b)\}$ and obtained qualitatively similar branching patterns for wide ranges of μ and μ'.

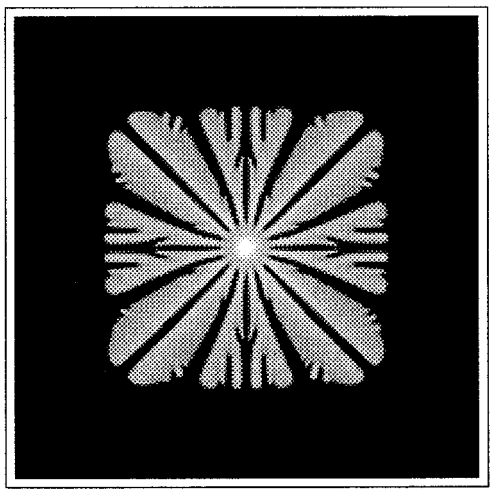

FIG. 6. Spatial pattern in the absence of stochastic fluctuation. $v_0 = 0.71$, $\sigma_0 = 1$, $\rho = 0$ and $\beta_M = 0.71$. The pattern is less branched than that in the presence of stochastic fluctuation [see Fig. 3(c)]. The anisotropy of the square lattice induces the branched patterns.

Our model equations (8) and (9) are susceptible to perturbation, even without stochastic fluctuation (i.e. $\Delta = 0$), so that they give rise to a branched pattern in the presence of minimal environmental anisotropy that comes from the square lattice used for simulation, as seen in Fig. 6. In reality, there should be considerable fluctuations in cell movements owing to their active motility and the local anisotropy in the agar medium. Thus these effects are incorporated into the diffusion term. As a result, we could obtain highly ramified patterns similar to those experimentally observed, except for concentric rings appearing on nutrient-rich semi-solid agar medium (the upper part of region C in Fig. 1). The latter case may involve some additional mechanisms or factors affecting bacterial movement, such as interconversions of bacteria between active and inactive states (Mimura & co-workers, personal communication).

Previously, Ben-Jacob *et al*. (1994a, b) presented a lattice model for bacterial movements. They experimentally observed finely branched DBM-like patterns with a round envelope on a semi-solid agar when the nutrient concentration was very low. To interpret this pattern, they proposed a special model termed "communicating walkers model" in which it is assumed that bacteria produce chemotactic signaling chemicals so as to drive other bacteria away. Although our model has no such assumption, it could generate fairly round envelope patterns with thin branches. In our model, bacteria move around most actively where both n and b are high, namely on the perimeter of the colony. This behavior may facilitate the leading fronts of colonies to expand outwards evenly, thereby substituting for the mechanism as proposed in the communicating walkers model in effect.

Although the bacterial colony on the agar plate grows in two dimensions, each tip elongates linearly in one dimension except for occasional branching. Thus, the velocity of tip elongation could be

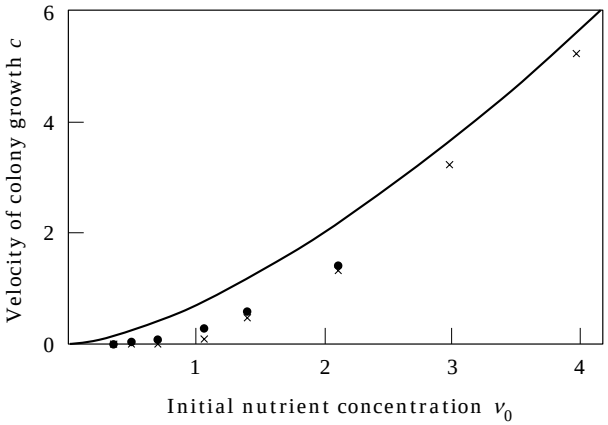

FIG. 7. Comparison of the growth velocities between one-dimensional and two-dimensional models. $\sigma_0 = 1$, $\rho = 1$ and $\beta_M = 0.71$, ×, one-dimensional simulation; ●, two-dimensional simulation; solid line, the velocity as given by eqn (14) for $K = v_0$.

approximated by a one-dimensional version of (1) and (2):

$$\frac{\partial n}{\partial t} = \frac{\partial^2}{\partial x^2} n - bn, \tag{11}$$

$$\frac{\partial b}{\partial t} = \frac{\partial}{\partial x}\left\{\sigma nb \frac{\partial}{\partial x} b\right\} + nb. \tag{12}$$

By simulating these equations, we found that the initial inoculum of bacteria evolves into a traveling wave. Thus, we calculate the speed of the wave front, which is illustrated in Fig. 7. As expected, the velocity obtained from the one-dimensional model is close to that from the two-dimensional. Although even the one-dimensional model, (11) and (12), is intractable as such, we may approximate it by the following equation with a single variable b, if we substitute σnb and nb in (12) by $\sigma_0 v_0 b$ and $v_0 b(1 - b/K)$, respectively:

$$\frac{\partial b}{\partial t} = \frac{\partial}{\partial x}\left\{\sigma_0 v_0 b \frac{\partial}{\partial x} b\right\} + v_0 b(1 - b/K). \tag{13}$$

The nutrient-limited growth of bacteria is represented by the logistic growth, $v_0 b(1 - b/K)$, where K is the saturated level of bacterial density which may be proportional to v_0. As a matter of fact, if diffusion terms are neglected in (11) and (12), we have $dn/dt + db/dt = 0$, and if we put $n + b = v_0$, a logistic equation $db/dt = v_0 b(1 - b/v_0)$ is derived. Although (13) includes a nonlinear diffusion term, it was analytically solved by Newman (1980). He showed that the solution of (13) starting from a localized initial distribution always evolves into a traveling wave, whose front advances at velocity

$$c = v_0(\sigma_0 K/2)^{1/2}. \tag{14}$$

If we put $K = v_0$, we have a relation $c = (\sigma_0/2)^{1/2} v_0^{3/2}$, which is plotted in the solid curve in Fig. 7. The curve tends to fit with the results of simulation as v_0 increases, though it gives overestimates when v_0 is small.

In the current study, we have used the functional form (5) for the rate of nutrient consumption by cells. However, when the nutrient concentration is sufficiently high as in region D in Fig. 1, where homogeneous compact colonies appear, we should use function (4). Thus, using (4) instead of (5) with other parameters kept the same, we carried out computer simulations as before. Resulting patterns were qualitatively similar to those shown in Fig. 3, as long as γ is relatively small (e.g. $v_0\gamma < 1$). However, as γ increases, the branch width becomes thickened and accordingly the degree of ramification decreases. The velocity of colony growth c is also lowered in increasing degrees as v_0 becomes higher.

Although the present analysis was focused on branched patterns observed by *B. subtilis*, a variety of colony patterns have been reported for other bacterial species. For example, complex spotty patterns are formed in *Escherichia coli* and *Salmonera typhimurium* (Budrene & Berg, 1991, 1995; Woodward *et al.*, 1995). In those cases, it was evidently shown that bacteria excrete chemoattractants which induce their strong aggregation. Diffusion-reaction models would also be applicable for these cases by introducing such specific features of bacterial behaviors (Keller & Segel, 1971a, b; Myerscough & Murray, 1992; Woodward *et al.*, 1995; Kawasaki *et al.*, 1995).

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