SIMULATION ANALYSES OF PATTERN FORMATION PROCESSES OF CHEMOTACTIC BACTERIAL COLONIES IN SEMI-SOLID MEDIA

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Abstract. *Many kinds of spatio-temporal patterns appear in various fields of engineering including chemical and biological engineering. Among such spatio-temporal patterns, analyses of patterns created by the so-called self-organization are very important as basic problems in engineering, mainly because analyses of the spatio-temporal patterns in phase transitions of polymeric materials are essential to develop new materials and the analyses of epidermal patterns of animals and seashell patterns shed light on mystery in biology. In this paper, focusing on the chemotactic bacterial colony patterns as the spatio-temporal patterns created by the self-organization, we study the influence of the random disturbance on the colony formations.*
Keywords: *Chemotaxis, stochastic reaction diffusion systems, E. coli, S. typhimurium, semi-solid media, colony formations, numerical simulations.*

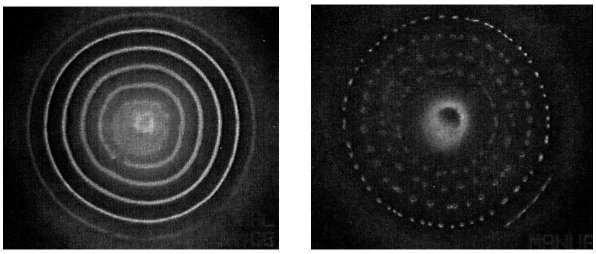
Introduction

Spatio-temporal patterns created by the self-organization [1]-[3] are often observed in various fields of engineering. In this paper, we study the bacterial colony patterns as the spatio-temporal patterns created by the self-organization. It is well known that some bacteria form complicate geometric spatio-temporal colonies. For example, bacterial species Bacillus subtilis create five kinds of colony patterns depending on two environmental conditions, concentrations of nutrient and hardness of agar in experiments [4], [5]. Among many kinds of bacteria, we consider ones such as Escherichia coli (*E. coli*) and Salmonella typhimurium (*S. typhimurium*) which move in the direction of increasing concentration of chemoattractant, asparate. This property of bacteria is called chemotaxis [6]. Because of chemotaxis, they form high-density aggregates, i.e, bacterial colonies. Taking into account the fact that there exist fluctuations more or less in the natural world, we propose the stochastic two components reaction diffusion equations as the model of the bacterial colony formation under chemotaxis. There are two types of culture of bacteria, one is culture in liquid media and the other is one in semi-solid media. In this paper, focusing on chemotactic bacterial colony formations in semi-solid media under random fluctuation, its stochastic modeling is considered. Since the colony formation processes of *E. coli* are much complicated than one of *S. typhimurium*, we make colony formation processes of *S. typhimurium* a particular study as the first step of the analyses of chemotactic bacterial colony formation. Using the proposed stochastic models, we study the influence of the fluctuations on the chemotactic bacterial colony formations by simulations.

The Model in Semi-solid Media

As the way of culturing the bacteria *E. coli* and *S. typhimurium* in experiments, there are cultures in liquid and semi-solid media (0.24% water agar). In liquid media, the bacteria *E. coli* and *S. typhimurium* form comparatively simple temporary bacterial colony patterns [6], [7], whereas in semi-solid media, they form more complex spatio-temporal colony patterns, which are radial spots, concentric ring and spotted ring patterns as shown in Figure 1, which are experimental results [8] in semi-solid media.

Even if the experimental conditions are the same, the pattern formation processes of the bacteria species *E. coli* and *S. typhimurium* are very different. From the initial inoculums at the center



(a) Continuous rings (b) Spotted rings

Figure 1. Colony patterns of *S. typhimurium* in semi-solid media [8].

of the region, *E. coli* first form the so-called swarm ring, continuous high-density ring and then they leave cluster of bacteria with angular variation behind the ring. On the other hand, *S. typhimurium* first form a low-density lawn and then create continuous concentric rings or spotted rings on the lawn, which expand outwards from the initial inoculums at the center. In this paper, we consider the bacterial colony formation of *S. typhimurium* in semi-solid media with fluctuation such as impurities in chemoattractant. We propose the stochastic model of the colony formation process of *S. typhimurium* in semi-solid media.

Letting $u(t,x)$ and $J(t,x)$ be the population density of bacteria, *S. typhimurium*, and be the flux of cells at time t and position x, it follows from a general conservation law that

$$\frac{\partial u(t,x)}{\partial t} + \nabla \cdot J(t,x) = f(u(t,x)) \text{ in } \Theta \times G \tag{1}$$

where $f(u(t,x))$ denotes the growth and death rate of bacteria and $\Theta = (0,T)$ and $G \subset R^n$.

The flux $J(t,x)$ of cells consists of diffusion and the chemotactic flows, $J_D(t,x)$ and $J_C(t,x)$, in such a way that

$$J(t,x) = J_D(t,x) + J_C(t,x) \tag{2}$$

Since diffusion of cells depends only on their density gradient and J_D denotes the diffusion contribution, we have

$$J_D(t,x) = -d_u \nabla u(t,x) \tag{3}$$

where d_u is a positive constant.

On the other hand, since the chemotaxis is the biological phenomenon that the bacteria move toward a higher concentration of the chemo-attractant, which is sort of like a negative diffusion, the chemotactic flow depends on concentration of chemoattractant and its spatial gradient and the interaction of cells. Therefore, denoting the attractant concentration at time t and position x by $v(t,x)$, we have

$$J_C(t,x) = u(t,x)\chi(v)\nabla v(t,x) \tag{4}$$

where $\chi(v)$ denotes the chemotaxis response, which is the function of the attractant concentration v.

Equations (2) to (4) yield that

$$J(t,x) = -d_u \nabla u(t,x) + u(t,x)\chi(v)\nabla v(t,x) \tag{5}$$

Some kinds of forms of the chemotaxis response function $\chi(v)$ have been proposed in the past. In this paper, we adopt the receptor law [6] below:

$$\chi(v) = \frac{a}{(b+v)^2} \tag{6}$$

where a and b are positive constants.

It follows from Eqs.(1), (5) and (6) that

$$\frac{\partial u(t,x)}{\partial t} = d_u \Delta u(t,x) - a\nabla \cdot \left(\frac{u(t,x)\nabla v(t,x)}{(b+v(t,x))^2} \right) + f(u(t,x)) \text{ in } \Theta \times G \tag{7}$$

Next, consider the modeling of time evolution of chemoattractant concentration $v(t,x)$. Since the chemoattractant diffuses with time and is also produced and consumed by the bacteria, we have

$$\frac{\partial v(t,x)}{\partial t} = d_v \Delta v(t,x) + g(u) - h(u,v) \text{ in } \Theta \times G \tag{8}$$

where $g(u)$ and $h(u,v)$ denote the production and the uptake rates of attractant by bacteria and d_v is a positive constant.

***Remark 2.1**:* The bacteria *E. coli* and *S. typhimurium* secrete the attractant under the existence of TCA (tricarboxylic acid) cycle intermediate, chemical stimulant such as succinate and fumarate. Therefore, the function $g(\cdot)$ in Eq.(8) depends on both of the bacteria density u and concentration of succinate or fumarate. From experimental results, we have knowledge that the consumption of chemical stimulant by *S. typhimurium* in semi-solid media is negligible. Furthermore, assuming that stimulant concentration is uniform in a whole region, we can consider that $g(\cdot)$ is the function of only the bacteria density *u*.

Consider the concrete forms of the functions f, g and h in Eqs.(1) and (8). As the growth and death rate $f(u)$ of bacteria in Eq.(1), we adopt the logistic form such that

$$f(u) = ru(t,x)(\delta - u(t,x)) \quad (9)$$

where r and δ are positive constants.

In disregard of saturation of the attractant concentration, a plausible form of the production rate $g(u)$ becomes:

$$g(u(t,x)) = \beta u(t,x)^2 \quad (10)$$

where β is a positive constant.

Since the consumption of the attractant by bacteria is proportional to both of the population density u and the attractant concentration v, the consumption rate $h(u,v)$ in Eq.(8) becomes

$$h(u,v) = \tau u(t,x) v(t,x) \quad (11)$$

where τ is a positive constant.

In the real situation, since fluctuations in the bacterial behaviors and substance concentration more or less are contained, taking this fact into consideration and modeling the randomness of fluctuations as the spatio-temporal white Gaussian noise [9], we propose the stochastic model:

$$\frac{\partial u(t,x)}{\partial t} = d_u \Delta u(t,x) - a\nabla \cdot \left(\frac{u(t,x)\nabla v(t,x)}{(b+v(t,x))^2} \right)$$

$$+ ru(t,x)(\delta - u(t,x)) + s_u u(t,x) \frac{\partial^2 w(t,x)}{\partial t \partial x} \text{ in } \Theta \times G \quad (12)$$

$$\frac{\partial v(t,x)}{\partial t} = d_v \Delta v(t,x) + \beta u(t,x)^2 - \tau u(t,x) v(t,x)$$

$$+ s_v v(t,x) \frac{\partial^2 z(t,x)}{\partial t \partial x} \text{ in } \Theta \times G \quad (13)$$

where $w(t,x)$ and $z(t,x)$ are mutually independent Brownian sheets, i.e., two parameters Wiener processes [9], s_u and s_v are constants.

Initial and boundary conditions are given by

$$u(0,x) = u_0(x), v(0,x) = v_0(x) \text{ on } G \quad (14)$$

$$\frac{\partial u(t,x)}{\partial \nu} = \frac{\partial v(t,x)}{\partial \nu} = 0 \text{ on } \Theta \times \partial G \quad (15)$$

where $\partial(\cdot)/\partial \nu$ denotes the outer normal derivative on the boundary ∂G.

Linear Stability Analysis of Deterministic Systems

In this section, we consider the linear stability of the deterministic equations with Eqs.(12) and (13):

$$\frac{\partial u(t,x)}{\partial t} = d_u \Delta u - \nabla \cdot (u\chi(v)\nabla v) + f(u) \text{ in } \Theta \times G \quad (16)$$

$$\frac{\partial v(t,x)}{\partial t} = d_v \Delta v + g(u) - h(u,v) \text{ in } \Theta \times G \quad (17)$$

where $\chi(v), f(v), g(u)$ and $h(u,v)$ are defined by Eqs.(6), (9), (10) and (11).

In the absence of diffusion and chemotaxis, linearizing Eqs.(16) and (17) around the nontrivial steady states (u^*, v^*) such that

$$u^* = \delta, v^* = \frac{\beta\delta}{\tau}, \quad (18)$$

i.e., setting as $u = \tilde{u} + \varepsilon u^*, v = \tilde{v} + \varepsilon v^*$, we have

$$\frac{\partial \tilde{u}}{\partial t} = d_u \Delta \tilde{u} - u^* \chi(v^*) \Delta \tilde{v} + f_u(u^*) \tilde{u} \quad (19)$$

$$\frac{\partial \tilde{v}}{\partial t} = d_v \Delta \tilde{v} + g_u(u^*) \tilde{u} - h_u(u^*, v^*) \tilde{u} - h_v(u^*, v^*) \tilde{v} \quad (20)$$

where subscripts of f, g and h denote the partial differentiation w.r.t. the corresponding subscripts.

Noting the boundary condition (15) and assuming that the spatial region G is square $(0,L) \times (0,L)$, set as

$$\tilde{u}(t,x) = \delta_1 e^{\lambda t} \cos(k_1 x_1) \cos(k_2 x_2) \quad (21)$$

$$\tilde{v}(t,x) = \delta_2 e^{\lambda t} \cos(k_1 x_1) \cos(k_2 x_2) \quad (22)$$

From Eqs.(15), (21) and (22), we have

$$k_1 = m\pi/L, k_2 = n\pi/L, (m, n = 1, 2, \cdots) \quad (23)$$

It follows from Eqs.(19) to (22) that the characteristic equation holds:

$$\lambda^2 + p(|k|^2)\lambda + q(|k|^2) = 0 \quad (24)$$

where

$$p(|k|^2) = (d_u + d_v)|k|^2 - f_u^* + h_v^* \quad (25)$$

$$q(|k|^2) = d_u d_v |k|^4 + A|k|^2 + B \quad (26)$$

and where

$$A = h_v^* d_u - f_u^* d_v - (g_u^* - h_u^*)u^* \chi^*, B = -f_u^* h_v^* \quad (27)$$

and $|k|^2 = k_1^2 + k_2^2$ and each $(\cdot)^*$ is calculated at the steady states u^* and v^*.

From the standard Turing Instability theory [6], in order to form the nonuniform spatio-temporal pattern, the following conditions are required:

(i) The system without diffusion and chemotaxis is asymptotically stable.

(ii) Under the existence of diffusion and chemotaxis, the real part of at least one solution of the characteristic equation (24) is positive.

Noting Eq.(18), we have

$$p(0) = (\tau + r)\delta > 0, \; q(0) = \tau r \delta^2 > 0 \quad (28)$$

therefore, the above condition (i) holds.

Let λ_1 and λ_2 be two solutions of Eq.(24) such that $\lambda_1 \geq \lambda_2$. Then, we have $\lambda_1 + \lambda_2 = -p(|k|^2) > 0$ from Eqs. (25) and (28). Therefore, if $\lambda_1 \lambda_2 = q(|k|^2) < 0$, the real part of λ_1 becomes positive. The condition (ii) is satisfied. Let k_a^2 and $k_b^2 (k_a^2 \leq k_b^2)$ be solutions of $q(|k|^2) = 0$, i.e.,

$$k_a^2 = \frac{-A - \sqrt{A^2 - 4Bd_u d_v}}{2d_u d_v}, k_b^2 = \frac{-A + \sqrt{A^2 - 4Bd_u d_v}}{2d_u d_v}$$

From Eqs.(23) and (26), the condition that $q(|k|^2) < 0$ holds in the range of $|k|^2 > 0$ and $k_a^2 < |k|^2 < k_b^2$ is derived in such a way that

$$A < 0, \; A^2 - 4Bd_u d_v > 0 \quad (29)$$

and the set S below is not empty

$$S = \{(m,n) \,|\, k_a^2 < \frac{\pi^2}{L^2}(m^2 + n^2) < k_b^2, (m,n = 1,2,\cdots)\} \quad (30)$$

In numerical simulations, all parameters are chosen to satisfy the diffusion-chemotaxis driven instability conditions (29) and (30).

Simulations

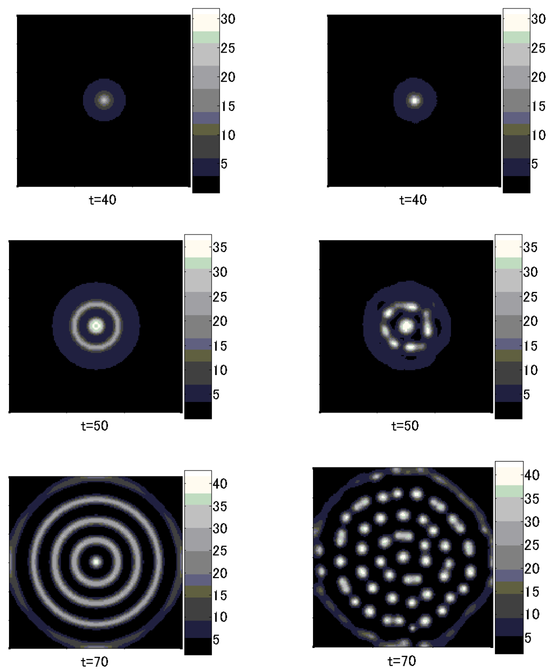
In this section, we study the influence of the random noise on chemotactic bacterial colony formations in the square region under the chemotaxis by numerical simulations.

Taking $G = (0,30) \times (0,30)$ and setting parameters and the initial attractant concentration as $d_u = 0.25$, $d_v = 1.0$, $\beta = 0.2$, $\delta = 20$, $\tau = 0.01$, $s_u = 0.04$, $s_v = 0.02$ and $v_0(x) = 0, ^\forall x \in G$ and changing the chemotaxis coefficient α in two ways, simulations are performed with different initial population density $u_0(x)$.

Case-1: Under the condition of $\alpha = 2.5$ and

$$u_0(x) = \begin{cases} 5, & x = (x_1, x_2) = (15,15) \\ 0, & \text{otherwise,} \end{cases} \quad (31)$$

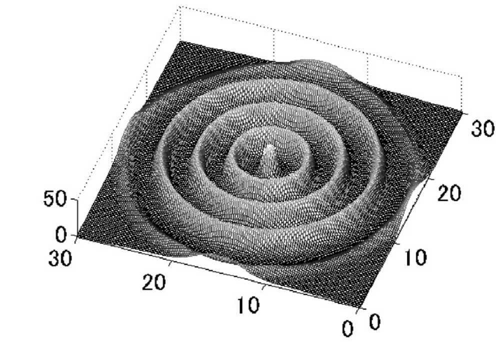
simulations are performed.



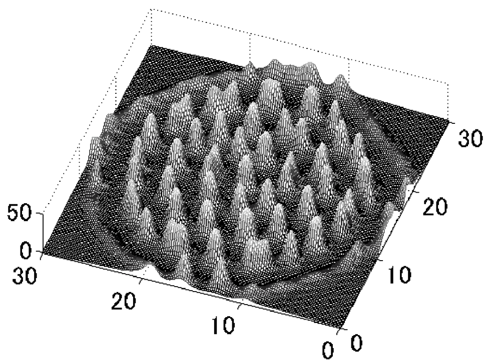
(a) Under the no noise (b) Under the noise

Figure 2. Time evolution of $u(t,x)$ in Case-1\.

Time evolution of the bacteria density $u(t,x)$ under the no noise and the noise is shown in Figure 2. In Figure 2, value of the population density $u(t,x)$ is shown as the gradations of white to black, for example, the region with white color denotes the high-density population one. It should be noted that scale of color in each figure changes with time, however, scale at the same time in the no noise and the noise cases is the same. In the no noise case, we can see that a low-density bacterial lawn spreads from the initial inoculums added to the center of the region. After that, the high-density ring appears on the lawn created at the first stage and concentric high density bacterial colonies are formed as shown in Figure 2(a), which is very similar to the exper- imental result in Figure 1.



(a) Under the no noise



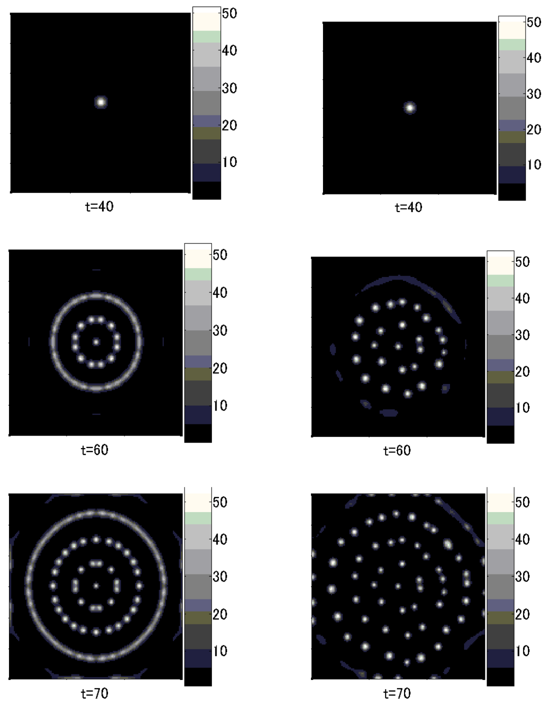
(b) Under the noise

Figure 3. Profile of $u(70, x)$ in Case-1.

On the other hand, in the noise case, the process at the first stage is similar to the no noise case, however, the created colonies are different from the no noise case in that the high-density concentric ring is broken by the noise and spotted concentric rings with higher density than Figure 2(a) appear as shown in Figure 2(b). In [10], we have found that the random noise promotes the colony formation in liquid media under chemotaxis. In other words, the random noise strengthens the effect of chemotactic property, so that concentric rings are broken and higher density spotted colonies are created. Simulation results show that enhancement of chemotaxis by the random noise holds in semi-solid media. In the Case-1, since a chemotactic property is not so strong, continuous rings are formed under the no noise and these continuous rings change into spotted rings under the noise because of enhancement of chemotactic property by the noise.

The profile of $u(70, x)$ under the no noise and the noise cases are shown in Figure 3.

Case-2: Setting the chemotaxis coefficient α as the larger value $\alpha = 3.0$ than the Case-1 and the other conditions are the same as in the Case-1, simulation results are shown in Figure 4.

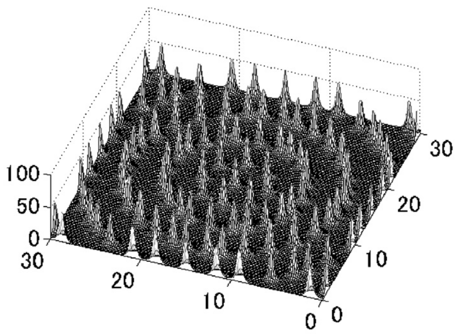


(a) Under the no noise (b) Under the noise

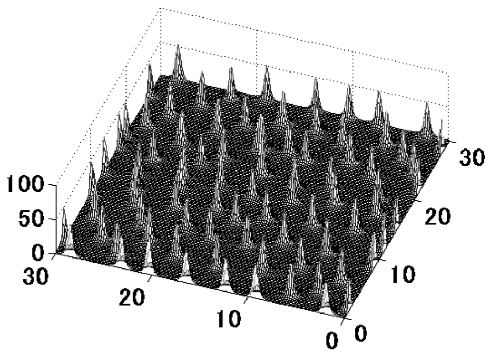
Figure 4. Time evolution of $u(t, x)$ in Case-2.

In the same manner as the Case-1, the low-density lawn is first created. After that, not continuous rings but spotted high-density rings are formed on the lawn in both of the no noise and the noise cases, mainly because the chemotactic property (opposite effect of diffusion) is stronger than the Case-1 and

bacteria have a strong trend toward aggregation. Since the random noise promotes the aggregation of bacteria, the spotted
rings under the noise are created at the earlier stage than under the no noise as shown in Figure 4. The 3-D views of population densities at time $t = 70$ under the no noise and the noise are shown in Figure 5. The spotted patterns are very similar to the experimental result in Figure 1. The distance between each spot on the same ring in the noise case is longer than one in the no noise case.



(a) Under the no noise



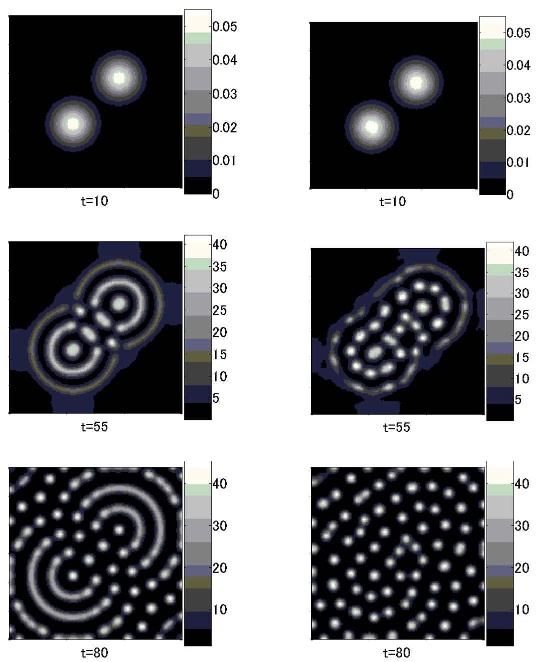
(b) Under the noise

Figure 5. Figure 3 Profile of $u(80, x)$ in Case-2.

Case-3: Setting the chemotaxis coefficient and other parameters at the same values as the Case-1, changing the initial attractant concentration in such a way that

$$u_0(x) = \begin{cases} 5, & x = (x_1, x_2) = (11,11), (19,19) \\ 0, & \text{otherwise}, \end{cases} \tag{32}$$

simulations are performed. Results are shown in Figure 6. Unlike the Cases-1 and -2, collisions of colonies occur in the Case-3 as shown in Figure 6. If there is no collision in the Case-3, colonies become continuous rings under the no noise case. In the no noise case, the continuous ring is formed until the collision occurs, but after collision of each colony, the destruction of continuous ring occurs at the collision point and the continuous rings become spotted high-density colonies. On the other hand, in the noise case, before collision, destruction of the ring occurs and the spotted rings are created. In this way, in the case where the chemotaxis property is not so strong, the influence of the random noise on the colony formation seems to be great and bacteria create the spotted colonies. The results in the Case-3 coincides with the experimental result that bacterial colonies never accrete each other.
The profile of $u(80, x)$ under the no noise and the noise cases is shown in Figure 7.

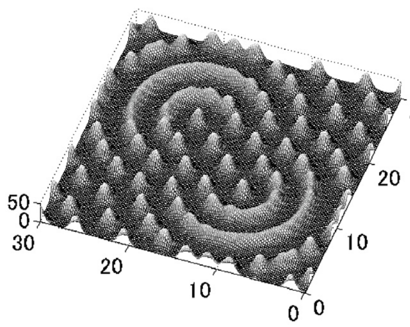


(a) Under the no noise (b) Under the noise

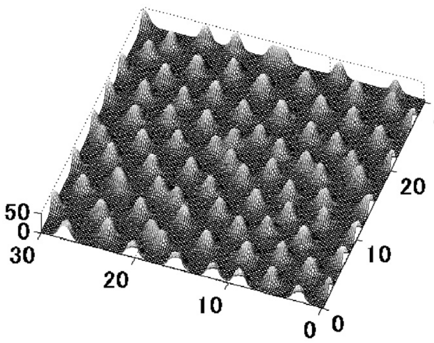
Figure 6. Time evolution of u in Case-3.

Conclusions

The stochastic model of chemotactic bacterial colony patterns in semi-solid media has been proposed. In liquid media, although we have already found that the random noise promotes the aggregation of colonies, simulation results by the



(a) Under the no noise



(b) Under the noise

Figure 7. Profile of $u(80, x)$ in Case-3.

proposed model have shown that the random noise plays the same role in semi-solid media as well as liquid media. We have knowledge that amalgamation of each bacterial colony never occur in the experiments. The simulation results coincide with this experimental result, which shows the efficiency of the proposed model.

As the future work, the analysis of colony formation process of bacteria species *E. coli* remains.

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