Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Theoretical Biology 225 (2003) 91–97
Journal of Theoretical Biology
www.elsevier.com/locate/jtbi

Growth dynamics of *Bacillus circulans* colony

Atsushi Komoto[a], Ken-ichi Hanaki[b], Shinya Maenosono[a], Joe Yuichiro Wakano[a], Yukio Yamaguchi[a], Kenji Yamamoto[a,b,*]

[a] *Department of Chemical System Engineering, School of Engineering, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8656, Japan*
[b] *Department of Medical Ecology and Informatics, Research Institute, International Medical Center of Japan, 1-21-1 Toyama, Shinjuku-ku, Tokyo 162-8655, Japan*

Received 8 July 2002; received in revised form 13 May 2003; accepted 21 May 2003

Abstract

We have investigated the growth dynamics of *Bacillus circulans* colony exhibiting the knotted-branching pattern by swarming on a hard agar medium. The knotted-branching pattern consists of many circular clusters, so-called subcolonies, and their trajectories. We analysed the processes of a subcolony because they are presumably the key elements for the formation of knotted-branching pattern. It was found that a subcolony has three processes, i.e. "generation", "growth", and "migration" by microscopic and time-resolved observations. An embryonic small subcolony (child subcolony) formed around an existing subcolony (parent subcolony) grows larger and migrates away from the parent subcolony. We proposed a simple model to explain the migration and the growth processes. It is assumed that the internal part of the subcolony is unfavorable for the bacteria and that the motion of the child subcolony on the agar medium can be modeled using a frictional force. The experimental data were quantitatively analysed in order to compare with models. Our models are consistent with the experimental results on following three points: (1) the radius of a subcolony increases linearly with the incubation time, (2) a subcolony stops just after formation and then starts to migrate suddenly, and (3) the trajectory of a subcolony predicted by the model agrees with the experimental one.

Keywords: Growth dynamics; *Bacillus circulans* colony; Knotted-branching pattern; Prediction of subcolony trajectory

1. Introduction

Recently, the mechanism of spontaneous pattern formation, i.e. self-organization, has been intensively investigated from both the fundamental and practical points of view (Sachs et al., 2001; Emmerich et al., 1999; Jin et al., 2001). On the other hand, self-organization is one of the characteristic features of biological systems (Kondo and Asai, 1995). It is thus important to study self-organizing pattern formation observed in biological systems. In many cases, however, the self-organization mechanisms in biological system are quite complex because there are a large number of elements playing a role in the given system (Kondo and Asai, 1995; Franks and Tofts, 1994). Through studying bacterial colonies, the diverse pattern formation mechanism can be understood. It has been studied during the last few decades because it is simple as compared with other biological systems (Budrene and Berg, 1991; Itoh et al., 1999; Ben-Jacob et al., 1997; Mimura et al., 2000; Cohen et al., 2000). Moreover, various kinds of mathematical models based on reaction–diffusion equations have been proposed (Mimura et al., 2000; Cohen et al., 2000; Ben-Jacob and Cohen, 2000 for the review article).

In particular, *Bacillus subtilis* (*B. subtilis*) (Matsushita, 1997) and *Proteus mirabilis* (*P. mirabilis*) (Watanabe et al., 2002) are well investigated, where the bacteria swarms on the agar plate and shows various colony patterns on the agar medium depending on the environmental conditions (i.e., the agar and nutrient concentration in the medium). There have been many reports on *B. subtilis* branching-patterned colonies (Matsushita, 1997; Ohgiwari et al., 1992). The similar behavior is also observed in the other bacterial strains (for example, see Ben-Jacob and Cohen, 2000 for the review article). The dynamics of the *B. subtilis* colony growth was also investigated (Wakita et al., 2001). It

*Corresponding author. Department of Medical Ecology and Informatics, Research Institute, International Medical Center of Japan, 1-21-1 Toyama, Shinjuku-ku, Tokyo 162-8655, Japan. Tel.: +81-3-3202-7181x2856; fax: +81-3-3202-7364.
E-mail address: backen@ri.imcj.go.jp (K. Yamamoto).

0022-5193/03/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0022-5193(03)00224-8

was reported that *B. subtilis* could move on the hard agar surface due to secretion of lubricant and a cooperative behavior (Ben-Jacob et al., 1994). In such a case, the bacterial mobility on the hard agar surface is an increasing function of the bacterial density. On the other hand, *P. mirabilis* forms a concentric-patterned colony (Watanabe et al., 2002; Matsuyama et al., 2000). On the hard agar surface, *P. mirabilis* forms a swarmer, which is a multinucleate and hyperflagellated cell, to increase the mobility (Belas, 1997). A number of mathematical models have been proposed to elucidate the pattern formation of bacterial colonies based on these experimental observations (Golding et al., 1998; Arouh, 2001). However, the validity of these models is limited due to the lack of quantitative experimental data related to the growth dynamics.

We have reported that *Bacillus circulans* (*B. circulans*) also exhibits diverse colony patterns depending on agar concentration (C_A) and nutrient concentration (C_N) as shown in Fig. 1(a) (Eiha et al., 2002). Unlike *B. subtilis*, *B. circulans* colony shows the knotted-branching pattern, which is the more complicated pattern containing many knots consisting of bacterial aggregates (described as a "subcolony"), on the hard agar surface. The knotted-branching pattern is a result of the growth and movement of the subcolonies, i.e. the trajectories of subcolonies. By forming the subcolony, *B. circulans* presumably acquires higher mobility on the hard agar surface as cooperative behavior.

Our ultimate goal is to understand the pattern dynamics exhibited in the colony growth of *B. circulans* and to build a mathematical model based on the experimental observations. In this paper, we analyse the elemental process for the colony growth considering that the subcolony is an important element for the formation of the knotted-branching pattern.

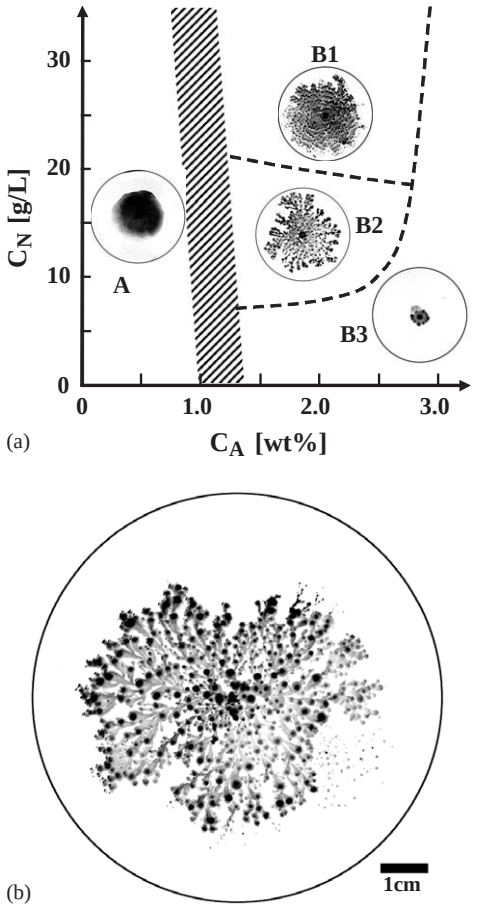

Fig. 1. (a) Morphology diagram of *B. circulans*. The colony patterns diversely change depending on the agar concentration C_A and the nutrient concentration C_N. These patterns are classified as follows: (A) disk-like, (B1) concentric-ring, (B2) knotted-branching, and (B3) Eden-like pattern. (b) The knotted-branching pattern of *B. circulans*. The photograph was taken after 5-day incubation. The colors were inverted by Adobe PhotoShop (Adobe Systems). The agar conditions are $C_A = 1.5$ wt% and $C_N = 15$ g/l.

2. Colony formation dynamics of *B. circulans*

Fig. 1(b) shows a colony of *B. circulans* colony on the hard agar medium (concentration of agar $C_A = 1.5$ wt%, and nutrient concentration $C_N = 15$ g/l). The details of the medium and the experimental procedures are described in the appendix. One can see many small circles in the interior of the colony which we call "subcolonies". Fig. 2 shows the transmission optical micrograph of an inner part of the subcolony taken by differential interference contrast microscopy. As seen in Fig. 2, the bacteria are closely packed and lose mobility. The internal part of the subcolony is presumably an unfavorable environment for the bacteria due to the high population density. The bacteria placed in the periphery of the subcolony might be active. The dynamics of the growth and the movement of subcolony are observed by time-resolved optical microscopy as shown in Fig. 3. The newborn subcolonies

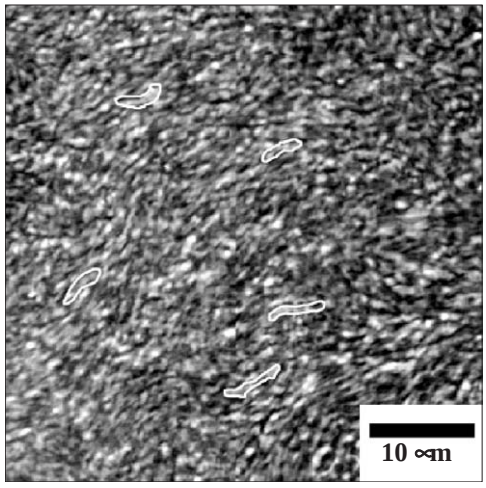

Fig. 2. Micrograph of the internal part of the subcolony. The white lines are guides for eye indicating individual bacteria strains. As seen in the image, the bacteria are closely packed.

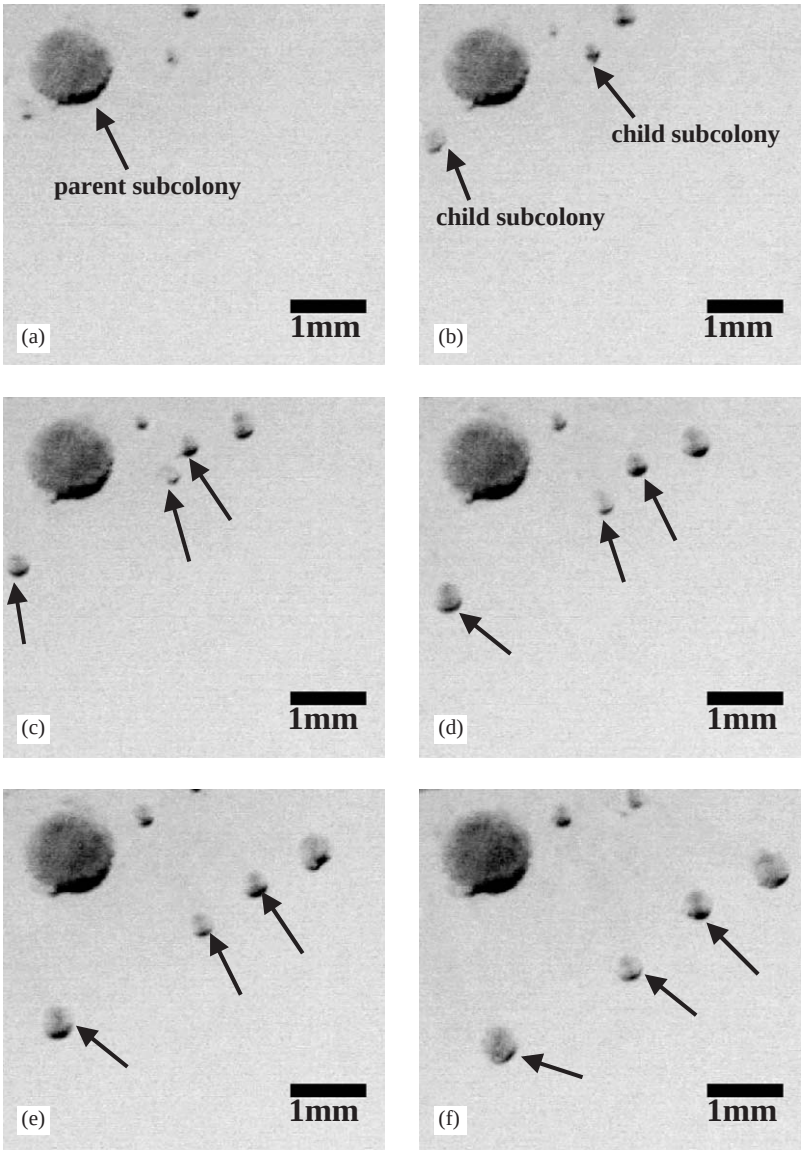

Fig. 3. Time series of enlarged photographs focusing on subcolonies. The arrows in Figs. 3(c)–(f) indicate child subcolonies migrating away from the parent subcolony. Each photograph was taken at every 30 min.

(child subcolonies) are formed at the periphery of the parent subcolony as seen in Figs. 3(a) and (b). After the formation of child subcolonies, they gradually grow and move away from the parent subcolony as shown in Figs. 3(c)–(f). We summarise that three elemental processes dominate the subcolony dynamics, i.e. "generation", "growth", and "migration". In this paper, we focus on two elemental processes: "growth" and "migration".

3. Model

3.1. Growth

From the experimental observation, the subcolony grows larger with incubation time. Here let us assume for simplicity that the shape of subcolony is disk-like and the bacterial density in the subcolony is spatially uniform constant. The cell-division rate for an individual bacterial cell is constant assuming that the nutrient concentration around the subcolony does not decrease significantly. However, the multiplication due to cell division occurred only in the outermost part of the subcolony from our observations. Thus, it is considered that the total increase in the distribution of bacteria is proportional to the perimeter of the subcolony. The mass balance is given by

$$\frac{\mathrm{d}}{\mathrm{d}t}(\rho\pi r^2) = 2\pi rk, \tag{1}$$

where ρ, r, and k are bacterial density, radius of subcolony, and the reproduction rate constant, respectively.

By assuming that the bacterial density is constant ($d\rho/dt=0$), we obtain from Eq. (1)

$$\frac{d}{dt}(\pi r^2) = \frac{2\pi k}{\rho} r. \quad (2)$$

Consequently, the area velocity of the subcolony is proportional to its radius. Thus we obtain

$$\frac{dr}{dt} = \frac{k}{\rho} = K \equiv const., \quad (3)$$

where K is a constant. Eq. (3) predicts that the radius of subcolony linearly relates to the incubation time.

3.2. Migration

The subcolony sinuates in the migration process and the resulting trajectory of the subcolony is not usually straight (see Fig. 1(b)). It is presumable that the migration direction is determined by attractive or repulsive chemotaxis, which is the bias of the bacterial movement according to the concentration gradient of chemical species. For example, such a phenomenon was experimentally studied in *Escherichia coli* colony (Budrene and Berg, 1995). However, the migration direction is not discussed in our model. We mainly discuss the one-dimensional mobility of the subcolony and describe a model based on the equation of motion.

The mass of the subcolony is proportional to the area because it is assumed that the subcolony is disk-like shaped and its bacterial density is spatially uniform. That is

$$m \propto \pi r^2, \quad (4)$$

where m represents the mass of the subcolony.

Next, we define impelling force. We previously confirmed that individual *B. circulans* cell swim inside of the soft agar medium (agar concentration <0.8 wt%) (Eiha et al., 2002). In our study, the colony growth rate on a hard agar medium (agar concentration $=1.5\%$), which was experimentally observed, was analysed by a set of reaction–diffusion equations and the diffusion coefficient of the bacteria was estimated. The estimated diffusion coefficient was much larger than that of a spherical particle of the same size in agarose gel. We suggested that it is because *B. circulans* has flagella and acquired much larger mobility than in the case of simple Brownian motion. It is natural to assume that the impelling force is an increasing function of the number of active flagella contained in the subcolony. Here we take two kinds of model into account to express the impelling force.

If we assume that the flagella located in the internal part of the subcolony are inactive, then the impelling force is proportional to the perimeter of the subcolony. If we assume that all flagella in the subcolony are active, the impelling force is proportional to the area of the subcolony. Thus, the impelling force F_i is described as

$$F_i \propto r^l \quad (l = 1, 2). \quad (5)$$

The subcolony finally stops migrating after the movement. However, the subcolony grows larger and child subcolonies are formed at the periphery as aforementioned after the subcolony stops migrating. This fact suggests that the stop of the subcolony is not due to the lack of nutrient but due to the existence of resistive force. We assume that the resistive force is caused by friction between the subcolony and the agar surface. Thus the resistive force F_r is proportional to the mass of the subcolony, described as

$$F_r \propto \pi r^2. \quad (6)$$

From Eqs. (4)–(6), the equation of motion for the subcolony is given by

$$A_1 r^2 \frac{dv}{dt} = A_2 r^l - A_3 r^2 \quad (l = 1, 2), \quad (7)$$

where A_1, A_2, and A_3 are positive constants and v represents the velocity of the subcolony.

3.3. Integration of the two models

Here we integrate the two aforementioned models, i.e. growth and migration processes, to derive the time dependence of the subcolony velocity v. Combining Eqs. (3) and (7), we obtain the relations between the velocity and the radius.

$l=1$:

$$v = B_1 \ln r - B_2 r + B_3. \quad (8)$$

$l=2$:

$$v = (B_1 - B_2) r + B_3, \quad (9)$$

where $B_1 = A_2/(A_1 K)$, $B_2 = A_3/(A_1 K)$, and B_3 are constants. Eq. (8), which corresponds to the case of the impelling force proportional to the perimeter of the subcolony, indicates that the velocity of the subcolony initially increases just after its formation because the impelling force is larger than the frictional force. Subsequently, the frictional force starts to dominate the migration as the subcolony grows, i.e. the radius of the subcolony increases. In short, it is predicted that the subcolony velocity v initially increases and then decreases with the subcolony growth. Eq. (9), which corresponds to the case of the impelling force proportional to the area of the subcolony, evidently indicates a linear dependence of the subcolony radius on the velocity. Since the velocity must finally reach zero, the sign of the pre-factor in Eq. (9) should be negative ($B_1 - B_2 < 0$).

4. Results and discussion

We experimentally measured the time evolution of the average radius of the subcolony $r(t)$ regarding they were circular-shaped. From Eq. (3), we obtain

$$r - r_i = K(t - t_i). \tag{10}$$

Here t_i and r_i are the time and the radius of the subcolony when the child subcolony becomes visible to the naked eye, respectively. Fig. 4 shows the linear time dependence of $r(t)$. This result is consistent with Eq. (10). The separation between the experimental data and the theoretical fit at late time could be due to the decrease in the reproduction rate caused by the nutrient starvation.

Here we compare the experimental data with Eqs. (8) and (9) regarding the migration process. The migration velocity of the subcolony was experimentally measured by chasing the position of the subcolony with using close-up pictures as shown in Fig. 3. However, the initial stage dynamics were observed by optical microscopy because the subcolony is too small to observe by the naked eye at the initial stage. According to the microscopic observation, it was confirmed that the child subcolony stands still for about 30 min after the generation and then it gradually starts to migrate. Fig. 5 shows the relation between the radius and the velocity of the child subcolony by analysing both the close-up observation and the microscopic observation (see the appendix for detail data). It was clarified that the velocity increases with the radius at small $r(t)$. As a result, we could conclude that the impelling force is created by the only flagella located at perimeter of the subcolony, i.e. $l = 1$.

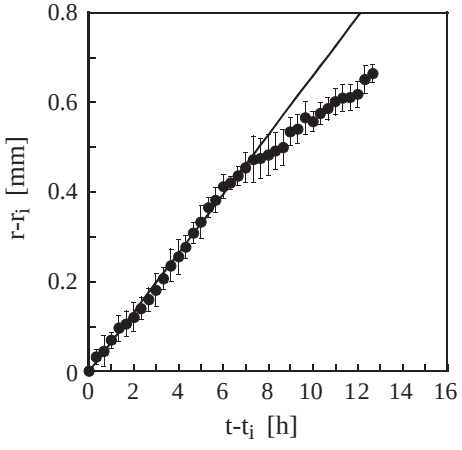

Fig. 4. Radius of the subcolony plotted versus incubation time. Here t_i and r_i are the time when the child subcolony becomes visible by the naked eye and the radius of the subcolony, respectively. The filled circles and the solid line represent experimental data and theoretical fit based on Eq. (10), respectively. The value of K obtained by fitting of experimental data.

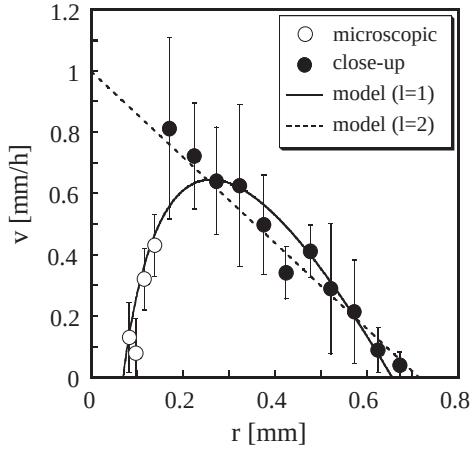

Fig. 5. Migration velocity of the subcolony plotted versus radius of the subcolony. Open circles and filled circles represent the experimental data from the microscopic and the close-up observation, respectively. Dashed and solid lines correspond to theoretical fits based on Eqs. (8) and (9), respectively. In the case of $l = 1$, the values of each constant are $B_1 = 1.1\,\mathrm{mm/h}$, $B_2 = 4.2\,\mathrm{h}^{-1}$, and $B_3 = 3.2\,\mathrm{mm/h}$, respectively. In the case of $l = 2$: $B_1 - B_2 = 1.4\,\mathrm{h}^{-1}$ and $B_3 = 1.4\,\mathrm{mm/h}$.

Combining Eqs. (3) and (8), we can obtain the equation for the trajectory of the subcolony is given by

$$x = C_1 r \ln r - C_2 r^2 + C_3 r + C_4. \tag{11}$$

Here x is the migration distance and $C_1 = B_1/K$, $C_2 = B_2/(2K)$, and $C_3 = (B_3 - B_1)/K$ are constants. We compared the trajectory of the subcolony which migrated almost radially with Eq. (11). In Figs. 6(a) and (b), the calculated and the experimentally observed trajectories are shown, respectively. This result also shows that our model is quantitatively consistent with the experimental result.

B. circulans exhibits the knotted-branching pattern on the hard agar medium. The origin of the knotted-branching pattern is the existence of the subcolonies. We believe that the subcolony is the key element for the *B. circulans* colony growth and analysed the dynamics of the subcolony formation process. According to the experimental and the theoretical analysis, it is possible to summarize that the subcolony formation could be divided into three processes: "generation", "growth", and "migration". We consider, in this paper, growth and migration processes and propose a simple physical model to elucidate the formation mechanism of the subcolony. Based on our analysis, the "active layer" exists at the periphery of the subcolony where the multiplication due to cell division occurs and the flagella are active. The model is very simple; however, it can quantitatively explain the shape of a branch, which is an important element of the knotted-branching pattern. The details of the "generation" process is unknown, but if we assume a small child sub-colony is generated near the parent and that sub-colony tends to move toward

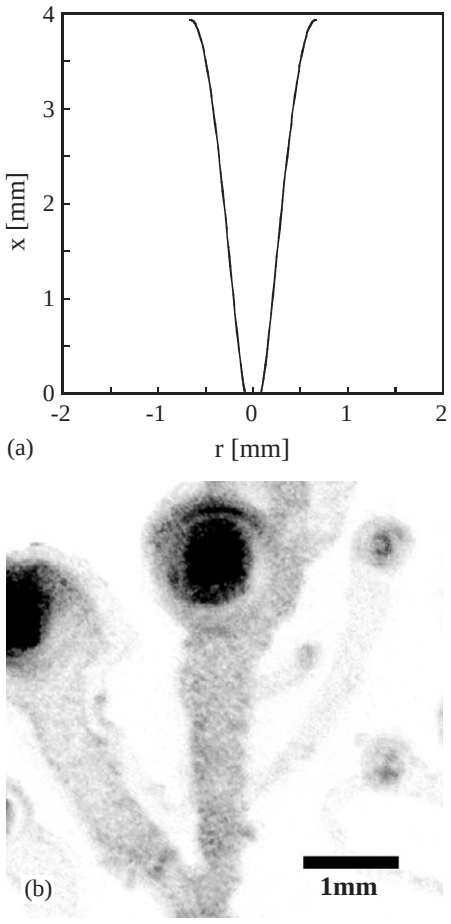

Fig. 6. The calculated (a) and the experimentally observed (b) trajectory of the subcolony. The values of each constant, which were used to draw the trajectory shown in Fig. 8(a), are $C_1 = 14$, $C_2 = 29\,\mathrm{mm}^{-1}$, $C_3 = 30$, and $C_4 = 0.61$ mm, respectively.

the nutrient rich locations, then the knotted-branching pattern is expected. To analyse it would be the fertile ground for the future studies.

Acknowledgements

We are grateful to Noriko Eiha for her help. This work was supported by the grants from the Ministry of Health, Labor and Welfare and the Ministry of Education, Science and Technology.

Appendix A

A.1. Experimental procedures

We used *Bacillus circulans*, which is a rod-shaped bacterium with a few flagella. The bacterial cells were incubated on the agar medium, which contains 10 g/l of Bacto Tryptone Peptone (Difco), 5 g/l of Bacto Yeast Extract (Difco), 1.5 wt% of Bacto agar (Difco) and 10 g/l of sodium chloride dissolved in pure water (the components of the liquid medium are the same as those of the agar medium without agar). This solution was autoclaved at temperature 120°C and pressure 2-atm pressure for 15 min, and then kept at 70°C in a water bath. Fifteen milliliters of the solution was poured into a Petri dish whose diameter was 86 mm and was cooled down to room temperature for about 1 hour. After the gelatin, the Petri dish was overturned and the agar medium was dried for 3 days. Then the weight of the dish decreased by 1.2–1.5 g. The liquid culture, whose bacterial density was about 10^8 cell/ml, was prepared by incubating bacteria at 37°C for 15–18 h. The droplet (1–2 μl) taken from the solution was inoculated at the center of the agar medium. Then the Petri dish was sealed with its cover by plastic tape to prevent the agar medium from drying. It was incubated at 37°C. The dynamics of the bacterial colony was observed by taking photographs every 10 min by a digital camera Nikon D1X (Nikon) equipped with a close-up lens (Micro-Nikon 60 mm lens, Nikon). Too small, and thus invisible to the naked eye, the subcolony was photographed by the digital camera through a microscope Olympus IX71 (Olympus) with the phase contrast filter. The size and the position of the subcolony were analysed from these photographs. The radius of the subcolony was estimated from its area. On the other hand, the migration velocity of the subcolony was experimentally measured by chasing the position of the subcolony. The average velocity of subcolony was plotted versus radius by converting original data into a discrete distribution. The class intervals for the radius are 50 μm for close-up and 20 μm for microscopic data. The averages of radius and velocity were calculated.

Table 1
Experimental data of Fig. 5

r (mm)	σ_r (mm)	v (mm/h)	σ_v (mm/h)
(a)			
0.084	0.006	0.131	0.113
0.098	0.005	0.079	0.113
0.116	0.004	0.322	0.101
0.139	0.003	0.432	0.102
(b)			
0.171	0.017	0.813	0.298
0.225	0.018	0.724	0.173
0.273	0.013	0.641	0.175
0.324	0.010	0.626	0.265
0.377	0.016	0.498	0.161
0.425	0.015	0.342	0.085
0.478	0.022	0.412	0.085
0.523	0.016	0.290	0.214
0.573	0.015	0.214	0.168
0.625	0.014	0.090	0.075
0.673	0.016	0.040	0.042

A.2. Experimental data

We show the experimental data, which compose Fig. 5. Tables 1(a) and (b) show the experimental data from the microscopic observation and from the close-up observation, respectively. In the table, r and v represent the averages of radius and velocity. And σ_r and σ_v represent the standard deviations of radius and velocity, respectively.

References

Arouh, S., 2001. Analytic model for ring pattern formation by bacterial swarmers. Phys. Rev. E 63, 031908.

Belas, R., 1997. *Proteus mirabilis* and other swarming bacteria. In: Shapiro, J.A., Dworkin, M. (Eds.), Bacteria as Multicellular Organisms. Oxford University Press, New York, pp. 183–219.

Ben-Jacob, E., Cohen, I., 2000. Cooperative self-organization of microorganisms. Adv. in Phys. 49, 395–554.

Ben-Jacob, E., Schochet, O., Tenenbaum, A., Cohen, I., Czirok, A., Vicsek, T., 1994. Generic modeling of cooperative growth patterns in bacterial colonies. Nature 368, 46–49.

Ben-Jacob, E., Cohen, I., Czirok, A., Vicsek, T., Gutnick, D.L., 1997. Chemo-modulation of cellular movement, collective formation of vortices by swarming bacteria, and colonial development. Physica A 238, 181–197.

Budrene, E.O., Berg, H.C., 1991. Complex patterns formed by motile cells of *Escherichia coli*. Nature 349, 630–633.

Budrene, E.O., Berg, H.C., 1995. Dynamics of formation of symmetrical patterns by chemotactic bacteria. Nature 376, 49–53.

Cohen, I., Ron, I.G., Ben-Jacob, E., 2000. From branching to nebula patterning during colonial development of the *Paenibacillus alvei* bacteria. Physica A 286, 321–336.

Eiha, N., Komoto, A., Maenosono, S., Wakano, J.Y., Yamamoto, K., Yamaguchi, Y., 2002. The mode transition of the bacterial colony. Physica A 313, 609–624.

Emmerich, H., Schleussner, D., Ihle, T., Kassner, K., 1999. Confinement effects in dendritic growth. J. Phys.: Condens. Matter 11, 8981–8993.

Franks, N.R., Tofts, C., 1994. Foraging for work: how tasks allocate workers. Anim. Behav. 48, 470–472.

Golding, I., Kozlovsky, Y., Cohen, I., Ben-Jacob, E., 1998. Studies of bacterial branching growth using reaction–diffusion models for colonial development. Physica A 260, 510–554.

Itoh, H., Wakita, J., Matsuyama, T., Matsushita, M., 1999. Periodic pattern formation of bacterial colonies. J. Phys. Soc. Jpn. 68, 1436–1443.

Jin, E.M., Givan, M., Newman, M.E.J., 2001. Structure of growing social networks. Phys. Rev. E 64, 046132.

Kondo, S., Asai, R., 1995. A reaction–diffusion wave on the skin of the marine angelfish *Pomacanthus*. Nature 376, 765–768.

Matsushita, M., 1997. Formation of colony patterns by a bacterial cell population. In: Shapiro, J.A., Dworkin, M. (Eds.), Bacteria as Multicellular Organisms. Oxford University Press, New York, pp. 366–393.

Matsuyama, T., Taguchi, Y., Nakagawa, Y., Itoh, H., Wakita, J., Matsushita, M., 2000. Dynamic aspects of the structured cell population in a swarming colony of *Proteus mirabilis*. J. Bacteriol. 182, 385–393.

Mimura, M., Sakaguchi, H., Matsushita, M., 2000. Reaction–diffusion modeling of bacterial colony patterns. Physica A 282, 283–303.

Ohgiwari, M., Matsushita, M., Matsuyama, T., 1992. Morphological changes in growth phenomena of bacterial colony patterns. J. Phys. Soc. Jpn. 61, 816–822.

Sachs, C., Hildebrand, M., Völkening, S., Wintterlin, J., Ertl, G., 2001. Spatiotemporal self-organization in a surface reaction: from the atomic to the mesoscopic scale. Science 293, 1635–1638.

Wakita, J., Shimada, H., Itoh, H., Matsuyama, T., Matsushita, M., 2001. Periodic colony formation by bacterial species *Bacillus subtilis*. J. Phys. Soc. Jpn. 70, 911–919.

Watanabe, K., Wakita, H., Itoh, H., Shimada, H., Kurosu, S., Ikeda, T., Yamazaki, Y., Matsuyama, T., Matsushita, M., 2002. Dynamical properties of transient spatio-temporal patterns in bacterial colony of *Proteus mirabilis*. J. Phys. Soc. Jpn. 71, 650–656.