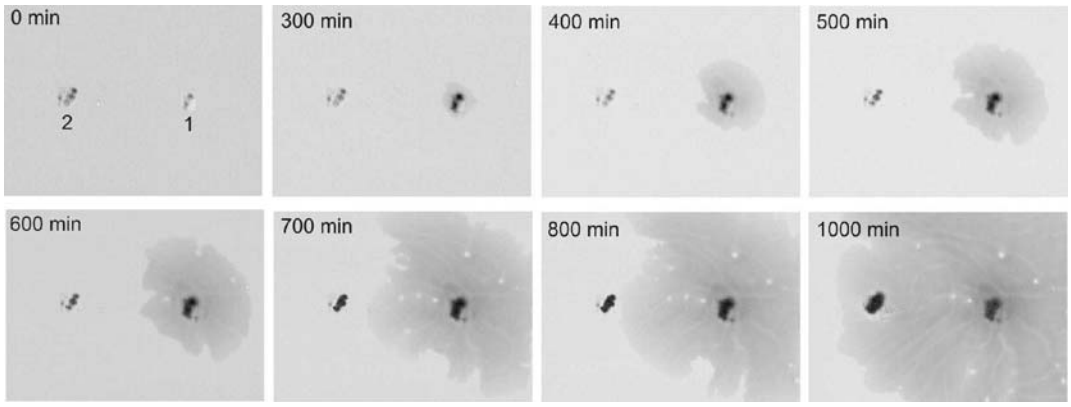

Figure 4.32: Snapshots of colonial growth on the agar plate with 6 g/l of agar concentration and 40 g/l of peptone concentration. B1 and B2 obtained from the same medium. The number 1 and 2 are corresponding to the bacteria of B2 and B1, respectively. The incubation temperature was 37°C. The first picture was taken after incubating for 2 hours.

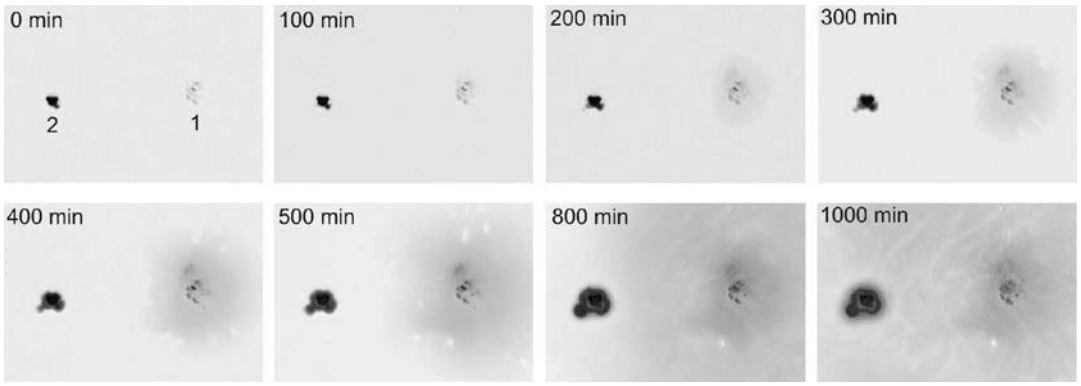

Figure 4.33: The snapshots of colonial growth on the agar plate with 6 g/l of agar concentration and 40 g/l of peptone concentration. B1 and B2 obtained from their isolated colonies. The incubation temperature was 37°C. The number 1 and 2 are corresponding with the bacteria of B2 and B1, respectively. The first picture was taken after incubating for 3 hours.

experiments, B1 and B2 were inoculated by streaking about 2.5 cm far form each other. The experimental results of 2 parts are shown in Fig. 4.32 and 4.33, respectively. The results of two parts represent that there is a lag phase in the period of about 6 hours. Then, the bacterial colony of B2 (labelled as number 1 in Figs. 4.32 and 4.33) grows and spreads

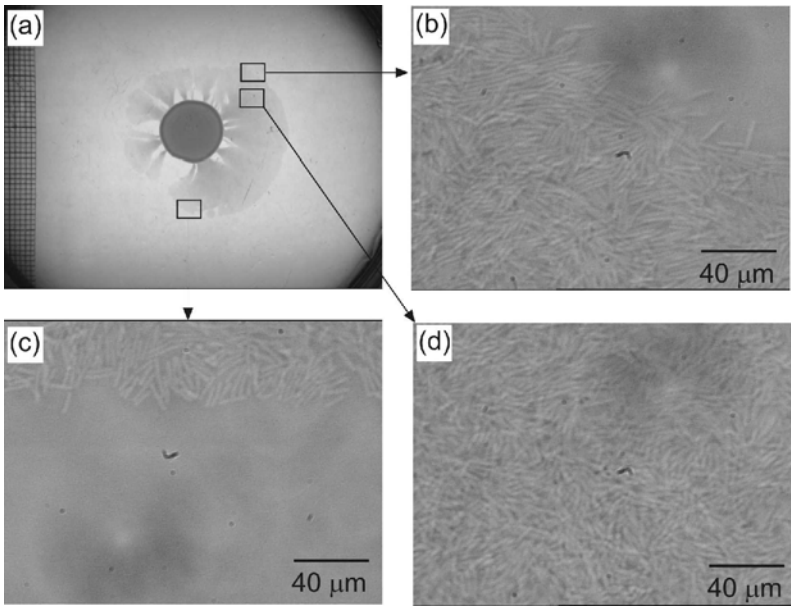

Figure 4.34: Microscopic observations of spreading colony of *Bacillus subtilis* TISTR 008 under the condition of 6 g/l of agar concentration and 40 g/l of peptone concentration. (a) is the picture of the colony taken by a CCD camera after incubation at the temperature of 37°C for 48 hours. (b), (c), and (d) are the pictures obtained from an optical microscope at magnification of 400. Initial cell density is 1.92×10^9 cfu/ml.

quickly, comparing to B1 (labelled as number 2). Finally, bacteria of B2 spread over the agar surface. In these results, we found that B2 are not form a compact colony before spreading, but tend to spread quickly after the lag time (about 6 hours).

4.3.5 Alignment of cells of the spreading colony

The microscopic observation of spreading colony by using an optical microscope is shown in Fig. 4.34. Macroscopically, the cell density represented by a gray level of spreading parts is lower than that of inside of colony clearly as shown in Fig. 4.34(a). Microscopic observations of spreading parts reveal that the alignment of bacterial cells is random (Figs. 4.34(b), (c) and (d)). We found that the bacterial colony in the spreading parts consist of a monolayer of cells. Comparing with the cells inside the colony, the swarming cells are very active and move fast.

4.3.6 Swarming

Swarming is a special form of bacterial surface translocation dependent on the

condition of the surface. This type of translocation was observed in the bacterial species of *B. subtilis*, *P. mirabilis*, *S. marcesens* [71]. It is known that the critical factors of swarming of bacterial cells are the concentration of agar and surface tension. D. B. Kearns et al. reported that *Bacillus subtilis* can exhibit swarming motility on the surface of the medium containing low agar concentration with 0.3 - 1.0% [72]. Corresponding to our work, the colony of *Bacillus subtilis* TISTR 008 is robustly spreading on the condition of soft medium (0.6% of agar concentration). M. Sharma et al. explained that the normal cells (vegetative cells) transition into hyper-agellated swarming ones as the swarming behavior occurred [71].

In 2004, M. B. Connelly et al. [73] studied the swarming Motility in Bacillus subtilis. Bacteria of *B. subtilis* with various strains were used in the study. They found that gene named Epr, a minor extracellular serine protease gene, plays an important role in swarming. They also found that if the strain of *Bacillus subtilis* is different, time for an initiated swarming is different as well.

It can be concluded that the swarming of cells is dependent on the concentration of agar and bacterial species.

5. CONCLUSION AND OUTLOOK

Under variations of agar and peptone concentrations, we found various colonial patterns: a fractal pattern, a fine branching pattern, a fingering pattern, a smooth circular pattern, and a spreading pattern. Microscopic observations reveal that the boundary of a smooth circular colony consists of bacterial cells connected with each other and aligned parallel with the circumference. The colonial front consists of bundles of chains of bacterial cells and these bundles meander crossing each other and form complex alignment in case of a fingering colony. Cell reproduction in limited space resulting in the formation of bundles of chains of cells is a cause of instabilities of colonial fronts.

Instability of propagating front is linearly increased with decreasing of peptone concentration and/or increasing of agar concentration. The proposed model can reproduce the experimental results of instabilities not only patterns, but also the roughness of colonial fronts.

The spreading or swarming colony is robustly obtained in the condition of soft medium with rich nutrient. Time for initiated swarming in *Bacillus subtilis* TISTR 008 is about 48-60 hours.

The outlook of this research is the study of swarming or spreading colony numerically. In order to describe such a behaviour, we suggest that there may have a critical population density of bacterial cells for the onset of spreading colony.

The other outlook is the study of pattern formation formed by cocci bacteria, such as *Staphylococcus aureus*. It is interesting because there is no report about patterns formed by this type of bacteria.

Additionally, reproduction of all patterns in morphological diagram of *Bacillus subtilis* TISTR 008 by using the proposed model can be considered to be a future work.

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Output จากโครงการวิจัยที่ได้รับทุนจาก สกว.

1. ผลงานตีพิมพ์ในวารสารวิชาการนานาชาติ
    - T. Somboon, C. Napasawad, C. Yoosook, and O.-U. Kheowan, “Instabilities in propagating front formed by *Bacillus subtilis* colony.”, Physica D, submitted.
    - T. Somboon, C. Napasawad, C. Yoosook, and O.-U. Kheowan, “Bifurcation of the bacterial translocation in a colony of *Bacllus subtilis* TISTR 008.”, Physica A, in preparation.

2. การนำผลงานวิจัยไปใช้ประโยชน์
    - เชิงพาณิชย์ –
    - เชิงนโยบาย –
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    - เชิงวิชาการ มีการพัฒนาทักษะการเขียนโปรแกรมเพื่อคำนวณแบบจำลองการเจริญเติบโตของแบคทีเรียของนักศึกษาระดับปริญญาโทและเอก โดยทักษะนี้สามารถนำไปปรับใช้กับการเจริญเติบโตในระบบอื่นๆ ได้อีกด้วย
3. อื่นๆ

    การเสนอผลงานในที่ประชุมวิชาการ
    - Titikan Somboon, Chanita Napasawad, Chalobon Yoosook, and On-Uma Kheowan, Onset of instabilities in propagating bacterial fronts. การประชุมนักวิจัยรุ่นใหม่ พบ เมธีวิจัยอาวุโส สกว. 13-15 ตุลาคม 2549, ณ โรงแรมรีเจนท์ ชะอำ จ.เพชรบุรี
    - Titikan Somboon, Chanita Napasawad, Chalobon Yoosook, and On-Uma Kheowan, Onset of instabilities in propagating bacterial fronts. การประชุมนักวิจัยรุ่นใหม่ พบ เมธีวิจัยอาวุโส สกว. 11-13 ตุลาคม 2550 ณ โรงแรมแอมบาสเดอร์ ชลบุรี จ.ชลบุรี
    - Titikan Somboon, Chanita Napasawad, Chalobon Yoosook, and On-Uma Kheowan, Bifurcation of the bacterial translocation in a colony of *Bacllus subtilis* TISTR 008. การประชุมนักวิจัยรุ่นใหม่ พบ เมธีวิจัยอาวุโส สกว. 16-18 ตุลาคม 2550 ณ โรวแรมฮอลิเดย์อินน์รีสอร์ท ชะอำ จ.เพชรบุรี

ภาคผนวก

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Title: Instabilities in propagating front formed by Bacillus subtilis colony

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Abstract: Depending on environmental conditions, colonies of bacterial species Bacillus subtilis TISTR 008 grown on the surface of agar mediums show patterns such as a smooth circular pattern or fingering pattern. The fingering forming or instability of colonial fronts is indicated by the roughnesses of the boundary of the bacterial colony. Hence, the instabilities can be determined quantitatively by means of the fractal dimension of the colony boundary. Experimental results show that the instabilities of propagating colonial fronts are linearly increased with decreasing of peptone concentration and/or increasing of agar concentration. Our microscopic observations reveal that cell reproduction in a limited space resulting in the formation of bundles of chains of bacterial cells causes a nonlinear diffusion and therefore the instability in propagating fronts. The proposed simulation model including the nonlinear diffusion successfully reproduces the experimental results both qualitatively and quantitatively. Furthermore, the instabilities can be discussed in terms of the ratio of diffusion coefficients for nutrient and bacterial cells. We found that the colony front can be stabilized, if this ratio decreases.

Dear Editors,

we are enclosing a manuscript by T. Somboon et al. entitled “Instabilities in propagating front formed by Bacillus subtilis colony, which we would like to submit to Physica D, Special Issue: Emerging Phenomena.

Pattern formation, fascinating phenomena in nonlinear systems, was found in various fields such as, chemistry, physics, and biology. Among these systems, the pattern formation of the bacterial colony is the simplest biological object for studying and understanding the pattern formation in biological systems. It is also easily controlled because its behavior depends directly on physical conditions, such as the nutrient concentration and hardness of the medium.

Although the diversity of colony patterns of Bacillus subtilis has been investigated intensively, however the detailed study about the transition from a simple smooth circular colony to a complicated structural colony is still unrevealed. Therefore, this is the goal of our work. Here we consider an expanding of the colony boundary as a propagating of bacterial front. If a planar front propagates with a uniform velocity, its will remain flat. Otherwise, the front will form a fingering shape, i.e., instability of propagating front. In this paper we focus on the transition from a stable to an unstable propagation of bacterial front. The instabilities of the propagating fronts under variations of medium hardness and nutrient concentration are studied, experimentally and numerically. The reaction-diffusion system with a nonlinear diffusion is proposed in order to explain and confirm the formation of colonial patterns. Finally, we discuss the instabilities in terms of the ratio of diffusion coefficients for nutrient and bacterial cells.

Due to the intensive and systematic investigations of these results we think that Physica D is the appropriate place for publication.

Sincerely yours,

On-Uma Kheowan

Bangkok, March 11, 2009

Instabilities in propagating front formed by Bacillus subtilis colony

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Abstract

Depending on environmental conditions, colonies of bacterial species *Bacillus subtilis* TISTR 008 grown on the surface of agar mediums show patterns such as a smooth circular pattern or fingering pattern. The fingering forming or instability of colonial fronts is indicated by the roughnesses of the boundary of the bacterial colony. Hence, the instabilities can be determined quantitatively by means of the fractal dimension of the colony boundary. Experimental results show that the instabilities of propagating colonial fronts are linearly increased with decreasing of peptone concentration and/or increasing of agar concentration. Our microscopic observations reveal that cell reproduction in a limited space resulting in the formation of bundles of chains of bacterial cells causes a nonlinear diffusion and therefore the instability in propagating fronts. The proposed simulation model including the nonlinear diffusion successfully reproduces the experimental results both qualitatively and quantitatively. Furthermore, the instabilities can be discussed in terms of the ratio of diffusion coefficients for nutrient and bacterial cells. We found that the colony front can be stabilized, if this ratio decreases.

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Instabilities in propagating front formed by *Bacillus subtilis* colony

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Depending on environmental conditions, colonies of bacterial species *Bacillus subtilis* TISTR 008 grown on the surface of agar mediums show patterns such as a smooth circular pattern or fingering pattern. The fingering forming or instability of colonial fronts is indicated by the roughnesses of the boundary of the bacterial colony. Hence, the instabilities can be determined quantitatively by means of the fractal dimension of the colony boundary. Experimental results show that the instabilities of propagating colonial fronts are linearly increased with decreasing of peptone concentration and/or increasing of agar concentration. Our microscopic observations reveal that cell reproduction in a limited space resulting in the formation of bundles of chains of bacterial cells causes a nonlinear diffusion and therefore the instability in propagating fronts. The proposed simulation model including the nonlinear diffusion successfully reproduces the experimental results both qualitatively and quantitatively. Furthermore, the instabilities can be discussed in terms of the ratio of diffusion coefficients for nutrient and bacterial cells. We found that the colony front can be stabilized, if this ratio decreases.

1. Introduction

Recently, much attention has been paid to pattern formation, which is fascinating phenomena in nonlinear systems. Pattern formation was found in various fields such as, chemistry, physics, and biology [1]. Spiral pattern in the Belousov-Zhabotinsky (BZ) reaction is a famous example in chemical systems [2]. Such a pattern can also be observed in biological systems, such as in an aggregation of slime mold *Dictyostelium discoideum* [3]. Patterns in nonlinear systems are created by an internal dynamics of the systems, this process of pattern formation is called self-organization. Such a process can only occur far from thermodynamic equilibrium [4]. Self-organizations occur in an open system, which has a flow of matter and energy between the system and environment that results in non-maximizing of entropy of the system. The complexity and diversity of self-organizing systems can be mathematically explained by using nonlinear equations, such as the reaction-diffusion equations [5]. The reaction-diffusion systems can be ap-

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plied to model and explain the complexity of pattern formation in biological systems in various scales, for example, calcium wave propagating in a rat retina [6], formation of the bacterial colony [7–10], and the Turing pattern on animal skins [11,12]. Among these systems, the pattern formation of the bacterial colony is the simplest biological object for studying and understanding the pattern formation in biological systems [13]. It is also easily controlled because its behaviour depends directly on physical conditions, such as the nutrient concentration and hardness of the medium [9].

In particular, bacteria species *Bacillus subtilis* is known to exhibit various colonial patterns such as, diffusion limited aggregation (DLA)-like, Eden-like, concentric ring-like, and disk-like, depending on the substrate softness and nutrient concentration [7,9,13,14]. Such patterns can be modelled by using reaction-diffusion system [15,16]. In the colonial formation, the complexity of the colonial growth generally increases as the environmental conditions become less favorable. The bacterial colony responds to adverse conditions by developing a strategy and organizing in a micro-level to cooperate the more efficiency of survival [17]. Various patterns can be observed, depending on both bacterial species and environmental conditions [18].

Although the diversity of colony patterns [7,9,13–16] of *Bacillus subtilis* has been investigated intensively, however the detailed study about the transition from a simple smooth circular colony to a complicated structural colony is still unrevealed. Therefore, this is the goal of our paper. Here we consider an expanding of the colony boundary as a propagating of bacterial front. If a planar front propagates with a uniform velocity, its will remain flat. Otherwise, the front will form a fingering shape, i.e., instability of propagating front. In this paper we focus on the transition from a stable to an unstable propagation of bacterial front. The instabilities of the propagating fronts under variations of medium hardness and nutrient concentration are studied, experimentally and numerically. The reaction-diffusion system modified from Mimura's model [16] with the nonlinear diffusion is proposed in order to explain and confirm the formation of colonial patterns. Finally, we discuss the instabilities in terms of the ratio of diffusion coefficients for nutrient and bacterial cells.

2. Experimental method

The used bacterial species was *Bacillus subtilis* TISTR 008. It was obtained from the Thailand Institute of Scientific and Technological Research. This bacterial species is commonly found in soil and able to form a protective endospore via a spore formation process under inappropriate living conditions. The experimental methods were carried out under 2 conditions: i) the variation of peptone concentration and ii) the variation of agar concentration. In the first case, the concentration of peptone were varied for 4 values: 10, 20, 30, 40 g/l, whereas the agar concentration was fixed at 6 g/l. For the later case, there were 5 concentrations of agar: 6, 8, 10, 12, 14 g/l, with fixed peptone concentration at 40 g/l. The temperature was kept constant at 37°C.

A solution for agar plate preparation consists of 5 g of NaCl, 2.397 g of K_2HPO_4, 0.68 g of KH_2PO_4, an amount of Bacto Peptone (Difco Laboratories), and Bacto Agar (Difco Laboratories) in 1 liter of distilled water. The final pH of the solution was 7.0 ± 0.2. The mixture was sterilized by using an autoclave at the temperature of 121°C for 15 minutes,

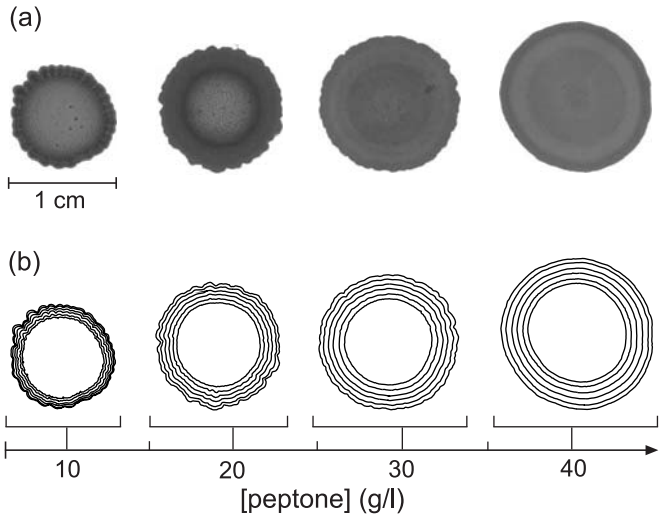

Figure 1. Colonial patterns (a) and the corresponding boundary contours (b) under the variation of peptone concentration. The agar concentration was fixed at 6 g/l. The snapshots (a) were taken at 60th hour, after inoculation. The innermost to outermost contours represent the fronts at 6th, 12th, 24th, 36th, 48th, and 60th hour, respectively. Incubated temperature was 37°C. Initial cell density was 2.23×10^9 cfu/ml.

then let it cool down to the temperature of 45°C. Because of the evaporation of water ($\sim$ 5 ml) in the mixture, the volume of the mixture was adjusted with sterile distilled water back to 1 liter. Then, 30 ml of the solution was immediately poured into a glass Petri dish (8.8 cm of diameter). The solution was solidified by keeping at the room temperature of 25°C for 1 hour. The thickness of agar was approximately 0.5 cm. Then, the agar plates were incubated at the temperature of 37.0±0.3°C and relative humidity of 49.0±2.0% for 24 hours to check the contamination.

Five microliters of liquid culture of bacteria was inoculated into the center of an agar plate. The value of the initial cell density calculated by using the method of viable count [19] was about $(2.0\pm0.3) \times 10^9$ cfu/ml. The inoculated agar plates were closed and sealed with parafilm to keep the humidity inside the agar plate approximately constant. Then, the agar plates were incubated at the temperature of 37±0.3°C and relative humidity of 49.0±2.0%.

Macroscopic colonial patterns were observed via a charge couple device (CCD) camera (SONY ExwaveHAD) with a magnification of 10 times. To observe a colonial pattern, colonial plates were taken out of the incubator to record an image. Then, it was immediately brought back to the incubator to limit the interrupted change of temperature, which affects the bacterial growth. For microscopic observations, an alignment of cells in a bacterial colony was observed by using a scanning electron microscope (SEM).

The roughnesses of colonial fronts were measured by applying the concept of fractal dimension [20–22]. In this work, we used the structured walk method to determine the fractal dimension of the colonial fronts. The length of the measuring sticks was varied in

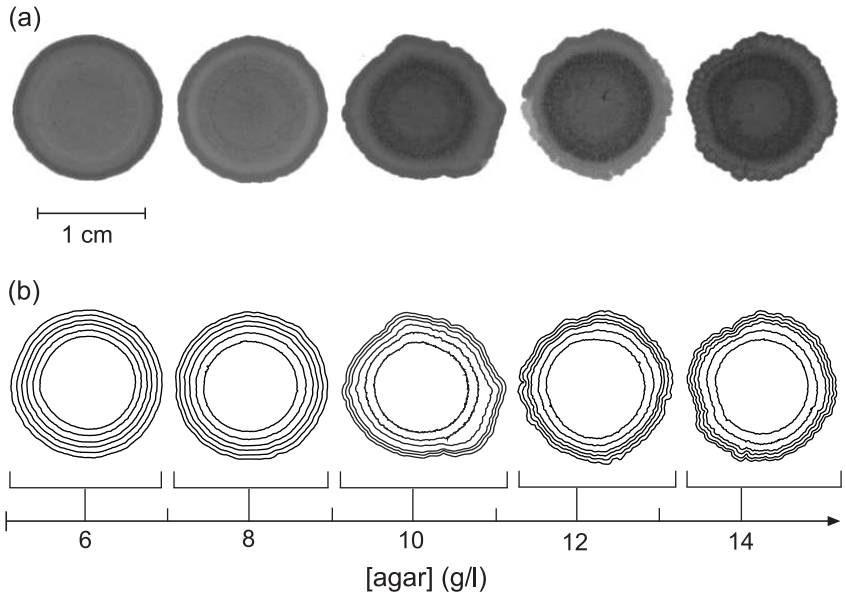

Figure 2. Colonial patterns (a) and the corresponding boundary contours (b) under the variation of agar concentration. The peptone concentration was fixed at 40 g/l. The snapshots (a) were taken at 60th hour, after inoculation. The innermost to outermost contours represent the fronts at 6th, 12th, 24th, 36th, 48th, and 60th hour, respectively. Incubated temperature was 37°C. Initial cell density was 1.85×10^9 cfu/ml.

the range of 1/3 to 1/100 of the colonial diameter.

3. Experimental Results

The experimental results can be divided into 3 parts: i) the instabilities under the variations of peptone concentration, ii) the instabilities under the variations of agar concentration, and iii) the microscopic observation of the bacterial front.

3.1. Variation of the peptone concentration

The instabilities of colonial front under the variation of peptone concentration are shown in Fig. 1. If the nutrient concentration is increased, with fixing of the agar concentration, the colonial front changes from a fingering shape to a smooth circular front as shown in Fig. 1(a). That is the amplitude of the fingering front decreases with increasing of nutrient concentration, and the front eventually becomes smooth when the nutrient concentration is sufficiently high. The growth rate of colony, indicated by the space between contours obtained with an equal time interval, (6 hours) is quite constant with time as shown in Fig. 1(b). However, the space between contours increases with increasing of the peptone concentration. This means that the growth rate is linear proportional to the peptone concentration. The measured growth rates are 25.0 ± 2.0, 57.3 ± 2.1, 68.7 ± 4.7, and

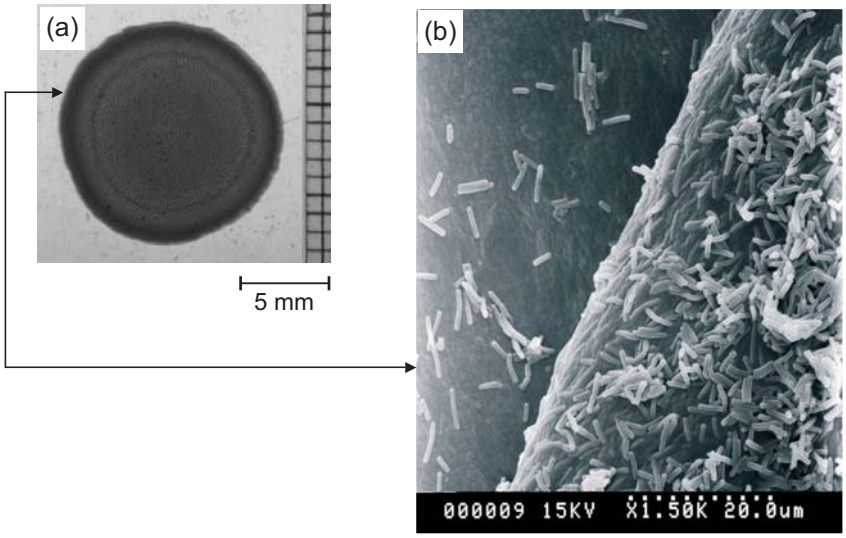

Figure 3. The microscopic observation of a smooth colony. (a) The picture of the colony taken by a CCD camera after incubation at the temperature of 37°C for 48 hours. The concentration of agar and peptone was 6 and 40 g/l, respectively. Initial cell density was 1.78×10^9 cfu/ml. (b) The SEM picture with the magnification of 1500 times.

77.6 ± 2.9 μm/hour for the peptone concentrations of 10, 20, 30, and 40 g/l, respectively. The linear relation between the growth rate and the peptone concentration is growth rate (μm/hour) = 1.692 ((μm/hour)(l/g))*[peptone] (g/l) + 14.850 (μm/hour).

3.2. Variation of the agar concentration

Instabilities of colonial front under the variation of agar concentration are shown in Fig. 2. If the agar concentration is increased (i.e., increasing of the medium hardness), the colonies change from a smooth front to a fingering front as shown in Fig. 2(a). For a rather high agar concentration about 10-14 g/l, the growth rate slows down after 24 hour, as the space between contours decreases dramatically, and the fingering front starts to grow, as shown in Fig. 2(b). However, the final sizes of colonies are not so different, compared to the experiments in Fig. 1, although the growth rates are not constant. This is because the nutrient concentration in this case is excess. Therefore, the population density of bacterial cells is high and the bacteria can move because of population pressure of cells in the colony [16].

3.3. Microscopic observations

In this work, the microscopic study was applied to understand an alignment of bacterial cells in the colonial front. The alignments of cells were investigated by using an SEM technique. The results can be divided into 3 types: i) the smooth circular front, ii) the rough colonial front under the condition of hard medium with rich nutrient, and iii) the

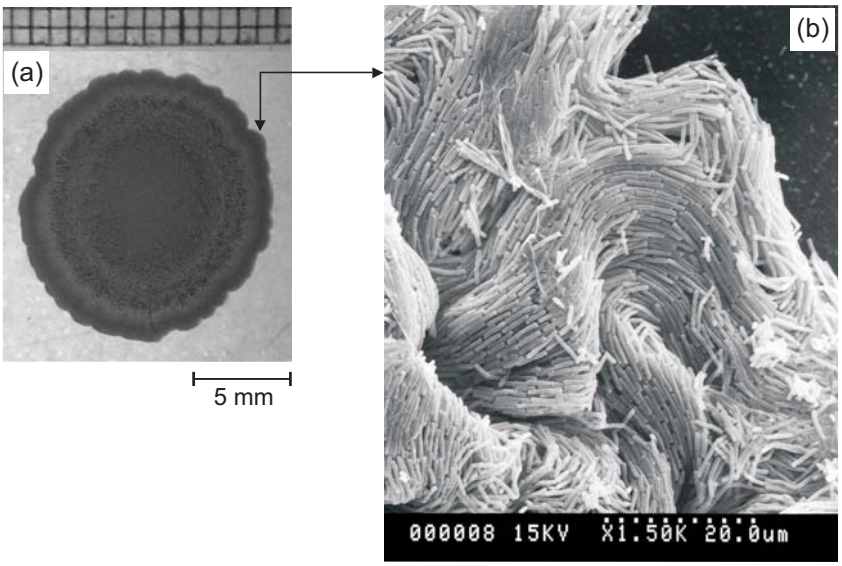

Figure 4. The microscopic observation of a fingering colony. (a) The picture of the colony taken by a CCD camera after incubation at the temperature of 37°C for 48 hours. The concentration of agar and peptone was 10 and 40 g/l, respectively. Initial cell density was 1.86×10^9 cfu/ml. (b) The SEM picture with the magnification of 1500 times.

rough colony under the condition of soft medium with poor nutrient.

The stable colonial front, smooth circular colony, shown in Fig. 3 was found on a soft medium (6 g/l of agar) with rich nutrient (40 g/l of peptone). The colonial front expands symmetrically and the reproduction of bacterial cells is quite high, because of soft agar medium with high nutrient concentration. For microscopic observation, the SEM image (Fig. 3(b)) reveals that the boundary of colonial front consists of bacterial cells connected with each other and aligned parallel to the circumference. In contrast, inside the colony (bottom right corner of Fig. 3(b)) the bacterial cells align randomly. Note that the scattering of single cells on the agar surface around the top left corner of Fig. 3(b) is not part of the dynamics, but it occurred during the process of the SEM sample preparation.

The unstable colonial front, a fingering pattern, at the condition of 10 g/l of agar and 40 g/l of peptone (hard agar medium with rich nutrient) is shown in Fig. 4. The microscopic observation (Fig. 4(b)) reveals that the colonial front consists of bundles of chains of bacterial cells. These bundles fold and meander crossing each other and form complex alignment. The microscopic result of the unstable colonial front at the condition of 6 g/l of agar and 20 g/l of peptone (soft agar medium with poor nutrient) brings out that again the front consists of bundles of chains of bacterial cells (the result is not shown here) similar to the result in Fig. 4.

From the microscopic investigation by using the SEM technique, it is plausible to discuss the stability of colonial front in terms of cell motility corresponding to the expansion rate of front ($\propto 1/[\text{agar}]$) and cell reproduction rate ($\propto [\text{peptone}]$). For the case of the stable

colonial front observed under the condition of soft medium and rich nutrient, the front expands symmetrically and forms a smooth circular shape. In this case, the expansion rate and cell reproduction rate are compatible. On the other hand, under the condition of hard medium the SEM images show that the colonial front consists of wavy bundles of chains of bacterial cells. In this case, the expansion rate is lower than that of the first case and does not match the growth rate. Under this reduction in cell motility, cells tend to consume nutrient and multiply themselves locally. Cell reproduction in the limited space results in the formation of bundles of chains of cells, which responds to macroscopic instability of colonial front. In general, cell motility is dependent on bacterial density, nutrient concentration, medium hardness, and on the growth rate of cells [9].

Kawasaki *et al.* remarked that bacterial cells can hardly move when either the concentration of nutrient or their cell density are low [15]. Under the condition of low nutrient level, bacteria tend to consume nutrient and multiply themselves locally, although the agar medium is soft. Hence they form wavy chains of cells similar to the result shown in Fig. 4(b). However, the density of bundles of chains in this case is lower than that in the condition of the hard medium with high nutrient because of lower nutrient concentration.

4. Numerical simulation

In order to confirm and describe the pattern formation and instabilities of colonial fronts obtained from the experiments, we use the modified reaction-diffusion model proposed by Mimura *et al.* [16]. In this work, a 9-point finite difference method [23] was applied to determine the value of cell density and nutrient concentration. Since the microscopic observations reveal that cells connected with each other and formed bundles of chains of cells. Therefore, it is plausible that at the local maxima of the bundle (high cell density) the front moves forward faster than that at the local minima (low density). We assume that the movements of cells is dependent on the density of active cells, locating mostly near the colony boundary. These active cells move, grow and multiply themselves, whereas inactive cells (inside the colony) do nothing at all but still alive. To apply this assumption, the nonlinear diffusion is used. We proposed a nonlinear diffusion of active cells D_u as following [17]:

$$D_u = d_1 u^k, \tag{1}$$

where an exponent, k refers to the hardness of the medium. u is the population density of active cells. The parameter d_1 is the diffusion of bacterial cells depending on an initial nutrient concentration, v_0. We assume that d_1 is a linear function of v_0 as following:

$$d_1 = b_1 v_0 + b_2, \tag{2}$$

where b_1 and b_2 are constants. This linear approximation agrees with the experimental results in Section 3.1. It should be noted that the parameters d_1 and k are respectively corresponding to the nutrient and agar concentrations in our experiments.

The used reaction-diffusion model [16] is shown as following:

$$\frac{\partial u}{\partial t} = \nabla(d_1 u^k \nabla u) + \varepsilon v u - \frac{a_0 u}{(1 + u/a_1)(1 + v/a_2)},$$

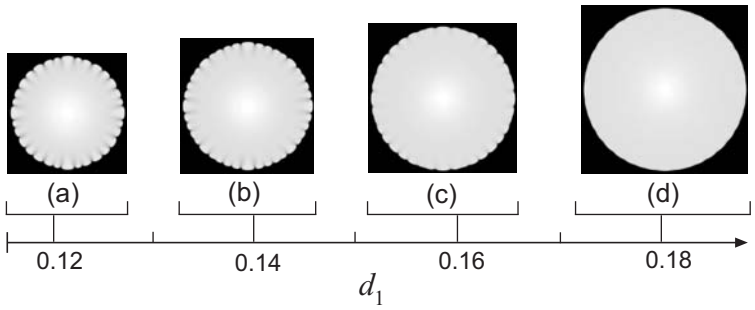

Figure 5. Patterns produced by numerical simulations under the variation of d_1. The values of v_0 were 1.00, 1.05, 1.10, and 1.15 for the simulation in (a)-(d), respectively. The value of k was fixed at 1.00. All patterns were captured at the time step of 800 t.u..

$$\begin{aligned} \frac{\partial v}{\partial t} &= D_v \nabla^2 v - vu, \\ \frac{\partial s}{\partial t} &= \frac{a_0 u}{(1+u/a_1)(1+v/a_2)}, \end{aligned} \tag{3}$$

where ε, a_0, a_1, a_2, and D_v are constants. $u(r,t)$ and $s(r,t)$ are the population densities of active and inactive bacteria, respectively. $v(r,t)$ is the nutrient concentration. $u(r,\, t) + s(r,\, t)$ is the total population density. Here r and t represent position and time, respectively. The used initial condition and boundary condition were obtained from Mimura *et al.* [16]. The numerical simulation was performed in two dimensional space (grid space = 0.5 s.u., array size = 800×800). We use $D_v = 1.00$, $\varepsilon = 1.00$, $a_0 = 1.00$, $a_1 = 0.000416$, $a_2 = 0.008333$, $b_1 = 4.0$, and $b_2 = 2.8$. The varied parameters are d_1 and k.

4.1. Numerical results under the variation of d_1

This simulation condition corresponds to the experiments under the variation of nutrient concentration, with soft agar medium. For a small value of d_1 (poor nutrient), we found a fingering pattern with high roughness as shown in Fig. 5(a). Note that white color represents high bacterial $(u + s)$ density, whereas black color indicates free agar medium. If the value of d_1 is increased, the fingering pattern transforms gradually to a smooth circular one as shown in Fig. 5(d). The final size of colony clearly increases with increasing of d_1. We found that the proposed model satisfactorily reproduces the experimental results in Fig. 1.

By applying the structured walk method, we found that the fractal dimension reflecting the instability of colonial fronts decreases linearly with increasing of d_1, corresponding to the experimental result of variation of peptone concentration as shown in Fig. 6. From this quantitative measurement we can conclude that the colony front can be stabilized, if the nutrient concentration is increased. In addition, the numerical results reproduce the experiments not only the patterns, but also their fractal dimension.

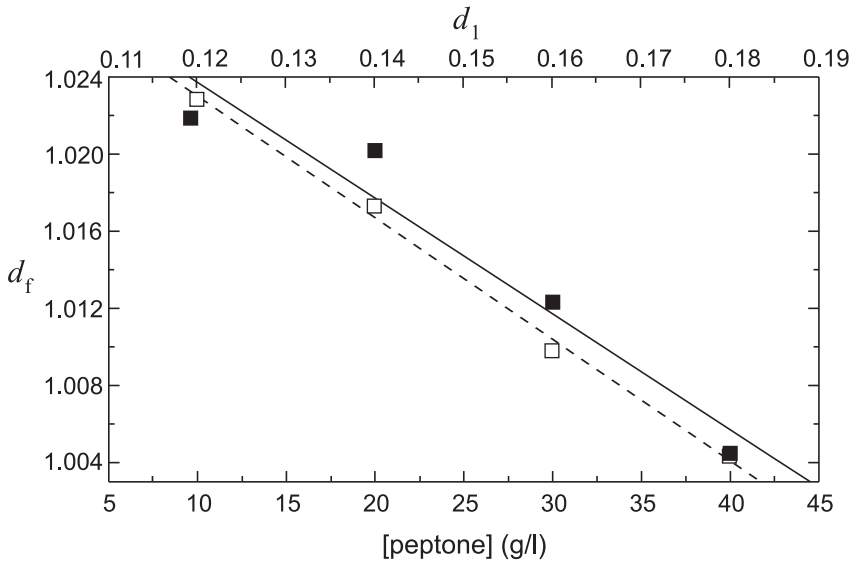

Figure 6. Fractal dimension, d_f of the bacterial fronts as a function of the nutrient concentration (i.e., [peptone] in experiments, indicated by filled squares and d_1 in simulations, indicated by open squares). The solid and dashed lines represent the linear fittings of experimental and numerical data, respectively.

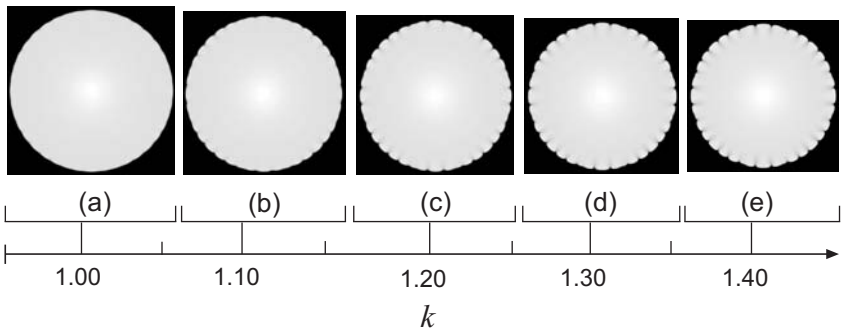

Figure 7. Patterns produced by numerical simulations under the variation of k. The value of v_0 and d_1 was fixed at 1.15 and 0.18, respectively. All patterns were captured at the time step of 800 t.u..

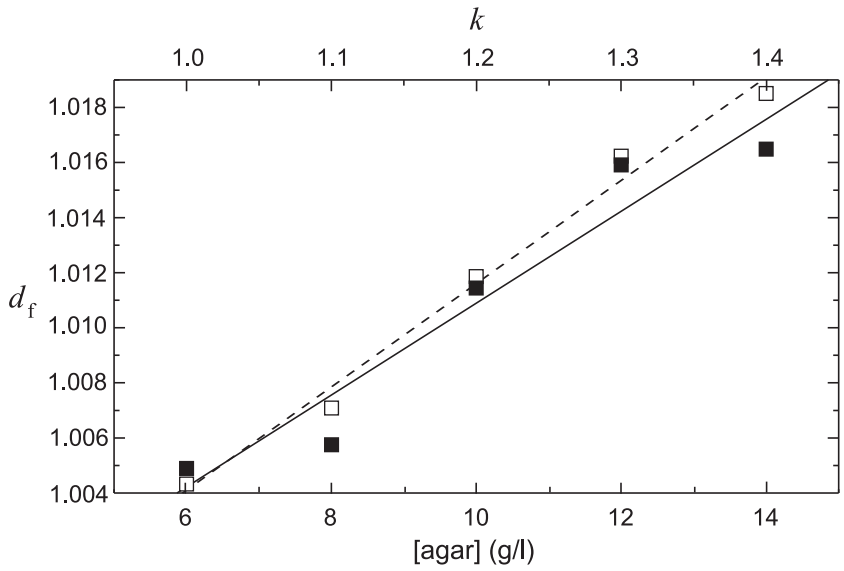

Figure 8. Fractal dimension, d_f of the bacterial fronts as a function of the medium hardness (i.e., [agar] in experiments, indicated by filled squares and k in simulations, indicated by open squares). The solid and dashed lines represent the linear fittings of experimental and numerical data, respectively.

4.2. Numerical results under the variation of k

In this part the values of d_1 and v_0 are fixed at 0.18 and 1.15, corresponding to the experiments under the variation of agar concentration, with rich nutrient. For a small value of k (soft medium), the colony represents a smooth circular pattern as shown in Fig. 7(a). The colonial pattern changes from a smooth circular front to a fingering pattern, when the value of k is increased. The final sizes of colony reduce only slightly with increasing of k. It reproduces the experimental results of variation of agar concentration shown in Fig. 2.

From the measurement of fractal dimension in Fig. 8, we found that the instabilities of colonial fronts linearly increase with increasing of k, corresponding to the results in experimental part. In this case the colony front destabilizes, if the medium hardness is increased. The detail of stabilization and destabilization of the colony front are discussed in Sec. 5.

5. Discussion

This section is devoted for the investigation of the instabilities of the colonial front. The instabilities in propagating reaction-diffusion fronts have been studied numerically by D. Horváth *et al.* [24] in the system of the iodate-arsenous acid reaction. They found that the instability occurs when the ratio of the diffusion coefficient for the reactant (iodate species) to that for the autocatalyst (iodide species) exceeds some critical value. This

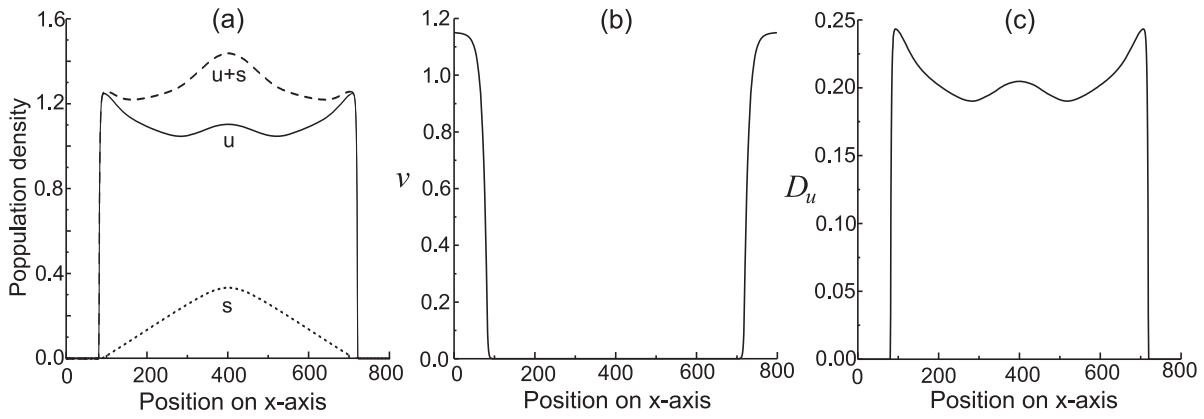

Figure 9. The example profiles of u, s, u+s, v, and D_u as a function of space. The data was obtained from the cross section (at y = 400) of the colony pattern shown in Fig. 7(e).

ratio is shown as following:

$$\delta = \frac{D_A}{D_B}, \tag{4}$$

where δ is the ratio of the diffusivity of reactant to that of autocatalyst. D_A and D_B are the diffusion coefficients of reactant and autocatalyst, respectively. The critical value of δ for the onset of instabilities of the propagating front is 2.9 [24]. The instabilities occur if δ is larger than this value.

Comparing the system of the iodate-arsenous acid reaction with the bacterial colony, here the active cells act as an autocatalytic species, since in the bacterial system a cell consumes the nutrient and then divides itself into 2 cells. The reactant in the chemical system corresponds to the nutrient in bacterial system. Thus, the occurrence of instabilities of colonial front should be also explained by using the ratio of diffusion coefficient in Eq. 4.

For the bacterial colony system, the ratio of diffusion coefficients for nutrient and bacterial cells is defined as

$$\delta = \frac{D_v}{D_u}, \tag{5}$$

where D_v and D_u are the diffusion coefficient of nutrient and active cells, respectively. Since the nutrient molecules are so small and can move around freely through water contained within the agar gel network [7], the value of D_v is taken to be constant (D_v = 1.00). Note that the diffusion coefficient of active cells, D_u in our work is nonlinear, whereas D_B in D. Horváth *et al.* [24] is linear.

In order to calculate the nonlinear diffusion D_u which is spacially nonuniform, we need to substitute $u(r,t)$ into Eq. 1. An example of the cross section profiles of the population densities u, s, u+s, and the nutrient concentration v are shown in Figs. 9(a-b). The maxima of u locate near the colony boundary, whereas the maxima of s locate in the

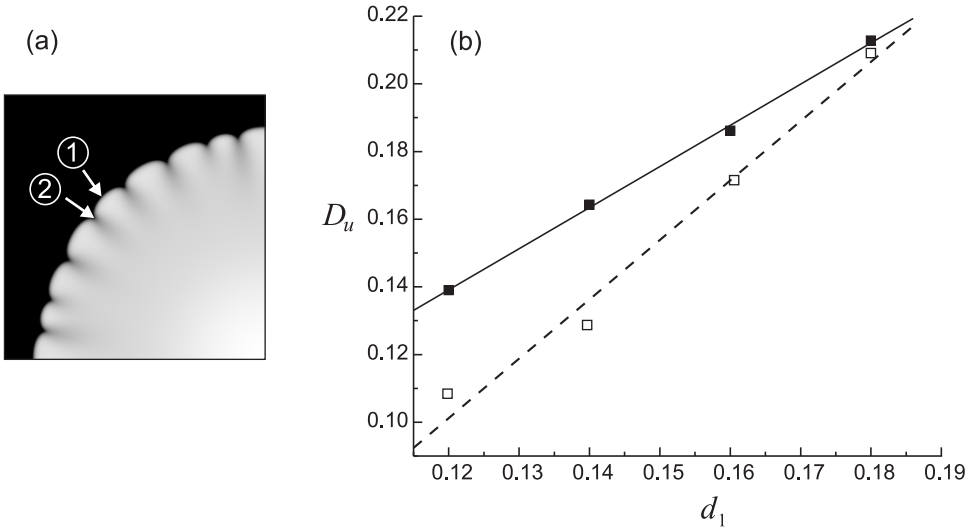

Figure 10. Diffusion coefficient of active cells as a function of d_1. The filled and open squares represent the diffusion coefficients obtained from position 1 (local maxima) and 2 (local minima) of the colony shown in (a), respectively. The solid and dashed lines indicate the linear fitting of the data from position 1 and 2, respectively.

middle. This means that most of the cells near the colony edge are active. Note that in this 2D model, the nutrient layer is very thin and the nutrient under the colony is consumed completely (Fig. 9(b)). The corresponding D_u profile is presented in Fig. 9(c). One can see that the maxima of D_u locate near the boundary of colony, according to the u profile in Fig. 9(a).

However, since the diffusion of the bacterial front is nonuniform as shown in Fig. 10. That is at the local maxima (position 1) the front diffuses fastest, whereas at the local minima (position 2) the front diffuses slowest. Therefore, the actual diffusion coefficient of the front can be determine as the average value of the two positions. For the case of variation of d_1 shown in Fig. 10, we found that the values of D_u increases with increasing of d_1.

After substituting the averaged D_u into Eq. 5, the ratio of diffusion coefficients, δ can be obtained. In our case $D_v = 1.00$ is constant. We found that the value of δ decreases with increasing of d_1 (result is not shown here). This decreasing in δ indicates that the colony front is stabilized, if d_1 is increased. This result agrees with the result of D. Horváth *et al.* [24]. However, the critical values of δ for the onset of the instabilities are slightly different, e.g., for a rather smooth colony in Fig. 5(d) the value of δ is 4.7, whereas D. Horváth *et al* reported that the critical value is 2.9. Actually it is quite difficult to compare the value of δ in our system which is nonlinear to the linear one of D. Horváth *et al.*

Finally we would like to discuss about the range of experimental parameters in comparison with the work of Matsushita *et al.* [7,9,14]. Note that although the concentration

range of agar (5 - 15 g/l) in this work and in Matsushita *et al.* are the same, but our nutrient concentration (10 - 40 g/l) is much higher than that of Matsushita *et al.* (0.2 - 10 g/l). Furthermore, we use different *Bacillus subtilis* strain, TISTR 008, whereas Matsushita *et al.* used *Bacillus subtilis* strain, OG-01. These differences can explain a slightly shift in our results and the results of Matsushita *et al.* However, we focus our study on the transition dynamics from a stable to an unstable propagation of bacterial front, not the diversity of colony patterns.

6. Conclusions

Our experimental results show that the instabilities of propagating front are linearly increased with decreasing of peptone concentration and/or increasing of agar concentration. The microscopic observations reveal that the cell reproduction in limited space resulting in the formation of meandering of bundles of cell chains causes a nonlinear diffusion and therefore the instabilities of propagating front. The proposed model successfully reproduces the experimental results both qualitatively and quantitatively. Furthermore, the instabilities can be discussed in terms of the ratio of diffusion coefficients for nutrient and bacterial cells. We found that the colony front can be stabilized, if this ratio is decreased, in agreement with the previous work.

Acknowledgments

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Bifurcation of the bacterial translocation in a colony of *Bacillus subtilis* TISTR 008.

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Abstract

An abrupt change in the colonial formation of *Bacillus subtilis* TISTR 008 is observed under the condition of soft agar medium with rich nutrient. At first state the bacteria form a thick colonial front which propagates slowly for about 48 hours, then suddenly they form a thin film which spreads quickly over the agar medium. From our experimental results we found that the behaviour in the second state is a swarming, which is a special form of bacterial surface translocation that a group of swarm cells at the rim of the bacterial colony rapidly spreads covering all available agar surface. At the swarming state, the bacteria change from flagellated cells to hyperflagellated ones and the bacterial colony consists of a monolayer of swarming cells which move around actively and randomly.

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I. INTRODUCTION

Depending on both bacterial species and environmental conditions, a bacterial colony can exhibit various colonial patterns [1–6]. The growth conditions are controlled by the initial concentration of the medium components. The agar concentration determines the consistency of the medium, which becomes harder as the amount of agar increases, and the nutrient concentration controls the bacterial reproduction. Depending on these two factors, the colony grows at a higher or lower rate, developing different kinds of patterns [7]. The complexity of the colonial growth normally increases as the environmental conditions become less favorable. The bacterial colony responds to adverse conditions by developing a strategy and organizing in a micro-level to cooperate the more efficiency of survival [1, 6]. Examples of these strategies are the production of extracellular wetting fluid [8] and the secretion of surfactants which change the surface tension resulting in swarming of bacterial cells [10].

Swarming is one type of surface translocation that bacterial cells group and rapidly move across a solid medium [9]. Rapid surface migration was preceded by a cell density dependent lag period, which could be eliminated if actively swarming cells were used as the inoculum. The leading edge of the swarm was characterized by multicellular rafts of highly flagellated cells [10]. As a collective bacterial process, swarming is often associated with biofilm formation and is linked to virulence factor expression in pathogenic bacteria [15]. As the swarming behavior occurred, the normal cells (vegetative cells) transition into hyper-flagellated swarming ones [9]. This type of translocation was observed in many bacterial species such as *B. subtilis*, *P. mirabilis*, *S. marcesens*. It is known that the critical factors of swarming of bacterial cells are the concentration of agar and surface tension [9, 14]. In addition, cell density is critical to swarming as the duration of the lag phase that precedes *P. mirabilis* migration is strongly influenced by inoculum density [14]. In 2004, M. B. Connelly et al. [15] studied the swarming motility in various strain of *Bacillus subtilis*. They found that gene named Epr, a minor extracellular serine protease gene, plays an important role in swarming. They also found that if the strain of *bacillus subtilis* is different, time for an initiated swarming is different as well.

In both Gram-negative and Gram-positive species of swarming bacteria, the transition to the swarming state is accompanied by dramatic changes in cell morphology: swarming cells become filamentous, multinucleoid and hyperflagellated. Flagella were implicated as

the motor for swarming motility by the dramatic increase in the density of flagella on the surface of cells at the swarm edge. Bacillus subtilis produces a lipopeptide antimicrobial surfactant called surfactin [10].

Swarming is a powerful means of rapidly colonising nutrient- rich environments, facilitating colony spread and accelerating biomass production. Several flagellated genera typically swarm, for example, Proteus, Bacillus [11]. Bacterial swimming motility is influential in many pathogenhost interactions, and several pathogens are additionally capable of multicellular swarming migration.

Swarming is neither a starvation response nor an obligatory development stage [14]. It is nonetheless a radical and reversible change in behaviour in response to the environment. The social nature of swarming indicates that extracellular and possibly cellcell signals are central stimuli, as are intracellular physiological parameters and contact with a surface. These signals might be sensed and transmitted by two-component regulatory systems, cytosolic regulators, and even cell-surface flagella [11].

The bacteria *Bacillus subtilis* is known to exhibit the swarming behaviour. They are commonly found in soil and considered to be a motile species [12]. It has been classified as an obligate aerobe. It is able to form a protective endospore via a spore formation process, if the conditions are not appropriated for the growth of bacterial cells, such as starvation and desiccation [13]. An endospore allows this bacterial species to tolerate extreme environmental conditions and is resistant to ultraviolet, gamma radiation, and chemical disinfectants. Normally, *Bacillus subtilis* is not considered as pathogen and toxicity.

In this work the swarming behaviour of the bacteria species *Bacillus subtilis* TISTR 008 under the condition of soft agar medium with rich nutrient is investigated. we focus on the spreading colony which have 2 state: a slow growth of a colony and a rapid one. We attempt to summarize the observed findings of the formation of the swarming colony and also show some experimental ideas for the investigation of the origin of the swarming growth.

II. EXPERIMENTAL METHOD

The used bacterial species was *Bacillus subtilis* TISTR 008 obtained from Thailand Institute of Scientific and Technological Research. A solution for agar plate preparation consists of 5 g of NaCl, 2.397 g of K_2HPO_4, 0.68 g of KH_2PO_4, 40 g of Bacto Peptone (Difco Labo-

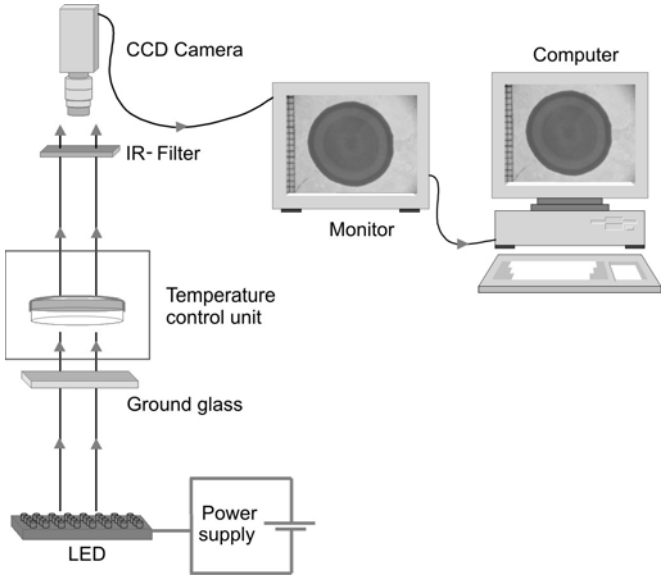

FIG. 1: Experimental setup for a macroscopic observation of colonial patterns.

ratories),and 6 g of Bacto Agar (Difco Laboratories) in 1 liter of distilled water. The final pH of the solution was 7.0 ± 0.2. The mixture was sterilized by using an autoclave at the temperature of 121°C for 15 minutes, then let it cool down to the temperature of 45°C. Because of evaporation of water in the mixture (∼ 5 ml), the volume of the mixture was adjusted with sterile distilled water back to 1 liter. Then, 30 ml of the solution was immediately poured into a glass Petri dish (8.8 cm of diameter). The solution was solidified by keeping at the room temperature of 25°C for 1 hour. The thickness of agar was about 0.5 cm. Then, the agar plates were incubated at the temperature of 37.0±0.3°C and relative humidity of 49.0±2.0% for 24 hours to check the contamination.

5 μl of liquid culture of bacteria was inoculated into the center of an agar plate. The value of the initial cell density calculated by using the method of viable count [16] was about (2.0±0.3) x 10^9 cfu/ml. The inoculated agar plates were closed and sealed with parafilm to keep the humidity inside an agar plate approximately constant. Then, the agar plates were incubated in a water jacket, in which the temperature are controlled by using a thermostat. The temperature inside is 37±0.3°C.

Colonial patterns were observed via a charge couple device (CCD) camera (SONY ExwaveHAD) with a magnification of 10 times. A schematic diagram of the experimental setup is shown in Fig.1. An array of infrared light-emitting diodes (IR-LED) is used as an IR light source because it emits a narrow-spectrum of IR light in one direction with a

narrow angle. It contrasts to a fluorescent lamp of a light box, which emits light in no specific direction. Thus, an image obtained from IR-LED is clearer than that from a light box. The generated infrared light passes through a ground glass used to obtain a homogeneous light, and then the bacterial colony in an agar plate. An infrared filter that allows only the transmission of the IR light was placed before the CCD camera. Then, the images were recorded on a computer hard disk. For microscopic observations, an alignment of cells in a bacterial colony was observed by using an optical microscope.

III. EXPERIMENTAL RESULTS

A. Morphological diagram

In order to investigate the morphological changes due to environmental conditions in colonial formation, two important parameters are varied: the peptone and agar concentrations. We found that the colony patterns grown on an agar surface change dramatically, if the concentrations of agar and nutrient are varied globally. They are classified into 4 distinct types, as shown in the morphological diagram in Fig.2: fingering colony in region A, fine branching colony in region B, fractal pattern in region C, and spreading colony in region D.

Region A: The colonies form a fingering front. The colonial patterns become compact. The colonies in this region are very dense obviously because of high nutrient concentration. The patterns in this region are similar to the Eden pattern in Matsushitas work [12].

Region B: When the nutrient concentration is low (10 g/l) and the medium is soft (6 g/l), the colonial fronts are not smooth and form a fine branching pattern. This pattern is also found when the medium is hard (10-14 g/l) and nutrient concentration is high (20-40 g/l). The bacterial cells in this region undergo cell division locally, similar to that in region A, but cell density is higher because of higher nutrient concentration. Therefore, the colonies in region B are darker than that in region A.

Region C: The colonies describe a fractal pattern. The nutrient concentration was fixed at 0.4 g/l. Cell division of bacterial cells in this region is low because of the limited nutrient. They only grow and undergo cell division locally. It seems that in this region the colonial patterns are not affected by the hardness of the medium.

Region D: The colonies form a spreading or a swarming pattern. The agar concentration

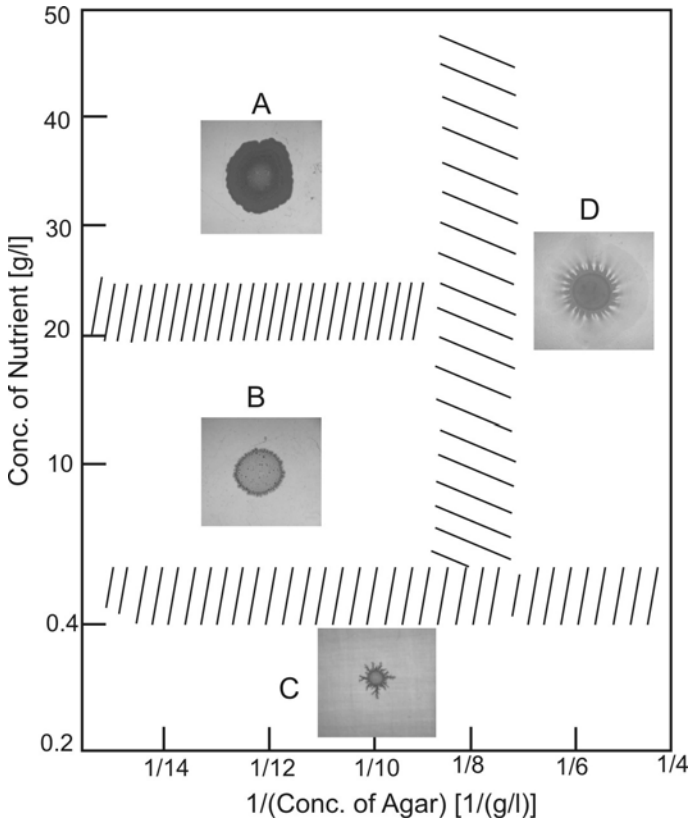

FIG. 2: Colonial patterns of *Bacillus subtilis* TISTR 008 under the variations of agar concentration and peptone concentration (all pictures were taken at 60 hours). Initial cell density was 1.76 x 10^9 cfu/ml. The incubation temperature was 37°C.

was fixed at 4 g/l (very soft agar medium). The colonial fronts spread quickly because of high moisture of the surface of medium, due to the vapour condensation, which increases the motility of cells.

B. Dynamics of a spreading colony

The dynamics of the spreading colony observed in region D are shown in Fig.3. The bacterial colony slowly grows and forms a compact colony with the smooth front for the period of 6-36 hours. Then, the front of the colony bursts and forms a sun-like pattern. The population density of bacterial cells may reach some critical values before bursting of the colonial boundary. After that, the bursting parts spread and then cover the agar surface quickly. Finally, the homogeneous thin-film of bacteria appears all over the agar surface at 72 hours. D. B. Kearns *et al.* reported that *Bacillus subtilis* can exhibit swarming

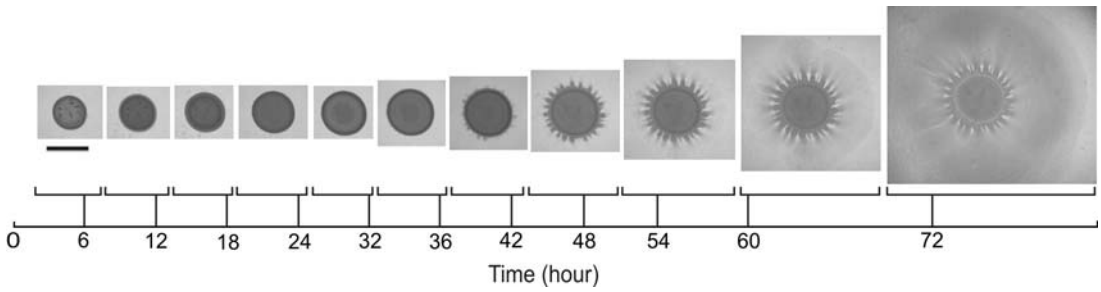

FIG. 3: Growth of a colony in the condition of 6 g/l of agar concentration and 40 g/l of peptone concentration. Incubation temperature was 37°C. Initial cell density was 2.23 x 10^9 cfu/ml. (scale bar: 1cm)

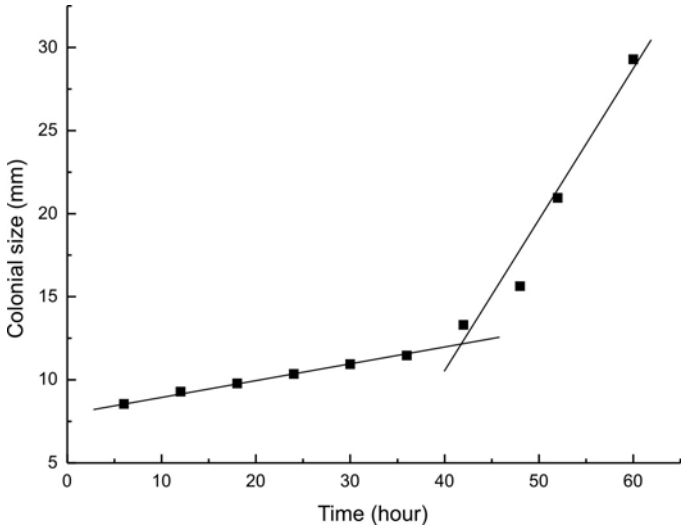

FIG. 4: Colonial size as a function of time.

motility on the surface of the medium containing low agar concentration with 0.3-1.0% [10]. Corresponding to our work, the colony of *Bacillus subtilis* TISTR 008 is robustly spreading on the condition of soft medium (0.6% of agar concentration) with rich nutrient (1-5% of peptone concentration). However, the number of the spreading colony is decreasing with decreasing of peptone concentration and the colony does not show a swarming behaviour when the peptone concentration is very low (0.4 g/l). It is reasonable because the activity of cells decreases with decreasing of nutrient [4].

The colonial size is investigated as a function of time as shown in Fig.3. The result shows that the colonial size slightly increase with time, then the colony grows quickly cover agar surface. There is a switch of propagation velocity of colonial front during the growth of

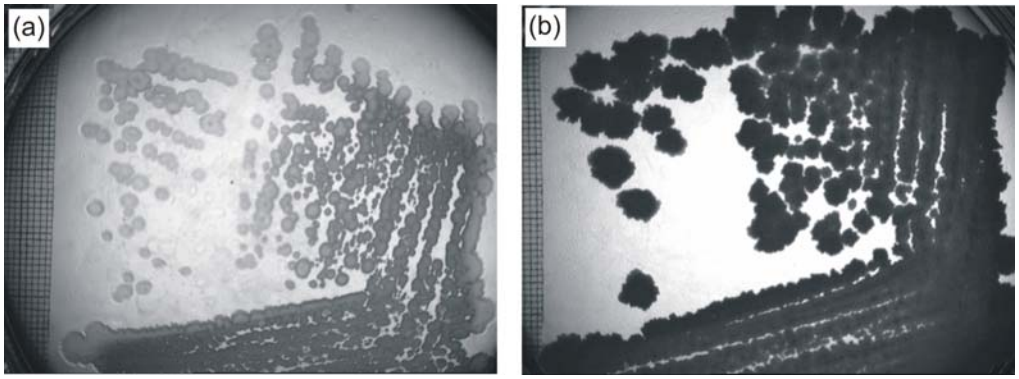

FIG. 5: Isolated colonies on the surface of tryptic soy agar (TSA) medium. (a) shows an isolated colonies obtained form the spreading part (B2). (b) presents an isolated colonies obtained form the stock (B1). The incubation temperature was 37°C. These pictures were taken at 24 hours after incubation.

colony. The velocity of colonial front, which is obtained by linear fitting of a slope in the graph shown in Fig.3, changes form 95.87±2.43 μm/hour to 921.20±130.75 μm/hour.

C. Characteristics of swarming cells

After the formation of the spreading colony is found, we try to understand and investigate how this behaviour occurs. The bacterial cells in the region of spreading part were streaked on TSA medium to isolate the colonies. The isolated colonies of bacterial cells of the spreading part are shown in Fig.5(a). Comparing with the isolated colonies from stock bacteria shown in Fig.5(b), we found a difference between these two types of isolated colonies. The isolated colonies of the spreading part are thinner than that of the stock. It is noted that bacterial cells of the stock and of spreading part are labelled as B1 and B2, respectively.

After reading publications of Ben-Jacob *et al.* [17, 18], the suspect of bacterial mutation arose. In their work, the sector formation of expanding bacterial colonies was studied. They mentioned that if the mutant bacteria have the same growth dynamics as the normal ones, they will be usually unnoticed after growing of the bacterial colony. Ben-Jacob *et al.* also suggest that if the bacterial mutants have different growth dynamics, the presence of mutant cells is indicated by the distinguished sector with a different growth pattern. In our results, we found different growth patterns, i.e., changing from the slow growth of the smooth colony to the spreading one. Therefore, in order to prove whether the onset of spreading formation

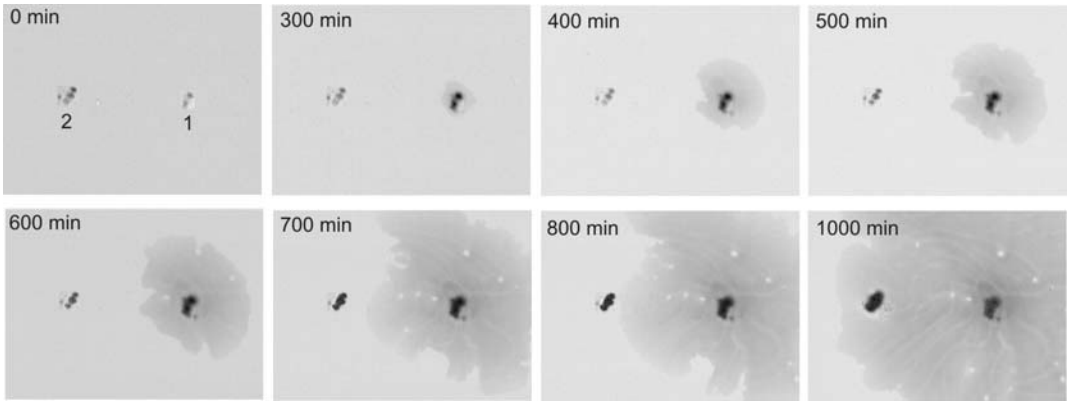

FIG. 6: Snapshots of colonial growth on the agar plate with with 6 g/l of agar concentration and 40 g/l of peptone concentration. B1 and B2 obtained from the same medium. The number 1 and 2 are corresponding to the bacteria of B2 and B1, respectively. The incubation temperature was 37°C. The first picture was taken after incubating for 2 hours.

occurred from the mutation of bacterial species or not, the 16s rDNA technique [19] and biochemical test were applied to classify the bacterial cells of B1 and B2. The result of DNA sequencing reveals that DNA sequences of B1 and B2 are not different. This means that species and strain of B1 and B2 are same. The results of biochemical test present that there are some differences, but the species of two types are concluded to be the same species. Thus, it can be concluded that there is no mutation in bacteria, when the spreading colony occurs.

In order to investigate the different characteristics of B1 and B2, two types of bacteria species were grown on the same medium. The experiments can be divided into 2 parts: i) B1 and B2 obtained from the same medium in the spreading part and the inside one, ii) B1 and B2 obtained from their isolated colonies on TSA medium. In these experiments, the agar and peptone concentrations are fixed at 6 and 40 g/l, respectively. In two experiments, B1 and B2 were inoculated by streaking about 2.5 cm far form each other. The experimental results of 2 parts are shown in Fig.6 and 7, respectively. The results of two parts represent that there is a lag phase in the period of about 6 hours. Then, the bacterial colony of B2 (labelled as number 1 in Figs.6 and 7) grows and spreads quickly, comparing to B1 (labelled as number 2). Finally, bacteria of B2 spread over the agar surface. In these results, we found that B2 are not form a compact colony before spreading, but tend to spread quickly after the lag time (about 6 hours).

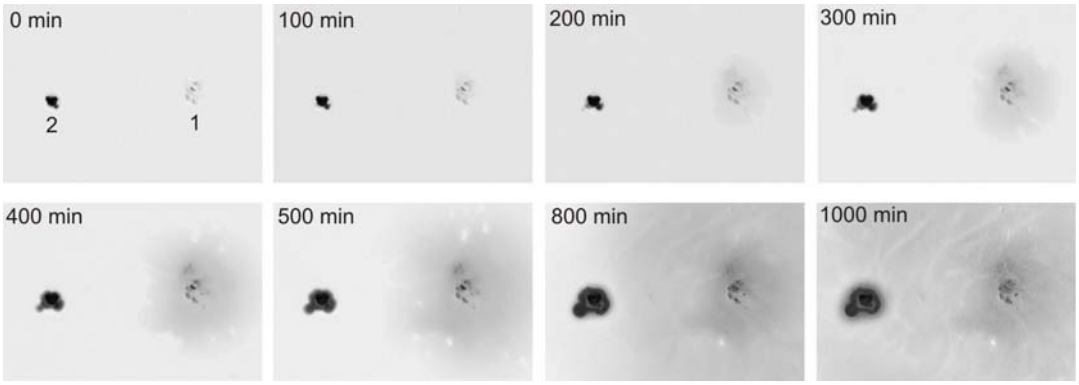

FIG. 7: The snapshots of colonial growth on the agar plate with with 6 g/l of agar concentration and 40 g/l of peptone concentration. B1 and B2 obtained from their isolated colonies. The incubation temperature was 37°C. The number 1 and 2 are corresponding with the bacteria of B2 and B1, respectively. The first picture was taken after incubating for 3 hours.

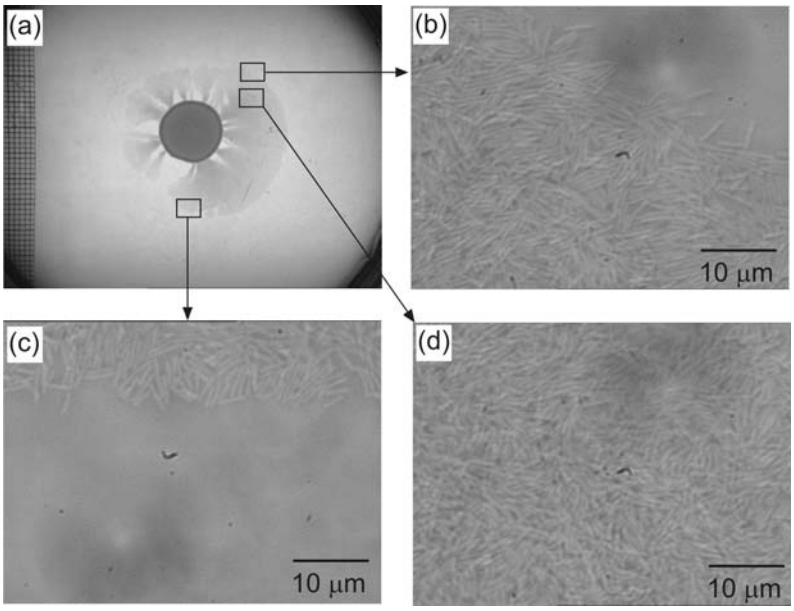

FIG. 8: Microscopic observations of spreading colony of *Bacillus subtilis* TISTR 008 under the condition of 6 g/l of agar concentration and 40 g/l of peptone concentration.

D. Microscopic observation

The microscopic observation of spreading colony by using an optical microscope is shown in Fig. 3. Macroscopically, the cell density represented by a gray level of spreading parts is lower than that of inside of colony clearly as shown in Fig. 3(a). Microscopic observations

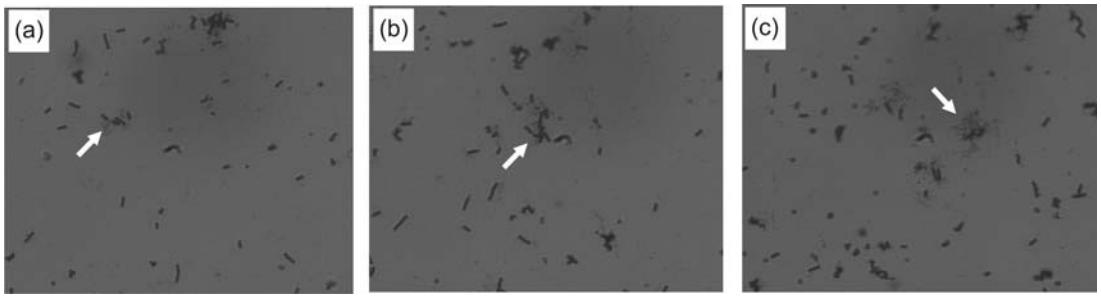

FIG. 9: Bacterial flagella at each state. (a), (b), and (c) represent prespreading state (flagella cells) at 24 hours, colonial bursting state at 36 hours, and spreading one (hyperflagella cells) at 60 hours, respectively.

of spreading parts reveal that the alignment of bacterial cells is random (Figs. 3(b), (c) and (d)). We found that the bacterial colony in the spreading parts consist of a monolayer of cells. Comparing with the cells inside the colony, the swarming cells are very active and move fast.

E. Hyperflagellated cells

The Leifson staining method [20] was used to investigate the swarming colony, since there is a report that the normal cells (vegetative cells) transition into hyper-flagellated swarming ones as the swarming behavior occurred [9, 15]. Thus, the bacterial cells in three states (prespreading, bursting, and spreading) were strained by using Leifson stain [20]. We found that the amount of flagella of bacterial cells increases and the bacterial cells change from flagelled cells to hyperflagelled ones as shown in Fig.9. The bacterial cells in the spreading state become peritrichous resulting in high motility of cells (see also in Fig.9(c)). We can conclude that the cause of swarming and switching in front velocity results from the change in cell phenotype, i.e., flagellated cells change to hyperflagellated ones.

IV. CONCLUSIONS

The spreading or swarming colony is robustly obtained in the condition of soft medium with rich nutrient. Under the condition of 6 g/l of agar concentration and 40 g/l of peptone concentration, an initiated time for swarming in *Bacillus subtilis* TISTR 008 is about 48-60

hours. The normal cells (vegetative cells) transition into hyper-flagellated swarming ones resulting in occurring of the swarming behavior. The outlook of this research can be the study of swarming or spreading colony numerically. In order to describe such a behaviour, we suggest that there may be a critical population density of bacterial cells for the onset of spreading colony and a function that describes the switch of a cell motility.

Acknowledgments

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