Dynamical Properties of Transient Spatio-Temporal Patterns in Bacterial Colony of *Proteus mirabilis*

Kazuhiko WATANABE, Jun-ichi WAKITA, Hiroto ITOH, Hiroshida SHIMADA, Sayuri KUROSU, Takemasa IKEDA, Yoshihiro YAMAZAKI, Tohey MATSUYAMA and Mitsugu MATSUSHITA

Department of Physics, Chuo University, Kasuga, Bunkyo-ku, Tokyo 112-8551

Department of Bacteriology, Niigata University School of Medicine, Niigata 951-8510

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Spatio-temporal patterns emerged inside a colony of bacterial species *Proteus mirabilis* on the surface of nutrient-rich semisolid agar medium have been investigated. We observed various patterns composed of the following basic types: propagating stripe, propagating stripe with fixed dislocation, expanding and shrinking target, and rotating spiral. The remarkable point is that the pattern changes immediately when we alter the position for observation, but it returns to the original if we restore the observing position within a few minutes. We further investigated mesoscopic and microscopic properties of the spatio-temporal patterns. It turned out that whenever the spatio-temporal patterns are observed in a colony, the areas are composed of two superimposed monolayers of elongated bacterial cells. In each area they are aligned almost parallel with each other like a two-dimensional nematic liquid crystal, and move collectively and independently of another layer. It has been found that the observed spatio-temporal patterns are explained as the moiré effect.

KEYWORDS: *Proteus mirabilis*, spatiotemporal patterns, stripe pattern, target pattern, spiral pattern, collective motion, moiré image

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1. Introduction

Recently much attention has been paid to pattern formation in biological systems along with the development of nonlinear dynamics and nonequilibrium statistical physics.\(^1,2\) Especially bacteria are one of main objects of the study due to the following reasons: (i) microbiological properties of bacteria are relatively clear, (ii) collective behavior of bacteria can be observed in the wide range of optical resolution from submicron to centimeter scales, and (iii) a variety of interesting patterns can be observed in the growth of a bacterial colony by varying environmental conditions. The reason (ii) is expected to be a clue to constructing a theoretical framework of the collective behavior of motile units and of pattern formation of the units whose scale is not distinguishable from the characteristic scale of the whole pattern.

Regarding the pattern formation of a bacterial colony on an agar substrate, concentrations of nutrient \(C_n\) and agar \(C_a\) are important control parameters for its morphology. In fact, for example, a bacterial species *Proteus* (*P.*) *mirabilis*, on which we focus our attention in this paper, exhibits various colony patterns when varying \(C_n\) and \(C_a\), as seen in the morphological diagram in Fig. 1. *P. mirabilis* is a flagellated rod-like bacteria and takes two states.\(^3-7\) One is a normally flagellated vegetative cell (1.5–2.0 \(\mu\)m in length) having 6–10 peritrichous flagella. The other is a hyperflagellated long swarmer cell (10–80 \(\mu\)m in length) with \(10^3–10^4\) peritrichous flagella. These two states are transformed into each other alternately through cell differentiation and dedifferentiation processes only when *P. mirabilis* is incubated on the surface of substrate medium such as agar gels.

From Fig. 1 it is found that colony patterns can be classified mainly into three types;\(^7\) cyclic spreading growth (region P), diffusion-limited growth (region Q), and three dimensional growth inside the agar medium (region R). The patterns observed in the regions Q and R are considered to be typical ones in the limiting cases where \(C_n\) is low and \(C_a\) is high, and where \(C_a\) is low, respectively. On the other hand, the region P is an intermediate one, where the motility and the transformation of the cell body are apparently considered to strongly influence the pattern formation. Actually the morphologies in the region P is classified into three subgroups: concentric-ring pattern (\(P_r\), homogeneous pattern (\(P_h\)), and transient spatio-temporal pattern (\(P_s\)).\(^8\) It is well-known that the concentric-rings observed in the region...
P, are well correlated circumferentially in this species. And in the region P, one observes target and spiral patterns as well which are commonly seen in chemical reaction system,9,10) liquid crystal system11,12) and so on. However, the dynamical aspects of the spatio-temporal patterns have not fully been understood.

In this paper we report the results of our detailed macroscopic, mesoscopic and microscopic observations of the spatio-temporal patterns in the region P, in Fig. 1. In the next section we briefly describe our experimental setup, and in §3 our experimental results are reported. We explain the mechanism of this pattern formation in §4, and the discussion follows in §5.

2. Experimental Procedures

The strain of P. mirabilis we used in this study is NPC3007. The agar substrate on which bacterial colonies grew was produced as follows. First, we prepared a solution containing 1% weight fraction of sodium chloride (NaCl). In the solution we further added specified amounts of Bacto Peptone (Difco, Detroit) and Agar (Eiken, Japan). Bacto Peptone is the nutrient of bacteria, and the amount of Agar controls the medium solidity, which influences the motility of bacterial cells. Their concentrations Cn and Ca are determined at this stage according to the amount of these two ingredients. We then adjusted pH of this solution to 7.1 by adding 1N sodium hydroxide (NaOH). The solution was autoclaved at 121°C for 15 min, and 20 ml of it was poured into a petri dish with 88 mm in diameter in order to make a thin gel medium with the thickness of about 4 mm. After cooling it in a refrigerator at 4°C for 10 min the agar plate was dried at 55°C for 60 min. In order to perform the colony formation in the P, region we fixed the values of Cn and Ca to 15 g/l and 6 g/l, respectively. 3 μl of the bacterial suspension whose optical density at 600 nm in wavelength was adjusted to 0.5 (corresponding to the population of about 3 × 10^6 viable cells) was inoculated at the center of the agar plate surface, and then incubated at 37°C.

Bacterial colonies were photographed by a digital camera C1400L (Olympus, Tokyo). The growth and spatio-temporal behavior of colonies were recorded with a time-lapse video SVT-S5100 (Sony, Tokyo) and CCD cameras CS572S (Sankei, Tokyo) and MC-780P (Texas Instruments, USA). Mesoscopic and microscopic behavior of bacteria was observed with the aid of a stereomicroscope (Leica, Tokyo) and a microscope DIAPHOT-TND (Nikon, Tokyo).

3. Experimental Results

3.1 Observed patterns

First we observed the time evolution of a bacterial colony in the region P, keeping the position for observation unchanged. Let us roughly show features of spatio-temporal patterns in the region P, About 5 h after inoculation of the bacterial suspension on an agar plate a colony starts to grow with a monolayer of vegetative cells, and spreads over the whole surface of the agar plate. About 8–9 h after the inoculation a few regions with bright and dark parts emerge and then spread on the whole plate. Figure 2 shows a typical pattern observed in the P, region. Bright and dark regions vary both spatially and temporally and this spatio-temporal behavior lasts about 3 h. The pattern finally vanishes and the colony comes to a quasi-static and variously patterned state. Therefore, this spatio-temporal pattern is transient and is quite different from those observed in other regions in that internal patterns of colonies in other regions are all static and do not change anymore.

As for the spatio-temporal patterns, the following four basic types can be observed: stripe, stripe with dislocation, target, and spiral. In Fig. 3 a typical stripe pattern is shown.
The width of stripes is 2–5 mm. Figure 4 shows a set of snapshots of stripe patterns with a dislocation taken at intervals of 30 s. Stripes are seen to appear near the boundary of the petri dish and move from right to left, while switching of stripes occurs at the fixed dislocation point. Considering that the pattern at $t = 120$ s is similar to that at $t = 0$ s with respect to the position of stripes and that the characteristic width of stripes is about 3.4 mm, the velocity of the motion of stripes can be estimated as $57 \mu \text{m/s}$ in this case.

Time evolution of a target pattern is shown in Fig. 5. Figure 5(a) is a set of five sequential snapshots at an interval of 20 s. Figure 5(b) indicates the one dimensional spatio-temporal pattern taken out along the white line in Fig. 5(c). The target pattern is seen to emerge from a fixed source and propagate outward with a period of 80 s. In the present system target patterns move not only outward but also inward, as shown in Fig. 6.

In Fig. 7 snapshots of a spiral pattern rotating outward are shown. As in the case of target patterns, there also exist spiral patterns rotating inward. Furthermore, interestingly, it was found that all spiral patterns are counterclockwise in our observation (see, e.g., Fig. 8).

From our observations described above, the following questions arise: (i) What do bright and dark regions reflect? (ii) Why does inward motion of target and spiral occurs in contrast with other physical systems such as BZ reaction and an electrohydrodynamic convection in liquid crystal system? (iii) Why do clockwise spirals not exist?

In the following subsections we report the detailed investigations in order to characterize the present spatio-temporal patterns and try to solve the above questions.
3.2 Spatio-temporal patterns

We investigated dynamical aspects of the patterns with a CCD camera macroscopically so as to grasp the properties of the bright and dark regions, as seen, e.g., in Fig. 2. As an elementary feature of the pattern, we noticed by a preliminary observation that this pattern changes immediately by altering our position for observation. Then we carried out the experiment as depicted schematically in Fig. 9. In this observation we rotated the petri dish so quickly that the pattern change due to bacterial movement hardly occurred. (In fact, one revolution was made within 5 s.) The result is shown in Fig. 10. Each figure is assigned rotation angle on the basis of the position of the first figure. It is confirmed from these figures that the patterns depend strongly on the position for observation. Especially when we make $\frac{\pi}{2}$ rotation, the patterns with bright and dark regions reversed are obtained [see, e.g., the patterns (a) and (c), or (b) and (d) in Fig. 10]. We can also notice that counterclockwise rotation causes such variation that a target pattern spreads outward. The crucial point in this observation is that the pattern (h) is almost the same as the pattern (a) in Fig. 10. Therefore, this spatio-temporal pattern has no direct relation with the motion or the local density of bacteria. We can rather conclude that these patterns are produced by some optical effect which reflects the microscopic states of the bacterial colony.

3.3 Microscopic and mesoscopic structures

In order to investigate the emergence of the spatio-temporal pattern, we further carried out the observation with higher resolution when the spatio-temporal patterns grow toward a homogeneous region where they do not appear yet. Figure 11(a) is a snapshot of the spatio-temporal region during its growth. Figure 11(b) is a magnification of the region where the spatio-temporal patterns do not appear yet, while Fig. 11(c) shows a magnified region where the spatio-temporal patterns have already emerged. It is found that there is clear distinction between these two Figs. 11(b) and 11(c) regarding the flow of bacteria. The flow of bacteria is displayed as the line of long strings, because each bacterial cell has a long string-like body (a swarmer cell) and tends to move along its own body. It is also found from Fig. 11(c) that the strings of bacteria are superimposed and cross each other. This fact means that there are at least two superimposed layers of bacteria and that the elongated bacteria in each layer are aligned almost parallel with each other like a two-dimensional liquid crystal and move independently of
the other layer. Therefore, there is a long-range correlation for the motion of bacteria within each layer and the collective motion of bacteria is like a two-dimensional fluid. On the other hand, as seen in Fig. 11(b), before the appearance of spatio-temporal patterns, the correlation of the direction of the bacterial flow is much shorter.

Figure 12 is a snapshot of the final structure seen after the spatio-temporal patterns have already finished. It is noted that the long strings which represent the collective motion of bacteria as shown in Fig. 11(c) are not observed. This vanishment is also consistent with the fact that the long-range collective motion of bacteria is one of the factors for the emergence of the spatio-temporal patterns and they are transient.

Let us now check the relation between the mesoscopic structures and the spatio-temporal patterns. Figure 13(a) shows a snapshot of the spatio-temporal pattern, and Fig. 13(b) the mesoscopic structures which are magnifications of four white square regions in (a). It was confirmed that all square regions have similar mesoscopic wrinkly structures to each other, although corresponding macroscopic regions have different brightness. Considering the fact that these wrinkly structures represent the local density fluctuation of bacterial cell population, the macroscopic spatio-temporal pattern turns out not to be the direct reflection of local density fluctuations of bacterial cells.

4. Summary and Mechanism

The features concerning macroscopic spatio-temporal patterns are summarized as follows:

(i) In the spatio-temporal patterns the following four basic types can be observed: traveling stripe, traveling stripe with fixed dislocation, expanding and shrinking target, and rotating spiral.

(ii) The pattern depends strongly on the position for observation. Namely, the pattern moves immediately if we change the position for observation.

(iii) The bright and dark region of the pattern are not caused by the local density fluctuation of bacterial cells.

(iv) From the microscopic observation of the pattern there
exist at least two superimposed layers of bacteria in the region where the spatio-temporal pattern emerges. The motion of bacteria in each layer is independent of that in other layers.

(v) The cell body of bacteria when they exhibit spatio-temporal patterns is a swimmer and looks like a long string. They move parallel along the long strings. Therefore, the group of bacteria in motion can be observed as periodic structure of the long parallel strings.

(vi) Time evolution of the spatio-temporal patterns is characterized by the velocity of interface between bright and dark regions. In our observation, the speed is estimated as about 100 \( \mu m/s \).

(vii) The speed of individual cells of bacteria when they exhibit spatio-temporal patterns varies depending on the state of collective bacterial flow. In the stagnation region observed mesoscopically as a wrinkly structure, the speed is estimated as about 10–15 \( \mu m/s \). On the other hand, the flow rate in the uniform flow region is about 50–80 \( \mu m/s \).

Let us now consider the mechanism for the formation of spatio-temporal patterns. The four basic types seen in the spatio-temporal pattern can be reproduced optically as moiré images.\(^{13,14}\) In fact, the following study about the moiré image has been done.\(^{14}\) If we superimpose two sheets where many straight lines aligning at regular intervals are drawn and move one of the two sheets, then a traveling stripe pattern whose characteristic scale is much larger than the interval of lines in the sheet can be observed as a moiré image. A pattern of stripes with dislocation is obtained if the dislocation line is included in either sheet. A target pattern is obtained when a straight line is slightly deformed. And a spiral pattern emerges if the tip of the dislocation line is deformed. Moreover, the motion of the target and spiral can be both inward and outward by changing the direction of the motion of the sheet. It is also noted that even if straight lines in the sheet are not deformed, the undulation of the sheet also brings about target and spiral patterns.

From this previous study\(^{14}\) it was found that (a) two layers with microscopic periodic structure, (b) deformation in the periodic structure, and (c) dislocation are essential factors for reproducing the four basic types of the spatio-temporal pattern in the case of the moiré effect. As for the bacterial colony system in the region \( P_s \), there are at least two layers of bacteria, and the motion of bacteria within one layer is independent of that in the other layer. The group of bacteria is represented by the long strings aligning parallel at almost regular intervals like a two-dimensional nematic liquid crystal and moves collectively like a two-dimensional fluid. And the bacterial layer is considered to be undulated due to the rough surface of the agar substrate. Furthermore, the existence of obstacles or irregularities on the surface of the agar substrate can prevent the motion of bacteria and may bring about the fixed dislocation point. Therefore, the bacterial colony system in the region \( P_s \) satisfies all essential requirements for the emergence of the four basic types as the moiré effect. Moreover, if there is a small gap between two superimposed layers, the moiré image moves rather freely according to the alteration of the position for observation. This phenomenon is also what we observed in the present system (Fig. 10). According to our observations, the speed of interface between bright and dark regions (\(< 100 \mu m/s\)) is larger than that of individual bacterial cells (10–80 \( \mu m/s \)). This is also consistent with the moiré effect: Since we see phase variation in the moiré effect, the speed of interface between bright and dark regions in a moiré pattern can easily exceed that of real material constituting the superimposed layers. It can, therefore, be concluded that the macroscopic spatio-temporal patterns observed in the region \( P_s \) is due to the moiré effect.

5. Discussion

In this paper experimental results of transient spatio-temporal patterns observed inside a colony of \( P. mirabilis \) is reported. The spatio-temporal pattern consists of bright and dark regions, and is considered as a moiré image. This implies that the brightness of the region does not correspond to the local cell density of bacteria. It is also confirmed that the pattern is not affected by the polarization of light. The pattern is composed of the four basic types: stripe, stripe with dislocation, target, and spiral. In the bacterial colony, target and spiral patterns propagate in both directions, i.e., inward and outward. This dynamical behavior is quite different from that of reaction-diffusion systems such as Belousov–Zhabotinsky reaction.\(^9\) In reaction-diffusion systems, the central point of target and spiral plays a role of a pacemaker of a periodic motion, and the other region around the center varies only by reaction and diffusion from the central point. In this case the central point is always a source or a pacemaker, which makes target and spiral propagate only outward. On the other hand, the dynamical behavior of the spatio-temporal patterns in the region \( P_s \) can be easily reproduced by a simple model based on the moiré effect. However, considering the fact that there is no spirals with clockwise rotation in our observation, the mechanism for the formation of the present spatio-temporal patterns cannot be explained by the simple moiré effect alone. As of now, the mechanism for the selection of only counterclockwise spirals is not clearly understood and an additional condition inherent to bacteria seems to be needed.

We consider the relation between three typical patterns in the \( P \) region (namely, \( P_s \), \( P_b \), \( P_r \)) and the motility of bacteria at a fixed \( C_a \). When \( C_a \) is high, the motility of bacteria becomes low and the regions where the density of bacterial cell are high emerges locally. In these local high density regions, if the density exceeds some threshold,\(^{4-6} \) bacteria are considered to transform their cell body to swimmers (cell differentiation) and start migrating. Then bacteria spread easier and the local density decreases. If the bacterial density decreases below the lower limit for motion of bacteria by faster spreading, bacteria are transformed back into the original vegetative cells having low motility by cell dedifferentiation. As a consequence, the bacteria move periodically, and the concentric ring pattern is formed. In the low \( C_a \) case, on the other hand, due to the high motility of bacteria they move collectively over a wide range and do not experience the above threshold dynamics for their motion. Instead, the long-range collective motion brings about a moiré image. Consequently, the threshold dynamics and the collective motion of bacteria are considered to be important factors for the determination of the morphology of the
bacterial colony in the region P.

In order to identify the microscopic origin of the spatio-temporal patterns, we are now carrying out further detailed observations microscopically. We hope that the relevance of the microscopic structures to the spatio-temporal pattern will be clarified in the future.

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