Spatiotemporal Patterns Produced by Bacteria

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Spatiotemporal patterns formed by a bacterial colony of *Proteus mirabilis* on an agar plate were observed. About half or one hour after the colony spread over the entire surface of the agar medium in a petridish, various patterns including target and spiral patterns appeared. They are very similar to those seen in other dissipative systems, such as chemical oscillations and electrohydrodynamic convective systems. Microscopic observations revealed that the collective motion of bacterial cells is responsible for the formation of these spatiotemporal patterns.

[Pattern formation, bacterial colony, spatiotemporal pattern, target pattern]

In this letter we report the formation of spatiotemporal patterns by bacterial colonies on an agar plate. So far, our attention has mainly been focused on the final quasistatic morphology of bacterial colonies because, with changing environmental conditions, bacterial colonies were found to form various patterns such as diffusion-limited-aggregation (DLA)-like, dense-branching-morphology (DBM)-like, Eden-like, concentric ring and spiral patterns. On the other hand, morphological analysis using a time-lapse video recording system has revealed that the dynamic (but very slow) evolution of bacterial colonies can produce spatiotemporal patterns such as target and spiral patterns, when we cultivate bacteria under particular environmental conditions.

First we explain our experimental procedures. We used a flagellated bacterium, *Proteus (P.) mirabilis* (NPC 3007). This species is well known to produce concentric-ring colonies; however the mechanism of ring formation remains unclear.

We prepared a solution containing 10 g of sodium chloride (NaCl) per 1 of distilled water. Bacto Peptone (Difco, Detroit) as a nutrient and Agar (Eiken, Japan) were added to the solution to form semisolid agar medium. To dissolve these ingredients completely, we heated the solution at 100°C for about 30 min. We then adjusted pH of the solution to 7.1 by adding sodium hydroxide (NaOH). The solution was autoclaved at 121°C for 15 min, then 20 ml of it was poured into a petridish with a diameter of 88 mm. After keeping at room temperature for 20 min and cooling in a refrigerator at 4°C for 10 min, we dried the medium at 55°C for 60 min. We point-inoculated the bacterium on the surface of the agar medium, and incubated it at 37°C. All these procedures are standard in the culture of bacteria.

A spatiotemporal pattern was observed when we inoculated the bacterium on the surface of the nutrient-rich semisolid agar medium. At first the bacterial colony spread homogeneously. The overall morphology of the growing colony resembled a simple disk, very similar to that observed in the D region for the bacterium *Bacillus subtilis*. In fact, the incubation conditions are very similar. About half or one hour after the colony spread over the entire surface of the agar medium, a spatiotemporal pattern was observed. At first a few small spatiotemporal patterns appeared in some regions. Then they grew, merged together and spread all over the agar medium surface, which became covered with the spatiotemporally varying pattern. This state lasts for about 4 hours. In Fig. 1 we show examples of photographs of the spatiotem-
poral pattern. Here the concentrations of nutrient and agar were \( C_n = 15 \text{ g/l} \) and \( C_a = 6 \text{ g/l} \), respectively. After the spatiotemporal pattern was blown out, a quasistatic pattern appeared. Thus, these spatiotemporal patterns are transient ones in a progression towards the final quasistatic states.

We occasionally observed the formation of target patterns, as shown in Fig. 2. Here Figs. 2(a)–2(c) are sequential photographs taken at an interval of 32 s. We see that a target pattern emerged inside the colony. This target pattern resembles other target patterns observed in dissipative systems such as chemical oscillations\(^{11,12}\) and electrohydrodynamic convections of nematic liquid crystals.\(^{13}\) Although these patterns look similar, the elements that form the systems are very different. The grids of rolls are the basic elements for the electroconvective system. The following experiment implies that the basic element of the present bacterial system is the collective flow of bacterial cells.

To investigate the mechanism that produces the spatiotemporal pattern, we performed microscopic observations and studied the movement of individual bacterial cells. First, we investigate a region before appearance of the spatio-temporal pattern. Figure 3 shows photographs of a bacterial colony taken through a microscope with the magnification of \( \times 4 \) for Fig. 3(a) and \( \times 40 \) for Fig. 3(b). From Fig. 3 we see that each bacterium moves rather randomly in a monolayer of individual bacterial cells.

Next, in Fig. 4 we show micrographs of the spatiotemporal pattern. In Fig. 4(a) the spatiotemporal pattern is reminiscent of clouds floating in the sky. The light and dark
areas seen in the figure are not, as might be imagined at first glance, due to the spatiotemporal variation of cell population density, because the increase of magnification to $\times 40$ revealed that the density is fairly homogeneous throughout the colony. This implies that the present spatiotemporal patterns are due to some optical interference effect. In fact, in Fig. 4(b) it appears that the bacterial colony consists of two superimposed monolayers of bacterial cells. In each layer bacterial cells move in a group in almost the same direction. This resembles the flow of liquid crystal molecules in one direction. However, the directions of the bacterial cell flow in the two layers can be different from each other. Thus, in some regions bacterial cells flow in slightly different directions between layers, while in other regions they flow in completely opposite directions.

Note that there is no superimposed layerlike structure or collective bacterial cell flow in a region in which the spatiotemporal pattern has not yet appeared (Fig. 3). From comparison of Figs. 3 and 4, the superimposed layerlike, collective flow of bacterial cells is considered to be responsible for the formation of macroscopic spatiotemporal patterns.

Although it is still unknown whether the mechanism that produces target patterns in the present bacterial system is the same as that in the liquid crystal convective system, it is clear that the collective motion of bacterial cells is responsible for the formation of the macroscopic spatiotemporal patterns. The present spatiotemporal patterns are clearly reminiscent of the moiré images seen in everyday life, for example when a lace curtain is folded. In the latter case also one can easily
observe spatiotemporal rippled and target patterns similar to those shown in Figs. 1 and 2, respectively.  

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References