

## Spatio-temporal Patterns in Colonies of Rod-shaped Bacteria

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In incubation experiments of bacterial colonies of *Proteus Mirabilis*, macroscopic spatio-temporal patterns, such as turbulent and unidirectional spiral patterns, appear in colonies. Considering only kinetic properties of rod-shaped bacteria, we propose a phenomenological model for the directional and positional distributions. As the average density increases, homogeneous states bifurcate sub-critically into nonuniform states exhibiting localized collective motion, and spiral patterns appear for sufficiently large density. These patterns result from interactions between the local bacteria densities and the order parameter representing collective motion. Our model can be described by reduced equations using a perturbative method for large density. The unidirectionality of spiral rotation is also discussed.

In systems of self-propelled particles, it is known that collective motion appears spontaneously with an increase in population. Collective motion has been reported to be observed as macroscopic spatio-temporal patterns in incubation experiments of bacterial colonies employing *Proteus Mirabilis*.<sup>1)-3)</sup> We propose a mathematical model to investigate the mechanism of collective motion that causes such patterns.

*Proteus* is a rod-shaped bacterium with many flagella. In dense states, swarms of *proteus* move like log rafts on the surface of a culture medium. Spatio-temporal patterns appear in a colony which has spread thinly on the surface of agar media.<sup>1)-3)</sup> They are typically turbulent, target and spiral patterns, which resemble those observed in reaction-diffusion systems. However, what we observe as such patterns is the directional order of bacteria visualized by exploiting certain optical properties. The bacteria density is approximately uniform in a colony. In addition, only counterclockwise spirals appear in colonies of *Proteus*, and this is believed to be caused by some biological factor, such as the rotation direction of the flagella.

We infer that a lack of nutrient and the growth of population plays no role in these phenomena because such patterns appear in nutrient-rich cultures, and because the density increases slowly. In this paper, we treat the spatial average of bacteria density,  $\bar{\rho}$ , as a control parameter and consider only kinetic properties. We focus on the numerical and analytical results for a 2-dimensional model here.

We express the density of bacteria moving in the direction of the angle  $\theta$  at the position  $\mathbf{x} \equiv (x, y)$  and time  $t$  as  $n(\theta, \mathbf{x}, t)$ , and define the local bacteria density  $\rho(\mathbf{x}, t) \equiv \langle n \rangle$  and the complex order parameter  $W(\mathbf{x}, t) \equiv \langle n e^{i\theta} \rangle \equiv |W| e^{i\Theta}$  using the directional average  $\langle \dots \rangle \equiv (1/2\pi) \int_0^{2\pi} d\theta \dots$ . Collective motion at a given position is characterized by the magnitude  $|W|$  and the direction  $\Theta$ . Quantitative features of the motion and interactions of bacteria in dense states have not been investigated thoroughly. We assume that, as in the case of low density, bacteria move linearly with an approximately constant speed  $v$  and change their direction randomly with a

constant probability  $\gamma_0$ , and introduce a local equilibrium distribution  $g(\theta, \mathbf{x}, t)$ . We describe the effect of short-range interactions approximately using a single relaxation time, as in the collision interval theory for Boltzmann equation. The continuity equation for  $n(\theta, \mathbf{x}, t)$  is given by  $\frac{\partial n}{\partial t} + \nabla \cdot \mathbf{j} = \gamma_0(g\rho - n)$ .

$\mathbf{j}(\theta, \mathbf{x}, t)$  represents the flux of bacteria moving in the direction of the angle  $\theta$ . This is expected to depend on the density gradient because ‘‘traffic jams’’ impede flows in dense states. It is assumed to be  $\mathbf{j} = \mathbf{v}(1 - \gamma^{-1}\mathbf{v} \cdot \nabla)n$  using the vector  $\mathbf{v} \equiv v(\cos\theta, \sin\theta)$  and a positive constant  $\gamma$ .

The local equilibrium distribution  $g(\theta, \mathbf{x}, t)$  is expected to become anisotropic with the occurrence of collective motion. We assume that  $g$  is a function of  $\theta$  and  $W(\mathbf{x}, t)$ . Stipulating the conservation of total number and the rotational symmetry, we can express  $g$  as the real part of a complex function  $G(aWe^{-i\theta})$  which satisfies  $G(0) = 1$ . Here we have introduced the complex constant  $a$  to set  $G'(0) = 1$ . Through an appropriate scaling of  $\mathbf{x}$ ,  $t$  and  $n$ , the constants  $v$  and  $\gamma$  are set to 1, and  $a$  is expressed as  $a \equiv e^{i\phi}$ . The continuity equation is written as

$$\frac{\partial n}{\partial t} = \frac{1}{\tau} \{ \rho \operatorname{Re} G(z) - n \} - \nabla \cdot \mathbf{v} n + (\nabla \cdot \mathbf{v})^2 n, \quad (1)$$

where  $z \equiv |W|e^{i(\Theta+\phi-\theta)}$  and  $\tau \equiv \gamma/\gamma_0$ . We first adopt the simplest function  $G(z) \equiv 1 + (1-b|z|^2)z$  containing only the lowest order terms in  $e^{\pm i\theta}$  in the Fourier expansion. The nonlinear term  $|z|^2z$  is necessary to prevent the divergence of solutions. We assume that  $b$  is a positive real constant. In this case,  $g(z)$  has the maximum at the angle  $\phi$  with respect to the direction of collective motion, and a major proportion of bacteria is headed in this direction through the local interactions.

In addition to the trivial uniform solution  $n = \bar{\rho}$ , there is the uniform oscillating solution with the frequency  $\omega \equiv (\tan\phi)/\tau$ ,

$$n = \rho + 2|W| \cos(\Theta - \theta) \equiv n_0, \quad |W| = \sqrt{\frac{\rho - \rho_c}{b\rho}}, \quad \Theta = \omega t + \text{const}. \quad (2)$$

for  $\rho > (\cos\phi)^{-1} \equiv \rho_c$ . This solution is unstable when  $(\rho - \rho_c)/\rho_c$  is small. The trivial uniform stationary state bifurcates sub-critically into nonuniform states exhibiting localized collective motion as the average density  $\bar{\rho}$  increases above  $\rho_c$ . As  $\bar{\rho}$  is sufficiently large,  $|W|$  approaches the constant  $1/\sqrt{b}$ , and the ratio of the density variation to the average,  $(\rho - \bar{\rho})/\bar{\rho}$ , decreases. Although  $\rho$  and the constant of  $\Theta$  are arbitrary constants in the above solution, they interact and change as slow modes.

We report the results for the case that  $0 < |\phi| \ll 1$  below. The collective motion rotates gradually in the direction determined by the sign of  $\phi$ . Such rotation may be caused by some biological factor. In fact, some experiments show that colonies grown radially from an incubated point form shapes with chirality whose direction is determined uniquely for each species.<sup>4)</sup>

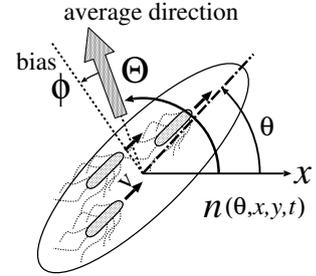


Fig. 1. 2-d model.

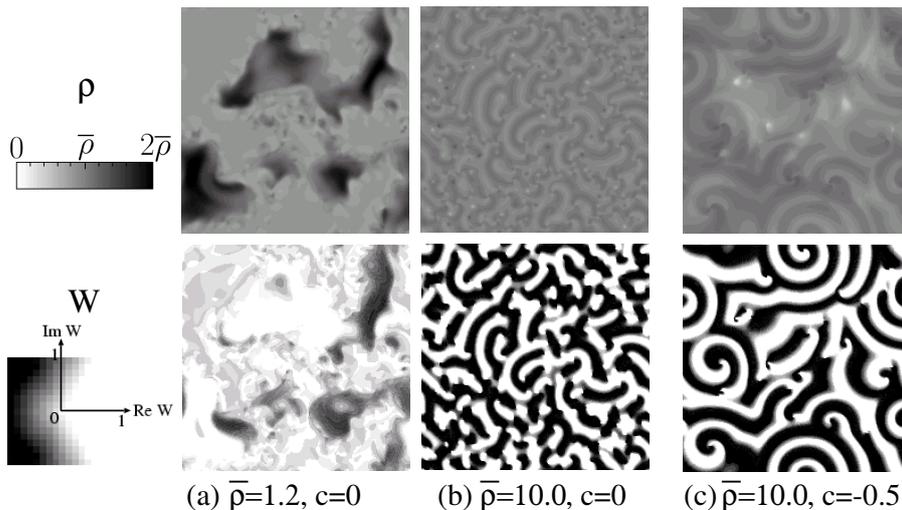


Fig. 2. Snapshots at  $t = 2500$  obtained from simulations with  $\tau = 0.1$ ,  $b = 1$  and  $\phi = 0.02$ . The system size is  $L = 400$ .  $\rho$  and  $W$  are displayed on the top and bottom, respectively.

Figures 2a and 2b display the results obtained from numerical simulations of Eq. (1) using periodic boundary conditions. The initial states are trivial uniform solutions added small white noise. The system settles into the trivial uniform state for  $\bar{\rho} < \rho_c$ , and non-uniform states appear with collective motion as  $\bar{\rho}$  increases. When  $(\bar{\rho} - \rho_c)/\rho_c$  is small, vortex motions appear in several localized regions with large population, and these regions slowly change shape. As  $\bar{\rho}$  is sufficiently large, collective motion containing many defects appears throughout the entire system, and spirals grow around these defects.

To investigate the growth of spirals, we used the special initial state,  $n = \bar{\rho}(1 + 2 \text{Re} W_0 e^{-\theta i})$  and  $W_0 = -0.1\{\cos(2\pi y/L) + i \cos(2\pi x/L)\}$ , which contains four topological defects. When  $\bar{\rho}$  is sufficiently large, both clockwise and counter-clockwise spirals grow as shown in Fig. 3a. We note that the two types of spirals make different density distributions. The growth of these spirals can be understood as a result of interactions between  $\rho$  and  $\Theta$ . Assuming that  $\tau \ll 1$  and  $\phi \sim O(\tau)$ , we can apply a reductive perturbation method to our model regarding the spatial interactions as a perturbation. We expand a solution as  $n = n_0(\rho, \Theta) + \delta n$  using the uniform oscillating solution  $n_0$ . Although we report the derivation elsewhere,<sup>5)</sup> when  $\rho$  is sufficiently larger than  $\rho_c$ , the reduced equations are approximated as

$$\frac{\partial \tilde{\rho}}{\partial t} = \frac{1}{2} \Delta \tilde{\rho} - \partial_{\perp} \Theta, \quad \frac{\partial \Theta}{\partial t} = \omega + \frac{1}{2} \Delta \Theta - \frac{1}{2} \partial_{\perp} \tilde{\rho} + \frac{1}{4} (\partial_{\perp}^2 - \partial_{\parallel}^2) \Theta, \quad (3)$$

where we have defined  $\partial_{\parallel} \equiv \cos \Theta \frac{\partial}{\partial x} + \sin \Theta \frac{\partial}{\partial y}$ ,  $\partial_{\perp} \equiv -\sin \Theta \frac{\partial}{\partial x} + \cos \Theta \frac{\partial}{\partial y}$  and  $\tilde{\rho} \equiv \sqrt{b}(\rho - \bar{\rho})$ . They describe the propagation of spirals, although the behavior of the spiral cores may not be faithfully reflected.  $\rho$  is affected by  $\Theta$  through the divergence term,  $\partial_{\perp} \Theta = \nabla \cdot (\cos \Theta, \sin \Theta)$ . Using the polar coordinate  $(r, \psi)$  with its origin at the center of a spiral, the phase of the spiral subtracted small periodic components is expected to be described as  $\Theta \simeq \Omega t - kr \pm \psi$ .  $\Omega$  and  $k$  are constants,

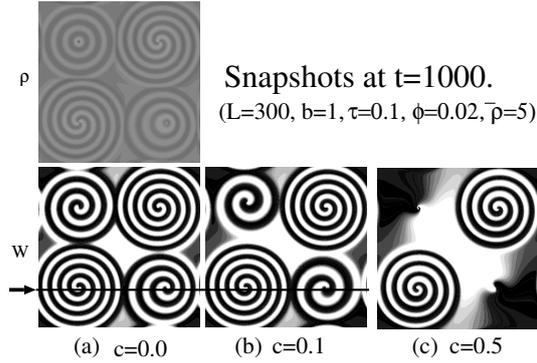
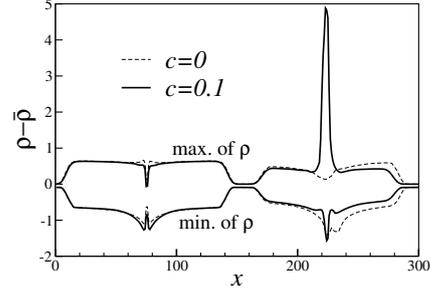


Fig. 3. Growth of spirals.

Fig. 4. Variation ranges of  $\rho$  for a given  $x$  and  $y = 75$  (indicated by the arrow in Fig. 3) obtained from the simulations from  $t = 500$  to  $1500$  with  $c = 0$  and  $0.1$ .

and its sign is determined by the rotation direction of spiral. For the two types of spirals,  $\partial_{\perp}\Theta \simeq \sin(\Theta - \psi)k \pm \cos(\Theta - \psi)/r$  has different periods with respect to  $\psi$ , which are  $0$  and  $\pi$  respectively.

In the model mentioned above, the two types of spirals grow in roughly symmetric shapes for sufficiently large  $\bar{\rho}$  although one of them dominates eventually due to mutual interactions. We find that, adopting the asymmetric equilibrium distribution  $G(z) = 1 + (1 - b|z|^2)(z - icz^2)$  with a real constant  $c$ , unidirectional spiral patterns similar to those observed in experiments appear, as shown in Fig. 2c. The uniform oscillating solution for this distribution is asymmetric with respect to  $\Theta$  although  $W$  is the same as in Eq. (2). Figs. 3b and 3c indicate that, as  $|c|$  increases, spirals with a concentric density profile are not able to grow. Its density profile changes largely near the center of the spirals as shown in Fig. 4. In this case the reduced equations lose the symmetry under the transformation  $(\Theta, \tilde{\rho}) \rightarrow (\Theta + \pi, -\tilde{\rho})$ . Particularly, the term  $c/\sqrt{b}\partial_{\perp}\Theta$  is added to the equation of  $\Theta$  in Eqs. (3). We infer that this term acts on this type of spirals as a disturbance with the same period of their rotation.

We have investigated the evolution of spatio-temporal patterns that result from the collective motion of bacteria. Our study was motivated by the experimental studies of *Proteus*.<sup>1)-3)</sup> In order to understand all morphologies observed in the experiments, it is necessary to consider the effects of the growth of population and nutrient concentration, which were ignored here. The author acknowledges M. Matsushita, A. Nakahara and C. Urabe for fruitful discussions.

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