Pattern Formation and Emergence of Collective Behavior in Bacterial Growth

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Abstract

Collective surface migration of bacterial cells on a growth medium can lead to interesting spatiotemporal patterns and dynamical behaviors. Studying the rich behavior of these swarming colonies can provide insight in how the multicellular organisms were originally formed. Bacterial swarming is an example of emergent behavior as a result of interplay between the collective interactions of a population of bacterial cells, colony growth, and hydrodynamic motions. Several models have been proposed to describe bacterial swarming some of which can qualitatively reproduce a variety of colony shapes observed in experiments. In this paper, some experimental work along with a theoretical model for the mechanism of pattern formation in bacterial swarming that captures some qualitative and quantitative aspects of this phenomenon is reviewed.

1 Introduction

Collective surface migration of bacterial cells on a growth medium can lead to interesting spatiotemporal patterns and dynamical behaviors. Bacterial swarming is a phenomenon emerging as a result of a complex network of interactions from a broad range of length and energy scales. Through a non-equilibrium process and a network of reversible interactions, bacterial cells self-assemble in an ordered but dynamical phase that can only happen with dissipation of energy. Understanding these dynamical interactions not only provides us with insight into the problem of bacterial growth, but also will have immediate applications in ecology, agricultural and biomedical sciences. In a broader perspective, however, a mathematical framework to study the dynamics of a system with a complex network of interation will have applications in disciplines from population dynamics to social and economical sciences[1].

Individual bacteria can swim by rotational motion of their flagella. The swarming motion of the colony is a result of interaction of bacterial cells with the surface of medium, with each other, and with the fluid motion in addition to individual swimming motion of each bacterium. Individual bacteria are not motile. There is a threshold cell density required for cells to move to swarming phase. At this density, bacterial cells go through a division of labor and act like parts of a multicellular organism. They differentiate in a swarming phenotype start moving and dedifferentiate. The differentiated cells are elongated cells with more flagella grown on their outer membrane. At this level, cells self-assemble to multicellular packs and form a complex structure as they grow. Near the edges, they form a monolayer of non-motile cells. Just behind this layer (closer to the center) the most motile cells move in a several layers. These cells collide with cells on the edge and they can substitute them or push the edge outward. Closer to the center, there are many layers of cells that are less mobile and form three dimensional structures [1].

The swarming process is very active and too complicated to be modeled in details. While swarming, individual cells change their phenotype, they regulated their flagella movement to move away or toward particular chemicals through chemotaxis, they engage in complicated signaling mechanism for quorum sensing to reaction to their local population density, they extract fluid from the medium to regulate the viscosity, and many other active processes that affect the swarming motion. However, some qualitative information can be extracted from simple models that can macroscopically describe the emergence of collective behavior and formation of patterns in these systems. To do so, we need to model the system with a simpler network consisting of only the interactions relevant to the emergence of the phenomenon we are interested and a coarse grained description of the system that averages out the irrelevant details of the process.

As a simple model, we can start with the sensory pathway of bacteria. It regulates the bacterial motility which defines a swimming behavior for the cell. The swimming of the cell causes a fluid flow pattern around the cell and as a result it feedbacks on the motion of the cell. Given this set of simple interactions, by taking the continuum limit of macroscopic variables such as velocity field of bacteria and their density, we can construct a model. Of course the missing loop in this model is the feedback from other cells and the hydrodynamical forces created as a collective effect acting on the motion of the cell. The non-linearity from the cell-cell interactions and fluid-cell interaction are most likely the source of a lot of interesting phenomena. Notice that in models like this, the chemotaxis is ignored, and it is replaced by the mechanical tendency of cells moving away from the populated area [2].

In order to study the behavior of the system under models like this, we need to understand the nature of each step of interaction in the model. In this paper, I will attempt to review some experimental work done to show the relevance of different interactions on models like this, as well as some numerical calculations of simple models that capture the emergence of interesting phenomena and formation of wide range of patterns.

2 Methods

The emergence of collective behaviors in swarming and formations of patterns in growing colonies is a result of an interplay of different levels of interactions in the system. To understand the emergence of these phenomena, we need to study the relevance each level of interaction to a particular phenomenon and then come up with a model with the relevant interactions. Since these interactions happen at different levels, different experimental techniques are used to study each interaction. In the following section I review some experiments done in this area followed by a model that can produce a phase diagram of pattern formation in growing bacterial colonies as well as some collective dynamical behavior in the growth process.

2.1 Experimental Work

One of the most elementary interactions in bacterial swarming is the swimming of individual cells by rotational movement of their flagella. The feedback of the flow resulted from this interaction on the swimming cell is a potentially important interaction. In the experimental work done by Drescher *et al.* , the velocity field is measured directly. In this study, the feedback from the hydrodynamics found to be an important factor in very short range interactions, but totally
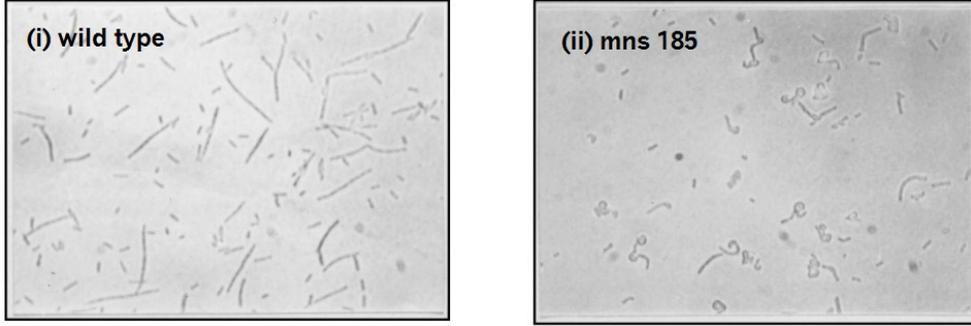
Figure 1: The mutant MNS185 on the left and the wild *Proteus mirabilis* on the right. The mutation changes the shape of bacteria so that they cannot align.[4].

dominated by the effect of intrinsic stochasticity of swimming process at length scales larger than a few microns. Therefore, even in the high density colonies that this interaction can be considered important, the asymptotic solutions to hydrodynamical models are irrelevant [3].

Cell-cell interaction is another factor to be considered. The alignment swarmers is one of the collective phenomena for which the cell-cell interaction becomes relevant. It is hypothesized that packing of bacteria with complementary shapes can be an origin of this alignment. In an experiment, Hay *et al.* used a transposon mutant, MNS185, to change the shape of *Proteus mirabilis*. As the result these cell were unable to align [4]. Figure 1 compares the shape of the mutants with the wild type. Also scanning electron microscopy (SEM) and Tunneling electron microscopy (TEM) images have shown the formation flagellar bundles between neighboring cells that contribute to formation of structures which is another example of cell-cell interaction. Reversal of flagellar motor is found to be responsible for the bundling/unbundling process [5].

Wetness of the surface and concentration of nutrients are important factors in determining the dynamics of swarming and formation of patterns which is an indication of importance of cell-surface interaction [1]. In harder plates, the dynamics is diffusion limited and fractal boundaries are observed. Flower like shapes, circular growth, and formation of branching patterns are observed in softer mediums. Interesting collective dynamical behavior such as jets and whirls are also common in softer plates [2].

Tracking algorithms are used in studying the velocity field of swarming cells. The largest correlation length and fastest moving cells are observed near the moving front the colony. Swimming cells undergo curvilinear translation and form vortices. They go through cycles of forming groups and dissipating and reforming groups [6]. Another interesting experiment is done showing that the swarming of the bacteria on soap film (which can simulate the two dimensional migration of bacteria across the surface of the growth medium) can lead to super

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diffusive movement of micron size beads suspending in the film [7].

2.2 Modeling and Numerical Simulation

In this section I briefly discuss the model presented by Lega *et al.* to model bacterial swarming[8]. In this model, the authors used a system of four couple differential equations each describing the evolution of one macroscopic variable. In the absence of chemotaxis, swarming bacteria can be modeled as a complex two phase flow which consists of bacterial particles and the fluid. The first equation is the a diffusion reaction equation describing the evolution of nutrient concentration S

$$\frac{\partial S}{\partial t} = R_S(S, N, W) + D^S \nabla^2 S, \tag{2.1}$$

where R_S is the rate of consumption of nutrients. Notice that the advection term is neglected in this model. Continuity equation can be modified to include reaction diffusion and advection terms to model the concentration of water (W)

$$\frac{\partial W}{\partial t} + \nabla \cdot (Wv) = R_W(S, N, W) + \nabla \cdot (D^W \nabla W) - \nabla \cdot (D^N \nabla N), \tag{2.2}$$

where v is the velocity field of the complex fluid, and N is the density of bacterial particles. The divergence term in the right hand side of the equation (2.2) comes from the fact that substituting v for v^W in continuity equation needs a correction that is proportional to the divergence of diffusive flux which is replaced by its Fick's law equivalent. The last term comes from the assumption that the net diffusive mass flux is zero, and therefore, the flux of water is negative the flux of bacterial particles. A similar equation can be written for the density of bacterial particles

$$\frac{\partial N}{\partial t} + \nabla \cdot (Nv) = R_N(S, N, W) + \nabla \cdot (D^N \nabla N). \tag{2.3}$$

Conservation of momentum can be written for each phase separately. Not only the total velocity field v can be calculated from v^W and v^N, but also writing separate equations gives us the opportunity to enter the force that the bacteria exert on the water phase and its reaction into the equations.

$$\frac{\partial}{\partial t} \left(\mathcal{Q} v^{\mathcal{Q}} \right) + \nabla \cdot \left(\mathcal{Q} v^{\mathcal{Q}} v^{\mathcal{Q}} \right) = \nabla \cdot \mathbf{T}^{\mathcal{Q}} + \mathbf{F}^{\mathcal{Q}} + R_{\mathcal{Q}} v^{\mathcal{Q}} \tag{2.4}$$

In this equation, $\mathcal{Q}$ can be either N or W. The stress tensor $\mathbf{T}$ can be written as the sum of a pressure term and a shear stress that is $\mu^{\mathcal{Q}} \nabla^2 v^{\mathcal{Q}} + \lambda^{\mathcal{Q}} \nabla \left(\nabla \cdot v^{\mathcal{Q}} \right)$. Notice that we can neglect the second term for water phase because of incompressibility assumption.

The force term includes the drag force between phases which is proportional to
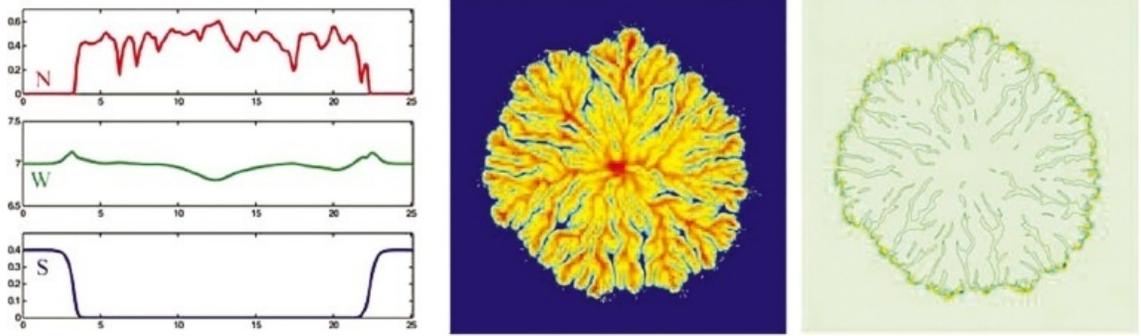
Figure 2: Emergence of branching pattern in a numerical calculation from the reaction diffusion model. The final distributions of S, N, and W are shown in the left. The colors in the middle picture represent the velocity field. The right picture shows the front boundry of moving bacteria[9].

their relative velocity, the force between agar gel and the phase, and the mechanical force of flagella on the water that helps the bacteria swim and its reaction. In principle the force of flagella should be calculated from the hydrodynamical models for flagellar motor. However, in this model, the authors decided to replace this term with a coarse grained average over the each bacterial contribution which results in a random force field. This is consistent with the experimental observation that intrinsic stocasticity of swimming process of bacterial cells dominates the hydrodynamical effects in distances as short as a few microns [3].

The next step is to complete the set of equations by finding the dependence of the coefficients in our differential equations to the variables N, S, and W. The reaction rates, R_S, R_W, and R_N, the diffusion coefficients D^S, D^W, and D^N, and finally the pressure term in the stress tensor all vary with N, S, and W. It is this dependence that makes these equations differential equations highly coupled. The authors, at this step, chose the simplest form of N, S, and W dependence for each variable phenomenologically.

Figure 3: Experimental picture of Bacillus subtilis coloniy growing in an agar plate forming a branching pattern [10].

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The numerical solution of this model shows emergence of interesting patterns varying in shape depending on the initial condition [9]. All the numerical simulation are done with initial N being a symmetric Gaussian function positioned at the center of the plate with periodic boundary conditions. The result shows that the velocity field can favor or hinder the development of branching on the boundary depending on the initial values of W and S [2]. Figure 2 shows an example of outcome of this simulation. This simulation produces a variety of different patterns observed in experiment. As an example, a similar pattern as Figure 2 is observed in an experiment done on Bacillus subtilis colonies on an agar plate shown in Figure 3[10].

3   Results and Discussion

Experimental work has shown the emergence of patterns on agar plates during the growth phase of bacterial colonies. Harder plates are diffusion limited and tend to form fractal boundaries while flower shapes, circular growth, and branching behavior has been observed on the softer mediums the colonies. Dynamical patterns such as whirls and jets have been also observed in the softer mediums. Swimming cells undergo curvilinear translation and form vortices. They go through cycles of forming groups and dissipating. The movement of these cells is shown to be more correlated where they are most motile [6]. Since individual bacteria are not motile, it is established that swarming is a collective behavior. In fact there is threshold cell density above which swarming emerges[1].

Effects of different levels of interaction on the emergent properties of growing colonies have been experimentally proven. The effect of water content and surface properties of the growth medium on the shape of growing boundary shows the importance of cell-surface interaction. SEM and TEM images of shows the cell-cell interaction through formation of flagellar bundles between neighboring cells [5]. The effect of packing of complementary cells on alignment is shown through mutation of cells to irregular shapes [4]. Feedback of hydrodynamics on individual cells is also studied experimentally and is shown to have effects in highly concentrated colonies [3]. The missing work in this area may be to study the effect of hydrodynamical feedback on motion of non-isolated cells. Also, tracking techniques can be used to collect enough data on velocity fields of growing colonies to study their scaling laws.

The numerical simulation of the reaction diffusion model has been successful to produce a wide range of patterns observed in experiment [8, 2, 9].The breaking of rotational symmetry of the system in this model is a result of instability in the front boundary of reaction diffusion equation with non-linear terms. This symmetry breaking results in formation of interesting pattern and sometimes circular spots with high density of bacteria. These results seem to depend only

on initial concentration of nutrients and water. Figure 4 shows the phase diagram of this model. According to this phase diagram branching happens at high water concentrations and low nutrient concentrations.

A more careful analysis of the results of this simulation shows that this model qualitatively predicts some dynamical collective behavior of these colonies such as emergence of whirls and jets observed in experiments. These jets and whirls are observed in different scales. Since in two dimensions, even in relatively small Reynolds numbers compared to turbulence regime, at least a partial cascade of energy transfer can be observed, it raises the question that whether these jets and whirls can be linked to the turbulence problem. The analysis of energy spectrum the velocity field can be the next step to answer this question.

Since the functional form of a lot of relevant coefficients are chosen phenomeno-logically, we can only expect that these simulations result in the same qualitative aspects of behaviors of colonies, and quantitative results can be obtained only with models with lots of free parameters that are measured experimentally that is only useful for particular bacterial colonies. However, it is still interesting that even the simplest choices of these functional forms captures all the ingredients
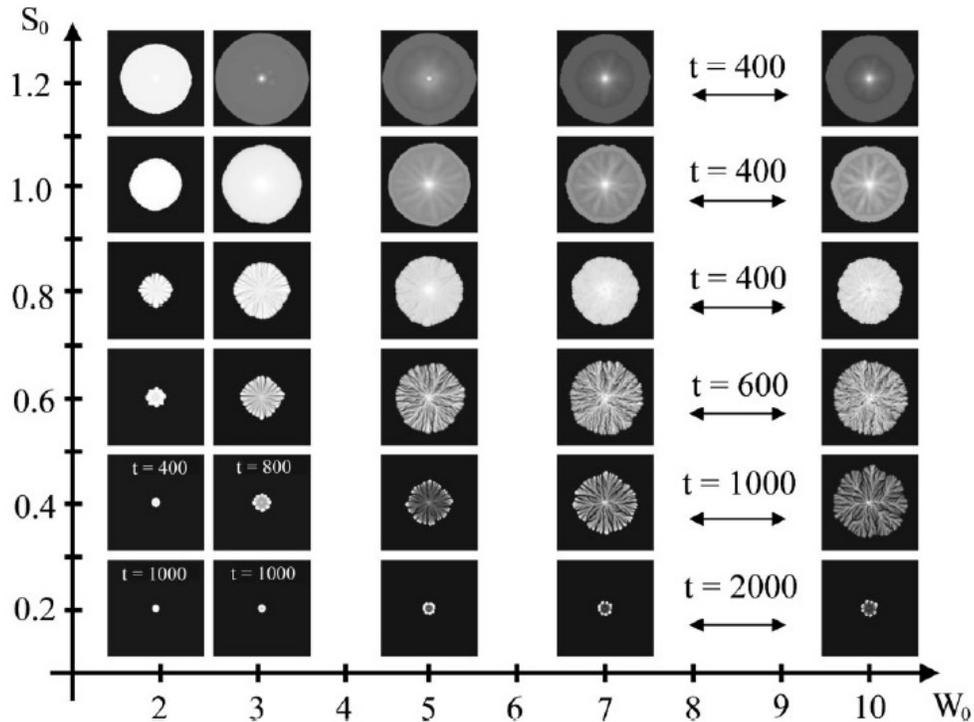
Figure 4: The phase diagram calculated numerically based the reaction diffusion model. The phase diagram of pattern formed from the growth of bacterial culture depends on intial concentration of nutriants and water[9].

to explain the formation of some of these collective behaviors. This robustness suggests that some of the collective phenomena observed in these systems may be universal and independent of details of the system. A more detail analysis is yet to be done to identify these universal variables.

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