Pattern Formation by Reproducible Random Walkers in Irreversibly Expandible Enclosure

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Based on microscopic observations, a reproducible random-walker model is presented to simulate the growth of a bacterial colony. Random walkers expand their territory by pushing the wall surrounding their colony. Numerical studies revealed that the model can explain our experimental results obtained using the bacteria species *Bacillus subtilis*.

Pattern formation of *in vitro* growing unicellular systems has recently attracted the attention of many scientists, because by changing environmental conditions, resulting colonies show various characteristic patterns, such as DLA (diffusion-limited aggregation)-like, Eden-like, DBM (dense-branching morphology)-like, ring and spiral patterns. However, most investigations were mainly on the statics of the final pattern of these bacterial colonies. In this letter we propose a reproducible random-walker model to study the dynamics of colony formation, and compare numerical results with those obtained experimentally.

Let us first describe our experiments. We used the flagellated bacteria *Bacillus subtilis*. We initially point-inoculated bacteria on the surface of an agar medium containing nutrient, and observed the growth of bacterial colonies using time-lapse video. Methods to prepare the agar medium are precisely described in our previous papers. We found that the bacterial colony spreads isotropically and homogeneously when bacteria are inoculated on a nutrient-rich soft agar medium (region D in ref. 4).

To investigate the dynamics of this homogeneously spreading colony, we applied a population dynamics approach to colony growth in the previous paper. By comparing the growth rate of the bacterial colony with numerical results of population dynamics, we found that the growth of the colony dynamics is consistent with the behavior of the solution to Fisher's equation,

$$\frac{\partial}{\partial t} b(x, t) = D \nabla^2 b(x, t) + [\epsilon - \mu b(x, t)] b(x, t),$$

which takes into account both the growth of individual bacterial cells and competition among them. Here, $b(x, t)$ is a bacterial population density at time $t$ at the spatial coordinate $x$, $D$ is a diffusion constant of bacteria, $\epsilon$ represents the growth rate of individual cells, and $\mu$ is a coefficient of competition among them.

Here, we try to improve the explanation from Fisher's equation, based on microscopic observations. We always observe, particularly in region D, that individual cells of bacteria move actively and randomly within their colony (see Fig. 2 in ref. 15). The inside of the colony seems to be full of water so that cells can swim there, although we have not confirmed this yet. On the other hand, they cannot move freely outside the colony, i.e., in a fresh region previously unoccupied. Some bacterial cells collide with the boundary of the colony, break through it often in groups, stop moving and return to where they came from. It appears as if these cells made trails on virgin
soil, which then became soaked with water. As cells repeat these processes, the boundary is gradually pushed outwards, and the size of the colony increases. This means that the spaces inside and outside a colony are different for bacterial cells. This situation cannot be described precisely by Fisher's equation. It may be interesting to simplify this situation into a model in which random walkers expand their territory by pushing the surrounding wall (interface).

Based on these microscopic observations, we propose a reproducible random-walker model (see refs. 16 and 17 for other recent studies on random-walker models). Here a random walker represents a bacterial cell. The bacterial colony is defined as a group of lattice sites which have already been occupied by random walkers. Namely, once the site is occupied by a random walker, that site is regarded as belonging to the colony even when it is not occupied by random walkers at later times. We also define the enclosure (interface) of the colony as a loop made of lattice sites surrounding the colony (see Fig. 1). Initially \((t=0)\), we set a random walker at the origin of a triangular lattice. We use \(\Delta t = 1/N(t)\) as the time interval, where \(N(t)\) is the total number of particles (random walkers) at time \(t\). The rules of the model are described as follows.

- One random walker is selected randomly, and time is added by \(\Delta t\).
- The selected walker moves to one of its neighboring sites when the following two conditions are satisfied.
  - the site is not occupied by another walker,
  - the site belongs to the colony.
- Otherwise the walker stays where it is. We assume that more than one walker cannot occupy the same site simultaneously. We also assume that walkers can move only inside their colony.
- To expand their territory, random walkers try to break down the wall surrounding their colony. When walkers collide with any unit wall site on the enclosure \(h\) times, the wall site is broken down, the wall shifts one lattice constant ahead, and any walker can move to this previous wall site.
- When a random walker has been selected \(\tau\) times after its last multiplication, the walker reproduces itself by producing one more walker at one of its neighboring sites. If the selected neighboring site is already occupied by another walker, the random walker does not produce a new walker until the site becomes empty. If the walker is selected to move before it produces the new walker, it will have a new chance to multiply itself at the new site.

A schematic illustration of the model is presented in Fig. 1.

This reproducible random-walker model with irreversibly expandable enclosure has two parameters, \(h\) and \(\tau\). \(h\) represents the hardness of the surrounding walls, and \(\tau\) is the characteristic time for multiplication. The value of \(\tau\) is related to the concentration of nutrient \(n\), because cells can multiply in a shorter period in a more nutrient-rich environment.

Figure 2 shows the morphological change of colony formation as we vary values of parameters \(h\) and \(\tau\) in this model. Here in Fig. 2, we present colony patterns for the cases \((h, \tau) = (4, 256), (16, 256), (4, 1024), \) and \((16, 1024)\). When the value of \(\tau\) is small (see patterns with \(\tau = 256\), i.e., when the colony grows in a nutrient-rich agar medium, the bacterial colony grows homogeneously with a smooth interface. On the other hand, as the
value of $\tau$ becomes larger (see patterns with $\tau=1024$), i.e., the agar medium contains less nutrient, the interface of the colony becomes rougher. We also find that the interface of the colony becomes smoother as the hardness of the wall, $h$, becomes higher. This smoothing is due to Monte Carlo averaging for the selection of wall sites.

Let us next study the dynamics of colony formation. We initially set a seed particle at the origin in our numerical simulation of the random-walker model. Figure 3 represents the growth of the radius of the colony, $r$, as a function of time $t$. We find that the radius $r$ starts to grow linearly with time after a certain period has passed. Therefore, there seems to be a transient period.

Next we compare our numerical results with experimental results. Figure 4 represents the growth of the radius $r$ of the bacterial colony as a function of time $t$. At first the colony does not grow at all. After this completely calm period, the bacterial population starts to increase by multiplication and translocation of cells. This experimental result is consistent with that obtained by numerical simulations of our random-walker model, except for the initial calm period.

We also study the case where seed particles are initially aligned on a line ($t=0$). We find that the growth rate $v$ of the line-inoculated colony is greater than that of the point-inoculated one. For example, the former is 1.24 times greater than the latter in our numerical simulation of the random-walker model. In our experiments, the former is 1.49 times greater than the latter. The dependence of the growth rate on the alignment dimension of seed particles cannot be explained by Fisher’s equation.

Finally, we investigate how the growth rate of the colony depends on the value of the characteristic time for multiplication, $\tau$, in our
model. Here the growth rate \( v \) is defined by the relation \( v \equiv dN/dr \). In Fig. 5 the growth rate \( v \) of initially point-inoculated colonies is represented double-logarithmically as a function of the inverse of \( \tau \). We find that \( v \) is expressed as

\[
v \propto (1/\tau)^{0.53}.
\]  

(2)

In our previous paper\(^5\) both the experimental investigation and numerical simulation of the Fisher’s equation showed that \( v \) is given by

\[
v \propto \sqrt{n},
\]  

(3)

where \( n \) is the nutrient concentration (see Fig. 7 in ref. 15). Assuming that the relation \( 1/\tau \sim n \) holds, i.e., nutrient-richer environment corresponds to the smaller value of \( \tau \), eq. (2) is consistent with eq. (3). This implies that even though the inside and outside of a colony are different environments for the bacterial cells, the growth rate of the boundary may still be consistent with that of Fisher’s equation except for some constant factor.

To summarize, based on microscopic observations, we proposed a reproducible random-walker model, the numerical results of which are in good agreement with results of experiments using the bacteria species *Bacillus subtilis*.

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References