Orientation field model for chiral branching growth of bacterial colonies

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We present a new reaction-diffusion model for chiral branching growth of colonies of the bacteria *Paenibacillus dendritiformis*. In our model the bacteria are represented by a density field with nonlinear diffusion and a complex scalar field which represents bacterial orientation. The orientation field introduces anisotropy into the flux of bacteria, representing self-propulsion along their long axes. The model can also reproduce tip-splitting growth of other strains (shorter bacteria) of the same species. The model can capture changes of small number of bacteria, thus it can be used to study the open question of transitions between tip-splitting and chiral dendritic growth.

Bacteria display various chiral properties. Mendelson et al. showed that long cells of *Bacillus subtilis* can grow in helices, in which the cells form long strings that twist around each other. They have also showed that the chiral characteristics affect the structure of the colony. Matsuyama et al. have found that colonies of *B. subtilis* can grow into tip-splitting patterns with a global rotation about the center of the colonies (global twist). Ben-Jacob et al. have found that some strains of *Paenibacillus dendritiformis* (see Ref. for identification of the bacteria) exhibit similar patterns with global twist. They have also found that other strains (referred to as *C* (chiral) morphotype) present different chiral properties, chiral dendritic growth with local twist (which they refer to as strong chirality).

Colonial patterns of *C* bacteria grown on semi-solid agar are characterized by chiral dendritic branching patterns, where the branches are narrow and are twisted with the same handedness (Fig. a). All colonies of this bacteria grown in similar conditions show the same handedness. Side branches are usually omitted to the convex side of the arced branches (see Refs. for morphologies and studies of *C* bacteria).

![Image](Image)

**FIG. 1.** Chiral colonial growth of *Paenibacillus dendritiformis*, strain *C*. a) Global view of a colony shows thin branches, all twisted with the same handedness. Colony is grown at 2g/l peptone level and 1.25% agar concentration. b) Optical microscope observations of branches of *C* colony, ×20 magnification of a colony at 1.6g/l peptone level and 0.75% agar concentration, the anti-clockwise twist of the thin branches is apparent. The curvature of the branches is the same throughout the growth.

2D chiral branching patterns are also observed in non-living systems. Shapes resembling Sea Horse, or S, are formed during deposition of thin films of fullerene-tetracyanoquinodimethane (C60-TCNQ) or pure TCNQ. Modeling of the system indicates that the apparent curvature of the branches is actually a strong bias in selection of splitting branches, and the branches themselves are not curved. The patterns of bacterial colonies share more resemblance with patterns formed during compression of monolayers of various chiral molecules at the air-water interface, and electro-chemical deposition under a uniform magnetic field. Various modeling attempts indicates that the processes forming these patterns are related to processes of solidification (see Refs. for the differences between the processes of solidification vs. colonial growth). A different model is required for chiral branching growth of bacterial colonies.

The Communicating Spinors model of Ben-Jacob et al. is an atomistic (discrete entities) model which describes chiral branching growth of bacterial colonies. The model presented here is not a mean field model of the spinors model. The two models complement each other and highlight different biological features. Each spinor in the spinors model represents a large groups of about 10⁸-10⁹ bacteria. This coarse graining makes simulations computationally feasible, but prevents modeling of processes which cannot be averaged over large groups (specifically, single-cell events). Continuous model is more appropriate for this purpose (when interpreting densities as probability densities). The model presented here can be used to study the effect of mutations and transitions on colonial morphologies, as will be shown elsewhere.

Detailed description of the materials and methods used in the experiments can be found in Ref. The bacterial colonies are grown on top of agar (semi-solid jelly) with peptone as a nutrient. During colonial growth bacteria are confined to within a layer of lubricating fluid, which is extracted from the agar by the bacteria themselves. Inside the layer the bacteria propel themselves in a random-walk like motion. Optical microscope observations indicate that the length of *C* bacteria is up to 50 times their width. In narrow branches, bacteria are aligned with their neighbors, and their movement is quasi 1D random walk along their long axis.
In order to capture the details of bacterial dynamics we define the bacterial density per angle $b(\vec{x}, \theta, t)$, where $\vec{x} \in \mathbb{R}^2$ is a position and $\theta \in [0, \pi]$ is an angle of orientation. We also use the bacterial density $B(\vec{x}, t)$, which is defined as the mean of $b$ over $\theta$. The equation for the bacterial dynamics is

$$\dot{b} = \nabla \cdot \{ D_b(B) \nabla b \} - \partial_\theta J_\theta + G(b, n)$$

(1)

where $\nabla$ operates in the spatial dimensions, $D_b$ is a non-constant diffusion coefficient, $J_\theta$ is the flux in the angle dimension which represents changes in bacterial orientation, $G$ is a reaction term representing growth and death, and $n$ is the nutrient concentration. We take the diffusion matrix

$$D_b(\theta) = R(\theta)^T \left( \begin{array}{cc} D_d & 0 \\ 0 & D_l \end{array} \right) R(\theta),$$

where $R(\theta)$ is a rotation matrix. $D_d$ is a constant coefficient for diffusion in the bacterial direction $\theta$ (due to self-propulsion forward and backward) and $D_l \leq D_d$ is a constant coefficient for diffusion in the lateral direction, due to fluctuations. Eq. (1) is invariant under rotation in space and translation in $\theta$ by the same angle.

It was shown that bacterial movement in a self-produced layer of fluid can be approximated by a non-linear diffusion, where the diffusion coefficient is proportional to the bacterial density to a power greater than one. The proportion constant is related to the agar dryness through the rate of absorption of the fluid into the agar. Hence we take $D_b(B) = B^k$ ($k \geq 2$), where the proportion constant is included in $D_d$ and $D_l$. We will assume that bacterial friction with the agar is proportional to the agar dryness, and as the bacterial velocity is inversely proportional to the friction, $D_d$ and $D_l$ are proportional to the agar dryness to the power $-2$.

Following we take a simple form for the growth function, $G(b, n) = b(n - \mu)$, where $\mu \geq 0$ is a rate of conversion into immobile sporulating cells. A more accurate form should have saturation for high values of $n/B$, but the linear form is a reasonable approximation for low levels of nutrients, as is the case in the bacterial colonies.

Bacteria change their orientation ($J_\theta$) in response to their neighbors orientation. It is convenient to use an auxiliary complex orientation field $p$, which is defined as $p(\vec{x}, t) = \frac{1}{2} \int_0^\pi b(\vec{x}, \theta, t) e^{-i \theta} d\theta$. The mean orientation of the bacteria is along the vector $\langle \pm \text{Re}(\sqrt{p}), \pm \text{Im}(\sqrt{p}) \rangle$.

If we assume that $\gamma = \gamma^0 |\vec{\alpha}|^2$, the branch’s boundary is identifiable by the divergence of $\nabla B$ (for the initial conditions specified below). The term $B^{k-1} \nabla B$ is always finite and can be used to identify the boundary (as $p$ is bound by $B$, it can replace $\nabla$ in the coefficient). $\gamma$ can be written as a function of $\Delta$, the angle between the branch’s boundary and the bacterial mean orientation:

$$\gamma(\vec{\alpha}, \beta) = \text{Re}[\gamma^0 |\beta| - \gamma^0 \beta (\vec{\alpha}^T \vec{M} \vec{\alpha}) / |\vec{\alpha}|]$$

$$= |\beta| \text{Re}(\gamma^0) \sin^2 (\Delta) + \text{Im}(\gamma^0) \sin (2\Delta)$$

(7)

where $\gamma^0$ is a complex constant. $\text{Re}(\gamma^0)$ is a measure of the anti-clockwise bias of the bacteria at the tip of the branch and $\text{Im}(\gamma^0)$ is a measure of the torque aligning the bacteria in parallel to the boundary. The rotation at the tip of the branch is also restrained by friction with the agar, and from the same reasoning that related $D_d$ and $D_l$ to the agar dryness, we deduce that $\text{Re}(\gamma^0)$ is inversely proportional to the agar dryness.

As initial conditions (in a circular 2D geometry), we set $n$ to have uniform distribution of level $n_0$, $B$ to have compact support at the center where it is positive, and the other fields to be zero everywhere. We solve the model numerically using a 2nd order explicit scheme. In order to reduce the implicit anisotropy of the scheme, we use

$$a(B, |p|) = \text{Re}(\gamma^0) + a(B, |p|) p + \gamma (B^{k-2} \nabla B, p) ip$$

$$\dot{n} = D_n \nabla^2 n - nB$$

$$\dot{s} = \mu B$$

(3)

(4)

(5)

where $n$ is scaled to units of bacterial mass and $D_n$ is its diffusion coefficient. $a$ and $\gamma$ are real valued functions, a decomposition into orthogonal elements of the derivation of $\partial_\theta J_\theta$ (we give here the functions $a$ and $\gamma$, not the original $J_\theta$). Here $D_1 = \frac{D_d + D_l}{2}$ and $D_2 = \frac{D_d - D_l}{4} \vec{M}$, where

$$\vec{M} = \left( \begin{array}{cc} 1 & i \\ i & -1 \end{array} \right)$$

(from here on we take all constants to be real, unless otherwise stated). Eqs. (4-5) are invariant under a rotation by $\phi$ and a multiplication of $p$ by $e^{-i2\phi}$.

For the co-alignment function $a$ we take

$$a(B, |p|) = -4D_\theta \nu (B - |p|)/(B + |p|)$$

(6)

The first term in the RHS results from linear diffusion of $b$ in the $\theta$ dimension (with $D_\theta$ the diffusion coefficient) and the second term is an alignment of non-align bacteria with the mean orientation (with $\nu$ being the rate of this co-alignment). The exact form of $\nu$ is not important, as long as $a(|p|)$ has at most one positive root with negative derivative in the range $[0, B]$ and $a(|p|)$ in non-positive outside the range $(0, B)$.

The rotation function $\gamma$ results from the only process that brakes left-right symmetry, the bacterial bias in their tumbling when they are placed in a thin layer of liquid. The bacteria are restrained by their neighbors, and the restraints on rotation are weakest near the boundary of the branches. Due to the non-linear diffusion, the branch’s boundary is identifiable by the divergence of $\nabla B$ (for the initial conditions specified below). The term $B^{k-1} \nabla B$ is always finite and can be used to identify the boundary (as $p$ is bound by $B$, it can replace $\nabla$ in the coefficient). $\gamma$ can be written as a function of $\Delta$, the angle between the branch’s boundary and the bacterial mean orientation:

$$\gamma(\vec{\alpha}, \beta) = \text{Re}[\gamma^0 |\beta| - \gamma^0 \beta (\vec{\alpha}^T \vec{M} \vec{\alpha}) / |\vec{\alpha}|]$$

$$= |\beta| \text{Re}(\gamma^0) \sin^2 (\Delta) + \text{Im}(\gamma^0) \sin (2\Delta)$$

(7)
tridiagonal lattice and multiply the bacterial diffusion coefficients by a quenched noise with mean 1. The quenched noise represent inhomogeneities of the agar surface. We show in Fig. 2 that the model can indeed reproduce the microscopic bacterial dynamics and the chiral branching patterns with the local twist.

![Image of branching patterns](Image)

**FIG. 2.** Results of numerical simulation of the OF model. 
(a) Global view of a simulated colony with a local twist of branches. Densities of $B + s$ are indicated by gray levels. Parameters values are $k = 3, D_A = 0.0625, D_I = 0, \mu = 0.1, D_\theta = 0.001, \nu = 0.5, \gamma^0 = 0.0075 - i0.006, D_n = 1$ and $\tau_0 = 1.5$. 
(b) A close look at the pattern of (a), showing the details of the curved branches.

The Non-Linear Diffusion model (NLD) for colonial growth can reproduce tip-splitting branching patterns of a related morphotype, $T$ morphotype, with shorter bacterial cells. The OF model reduces to the NLD model if the self-propulsion is not primarily along the bacterial long axis ($D_A \approx D_I$), if the bacteria do not tend to co-align ($\nu \leq 4D_\theta$ or $(\nu - 4D_\theta) \ll (\nu + 4D_\theta)$), or if bacteria at the tip of the branch tend to rotate too freely ($|\gamma^0| (\nu - 4D_\theta) \gg |D_2|$). In all these cases $|\text{Re}(D_2 \nabla p)| \ll |D_1 \nabla B|$ everywhere and the OF model produce tip-splitting branching patterns.

The response of the simulated growth to initial food concentration and agar dryness is shown in Fig. 3. In agreement with experimental observations, the main effect of the parameters is on the global density of branches, while the curvature of the branches is only weakly affected.

**FIG. 3.** The response of simulated colonies to experimental control parameters. Figures (a) and (b) present change in initial food concentration with $n_0 = 1$ and $n_0 = 2$ in (a) and (b) respectively. All other parameters are the same as in Fig. 2a. The expansion time of the colonies, normalized by the expansion time in Fig. 2a, is 2.9 and 0.4 in (a) and (b) respectively. Figures (c) and (d) present change in agar dryness $A$. $A$ is related to the model’s parameters with $\text{Re}(\gamma^0) = 0.009/A$ and $D_A = 0.09/A^2$. In (c) and (d) $A = 1$ and $A = 1.5$ respectively, with all other parameters are the same as in Fig. 2a, where $A = 1.2$. The normalized expansion time of the colonies is 0.8 and 1.2 in (c) and (d) respectively.

It was shown that many features of the colonial patterns are explained when food chemotaxis and chemotactic signaling (a chemotactic response to a material emitted by the bacteria themselves) are modeled.

In order to include chemotaxis in our model we bias the spatial flux of $b$ in Eq. (1) by adding a chemotaxis term: $B^k \tilde{b}_n(n)D_0(\theta)\nabla n$ (here we take food chemotaxis as an example). $D_0(\theta)\nabla n$ is the spatial derivative of the food concentration along the direction of movement of the bacteria. $\zeta_n(n)D_0(\theta)\nabla n$ is the derivative as sensed by the bacteria, where $\zeta_n(n)$ can be, for example, the “receptor law” or a constant. The chemotaxis is attractive for positive values of $\zeta_n(n)$ and repulsive otherwise.

The bacterial flux due to food chemotaxis translates in the OF model into additional flux terms in Eqs. (2). To the flux of $B$ in Eq. (2) we add the term $B^k \zeta_n(n)[BD_1I + 2 \text{Re}(pD_2)] \nabla n$ (where $I$ is the unit matrix) and to the flux of $p$ in Eq. (3) we add the term $B^k \zeta_n(n)[D_2B + pD_1I] \nabla n$.

If a repulsive signaling material $r$ is emitted by sporulating cells then Eqs. (2) are affected in a similar manner, with $\zeta_r(r)$ replacing $\zeta_n(n)$ and $\nabla r$ replacing $\nabla n$. $\zeta_r(r)$ is negative for a repulsive signal. The equation for the dynamics of the signaling material is

$$\dot{r} = D_r \nabla^2 r + \Gamma_r s - \lambda_B Br - \lambda_r r$$

where $\Gamma_r$, $\lambda_B$, $\lambda_r$ are non-negative constants. $\Gamma_r$ is the rate of chemical production by sporulating bacteria, $\lambda_B$ is the rate of chemical digestion by bacteria and $\lambda_r$ is the rate by which the chemical decompose.

Both food chemotaxis and repulsive chemotactic signaling increase the expansion rate of the colonies. Food chemotaxis does not affect the colonial patterns significantly, while chemotactic signaling does (Fig. 4). For short bacteria, the colonial pattern becomes less rami-fied, with radial branches and circular global envelope. For long bacteria, the global envelope becomes circular and the branches acquire an outward bias, changing their appearance from an arc-like to a hook-like appearance.

In Fig. 4 we also show the phenomena of global twist. For parameters representing bacteria with intermediate length, repulsive chemotactic signaling can impose global twist on an otherwise tip-splitting pattern.
The twist of the branches is relative to the center of the colony, not to the local orientation of the branch. The global nature of the twist is evident by using the “de-chiraling” method presented by Ben-Jacob et al. in Ref. (see Fig. 4d). In Ref. Ben-Jacob et al. were able to obtain patterns with a global twist, using the NLD model and applying a rotation operator on the chemotaxis term. As they argue themselves, such operator is inconsistent with the known biological facts and a more detailed model – like the OF model – is required in order to model any type of chirality in the bacterial colonies.

In this letter we present a reaction-diffusion model which accounts for the various morphologies presents by colonies of *P. dendritiformis*. We focus on chiral features of the colonial patterns, but we are also able to model tip-splitting patterns. We successfully simulated intermediate growth patterns (not shown here). The aim of developing such model is to aid in the study of transition between the two types of growth. Our model is able to handle events of minute density such as a mutation in a single bacterium, and it will be used in following studies of transitions between morphologies and morphotypes.

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