Modeling Branching and Chiral Colonial Patterning of Lubricating Bacteria

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Abstract

In nature, microorganisms must often cope with hostile environmental conditions. To do so they have developed sophisticated cooperative behavior and intricate communication capabilities, such as: direct cell-cell physical interactions via extra-membrane polymers, collective production of extracellular “wetting” fluid for movement on hard surfaces, long range chemical signaling such as quorum sensing and chemotactic (bias of movement according to gradient of chemical agent) signaling, collective activation and deactivation of genes and even exchange of genetic material. Utilizing these
capabilities, the colonies develop complex spatio-temporal patterns in response to adverse growth conditions. We present a wealth of branching and chiral patterns formed during colonial development of lubricating bacteria (bacteria which produce a wetting layer of fluid for their movement). Invoking ideas from pattern formation in non-living systems and using “generic” modeling we are able to reveal novel survival strategies which account for the salient features of the evolved patterns. Using the models, we demonstrate how communication leads to self-organization via cooperative behavior of the cells. In this regard, pattern formation in microorganisms can be viewed as the result of the exchange of information between the micro-level (the individual cells) and the macro-level (the colony). We mainly review known results, but include a new model of chiral growth, which enables us to study the effect of chemotactic signaling on the chiral growth. We also introduce a measure for weak chirality and use this measure to compare the results of model simulations with experimental observations.

1 Introduction

Among non-equilibrium dynamical systems, living organisms are the most challenging ones that scientists can study. A biological system constantly exchanges material, energy and information with the environment as it regulates its growth and survival. The energy and chemical balances at the cellular level involve an intricate interplay between the microscopic dynamics and the macroscopic environment, through which life at the intermediate mesoscopic scale is maintained [3]. The development of a multicellular structure requires non-equilibrium dynamics, as microscopic imbalances are translated into the macroscopic gradients that control collective action and growth [66].

Much effort is devoted to the search for basic principles of organization (growth, communication, regulation and control) on the cellular and multicellular levels [39, 42, 72, 32, 54, 73, 40, 82].
Our approach is to use the successful conceptual framework for pattern formation in non-living systems as a tool to unravel their significantly more complex biological counterparts. Of critical importance is the choice of starting point, i.e. the choice of which phenomena to study: it has to be simple enough to allow progress, but also well motivated by the significance of the results. Cooperative microbial behavior is well suited for these requirements, as we explain below. We focus on two examples: the branching growth in bacterial colonies of *P. dendritiformis* var. *dendron*, and the chiral growth of *P. dendritiformis* var. *chiralis*. Examples of these patterns are shown in Fig. 1.

Traditionally, bacterial colonies are grown on substrates with a high nutrient level and intermediate agar concentration [35]. Such “friendly” conditions yield colonies of simple compact patterns, which fit well the contemporary view of bacterial colonies as a collection of independent unicellular organisms (non-interacting “particles”). However, bacterial colonies in nature must regularly cope with hostile environmental conditions [72, 67]. When hostile conditions are created in a petri dish by using a very low level of nutrients, a hard surface (high concentration of agar), or both, very complex patterns are observed.

Drawing on the analogy with diffusive patterning in non-living systems [39, 47, 13, 5, 6], we can state that complex patterns are expected. The cellular reproduction rate that determines the growth rate of the colony is limited by the level of nutrients available for the cells. The latter is limited by the diffusion of nutrients towards the colony (for low nutrient substrate). Hence colony growth under certain conditions should be similar to diffusion limited growth in non-living systems as mentioned above [13, 4, 6]. The study of diffusive patterning in non-living systems teaches us that the diffusion field drives the system towards decorated (on many length scales) irregular fractal shapes [70, 49, 71, 81, 28]. Indeed, bacterial colonies can develop patterns reminiscent of those observed during growth in non-living systems [29, 52, 30, 54, 16, 14, 19, 15, 55, 56].

But, this is certainly not the end of the story. In fact, the colonies exhibit far richer behavior. This is ultimately a re-
flection of the additional levels of complexity involved [14, 19, 18, 17, 11, 12, 25, 9]. The building blocks of the colonies are themselves living systems, each having its own autonomous self-interest and internal degrees of freedom. Yet, efficient adaptation of the colony to adverse growth conditions requires self-organization on all levels which can only be achieved via cooperative behavior of these individual cells. Thus, pattern formation at the colony level may be viewed as the outcome of a dynamical interplay [14, 19, 15] between the micro-level (the individual cell) and the macro-level (the colony). For this interplay to work, the effects of changes at the micro-level must make themselves felt at the macro-level. This is why the notion of a singular perturbation, discovered to be a key for understanding pattern selection in non-living systems, will be of even more importance here.

This manuscript is mainly a review of our modeling studies. Yet we include some important new results: 1. Explanations and modeling of chemotaxis during chiral growth. 2. Modeling and characterization of weak chirality (global weak twist) during branching growth.

For completeness, section 2 includes a brief description of the necessary biological background needed to justify our models of the growth.

How should one approach the modeling of the complex bacterial patterning? With present computational power it is natural to use computer models as a main tool in the study of complex systems. However, one must be careful not to be trapped in the "reminiscence syndrome", described by J. D. Cowan [30], as the tendency to devise a set of rules which will mimic some aspect of the observed phenomena and then, to quote J. D. Cowan "They say: 'Look, isn’t this reminiscent of a biological or physical phenomenon!' They jump in right away as if it’s a decent model for the phenomenon, and usually of course it’s just got some accidental features that make it look like something.” Yet the reminiscence modeling approach has some indirect value. True, doing so does not reveal (directly) the biological functions and behavior. However, it does reflect understanding of
geometrical and temporal features of the patterns, which indirectly might help in revealing the underlying biological principles. Another extreme is the "realistic modeling" approach, where one constructs a model that includes in details all the known biological facts about the system. Such an approach sets a trajectory of ever including more and more details (vs. generalized features). The model keeps evolving to include so many details that it loses any predictive power.

Here we try to promote another approach – the "generic modeling" one \[40, 18, 4, 43\]. We seek to elicit, from the experimental observations and the biological knowledge, the generic features and basic principles needed to explain the biological behavior and to include these features in the model. We will demonstrate that such modeling, with close comparison to experimental observations, can be used as a research tool to reveal new understanding of the biological systems.

Generic modeling is not about using sophisticated, as it may, mathematical description to dress pre-existing understanding of complex biological behavior. Rather, it means a cooperative approach, using existing biological knowledge together with mathematical tools and synergetic point of view for complex systems to reach a new understanding (which is reflected in the constructed model) of the observed complex phenomena.

The generic models can yet be grouped into two main categories: 1. Discrete models such as the Communicating Walkers models of Ben-Jacob et al. \[18, 12, 9\] and the Bions model of Kessler and Levine \[40, 41\]. In this approach, the microorganisms (bacteria in the first model and amoebae in second) are represented by discrete, random walking entities (walkers and bions, respectively) which can consume nutrients, reproduce, perform random or biased movement, and produce or respond to chemicals. The time evolution of the chemicals is described by reaction-diffusion equations. 2. Continuous or reaction-diffusion models \[43, 48\]. In these models the microorganisms are represented via their 2D density, and a reaction-diffusion equation of this density describes their time evolution. This equation is coupled to the other reaction-diffusion equations for
the chemical fields. In the context of branching growth, this idea has been pursued recently by Mimura and Matsushita et al. [62, 53], Kawasaki et al. [38], Kitsunezaki [14] and Kozlovsky et al. [15]. A summary and critique of this approach can be found in [68] and [31].

In section 3 we present the continuous modeling of the branching growth. In section 4 the chiral growth is modeled using the “atomistic” Communicating Spinors model, which enables us to model chemotaxis response. It is the first time that the chemotaxis effect on chiral growth has been studied. The actual study of chemotaxis, both in the chiral growth and the branching growth is done in section 5.

Section 6 is devoted to the studies of weak chirality. The phenomenon is modeled using both continuous and discrete models. We introduce a measure for weak chirality which enables a more crucial comparison between the models’ results and the observed patterns. Good agreement was found.

Conclusions are presented in Section 7. We explain that the weak chirality phenomenon is general, and show examples of weak chirality during growth of the chiral morphotype and the vortex morphotype. In the latter it results from a different mechanism, and indeed the twist is not linear with the radius of growth.

2 Observations and Biological background

Following the experimental observations which are explained in this manuscript, we will describe the most relevant information for the understanding and modeling of the observed colonial patterning. We base relevancy on our previous experience and we concentrate on bacterial movement.
2.1 Experimental observations: branching growth of bacterial colonies

2.1.1 Macroscopic Observations

Some additional examples of the patterns exhibited by colonies of the $T$ morphotype are shown in figures 2, 3 and 4. For intermediate agar concentrations (about 1.5% – 1.5g in 100ml), at very high peptone levels (above 10g/l) the patterns are compact (Fig. 3a). At somewhat lower but still high peptone levels (about 5-10g/l) the patterns exhibit quite pronounced radial symmetry and may be characterized as dense fingers (Fig. 3b), each finger being much wider than the distance between fingers. For intermediate peptone levels, branching patterns with lower fractal dimension (reminiscent of electro-chemical deposition) are observed (Fig. 3c). The patterns are “bushy”, with branch width smaller than the distance between branches. As the peptone level is lowered, the patterns become more ramified and fractal-like. Surprisingly, at even lower peptone levels (below 0.25g/l for 2% agar concentration) the colonies revert to organized structures: fine branches forming a well defined global envelope. We characterize these patterns as fine radial branches (Fig. 3d). For extremely low peptone levels (below 0.1g/l), the colonies lose the fine radial structure and again exhibit fractal patterns (Fig. 4). For high agar concentration the branches are very thin (Fig. 4b).

At high agar concentration and very high peptone levels the colonies display a structure of concentric rings (Fig. 5). At high agar concentrations the branches also exhibit a global twist with the same handedness (weak chirality), as shown in Fig. 6. Similar observations during growth of other bacterial strains have been reported by Matsuyama et al. [56, 55]. We referred to such growth patterns as having weak chirality, as opposed to the strong chirality exhibited by the $C$ morphotype.

A closer look at an individual branch (Fig. 7) reveals a phenomenon of density variations within the branches. These 3-dimensional structures arise from accumulation of cells in lay-
ers. The aggregates can form spots and ridges which are either scattered randomly, ordered in rows, or organized in a leaf-veins-like structure. The aggregates are not frozen; the cells in them are motile and the aggregates are dynamically maintained.

2.1.2 Microscopic Observations

Under the microscope, cells are seen to perform a random-walk-like movement in a fluid. This fluid is, we assume, excreted by the cells and/or drawn by the cells from the agar [18, 17]. The cellular movement is confined to this fluid; isolated cells spotted on the agar surface do not move. The boundary of the fluid thus defines a local boundary for the branch (Fig. 8). Whenever the cells are active, the boundary propagates slowly as a result of the cellular movement pushing the envelope forward and production of additional wetting fluid. Electron microscope observations reveal that these bacteria have flagella for swimming.

The observations reveal also that the cells are active at the outer parts of the colony, while closer to the center the cells are stationary and some of them sporulate (form spores) (Fig. 9). It is known that certain bacteria respond to adverse growth conditions by entering a spore stage until more favorable growth conditions return. Such spores are metabolically inert and exhibit a marked resistance to the lethal effects of heat, drying, freezing, deleterious chemicals, and radiation.

At very low agar concentrations (below 0.5%) the bacteria swim inside the agar and not on its surface. Between 0.5% and 1% agar concentration some of the bacteria move on the surface and some inside the agar.

2.2 Chiral patterns

Chiral asymmetry (first discovered by Louis Pasteur) exists in a whole range of scales, from subatomic particles through human beings to galaxies, and seems to have played an important role in the evolution of living systems [33, 3]. Bacteria display various chiral properties. Mendelson et al. [58, 60, 61, 59] showed
that long cells of *B. subtilis* can grow in helices, in which the cells form long strings that twist around each other. They have shown also that the chiral characteristics affect the structure of the colony. Ben-Jacob *et al.* [14, 19, 12] have found yet another chiral property – the strong chirality exhibited by the $C$ morphotype. Here, the flagella handedness acts as a microscopic perturbation which is amplified by the diffusive instability, leading to the observed macroscopic chirality. This appears to be analogous to the manner in which crystalline anisotropy leads to the observed symmetry of snowflakes [3]; more about this later.

2.2.1 A Closer Look at the Patterns

$C$ morphotype exhibits a wealth of different patterns according to the growth conditions (Fig. 10). As for $T$ morphotype, the patterns are generally compact at high peptone levels and become ramified (fractal) at low peptone levels. At very high peptone levels and high agar concentration, $C$ morphotype conceals its chiral nature and exhibits branching growth similar to that of $T$ morphotype.

Below 0.5% agar concentration the $C$ morphotype exhibits compact growth with density variations. These patterns are almost indistinguishable from those developed by the $T$ morphotype. In the range of 0.4%-0.6% agar concentration the $C$ morphotype exhibits its most complex patterns (Fig. 11). Surprisingly, these patterns are composed of chiral branches of both left and right handedness. Microscopic observations reveal that part of the growth is on top of the agar surface while in other parts the growth is in the agar. Our model of the chiral growth explains that indeed growth on top of the surface and in the agar should lead to opposite handedness.

Optical microscope observations indicate that during growth of strong chirality the cells move within a well defined envelope. The cells are long relative to those of $T$ morphotype, and the movement appears correlated in orientation (Fig. 12). Each branch tip maintains its shape, and at the same time the tips keep twisting with specific handedness while propagating. Elec-
tron microscope observations do not reveal any chiral structure on the cellular membrane [17].

2.3 Biological background

2.3.1 Bacterial movement

In the course of evolution, bacteria have developed ingenious ways of moving on surfaces. The most widely studied and perhaps the most sophisticated translocation mechanism used by bacteria is the flagellum [27], but other mechanisms exist as well [34]. Swimming is a solitary movement done in liquid. A swimming bacterium runs nearly straight runs, interrupted by short periods of tumbling. Tumbling event is a random rotation in one location. The direction of the next run is dictated by the final orientation of the tumbling bacterium.

A swimming bacterium propagates itself by rotating a bundle of flagella. Each flagellum is an helical protein filament which is hooked to a molecular engine transversing the bacterial membrane. The engines of all flagella rotate synchronously clockwise or counterclockwise. When the bacterium turn them counterclockwise, the flagella form an aligned bundle and push the bacterium forward. When they turn clockwise, the flagella disjoin and the bacterium tumbles.

In our experiments, the bacteria do not swim in a presupplied liquid, but in a layer of fluid on the surface of the agar. The bacterial cells move individually and at random in the same manner as flagellated bacteria move in wet mounts (i.e., nearly straight runs separated by brief tumbling). Swimming takes place only in sufficiently thick surface fluid. Microscope observations reveal no organized flow-field pattern.

Based on microscope observations of movement and electron microscope observations of flagella we identify the movement of $\mathcal{C}$ and $T$ morphotype as swimming. Cells tumble about every $\tau_T \approx 1 - 5 \text{ sec}$ depending on external conditions. The speed of the bacterium between tumbling events is very sensitive to conditions such as the liquid viscosity, temperature and pH level.
Typically, it is of the order of 1-10 $\mu$m/sec.

Swimming can be approximated by a random walk with variable step size [20]. At low bacterial densities the random walk can be described by a diffusion equation with a diffusion coefficient $D_b \equiv v^2 \tau_T = 10^{-8} - 10^{-5} cm^2/sec$. Low bacterial densities means that the mean free path between bacterial collisions $l_c$ is longer than the tumbling length $l_T \equiv v \tau_T$, thus collisions between the bacteria can be neglected. The mean free path (or collision length) is

$$l_c \propto \begin{cases} \rho^{-\frac{1}{3}} & \text{in 3 dimensions} \\ \sigma^{-\frac{1}{2}} & \text{in 2 dimensions} \end{cases}$$ (1)

where $\rho$ is the 3D bacterial density and $\sigma$ is the 2D density – the projection of $\rho$ on the surface.

At high densities ($l_c < l_T$), the collisions cannot be neglected. In attempt to approximate the dynamics in those conditions, one may want to consider the time of straight motion to be $l_c/v$ instead of $\tau_T$. Hence $D_b$ depends on the bacterial density to yield

$$D_b \propto \begin{cases} v \rho^{-\frac{1}{3}} & \text{in 3D} \\ v \sigma^{-\frac{1}{2}} & \text{in 2D} \end{cases}.$$ (2)

This approximation is valid under the assumptions that a collision event is identical to a tumbling event (abrupt uncorrelated change in direction of motion), that a tumbling event is independent of the collisions, and that the speed between such events is not affected by their frequency.

The assumption that a collision event is like a tumbling event poses many problems. Even if the bacteria do not activate special response to collision it is unrealistic to assume that collisions are elastic, or that the flagella adopt immediately to the new orientation which changes during collisions. Thus it is reasonable to assume strong correlation between the cell’s orientation before collision and the cell’s orientation after collision. In addition, the orientation after the collision should be biased according with the average direction of motion of the surrounding bacteria, as they carry the liquid with them. The important
parameter is not the collision length $l_c$ but re-orientation time $\tau_r$. The re-orientation time is the time it takes a bacterium to lose memory of its initial orientation, i.e. the time span on which the final orientation has effectively no correlation with the initial orientation. At low densities the re-orientation time $\tau_r$ is equal to the tumbling time $\tau_T$. As the density rises and the collisions become more frequent, $\tau_r$ decrease. $\tau_r$ defines the densities above which the constant diffusion coefficient $D_b \equiv v^2 \tau_T$ is not a good approximation. It is quite possible that these densities are high enough so as to make the velocity and even the type of motion dependent on bacterial density, making relation (2) irrelevant. In any case, high cellular densities does mean an effective decrease in the diffusion coefficient related to the bacterial movement.

When swimming in an unstirred liquid, very low cellular densities also effect the movement. The bacteria secrete various materials into the media and some of them, e.g. enzymes and other polymers, significantly change the physical properties of the liquid making it more suitable for bacterial swimming. The secretion of these materials depend on cellular density, thus at not-too-high densities the speed of swimming rise with the cellular density. Hence the diffusion coefficient related to the bacterial movement should be a non-monotonic function of the bacterial density. Moreover, the specific functional form might depend on the specific bacterial strain.

In other conditions there is similar but more pronounced effect. On semi-solid surface the bacteria cannot swim at all inside the agar and they have to produce their own layer of liquid to swim in it. To produce such fluid the bacteria secrete lubricant (wetting agents). Other bacterial species produce known extracellular lubricants (such as surfactants, see [80, 51, 26, 57] and references therein, or the extracellular slime produced by *Proteus mirabilis* [78]). These are various materials (various cyclic lipopeptides were identified) which draw water from the agar. The composition and properties of the lubricant of *P. dendritiformis* is not known, but we will assume that higher concentration of lubricant is needed to extract water from a
dryer agar, and that the lubricant is slowly absorbed into the agar (or decomposes). A single bacterium on the agar surface cannot produce enough fluid to swim in it, thus the bacteria cannot break out of the layer fluid and the branches of a T or C colony can be defined by this fluid. Whenever bacteria enter the shallower parts of the layer, at the edge of the branch, they become sluggish, indicating that the depth of the layer effects the bacterial movement. It can be argued (see section 3.2) that in such cases the bacterial speed is related to the bacterial density by a power law (at least in low densities). Not only the diffusion coefficient related to the bacterial movement is a non-monotonic function of the bacterial density (as in a liquid agar), but it is also vanishes for extremely low densities. In this case it is clear that the specific functional form depend on the specific bacterial strain (B. subtilis, for example, cannot move at all under such conditions).

2.3.2 Chemotaxis in swimming bacteria

Chemotaxis means changes in the movement of the bacteria in response to a gradient of certain chemical field [1, 21, 46, 20]. The movement is biased along the gradient either in the gradient direction or in the opposite direction. Usually chemotactic response means a response to an externally produced field, like in the case of chemotaxis towards food. However, the chemotactic response can be also to a field produced directly or indirectly by the bacterial cells. We will refer to this case as chemotactic signaling. The bacteria sense the local concentration $R$ of a chemical by membrane receptors binding the chemical’s molecules [1, 46]. It is crucial to note that when estimating gradients of chemicals, the bacterial cells actually measure changes in the receptors’ occupancy and not in the concentration itself. When put in continuous equations [63, 31], this indirect measurement translates to measuring the gradient

\[
\frac{\partial}{\partial x} \frac{R}{(K + R)} = \frac{K}{(K + R)^2} \frac{\partial R}{\partial x} \quad (3)
\]
where $K$ is a constant whose value depends on the receptors’ affinity, the speed in which the bacterium processes the signal from the receptor, etc. This means that the chemical gradient times a factor $K/(K + R)^2$ is measured, and it is known as the “receptor law” [63].

In a continuous model, we incorporate the effect of chemotaxis by introducing a chemotactic flux $\vec{J}_{chem}$:

$$\vec{J}_{chem} \equiv \zeta(\sigma)\chi(R)\nabla R$$  \hspace{1cm} (4)

$\chi(R)\nabla R$ is the gradient sensed by the bacteria (with $\chi(R)$ having the units of 1 over chemical’s concentration). $\chi(R)$ is usually taken to be either constant or the “receptor law”. $\zeta(\sigma)$ is the bacterial response to the sensed gradient (having the same units as a diffusion coefficient times the units of the bacterial density $\sigma$). It is positive for attractive chemotaxis and negative for repulsive chemotaxis.

Ben-Jacob et al. argued [8, 23, 7, 6] that for the colonial adaptive self-organization the bacteria employ three kinds of chemotactic responses, each dominant in different regime of the morphology diagram (the claim was made for $T$ morphotype, but the same hold for their relatives $C$ morphotype). One response is the food chemotaxis mentioned above. It is expected to be dominant for only a range of nutrient levels (see the “receptor law” below). The two other kinds of chemotactic responses are signaling chemotaxis. One is long-range repulsive chemotaxis. The repelling chemical is secreted by starved bacteria at the inner parts of the colony. The second signal is a short-range attractant. The length scale of each signal is determined by the diffusion constant of the chemical agent and the rate of its spontaneous decomposition.

Amplification of diffusive Instability Due to Nutrients Chemotaxis: In non-living systems, more ramified patterns (lower fractal dimension) are observed for lower growth velocity. Based on growth velocity as function of nutrient level and based on growth dynamics, Ben-Jacob et al. [18] concluded that in the case of bacterial colonies there is a need for mechanism that can both
increase the growth velocity and maintain, or even decrease, the fractal dimension. They suggested food chemotaxis to be the required mechanism. It provides an outward drift to the cellular movements; thus, it should increase the rate of envelope propagation. At the same time, being a response to an external field it should also amplify the basic diffusion instability of the nutrient field. Hence, it can support faster growth velocity together with a ramified pattern of low fractal dimension.

**Repulsive chemotactic signaling:** We focus now on the formation of the fine radial branching patterns at low nutrient levels. From the study of non-living systems, it is known that in the same manner that an external diffusion field leads to the diffusion instability, an internal diffusion field will stabilize the growth. It is natural to assume that some sort of chemotactic agent produces such a field. To regulate the organization of the branches, it must be a long-range signal. To result in radial branches it must be a repulsive chemical produced by bacteria at the inner parts of the colony. The most probable candidates are the bacteria entering a pre-spore stage.

If nutrient is deficient for a long enough time, bacterial cells may enter a special stationary state – a state of a spore – which enables them to survive much longer without food. While the spores themselves do not emit any chemicals (as they have no metabolism), the pre-spores (sporulating cells) do not move and emit a very wide range of waste materials, some of which unique to the sporulating cell. These emitted chemicals might be used by other bacteria as a signal carrying information about the conditions at the location of the pre-spores. Ben-Jacob et al. [18, 17, 23] suggested that such materials are repelling the bacteria ('repulsive chemotactic signaling') as if they escape a dangerous location.

### 2.3.3 Food Consumption, Reproduction and Starvation

*P. dendritiformis*, like most bacteria, reproduce by fission of the cell into two daughter cells which are practically identical
to the mother cell. The crucial step in the cell division is the replication of the genetic material and its sharing between the daughter cells. Haste replication of DNA might lead to many errors – most organisms limit the rate of replication to about 1000 bases per second. Thus the reproduction must take at least minimal reproduction time $\tau_R$. This reproduction time $\tau_R$ is about 25 min in Bacilli.

For reproduction, as well as for movement and other metabolic processes, bacteria and all other organisms need influx of energy. Any organism which does not get its energy directly from sunlight (by photo-synthesis) needs an external supply of food. In the patterning experiments the bacteria eat nutrient from the agar. As long as there is enough nutrient and no significant amount of toxic materials, food is consumed (for cell replication and internal processes) at maximal rate $\Omega_c$. To estimate $\Omega_c$ we assume that a bacterium needs to consume an amount of food $C_R$ of about $3 \times 10^{-12} g$. It is 3 times its weight – one quanta for doubling body mass, one quanta used for movement and all other metabolic processes during the reproduction time $\tau_R$, and one quanta is for the reduced entropy of making organized cell out of food. Hence $\Omega_c$ is about $2 fg/sec$ ($1 fg = 10^{-15}$ gram).

If nutrient is deficient for a long enough period of time, the bacterial cells may enter a special stationary state – a state of a spore – which enables them to survive much longer without food. The bacterial cells employ very complex mechanisms tailored for the process of sporulation. They stop normal activity – like movement – and use all their internal reserves to metamorphose from an active volatile cell to a sedentary durable ‘seed’. While the spores themselves do not emit any chemicals (as they have no metabolism), the pre-spores (sporulating cells, see Fig. 9) do not move and emit a very wide range of waste materials, some of which unique to the sporulating cell. These emitted chemicals might be used by other cells as a signal carrying information about the conditions at the location of the pre-spores. Ben-Jacob et al. [18, 17, 23] suggested that such materials are repelling the bacteria (‘repulsive chemotactic signaling’) as if they escape a dangerous location.
When bacteria are grown in a petri dish, nutrients are usually provided by adding peptone, a mixture including all the amino acids and sugars as source of carbon. Bacteria which are not defective in synthesis of any amino acid can grow also on a minimal agar in which a single source of carbon and no amino acids are provided. Such growth might seem to be easier to model as the growth is limited by the diffusion of a single chemical. However, during growth on minimal agar there is usually a higher rate of waste products accumulation, introducing other complications into the model. Moreover many of our strains are auxotrophic i.e. defective in synthesis of some amino acids and need an external supply of it. Providing the bacteria with these amino acids and only a single carbon source might pose us the question as to what is the limiting factor in the growth of the bacteria. For all those reasons we prefer to use peptone as nutrient source.

We said that if there is ample supply of food, bacteria reproduce in a maximal rate of one division in $\tau_R$. If the available amount of food is limited, bacteria consume the maximum amount of food they can. In the limit of low bacterial density, the available amount of food over the tumbling time $\tau_T$ is the food contained in the area $\tau_T \sqrt{D_b D_n}$, where $D_b$ and $D_n$ are the diffusion coefficients of the bacteria and the food, respectively. Hence the rate of food consumption is given by $n \sqrt{D_b D_n}$ (weather $D_b$ is constant or not).

In a continuous model, reproduction of bacteria translates to a growth term of the bacterial density which is $\sigma$ times the eating rate per bacteria. In the limit of high nutrient it is $\sigma/\tau_R$, and in the limit of low nutrient it is proportional to $n \sigma$. This brings to mind Michaelis-Menten law of $\frac{K}{1 + \gamma n} n \sigma$ with $K, \gamma$ constants. Many authors take only the low nutrient limit of this expression, $K n \sigma$, although it is not biologically established that the bacteria in the experiments are limited by the availability of food and not by their maximal consumption rate.
3 Continuous models for the branching growth of $T$ morphotype

3.1 The Lubricating Bacteria Model

The Lubricating Bacteria model is a reaction-diffusion model for the bacterial colonies of the $T$ morphotype \[11, 15\]. This model includes four coupled fields. One field describes the bacterial density $b(\vec{x}, t)$, the second describes the height of lubrication layer in which the bacteria swim $l(\vec{x}, t)$, a third field describes the nutrients $n(\vec{x}, t)$ and the fourth field is the stationary bacteria that “freeze” and begin to sporulate $s(\vec{x}, t)$ (see section 2.3.2).

We first describe the dynamics of the bacteria and of the nutrient. The two reaction-diffusion equations governing those fields are:

\[
\frac{\partial b}{\partial t} = \text{movement} + \Gamma_b(b, n)
\]
\[
\frac{\partial n}{\partial t} = D_n \nabla^2 n - g(n, b)
\]

where $\Gamma_b(b, n)$ is the bacterial reproduction term. The nutrient diffusion is a simple diffusion process with a constant diffusion coefficient $D_n$. The bacteria consume nutrients at the rate $g(n, b)$ which is taken to be:

\[g(n, b) = nb\]

This approximate term is correct at the limit of low nutrient level and low bacterial density.

The nutrient consumed by bacteria serves as an energy source and as a precursor for synthesis of macromolecules. There is probably a minimum amount of energy necessary to maintain cell structure and integrity, called maintenance energy, and nutrients used to supply the maintenance energy are not available for cell growth. We assume that those nutrients are required at a constant rate $\mu$. Bacteria then cannot utilize all the nutrients
for reproduction:
\[
\Gamma_b(b, n) = g(n, b) - \mu b \tag{7}
\]

There is no explicit term for sporulation in (5). Instead the reproduction term \(\Gamma_b\) can be used also to model sporulation. Sporulation is initiated by starvation so it is complementary to reproduction. When \(\Gamma_b > 0\) bacteria reproduce and so sporulation is excluded. In the other case when \(\Gamma_b < 0\) bacteria reduce in number. This should represent sporulation. Note that \(\Gamma_b < 0\) when the nutrient level is low so indeed we can claim that bacteria are starved. This simple sporulation scheme has its limitations. Its rate is set by the nutrient consumption and effects such as density dependence are neglected. We represent the sporulating bacteria by the field \(s(\vec{x}, t)\), whose time evolution is:

\[
\frac{\partial s}{\partial t} = \begin{cases} 
0 & \text{if } \Gamma_b > 0 \\
-\Gamma_b & \text{if } \Gamma_b < 0 
\end{cases} \tag{8}
\]

In other continuous models the bacterial reproduction and the sporulation process are modeled differently [44, 38, 53, 31]. The bacterial reproduction is proportional to the nutrient consumption rate and sporulation is an independent process that proceeds at a rate \(\mu\) which could depend on other variables such as the bacterial density or the nutrient concentration. Note that if those modifications are applied to this model, the functional form of the bacterial reaction terms (5), (7) will not change. The equation for the field \(s\) (8) will change to:

\[
\frac{\partial s}{\partial t} = \mu b \tag{9}
\]

Since the dynamics of the other variables are separated from \(s\), its different dynamics are not significant. Moreover, the modification of the equation for \(s\) is minor. The difference is only in the biological interpretation of the terms.

We now turn to the bacterial movement. In a uniform layer of liquid, bacterial swimming is a random walk with a variable step length and can be approximated by diffusion. The layer
of lubricant is not uniform, and its height affects the bacterial movement. An increase in the amount of lubricant decreases the friction between the bacteria and the agar surface. We suggest that the bacterial movement depends on the local lubricant height through a power law with the exponent $\gamma > 0$:

$$\text{movement} = \nabla \cdot (D_b l^\gamma \nabla b)$$  \hspace{1cm} (10)

where $D_b$ is a constant with dimensions of a diffusion coefficient. $D_b$ is related to the fluid’s viscosity and the dryness of the agar might affect this viscosity. Gathering the various terms gives the partial model:

$$\begin{align*}
\frac{\partial b}{\partial t} &= \nabla \cdot (D_b l^\gamma \nabla b) + nb - \mu b \\
\frac{\partial n}{\partial t} &= D_n \nabla^2 n - nb \\
\frac{\partial s}{\partial t} &= -\min (nb - \mu b, 0) \hspace{1cm} (11)
\end{align*}$$

It is possible to define dimensionless time and space variables $t' = t\mu$ and $\tilde{x}' = \tilde{x}\sqrt{\mu/D_n}$. In those units the parameters $D_n$ and $\mu$ are equal to 1.

We model the dynamics of the lubricating fluid also by a reaction diffusion equation. There are two reaction terms: production by the bacteria and absorption into the agar. The dynamics of the field are:

$$\frac{\partial l}{\partial t} = -\nabla J_l + f_l(b, n, l) - \lambda l$$  \hspace{1cm} (12)

where $\tilde{J}_l$ is the fluid flux, $f_l(b, n, l)$ is the fluid production term and $\lambda$ is the absorption rate of the fluid into the agar.

We assume that the fluid production depends on the bacterial density. As the production of lubricant probably demands substantial metabolic efforts, it should also depend on the nutrient’s level. We take a simple form where the production depends linearly on the concentrations of both the bacteria and the nutrient. The exact relation should depend on the synthetic
pathway of the active agents composing the lubricant. If they are, for example, secondary metabolites, then their production does not depend on the current nutrient level, but on the prior accumulation of primary metabolites. However, the model is not sensitive to the exact dependence of the lubricant production on the nutrient’s level since the lubricant production is important at the front of the expanding colony, where the nutrient level is close to the initial level. It is reasonable that the bacteria produce lubricant up to a height, denoted as $l_M$, which is sufficient for their swimming motion. We therefore take the production term to be:

$$f_l(b, n, l) = \Gamma bn(l_M - l)$$

where $\Gamma$ is the production rate.

We turn to the flow of the lubricating fluid. The physical problem is very complicated. However a simplified model will suffice. We model the lubricant flux as a non-linear diffusion process:

$$\vec{J}_l = -D_l \nu \nabla l$$

where $D_l$ is a constant with dimensions of a diffusion coefficient. The diffusion term of the fluid depends on the height of the fluid to the power $\nu > 0$. The nonlinearity causes the fluid to have a sharp boundary at the front of the colony, as is observed in bacterial colonies. The equation for the lubricant field is:

$$\frac{\partial l}{\partial t} = \nabla \cdot (D_l \nu \nabla l) + \Gamma bn(l_M - l) - \lambda l$$

The functional form of the terms that we introduced are simple and plausible, but they are not derived from basic physical principles. Therefore we do not have quantitative relations between the parameters of those terms and the physical properties of the agar substrate. However we can suggest some relations. In the experiments, the agar concentration is controlled. Higher agar concentration gives a drier and more solid substrate. We shall try to find what are the effects on the lubricant layer. We recall that the lubricant fluid is composed from water and active components such as surfactants. A drier agar can increase the
absorption rate $\lambda$. Alternatively it can diminish the amount of water extracted by the active components. Then either the lubricant layer will be thinner or the bacteria will have to produce more of the active components. The former case should decrease $D_b$ while the latter should decrease the production rate $\Gamma$. In both cases the composition of the lubricant fluid will change as the concentration of the active components will increase. The lubricant fluid should become more viscous, with the effect of $D_b$ and $D_l$ decreasing.

Equation (15) together with equations (11) form the Lubricating Bacteria model. For the initial conditions, we set $n$ to have a uniform distribution of level $n_0$, $b$ to be zero everywhere but in the center, and the other fields to be zero everywhere.

Our results show that the model can reproduce branching patterns, similar to the bacterial colonies. In the experiments there are two control parameters: the agar concentration and the initial nutrient concentration. First we examine the effect of changing the initial nutrient concentration $n_0$. As Fig. 13 shows, the model produced a dense circular colony when $n_0$ was large. The pattern became more branched and ramified as $n_0$ decreased until $n_0$ was close to 1, which is the minimal value of $n_0$ to support growth.

Changing the agar concentration affects the dynamics of the lubricant fluid. Previously we demonstrated that a higher agar concentration relates to a larger absorption rate $\lambda$ and to lower production rate $\Gamma$ and lower diffusion coefficients $D_l$ and $D_b$. In Fig. 14 we show patterns obtained with different values of the parameters $\Gamma$ and $\lambda$. As we expected, increasing $\lambda$ or decreasing $\Gamma$ produced a more ramified pattern, similar to the effect of a higher agar concentration on the patterns of bacterial colonies. Similar effects are obtained by decreasing $D_b$.

### 3.2 The Non-Linear Diffusion Model

Under certain assumptions, the Lubricating Bacteria model can be reduced to the non-linear diffusion model of Kitsunezaki [44] and Cohen [22]. All the additional assumptions needed are
about the dynamics near zero bacterial density:

1) The lubricant height \( l \) is much smaller than \( l_{\text{max}} \), so that the production of the lubricant can be assumed to be independent of its height

2) The production of lubricant is proportional to the bacterial density to the power \( \alpha > 0 \) (in the simplest case taken above \( \alpha = 1 \))

3) The absorption of the lubricant is proportional to the lubricant height to the power \( \beta > 0 \) (in the simplest case taken above \( \beta = 1 \)).

4) Over the bacterial length scale, the two above processes are much faster than the diffusion process, so the lubricant height is proportional to the bacterial density to the power of \( \beta/\alpha \).

5) The friction is proportional to the lubricant height to the power \( \gamma < 0 \).

Given these assumptions, the lubricant field can be removed from the dynamics and be replaced by a density dependent diffusion coefficient. This diffusion coefficient is proportional to the bacterial density to the power \( k \equiv -2\gamma\beta/\alpha > 0 \).

The resulting model is:

\[
\begin{align*}
\frac{\partial b}{\partial t} &= \nabla (D_0 b^k \nabla b) + nb - \mu b \\
\frac{\partial n}{\partial t} &= \nabla^2 n - bn \\
\frac{\partial s}{\partial t} &= \mu b
\end{align*}
\]

For \( k > 0 \) the 1D model gives rise to a front “wall”, with compact support (i.e. \( b = 0 \) outside a finite domain). For \( k > 1 \) this wall has an infinite slope. The model exhibits branching patterns for suitable parameter values and initial conditions, as depicted in Fig. 15. Increasing initial levels of nutrient leads to denser colonies, similar to the observed patterns.
4 The Communicating Spinors Model for the chiral growth of $C$ morphotype

The Communicating Spinors Model was developed to explain the chirality of the $C$ morphotype colonies. Our purpose is to show that the flagella handedness, while acting as a singular perturbation, leads to the observed chirality. It does so in the same manner in which crystalline anisotropy leads to the observed symmetry of snowflakes [5].

It is known [27, 79, 74] that flagella have specific handedness. Ben-Jacob *et al.* [12] proposed that the latter is the origin of the observed chirality. In a fluid (which is the state in most experimental setups), as the flagella unfold, the cell tumbles and ends up at a new random angle relative to the original one. The situation changes for quasi 2D motion – motion in a "lubrication" layer thinner than the cellular length. We assume that in this case, of rotation in a plane, the tumbling has a well defined handedness of rotation. Such handedness requires, along with the chirality of the flagella, the cells’ ability to distinguish up from down. The growth in an upside-down petri-dish shows the same chirality. Therefore, we think that the determination of up vs. down is done either via the vertical gradient of the nutrient concentration or via the vertical gradient of signaling materials inside the substrate or via the friction of the cells with the surface of the agar. The latter is the most probable alternative; soft enough agar enables the bacteria to swim *below* the surface of the agar which leads to many changes in the patterns, including reversing the bias of the branches.

To cause the chirality observed on semi-solid agar, the rotation of tumbling must be, on average, less than $90^\circ$ and relative to a specific direction. Co-alignment (orientational interaction) limits the rotation. We further assume that the rotation is relative to the local mean orientation of the surrounding cells.

To test the above idea, we included the additional assumed features in the Communicating Walkers model [18], changing
it to a ‘Communicating Spinors’ model (as the particles in the new model have an orientation and move in quasi-1D random walk). The Communicating Walkers model \[18\] was inspired by the diffusion-transition scheme used to study solidification from supersaturated solutions \[76, 77, 75\]. The former is a hybridization of the “continuous” and “atomistic” approaches used in the study of non-living systems. Ben-Jacob et al. have presented in the past a version of the Communicating Spinors model for the chiral growth \[12\]. The model we present here is closely related to a previous model of the chiral growth, but it differs in two features. The first is the orientation field (see below), which was discontinuous piecewise constant and in this model it is continuous piecewise linear. The second difference is the definition of a single run of a spinor (the stretch between two tumbling events), which was defined as one run per one time unit (i.e. each step is a run) and now is defined as variable number of steps in the same direction.

The representation of bacteria as spinors allows for a close relation to the bacterial properties. The bacterial cells are represented by spinors allowing a more detailed description. At the end of the growth of a typical experiment there are $10^8 - 10^9$ bacterial cells in the petri-dish. Thus it is impractical to incorporate into the model each and every cell. Instead, each of the spinors represents about $10 - 1000$ cells, so that we work with $10^4 - 10^6$ spinors in one numerical “experiment”.

Each spinor has a position $\vec{r}_i$, direction $\theta_i$ (an angle) and a metabolic state (‘internal energy’) $E_i$. The spinors perform an off-lattice constrained random walk on a plane within an envelope representing the boundary of the wetting fluid. This envelope is defined on the same tridiagonal lattice where the diffusion equations are solved. To incorporate the swimming of the bacteria into the model, at each time step each of the active spinors (motile and metabolizing, as described below) recalculate its direction $\theta'_i$ and moves a step of size $d$ to this direction.

The direction in which each spinor moves is determined in two steps; first the spinor decides whether it should continue the
current run, that is to continue in the same direction $\theta'_i = \theta_i$. In
the basic version of the model (see Sec. 5.3 for extension of the
model) the decision is random with a specific probability $p$ to
continue the run. The resulting runs have geometric distribution
of lengths, with mean run length of $d/p$. Once a spinor decides
to change direction, the new direction $\theta'_i$ is derived from the
spinor’s previous direction by

$$\theta'_i = P(\theta_i, \Phi(\vec{r}_i)) + Ch + \xi + \omega$$

(19)

$Ch$ and $\xi$ represent the new features of rotation due to tumbling.
$Ch$ is a fixed part of the rotation and $\xi$ is a stochastic part,
chosen uniformly from an interval $[-\eta, \eta]$ ($\eta$ constant). $\omega$ is an
orientation term that takes, with equal probabilities, one of the
values 0 (forward direction) or $\pi$ (backward direction). This
orientation term gives the spinors their name, as it make their
re-orientation invariant to forward or backward direction. $\Phi(\vec{r}_i)$
is the local mean orientation in the neighborhood of $\vec{r}_i$. $P$ is a
projection function that represents the orientational interaction
which acts on each spinor to orient $\theta_i$ along the direction $\Phi(\vec{r}_i)$.
$P$ is defined by

$$P(\alpha, \beta) = \alpha + (\beta - \alpha).$$

(20)

Once oriented, the spinor advances a step $d$ in the direction
$\theta'_i$, and the new location $\vec{r}'_i$ is given by:

$$\vec{r}'_i = \vec{r}_i + d \cos \theta'_i, \sin \theta'_i$$

(21)

The movement is confined within an envelope which is defined
on the tridiagonal lattice. The step is not performed if $\vec{r}'_i$ is
outside the envelope. Whenever this is the case, a counter on
the appropriate segment of the envelope is increased by one.
When a segment counter reaches $N_c$, the envelope advances one
lattice step and a new lattice cell is occupied. Note that the
spinor’s direction is not reset upon hitting the envelope, thus it
might ”bang its head” against the envelope time and time again.
The requirement of $N_c$ hits represent the colony propagation
through wetting of unoccupied areas by the bacteria. Note that
$N_c$ is related to the agar dryness, as more wetting fluid must be produced (more “collisions” are needed) to push the envelope on a harder substrate.

Next we specify the mean orientation field $\Phi$. To do so, we assume that each lattice cell (hexagonal unit area) is assigned one value of $\Phi(\vec{r})$, representing the average orientation of the spinors in the local neighborhood of the center of the cell. The value of $\Phi$ is set when a new lattice cell is first occupied by the advancement of the envelope, and then remains constant. We set the value of $\Phi(\vec{r})$ to be equal to the average over the orientations of the $N_c$ attempted steps that led to the occupation of the new lattice cell. The value of $\Phi$ in any given point inside the colony is found by linear interpolation between the three neighboring centers of cells. Clearly, the model described above is a simplified picture of the bacterial movement. For example, a more realistic model will include an equation describing the time evolution of $\Phi$. However, the simplified model is sufficient to demonstrate the formation of chiral patterns. A more elaborate model will be presented elsewhere [24].

Motivated by the presence of a maximal growth rate of the bacteria even for optimal conditions, each spinor in the model consumes food at a constant rate $\Omega_c$ if sufficient food is available. We represent the metabolic state of the $i$-th spinor by an ‘internal energy’ $E_i$. The rate of change of the internal energy is given by

$$\frac{dE_i}{dt} = \kappa C_{\text{consumed}} - \frac{E_m}{\tau_R},$$

where $\kappa$ is a conversion factor from food to internal energy ($\kappa \approx 5 \cdot 10^3 \text{cal/g}$) and $E_m$ represent the total energy loss for all processes over the reproduction time $\tau_R$, excluding energy loss for cell division. $C_{\text{consumed}}$ is $C_{\text{consumed}} \equiv \min(\Omega_C, \Omega'_C)$, where $\Omega'_C$ is the maximal rate of food consumption as limited by the locally available food (Sec. 4). When sufficient food is available, $E_i$ increases until it reaches a threshold energy. Upon reaching this threshold, the spinor divides into two. When a spinor is starved for long interval of time, $E_i$ drops to zero and the spinor “freezes”. This “freezing” represents entering a pre-
spore state (starting the process of sporulation, see section 5.2).

We represent the diffusion of nutrients by solving the diffusion equation for a single agent whose concentration is denoted by \( n(\vec{x}, t) \):

\[
\frac{\partial n}{\partial t} = D_n \nabla^2 C - b C_{\text{consumed}},
\]

where the last term includes the consumption of food by the spinors (\( b \) is their density). The equation is solved on the same tridiagonal lattice on which the envelope is defined. The length constant of the lattice \( a_0 \) must be larger than the size of the spinors' step \( d \). The simulations are started with inoculum of spinors at the center and a uniform distribution of the nutrient. Both \( \Phi \) and the spinors at the inoculum are given uniformly distributed random directions.

Results of the numerical simulations of the model are shown in Fig. 16. These results do capture some important features of the observed patterns: the microscopic twist \( Ch \) leads to a chiral morphology on the macroscopic level. The growth is via stable tips, all of which twist with the same handedness and emit side-branches. The dynamics of the side-branches emission in the time evolution of the model is similar to the observed dynamics.

For large noise strength \( \eta \) the chiral nature of the pattern gives way to a branching pattern (Fig. 17). This provides a plausible explanation for the branching patterns produced by \( C \) morphotype grown on high peptone levels, as the cells are shorter when grown on a rich substrate. The orientation interaction is weaker for shorter cells, hence the noise is stronger.

5 The effect of chemotaxis

So far, we saw that the models can reproduce many aspects of the microscopic dynamics and the patterns in some range of nutrient level and agar concentration, but at least for the \( T \)-like growth, other models can do the same [31, and reference there in]. We will now extend the Non-Linear Diffusion model and the Communicating Spinors model to test for their success
in describing other aspects of the bacterial colonies involving chemotaxis and chemotactic signaling (which are believed to by used by the bacteria [8, 23, 7, 6]).

5.1 Chemotaxis in the Non-Linear Diffusion Model

As we mentioned in section 2.3.2, in a continuous model we incorporate the effect of chemotaxis by introducing a chemotactic flux $\vec{J}_{\text{chem}}$:

$$\vec{J}_{\text{chem}} \equiv \zeta(b) \chi(R) \nabla R$$  \hspace{1cm} (24)

$\chi(R)\nabla R$ is the gradient sensed by the bacteria (with $\chi(R)$ having the units of 1 over chemical’s concentration). $\chi(R)$ is usually taken to be either constant or the “receptor law”. $\zeta(b)$ is the bacterial response to the sensed gradient (having the same units as a diffusion coefficient times the units of the bacterial density $b$). In the Non-Linear Diffusion model the bacterial diffusion is $D_b = D_0 b^k$, and the bacterial response to chemotaxis is $\zeta(b) = \zeta_0 b (D_0 b^k) = \zeta_0 D_0 b^{k+1}$. $\zeta_0$ is a constant, positive for attractive chemotaxis and negative for repulsive chemotaxis.

We claimed that the fine radial branching patterns at low nutrient levels result from repulsive chemotactic signaling. The equation describing the dynamics of the chemorepellent contains terms for diffusion, production by pre-spores, decomposition by active bacteria and spontaneous decomposition:

$$\frac{\partial R}{\partial t} = D_R \nabla^2 R + s \Gamma_R - \Omega_R b R - \lambda_R R$$  \hspace{1cm} (25)

where $D_R$ is a diffusion coefficient of the chemorepellent, $\Gamma_R$ is an emission rate of repellent by pre-spores, $\Omega_R$ is a decomposition rate of the repellent by active bacteria, and $\lambda_R$ is a rate of self decomposition of the repellent.

Fig. 18 demonstrates the effect of repulsive chemotactic signaling. In the presence of repulsive chemotaxis the pattern becomes much denser with a smooth circular envelope, while the branches are thinner and radially oriented.
5.2 Chemotaxis in the Communicating Spinors Model

The colonial patterns of $C$ morphotype (e.g. Fig. 16) are rarely as ordered as the simulated patterns of the Communicating Spinors model. For example, the branches of the observed colonies usually have varying curvature. In the simulations of $C$ morphotype shown in Fig. 16 all the branches have a uniform curvature. One of the reasons for this difference is the simplifications taken during the model’s development. A more elaborate model that we will present [24] will be a better description of the colony. However, some of the observed features can be explained in the context of the Communicating Spinors model. In some of the observed patterns (Fig. 11b), the curvature of the branches has a distinct relation to the branch’s radial orientation (the orientation relative to the radial direction): the curvature is smaller when the branch is in the radial orientation and larger when the branch is orthogonal to that orientation. This brings to mind the radial organization of branches in the $T$ morphotype, and indeed we were able to explain the chiral pattern with the aid of the same concept – repulsive chemotaxis.

Chemotaxis was introduced in previous versions of the Communicating Walkers model by varying, according to the chemical’s gradient, the probability of moving in different directions [18, 6]. Modulating the directional probability is not the way bacteria implement chemotaxis – they modulate the length of runs. However, the growth of $T$ morphotype is insensitive to the details of the movement. Modulating the directional probability is as good an implementation of chemotaxis as many other implementations (it was chosen for computational convenience). The pattern of the $C$ morphotype is based on amplification of microscopic effects (singular perturbation) such as the left bias in the bacterial tumbling. Small differences in the microscopic dynamics of chemotaxis might affect the global pattern. Indeed we found that modulating the directional probability yield unrealistic results in the simulations of $C$ morphotype. We had to resort to the bacterial implementation of chemotaxis – modu-
lating the length of runs according to the chemical’s gradients.

When modulating the length of runs of walkers or spinors one must be careful not to change the particles’ speed. Such change is not observed in experiments and it has far reaching effects on the dynamics. Changing the particles’ speed is like changing the diffusion coefficient of the bacterial density field, a change that can have undesirable effects on the pattern.

Modulating the length of spinors’ runs without changing their speed can be done by modulating the number of steps that compose a single run (that was our motivation for dividing the runs into steps). Since the mean number of steps in a run is determined by the reorientation probability \( p \), chemotaxis should modulate this probability. For chemotaxis, the probability of changing direction by the \( i \)-spinor in one time step is (for a repellent \( R \)):

\[
p^* = p + \chi(R) \partial_{\theta_i} R
\]

where \( R \) is measured at the spinor position \( \vec{r}_i \), \( \chi(R) \) is the same as in the continuous model (either constant or the "receptor law") and \( \partial_{\theta_i} \) is directional derivative in the spinor’s direction \( \theta_i \). \( p^* \) is truncated to within the range \([0, 1]\) as it is a probability. The length of the resulting runs will depend on the runs’ direction, where a spinor moving up the gradient of the repellent will have shorter mean run length than the same spinor moving down the gradient.

The production and dynamics of the repulsive chemotactic signaling in the Communicating Spinors model is the same as in the Non-Linear Diffusion model, see Eq. (25) (with \( s \) representing the density of spinors that “freezed”). The patterns resulting from including repulsive chemotaxis in the model have indeed branches with variable curvature, as can be seen in Fig. 19. The curvature is smaller for branches in the radial direction. Food chemotaxis also varies the branches’ curvature, but in a less ordered manner, not similar to the observed bacterial patterns.

Under different growth conditions the \( C \) morphotype can produce very different patterns. As mention above, if the agar
is soft enough the bacteria can move inside it. In such case, the bias in the bacterial movement might change or even reverse, and it is manifested in the curvature of the branches. Widely changing curvature of the branches can be seen in Fig. 11a. The agar hardness was tuned such that in the beginning of the growth the bacteria could swim inside the agar, but they are forced to swim on the agar by the end of the growth due to the marginal water evaporation during the growth. In Fig. 20 we demonstrate the models’ ability to explain such patterns by changing the spinors’ bias $Ch$ during the simulation. $Ch$ is set to be a continuous random function of the colonial size, which is constrained only at the beginning and end of growth to have certain values. The function for $Ch$ is the same in all the images of Figs. 20, only that in various types of chemotaxis are used. As can be seen, repulsive chemotactic signaling is needed to explain the observed bacterial patterns.

6 Weak chirality in $T$ morphotype

Colonies of $T$ morphotype grown on hard substrate (above 2.0% agar concentration) exhibit branching patterns with a global twist with the same handedness, as shown in Figs. 6 and 21. Similar observations during growth of other bacterial strains have been reported by Matsuyama et al. [56, 55]. We refer to such growth patterns as having weak chirality, as opposed to the strong chirality exhibited by the $C$ morphotype. In [12], Ben-Jacob et al. proposed that, in the case of $T$ morphotype, it is the high viscosity of the "lubrication" fluid during growth on a hard surface that replaces the cell-cell co-alignment of the $C$ morphotype that limit the rotation of tumbling. They further assumed that the rotation should be relative to a specified direction. They used gradient of a chemotaxis signaling field (specifically, the long-range repellent chemotaxis) as a specific direction, rather than the local mean orientation field which is used in the case of $C$ morphotype. It was shown in [12] that inclusion of the above features in the Communicating Walkers
model indeed leads to a weak chirality which is highly reminiscent of the observed one. The idea above also provides a plausible explanation to the observations of weak chirality by Matsuyama et al. [56] in strains defective in production of “lubrication” fluid.

6.1 Weak chirality – the Non-Linear Diffusion Model

In the reaction-diffusion model, weak chirality can obtained by modifying the chemotactic mechanism and causing it to twist: We alter the expression for the chemotactic flux $\vec{J}_{\text{chem}}$ (Eq. 4) so that it is not oriented with the chemical gradient ($\nabla R$) anymore. Instead it is oriented with a rotated vector $\hat{R}(\theta)\nabla R$, where $\hat{R}(\theta)$ is the two-dimensional rotation operator and $\theta$ is the rotation angle. The chemotactic flux is thus written:

$$\vec{J}_{\text{chem}} = \zeta(b)\chi(R)(\hat{R}(\theta)\nabla R)$$  \hspace{1cm} (27)

The effect of rotating the repulsive chemotaxis, as depicted in Fig. 22, is to make the pattern chiral, with the degree of chirality determined by the rotation angle $\theta$.

One must note that adding a similar rotation to the food chemotaxis does not have the same effect, because the nutrient gradients do not possess the long-range, radial nature of the chemorepellent gradients.

6.2 Weak chirality – the Communicating Spinors Model

As was demonstrated in section 4, the Communicating Spinors model is robust enough to reproduce patterns of $T$ morphotype, as well as patterns of $C$ morphotype. Here we use it to model patterns of weak chirality. Two simulated $T$-like colonies are shown in Fig. 23. Fig. 23a shows colony with radial branches while Fig. 23b shows colony with weak chirality and thinner branches. In the two simulations the spinors have exactly the
same response to chemotaxis and the same bias $Ch = 9^\circ$. The two runs differ in the freedom of rotation $\eta$; in 23a the spinors can rotate freely ($\eta = 180^\circ$) while in 23b the spinors rotation is somewhat limited ($\eta = 35^\circ$, while for the colony of strong chirality $\eta = 5^\circ$).

It seems that both models – the Non-Linear Diffusion model and the Communicating Spinors model – can capture the essential features of the observed weak chirality. Yet a closer examination reveals that the description of the two models is incompatible. In the Non-Linear Diffusion model the bias from the direction of the gradient is through the chemotaxis process. The spinors, like the bacteria, cannot modulate their runs as a function of the difference between their direction and that of the gradient; they do not know what is the direction of the gradient, only the directional derivative along their path. As was demonstrated in figure 23, one of the key features for the weak chirality in the spinors model is the correlation in orientation of the spinors (through $\Phi$). In fact in this model the twist of the branches stems from the deviation of the runs’ direction from the orientation of neighboring spinors. The twist of the branches is related only indirectly, through the neighbors’ orientation, to the chemorepellent’s gradient. A continuous model of such process should include information about the mean orientation of the bacterial cells. It should include chemotaxis without rotation, anisotropic diffusion (smaller diffusion coefficient in orientations orthogonal to the mean orientation of neighbors) and a rotation on the diffusion operator. Such a model will be presented elsewhere [24].

The discrete spinors model allows for a detailed representation of the bacterial properties. The macroscopic dynamics and resulting patterns, however, are similar in both models – apparently the growth does not amplify the difference in the microscopic dynamics. Thus the ‘unrealistic’ microscopic description of the Non-Linear Diffusion model does not rule it out as an approximation to the growth dynamics of bacterial colonies with weak chirality.
6.3 Chirality measure

All through this manuscript we referred to chirality as a two-valued property – either the pattern is chiral or it is not. There are various attempts in the literature to quantify chirality with a continuous measure. See for example the method of Avnir et al. [83, 37], who applied their method also to large disordered objects. While this method is general and can quantify with a single number the measure of asymmetry of any given object, it is not quite satisfactory for our purpose. We would like to know the time evolution of the chirality of a colony, and not just “mean” chirality given by a single number. We sacrifice the generality of the measure to that end.

Since the growth velocity of the colonies (both experimental and simulated) is constant, we measure the chirality as a function of radius instead of function of time. Thus we can work on chirality of an image, not of a process. The image can be a scanned picture of the real colony or a result of a computer simulation. We look for a mapping of the image to a new one, which in some sense does not distinguish left from right (the ambiguity stems from the fact that a large random object will not have, in general, reflection symmetry, thus there is no trivial definition for chirality of such objects). The mapping is defined by:

\[(r, \theta) \rightarrow (r, \theta + \Delta \theta(r))\] (28)

where each point in the image is described by the polar coordinates \((r, \theta)\), measured from the center of the colony. Thus, each point is rotated by an increment \(\Delta \theta\) which depends on the radius \(r\) (i.e. the distance from the center).

Working on many experimental patterns, as well as simulated patterns, we have learned that in most cases a linear dependence of \(\Delta \theta\) on \(r\) is sufficient to give quite satisfactory results, that is, to transform a chiral pattern into a “normal” branching pattern. The rotating angle is thus written:

\[\Delta \theta(r) = \left(\frac{r}{r_{max}}\right) \theta_{max}\] (29)
where $r_{\text{max}}$ is the radius of the colony, and $\theta_{\text{max}}$ is the rotation angle at that radius.

The fact that this linear angular mapping suffices to “de-chiral” the simulated patterns may not be of much importance (in the case of the continuous model, at least, this is almost a direct result of the way in which we introduce the weak chirality). The same transformation works for images of real colonies of $T$ morphotype, but does not work for chiral colonies of other bacteria (see Sec. 7). This strengthens our belief in the models.

7 Conclusions

We first briefly reviewed experimental observations of colonial patterns formed by bacteria of the species $Paenibacillus dendritiformis$. We described both the tip-splitting growth of the $T$ morphotype and the chiral, twisted-branches growth of $C$ morphotype. Both colonial patterns and optical microscope observations of the bacteria dynamics were presented.

In this manuscript we presented observations of various forms of chiral patterns in bacterial colonies. Our goal was to explain the various aspects of chirality. We used two types of models: continuous reaction-diffusion models which deal with bacterial density, and a hybrid semi-discrete model which deals with properties of the individual bacterium. From a comparison of the models’ simulation and experimental observations we conclude that chemotactic signaling plays an important part in the development of colonies of the two types. We also estimate how sensitive the growth is to the details of the microscopic dynamics, demonstrating that the more 'complex' the pattern is, the more sensitive the growth is to the small details.

We would like to note that the $P. \ dendritiformis$ is not the only bacteria whose colonies exhibit chirality. Ben-Jacob et al. discussed in \textbf{[9]} the formation of colonies of $Paenibacillus vortex$, where each branch is produced by a leading droplet and emits side branches, each with its own leading droplet. Each leading droplet consists of hundreds to millions of bacterial cells that
circle a common center (a vortex) at a cellular speed of about 10\(\mu\)m/s. In Fig. 24 we show a colonial pattern of these bacteria. The chirality we termed ‘weak chirality’ is evident in this figure. In this case the chirality is not related to the handedness of the flagella, but to the rotation of the vortices. When “pushed” by repulsive chemotaxis, Magnus force acts on the vortices and drive them side-ways from the radial direction of the chemorepellent’s gradient. This difference in mechanisms is expressed in the global pattern: the colonial patterns of \(P.\ vortex\) cannot be “de-chiraled” by the transformation (linear angular mapping) that “de-chiral” the \(T\) morphotype. The fact that the models for weak chirality match in this respect the the weak chirality of \(T\) morphotype and not the ‘weak chirality’ of \(P.\ vortex\) is another support for their success in describing the bacterial colonies.

We hope we have convinced the reader that chirality in patterns of bacterial colonies gives important clues about the underlying dynamics. The processes leading to such patterns are more complex than those leading to non-twisted branching patterns. The chiral patterns are more sensitive to the underlying dynamics and as such they require more accurate models. This reflects on the success of the models we presented as being a good description of the colonies.

**Acknowledgments**

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**Figure Captions**

1. (a) Typical example of branching growth of the *Paenibacillus dendritiformis* var. *dendron* (referred to as *T* morphotype [図]) for 0.5 g/l peptone and 1.75 % agar concentration. (b) Chiral growth of the *Paenibacillus dendritiformis* var. *chiralis* (referred to as *C* morphotype [図]) for 2.5 g/l peptone and 1.25 % agar concentration.

2. Patterns exhibited by the *T* morphotype as function of peptone level (increasing from left to right) and agar concentration (1.5% bottom row, 2% middle row, 2.5% top row).

3. Examples of typical patterns of *T* morphotype for intermediate agar concentration. (a) Compact growth for 12g/l peptone level and 1.75% agar concentration. (b) Dense fingers for 3g/l peptone and 2% agar. (c) Branching fractal pattern for 1g/l peptone and 1.75% agar. (d) A pattern of fine radial branches for 0.1g/l peptone and 1.75% agar.

4. Colonial patterns of *T* morphotype. (a) Fractal pattern for 0.01g/l peptone level and 1.75% agar concentration. (b) Dense branching pattern for 4g/l peptone and 2.5% agar. Note that the branches are much thinner than those in Fig. 3b, i.e. the branches are thinner for higher higher agar concentrations.

5. (a) Pattern of concentric rings superimposed on a branched colony for 2.5g/l peptone level and 2.5% agar concentration. (b) Concentric rings in a compact growth for 15g/l peptone level and 2.25% agar concentration.
6. Weak chirality exhibited by the $T$ morphotype during growth on 4g/l peptone and 2.5% agar concentration.

7. Density variations within branches of a colony of $T$ morphotype. Optical microscope observations ×50.

8. Closer look on branches of a colony. a) ×20 magnification shows the sharp boundaries of the branches. The width of the boundary is in the order of micron. b) Numarsky (polarized light) microscopy shows the height of the branches and their envelope. What is actually seen is the layer of lubrication fluid, not the bacteria. c) ×50 magnification shows the bacteria inside a branch. Each bar is a single bacterium. There are no bacteria outside the branch.

9. Electron microscope observation of $T$ bacteria. Round or oval shapes with bright center are spores. Elongated shapes are living cells. The cells engulfing oval shapes are pre-spores.

10. Patterns exhibited by the $C$ morphotype for different growth conditions. a) Thin disordered twisted branches at 0.5g/l peptone level and 1.5% agar concentration. b) Thin branches, all twisted with the same handedness, at 2g/l peptone level and 1.25% agar concentration. c) Pattern similar to (b) but on softer agar: 1.4 g/l peptone level and 0.75% agar concentration. d) Four inocula on the same plate, conditions of 1g/l peptone level and 1.25% agar concentration.

11. In agar soft enough for the bacteria to swim in it, the branches lose the one-side handedness they have on harder agar. The two colonies of (a) and (b) are of 5g/l peptone level and 0.6% agar concentration. The two patterns are of two stains of the $C$ morphotype, strains whose patterns are indistinguishable on harder agar. c) Closer look (×10 magnification) on a colony grown at 8g/l peptone level and 0.6% agar concentration.
12. Optical microscope observations of branches of C morphotype colony. a) ×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 almost constant throughout the growth.
b) ×10 magnification of a colony at 4g/l peptone level and 0.6% agar concentration at branches are not thin, but have a feathery structure. The curvature of the branches varies, but it seems that at any given stage of growth the curvature is similar in all branches. That is, the curvature is a function of colonial growth. c) ×500 magnification of a colony at 1.6g/l peptone level and 0.75% agar concentration. Each line is a bacterium. the bacteria are long (5-50µm) and mostly ordered.

13. Effect of varying the initial nutrient concentration $n_0$ on colony pattern. $n_0$ increases from left to right: 1.2 (a), 1.4 (b), 1.7 (c), 2 (d), 3 (e), 6 (f). The minimal value of $n_0$ to support growth is 1.

14. Effect of varying $\lambda$, the fluid absorption rate, on colony pattern. The fluid production rate $\Gamma$ is 1 in the upper row and 0.3 in the lower row. In both rows $\lambda$ increases from left to right: $\lambda = 0.03$ (left), $\lambda = 0.1$ (center), $\lambda = 1$ (right) The patterns become more ramified as $\lambda$ increases. Decreasing $\Gamma$ also produces a more ramified pattern. The other parameters are: $D_0 = D_1 = 1, n_0 = 1.5$

15. Growth patterns of the Non-Linear Diffusion model, for different values of initial nutrient level $n_0$. Parameters are: $D_0 = 0.1, k = 1, \mu = 0.15$. The apparent 6-fold symmetry is due to the underlying tridiagonal lattice.

16. A morphology diagram of the Communicating Spinors model for various values of $N_c$ and initial $n$ concentration $n_0$. $Ch = 6^\circ, \eta = 3^\circ, d = 0.2, p = 0.5$.

17. When the noise $\eta$ is increases to $\eta = 180^\circ$ the tumbling of the spinors becomes unrestricted. Their movement be-
comes like that of the $T$ bacteria and accordingly the simulated colonial pattern is like that of $T$ morphotype. On the left $\eta = 3^o$, on the right $\eta = 180^o$.

18. Growth patterns of the Non-Linear Diffusion model with food chemotaxis (left, see section 2) and repulsive chemotactic signaling (right) included. $\chi_{0f} = 3, \chi_{0R} = 1, D_R = 1, \Gamma_R = 0.25, \Omega_R = 0, \Lambda_R = 0.001$. Other parameters are the same as in figure 13. The apparent 6-fold symmetry is due to the underlying tridiagonal lattice.

19. The effect of repulsive chemotactic signaling on the Communicating Spinors model. a) Without chemotaxis. b) With repulsive chemotaxis. The Spinors are repelled from the inner parts of the colony. The resulting curvature of the branches is reduced when they are in the radial direction. In spite the reversed handedness, the pattern resemble Fig. 13b.

20. The snake-like branches observed in Fig. 11a can be reproduced by the Communicating Spinors model. $Ch$ is a continuous function of the colony’s radius (the same function in a, b, and c). Maximal value of $Ch$ is $8^o$, minimal value is $-2^o$. (a) With repulsive chemotactic signaling. (b) Without chemotaxis. (c) With food chemotaxis. The best resemblance to the observed colony is obtained with repulsive chemotactic signaling.

21. Weak chirality (global twist of the branches) exhibited by the $T$ morphotype for a peptone level of $0.25g/l$ peptone level and agar concentration of $1.75\%$.

22. Growth patterns of the Non-Linear Diffusion model with a “squinting” repulsive chemotactic signaling, leading to weak chirality. Parameters are as in the previous picture, $\theta = 43^o$.

23. Weak chirality of the $T$ morphotype is modeled by the Communicating Spinors model. Both simulations are with
repulsive chemotactic signaling and with bias in the walkers rotation. a) With free rotation ($\eta = 180^\circ$) The pattern is branched, without apparent chirality expressed. b) With constrained rotation ($\eta = 35^\circ$) weak chirality is expressed.

24. A colony of Paenibacillus vortex on 10 g/l peptone level and 2% agar concentration. The dots at the tips of the branches are bacterial vortices – each is composed of up to millions of bacterial cells rotating around a common center. The twist of the branches results from a Magnus force induced by repulsive chemotactic signaling.
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