Models of Self-Organizing Bacterial Communities and Comparisons with Experimental Observations

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Abstract. *Bacillus subtilis* swarms rapidly over the surface of a synthetic medium creating remarkable hyperbranched dendritic communities. Models to reproduce such effects have been proposed under the form of parabolic Partial Differential Equations representing the dynamics of the active cells (both motile and multiplying), the passive cells (non-motile and non-growing) and nutrient concentration. We test the numerical behavior of such models and compare them to relevant experimental data together with a critical analysis of the validity of the models based on recent observations of the swarming bacteria which show that nutrients are not limiting but distinct subpopulations growing at different rates are likely present.

Key words: Dendritic patterns, *Bacillus subtilis* swarming, Reaction-diffusion equations, Cell community growth.
AMS subject classification: 35K55, 65M60, 92C17

1. Introduction

Communities of cells can exhibit remarkable patterns which have attracted the attention of scientists for many years. They result from highly complex but poorly understood interactions between cells and internal regulatory networks, which involve both chemical signaling and the effects of

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physical factors. Numerous models have been used in attempts to represent some of these bio-physico-chemical effects and to describe the resulting self-organizing patterns.

Different biophysical factors involved in pattern formation have given rise to various types of modelling. One class of model concerns auto-chemotaxis (attraction of cells by a chemical substance emitted by the cells themselves) and gives rise to a Fokker-Planck equation that is commonly called the Keller-Segel system after the seminal work [12]. This model is mathematically very challenging and has motivated numerous studies (see [3, 23] and the references therein); in particular this class of model typically leads to cell aggregation in one or several discrete spots (blow-up of the system as a Dirac mass solution). Several such models and numerical results are presented in [22].

Other models are based on the multiplication of cells resulting from nutrients initially present in the medium and consumed by the expanding community, combined with active and random motion of bacteria. This approach has also been widely used and can generate dendritic patterns in the absence of oriented drift (preferred direction of motion) in contrast to the Keller-Segel model which describes cells moving preferentially towards higher concentrations of the chemo-attractant. Many additional factors have been incorporated into models, such as the observed higher motility of cells at the tip of the dendrites (region of higher population density and higher nutrient concentration) in [11], a surfactant secreted by the cells that may change the liquid surface and thus the migration speed of cells [15, 6], or differentiation from swimmers to swarmers for *Proteus mirabilis* as modelled in [4, 5].

We are interested in models that can explain the dendritic patterns exhibited by swarming communities of *Bacillus subtilis*, taking into account possible biophysical effects arising during the migration. We summarize some recent experimental observations in Section 2., then we review several models and show numerical simulations (Section 3.). Finally we discuss in Section 4. the similarities and differences between simulations and experimental results.

2. Experimental results

Bacillus subtilis is a non-pathogenic but important constituent of soil and the plant rhizosphere. This bacterial species is also one of the major model organisms used in the laboratory throughout the world to study fundamental questions concerning bacterial growth, metabolism, physiology and behaviour. *B. subtilis* is now an important model for studying the life style and social behaviour of bacteria as large communities – the normal form of most bacteria in nature. A particularly remarkable form of such community growth is the ability of *B. subtilis* to 'swarm' over the surface of low concentration agar (0.7%-1%). Swarming is a process of rapid mass migration of cells over a surface, involving a co-operative interaction between cells but not necessarily involving cell aggregation. We are studying the swarming of strains of *B. subtilis* over a fully defined medium (B-medium) in a Petri dish (a swarm plate), in which the bacteria migrate from a central inoculum as hyper-branching dendrites, forming radiating patterns covering several square centimeters in a few hours ([9, 10]).

The presence of flagella and the secretion of a surfactant (surfactin) by the bacteria, plus the products of at least 15 genes, are absolutely essential for swarming. Following inoculation of the plate with 10^4 cells, the bacteria multiply with an estimated generation time (doubling time) of about 90 min, compared to 110 min when cells are growing in a classical shaking liquid culture. After 11–12 h of growth, the inoculum forms the mother colony (MC), approximately 30 μm thick, 2 mm in diameter. This growth period is presumably necessary to build a critical mass and an accumulation of a chemical signal sufficient to trigger in some cells the ability to form dendrites. The first visible sign of initiation of swarming is the appearance of a transparent zone of surfactin spreading outwards from the MC of a transparent zone of surfactin. Approximately one hour later, hemispherical 'buds' approximately 800 μm in diameter, abruptly appear (burst) from the edge of the MC. These form the heads (tips) of the rapidly elongating 10-14 primary dendrites. Surfactin production is essential for formation of the pre-dendritic buds and experiments suggest that its presence modifies the surface of the agar gel, presumably by inducing the formation of a thin layer of fluid close to the agar surface [1]. Flagella, whose deployment presumably depends on an appropriate fluid film on the agar surface, are essential for a later stage in the development of the bud and for driving dendrite migration (see below). Importantly, the entire process of bud formation and elongation of the radiating dendrites, up to lengths of 1.5 cm, occurs as a monolayer of cells. The cells in dendrites are distributed in an irregular mesh-like organization, including closely packed but clearly separated cells. Dendrites can be divided into two distinct regions, a long stem containing largely non-motile cells, which remarkably are maintained at an overall constant population density, and the extreme 1 mm at the tip where the population density increases sharply. This tip region contains hyper-motile cells that we term *swarmers*, which appear to constitute the 'motor' for elongation. Dead cells in dendrites or the MC are rare, perhaps less than 1%.

When dendrites reach approximately 1.5 cm in length, equivalent to 5-6 h following emergence of pre-dendrite buds, a dramatic switch from monolayered to multilayered dendrites slowly spreads progressively from the base of dendrites, as the swarm begins to develop the classical biofilm form (at least 50 μm thick). These observations demonstrate that nutrients are in great excess for at least 24 h encompassing the swarming process and subsequent maturation of the bilayer. Moreover, we have shown that diluting the nutrients in the swarm plates at least 4-fold, prior to inoculation has little obvious effect on the pattern of swarming (unpublished data).

Recent studies [8], using genetic analysis and fluorescent microscopy to measure the level of production (expression) from the gene encoding the major flagellum subunit *in situ*, have identified a specific subpopulation of hyper-flagellated cells ('swarmers'). These are dominant in the formation of buds and then subsequently spearhead dendrites in the tips. These hyper-motile cells are in contrast to the cells forming the stem of dendrites that we term 'supporters'. We propose (manuscript in preparation) that supporters contribute to dendrite elongation by growth and division (multiplication), while swarmers actively drive extension from the tip, generating hydrodynamic forces dependent upon their hyper-motility. Thus, migration of the swarm front results from the co-operative action of two sub-populations to promote dendrite extension. In contrast to these mechanical forces however, we note that the piloting mechanism or guidance system that ensures *radial* migration likely depends on a self-generated chemical gradient, but so far this explanation remains speculative.

We shall now summarize a number of notable features of the migration process and consequent pattern development that should be taken into account when constructing mathematical models, if these are to describe the swarming process adequately. Migrating dendrites on encountering a large obstacle (like a cover slip or an *E. coli* colony) are induced to make a 90° turn but then in most cases these return to the original radial direction. In addition, dendrites rarely merge and appear to avoid each other. This behaviour is consistent with the diffusion of a chemical repellent generated by the cells able to keep dendrites well separated. Significantly, the limited number of primary dendrites established at the initiation of swarming are usually supplemented at much later times by additional dendrites arising from the MC. This might indicate that the differentiation event to form swarmers (capable of breaking out of the MC) is based on a stochastic process that can occur repeatedly over time. In relation to the branching process, we have observed that branching can occur by tip splitting. However, a significant number of branches abort and remain very short and are often restricted to one side of the dendrite stem. All new branches tend to quickly adopt a radial direction during subsequent elongation. Importantly, while the overall frequency of branching increases towards the edge of the swarm plate, dendrite stems progressively become thinner (Fig. 1).

A surprising characteristic of the swarming process is the paradox that while cells are expected to grow and divide exponentially, as all cells do in a liquid culture, the rate of swarming migration remains constant. This, combined with the constant population density over most of the dendrite, clearly indicates that not all cells in the dendrites can be growing at the same rate. This would be an extremely unusual behaviour for a bacterial population and it is important to establish now which subpopulations may be subject to growth rate control.

3.   Models for self-organizing communities

The mathematical description is performed in two steps. First, a model is proposed based on the major supposed mechanisms that drive the migration of the cells. We give two such examples: one is based on nutrient consumption, the other on chemoattraction and chemorepulsion. Secondly, a numerical simulation is performed that allows us to visualize the approximate solution.

Throughout this section we consider a general situation where the cell community lives in a domain Ω. For numerical simulations we consider only the case of a disk that represents the usual experimental domain (a Petri dish). The initial state, mimicking the experimental device, is set with an initial value for the bacterial population density representing an 'inoculum' in the center of the disk.

3.1.   Reaction-diffusion models (nutrient only)

The nutrient-based models used to generate dendritic patterns of cell communities are mostly formulated in terms of three quantities
• the population density $u(x, t)$ of active cells at the location $x \in \Omega$. Under the effect of their
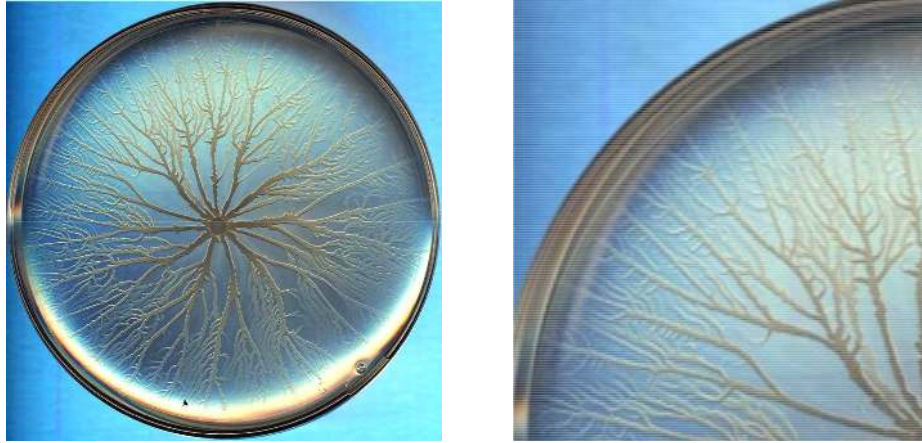
Figure 1: An experimental swarming pattern displayed by *B. subtilis* 168 on B-medium. Bacteria were inoculated in the centre of the plate and incubated for 24 h. Dendrites elongate radially from the central mother colony (approximately 3.5 mm per h) and begin branching after 1.5 cm. Highly reproducible patterns are obtained, characterized by increased frequency of progressively thinner branches. Dendrites generally appear to evade each other and rarely fuse. Side branches tend to be biased to one side and rather frequently abort after a relatively short distance. Interestingly, this motif, termed domain branching, is observed in mouse lung tissue [18]. Side branches frequently commence at $40 - 90^o$ to the main branch, but then adopt a radial direction.

flagella, active cells undergo a random movement resulting in a diffusion of intensity d_u, and they multiply according to the nutrient available locally
• the nutrient concentration $v(x, t)$ diffuses according to Einstein's rule and, because the nutrient is limited, it can diminish locally due to its consumption by multiplying cells
• the population density of passive cells $w(x, t)$ are assume to be stationary and non-multiplying. Active cells become passive according to some rules that differ from one model to the other. Passive cells do not move nor multiply, and do not revert to the active form, but accumulate in the domain.

These assumptions lead to write general systems of the form

$$
\begin{cases}
\dfrac{\partial}{\partial t}u(x,t) - d_u\Delta u(x,t) = u[vf(u,v) - g(u,v)], \\[2mm]
\dfrac{\partial}{\partial t}v(x,t) - d_v\Delta v(x,t) = -uvf(u,v), \\[2mm]
\dfrac{\partial}{\partial t}w(x,t) = ug(u,v).
\end{cases}
\tag{3.1}
$$

Such systems have first been introduced to model chemical reactions. A simple example is the
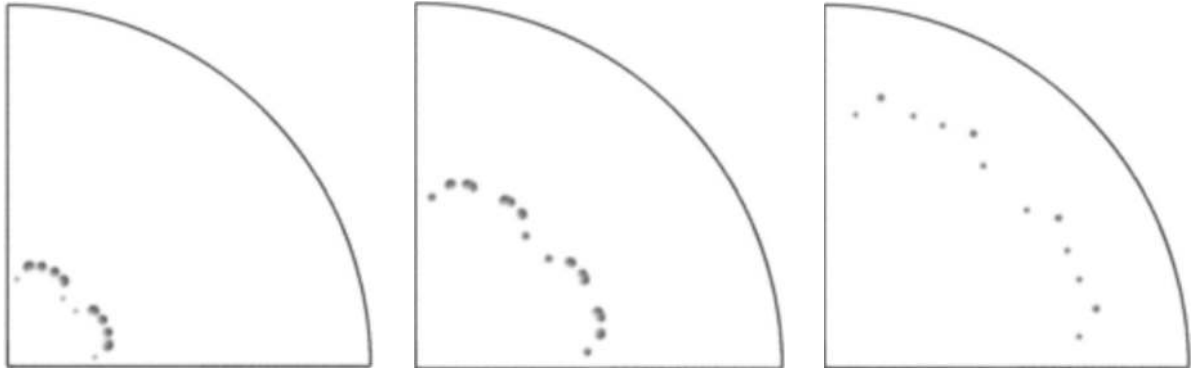
Figure 2: Dynamics of community growth as given by a numerical simulation of the Gray-Scott model (3.2) with $n = 1$. Active cells modelled by the component $u(x, t)$ are shown (total number of cell presented in Fig. 3). Three different successive times are presented. These cells are positioning where nutrient is available.
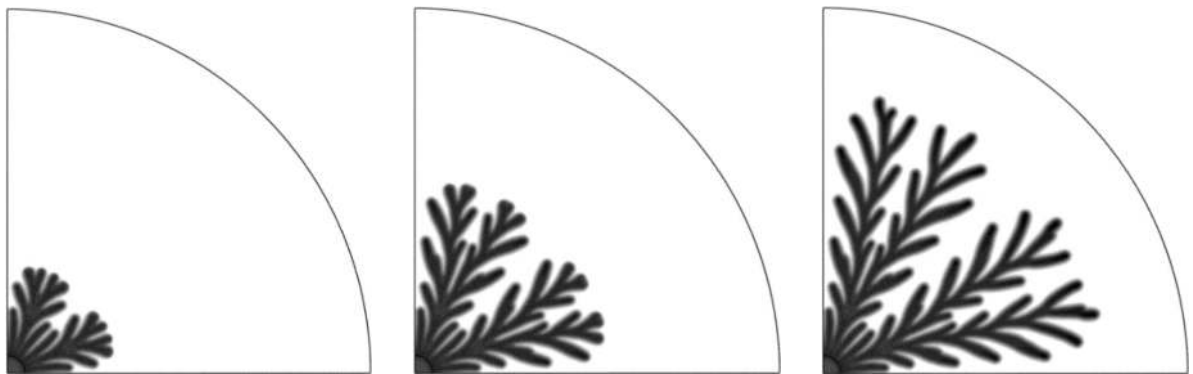

Figure 3: Same model as in Figure 2, the total population density $u + w$ is shown. The dendritic pattern is determined by the passive cells in this type of model.
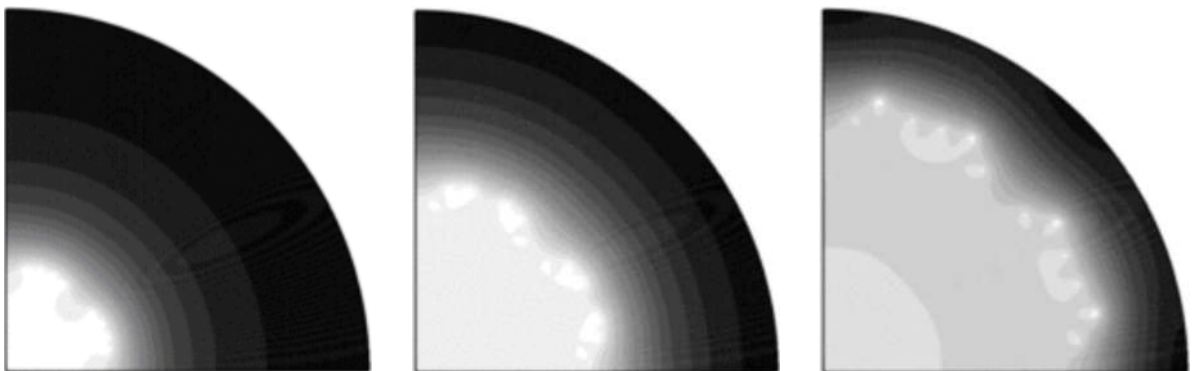

Figure 4: Same model as in Figure 2, the nutrient concentration v is shown (dark represents high concentration). While moving outwards towards undepleted nutrient, the active bacteria consume the nutrient.

Gray-Scott system [7] is a simple and classical example which writes as

$$\begin{cases} \dfrac{\partial}{\partial t}u(x,t) - d_u\Delta u(x,t) = u[u^n v - \mu], \\[2mm] \dfrac{\partial}{\partial t}v(x,t) - d_v\Delta v(x,t) = -u^{n+1}v, \\[2mm] \qquad\qquad \dfrac{\partial}{\partial t}w(x,t) = \mu u(x,t). \end{cases} \qquad (3.2)$$

Here $n \geq 0$ is an integer related to the mass action law for the molecules undergoing the chemical reaction and $\mu > 0$. Variations around this model can also be interpreted in terms of bacterial motion as proposed in Kessler and Levine [13], and Golding et al [6]; they replace the growth term $u^n v$ by $h(u)v$ where $h(\cdot)$ is a truncation function for small values of u and $h \approx 1$ for large values.

The Gray-Scott model explains the instability that generates the digitation process. It is related to concentration effects of the equation on active cells; its solution u exhibits high values on the tip of the dendrite and move outwards where nutrients are replete (Figure 2). These concentration points are traveling pulses that undergo secondary instabilities which explain their branching, see [21, 14]. They leave behind them the column of passive bacteria forming the dendritic pattern shown in Figure 3. In order to run simulations on the normalized unit disk, we have used parameters given by

$$n = 1, \qquad d_u = 6.25 \ 10^{-8}, \qquad d_v = 100 d_u, \qquad \mu = 0.01, \qquad v_0 = 1,$$

where v_0 is the initial constant value of v.

Rather than a limitation on growth for small values of u as in the Kessler and Levine model, Mimura et al [19] proposed a limitation on the transition rate to the passive state for large values of u or v. The choice of the reaction terms f and g in the general system (3.1) is then given by

$$\begin{cases} \dfrac{\partial}{\partial t}u(x,t) - d_u\Delta u(x,t) = u\left[v - \dfrac{\mu}{(a+u)(b+v)}\right], \\[3mm] \dfrac{\partial}{\partial t}v(x,t) - d_v\Delta v(x,t) = -uv, \\[3mm] \qquad\qquad \dfrac{\partial}{\partial t}w(x,t) = \dfrac{\mu u}{(a+u)(b+v)}. \end{cases} \qquad (3.3)$$

The resulting dendritic patterns differ slightly from those obtained with the Gray-Scott model, but the underlying mechanism is very similar. Figure 5 shows the active cells and the total population. Simulations were run in the unit disk and the parameters we have used for model (3.3) are

$$d_u = 1.4 \ 10^{-7}, \qquad d_v = 20 \ d_u, \qquad a = 1/2400, \qquad b = 1/120, \qquad \mu = ab, \qquad v_0 = 0.087.$$

Other biophysical processes can be taken into account in this class of models in order to explain the behaviour of specific bacterial communities. The effect of surfactant has, for instance, been analysed in [15] and affects the diffusion term by allowing variation in cell motility of cells depending on the height of the surface liquid. The influence of the reaction terms and analogy with phase transitions are studied in [26]. The references [6, 22] also contain several related models.
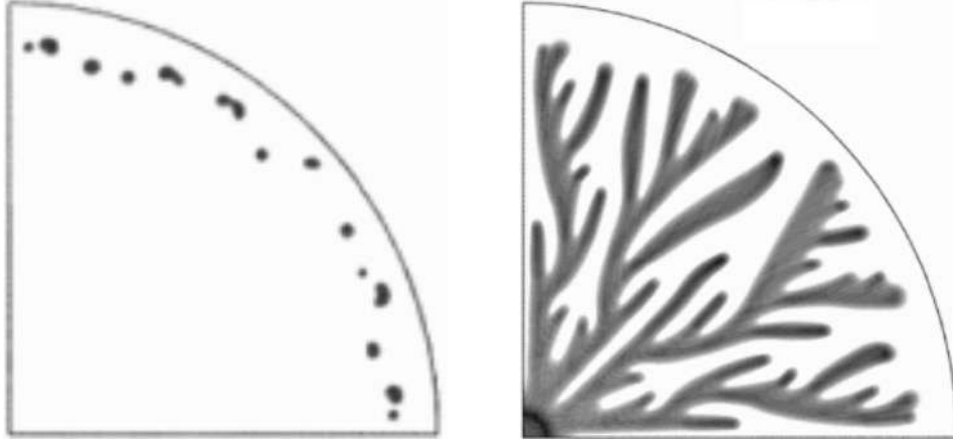
Figure 5: Dynamics of the dendritic expansion as given by a numerical simulation of the Mimura model (3.3). Left: active cells modeled by the component $u(x,t)$. Right: total population $u + w$.

3.2.   Chemoattraction, chemorepulsion and Fokker-Planck terms

When the bacteria emit a chemoattractant or chemorepellent terms must be included in the reaction diffusion systems described above. Assuming that the medium is rich enough so that the nutrient is not limiting, we arrive at systems of the form

$$\begin{cases} \dfrac{\partial}{\partial t}u - d_u\Delta u + \mathrm{div}[(\dfrac{\alpha}{(1+c_a)^2}\nabla c_a - \eta\nabla c_r)] = u[g(u,c_r) - f(u)], \\[2ex] \hspace{6cm} \dfrac{\partial}{\partial t}w = uf(u), \\[2ex] \hspace{2cm} \dfrac{\partial}{\partial t}c_a - d_a\Delta c_a + uc_a = \beta u^2, \\[2ex] \dfrac{\partial}{\partial t}c_r - d_r\Delta c_r + \gamma(u+w)c_r = \chi w^2. \end{cases} \tag{3.4}$$

Here c_a and c_r represent the concentration of chemoattractant and chemorepellent respectively. These are assumed to diffuse according to Einstein's rule with coefficients d_a and d_r, they are degraded with the rates assumed to be u and $\gamma(u+w)$ here, and they are secreted by the cells with rates β and χ. Their actions are represented by Fokker-Planck terms in the equation for u, together with the Keller-Segel model mentioned earlier.

In this combination of reaction-diffusion equations together with drift terms, the latter represent chemoattraction/chemorepulsion and have tendency to dominate the dynamics. Their hyperbolic character change the time scales, infer stronger aggregation effects on active cells and this yields much more dynamical profiles (thiner structures that move faster). This can be seen in Figure 6 where we have assumed a short-range attraction on active cells (u component) and long-range repulsion on passive cells (w component). The parameter values for this figure are

$$d_u = 0.1, \quad d_a = 1., \quad d_r = 10., \quad \alpha = 1.5, \quad \eta = 0.12, \quad \beta = 0.2, \quad \gamma = 5, \quad \chi = 1,$$
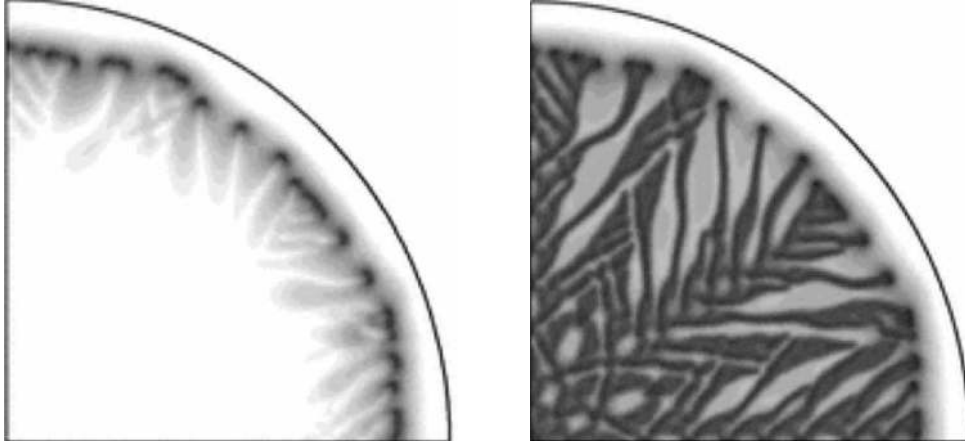
Figure 6: Dynamics of community expansion as given by a numerical simulation of a model (3.4), including cell population densities, chemoattractant and chemorepellent factors, but no nutrient limitation. Left: active cells modeled by the component $u(x,t)$. Right: total population $u + w$. Compared to Figures 3 and 5, the oriented drift creates more dynamical patterns where active spots can cross each other leading to an interwoven pattern.

$$f(u) = \rho(u - \mu \ln(u)), \qquad \rho = 0.025, \qquad \mu = 2.718,$$

$$g(u, c_r) = \delta \frac{\beta_r^2 + c_r^2}{\beta_r^2 + \alpha_r c_r^2}, \qquad \delta = 20, \quad \alpha_r = 50, \quad \beta_r = 5.$$

3.3. Numerical method

The numerical solutions we have presented are based on the mixed finite element method (with Raviart-Thomas elements of lowest degree often denoted by RT_0) that has been described in [16, 17]. One of the major ingredients is a change of unknown functions to handle the several orders of magnitude in population densities that can occur in different areas of the computational domain. This is run on an unstructured triangular grid as shown in Figure 7 which allows the instabilities to progress without preferred direction, in contrast to rectangular grids which have the tendency to create mesh effects.

For numerical purposes the domain is the unit disk (or a sector of it) and the diffusion coefficients are small. This forces us to use fine grids (finer as the diffusion coefficients become smaller).

The dendritic patterns are very dependent on the grid used for computing and convergence is reached with very fine grids. For this reason the numerical convergence has only been reached for relatively moderate values of d_u and of the ratio d_v/d_u.
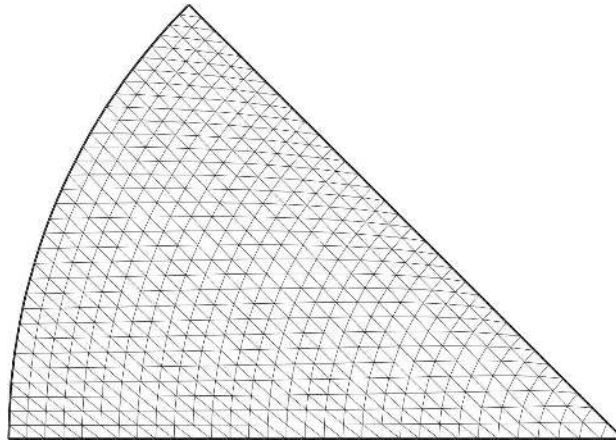
Figure 7: An example of the triangular mesh with 1024 elements. The meshes used for the numerical solutions are obtained by successive subdivisions with similar triangles and adjustment of the vertices to the curved boundary. Finer grids with up to 600.000 elements have been used.
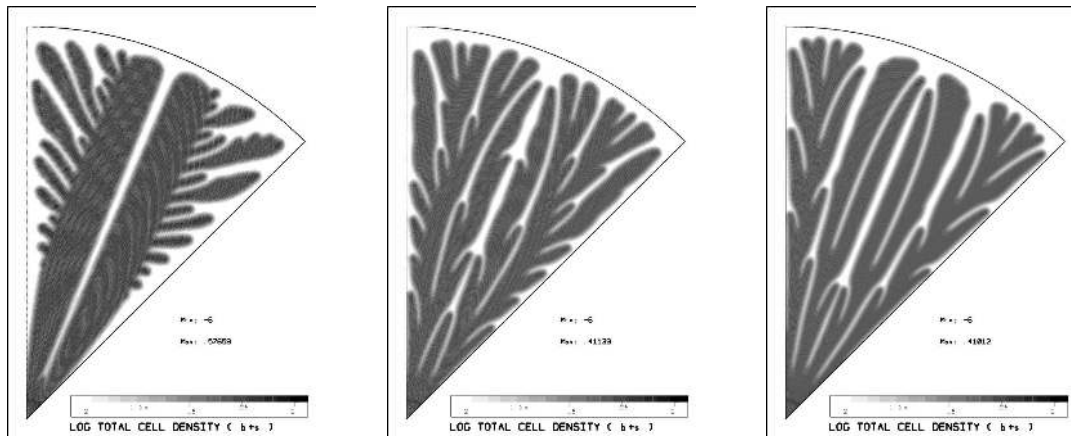

Figure 8: Example of numerical simulations of the Gray-Scott model (3.2) with the same set of parameters as in Fig. 2–4 , except we have used the ratio $d_v = 20d_u$ (this makes the pattern denser compared to the ratio 100 used previously), using different triangular meshes; from left to right: 65536 elements, 262144 elements, 589824 elements.

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4. Critical review of the correspondence between experiments and models

Whereas the models presented here clearly produce dendrites of various shapes, a critical analysis of the experimental data reveals that many features cannot be explained by these models. Moreover, as we will now discuss, these observations make it clear that an entirely new class of models will be needed for a detailed description of swarming in *Bacillus subtilis* and probably other bacteria.

The major point concerns the mechanism of branch formation. The models (3.1), (3.2), and (3.3), as well as most of the models found in the literature, suppose that the proliferation of the bacteria is limited by the availability of a chemical nutrient which satisfies a diffusion equation. In some situations described in the literature this appears to be true, and convincing agreement between patterns from models and experiments has been obtained [6]. This however, may be an illusion. In all standard microbiological media, including Peptone - a so called poor medium employed in many early swarming studies - in which indeed the growth rate of cells is slow because the amino acids constituting the carbon source are not energy rich, many generations of growth can nevertheless be supported before nutrients become limiting. For the swarming experiments described in our studies (see Section 2.), certainly, nutrients do not become limiting for growth, as shown by the continued visible increase in cell numbers for many hours after completion of the swarming process. Similarly, swarming in *Proteus mirabilis* is not controlled by nutrient limitation [4, 24].

The importance of this experimental observation stems from the fact that a gradient in nutrient concentration is required in these models to create dendrites, since this promotes faster growth of bacteria in the tips that have easier access to the nutrients. In the absence of nutrient limitation, a different physical or chemical effect must pilot the outward migration of bacteria leading to branch formation. Two different potential alternative mechanisms can be envisaged for *Bacillus subtilis*.

A first hypothesis is suggested by the fact that mutants with reduced surfactin production swarm slowly or do not exhibit swarming. Moreover, the swarming process is accompanied by spatial gradients of surfactin concentration. Since surfactin is a surfactant, concentration gradients give rise to Marangoni forces, which have been shown to create branching patterns in the spreading of liquid droplets [25]. Moreover, many models to explain swarming assume that the surface of the agar is covered by a thin liquid film [6, 2]. However, the reality is more complex since a simple experiment shows that a droplet of pure water deposited on the surface of the agar gel does not spread over the surface, but remains sessile [1]. This obviously implies that the surface of the agar cannot be covered by a continuous liquid film. Nevertheless, in the presence of surfactin, a very thin film develops in the vicinity of the deposited droplet and expands slowly (the covered distance is approximately $\sim t^{0.45}$ [1]). While the presence of this film is necessary for swarming, presumably because this allows the movement of the bacteria, it is unlikely to provide a mechanism for the formation of dendrites. Indeed, the thickness of the film is close to that of prewetting films and these have never been reported to lead to the formation of dendrites on a solid liquid interface. Furthermore, the results of [1] clearly indicate that the agar surface has a quite complex structure and that the wetting phenomena at this surface are not well understood. Therefore, a

better understanding of these wetting properties is needed before they can be incorporated into new models, even at a qualitative level.

A second hypothesis, which was the basis for formulating models (3.4), is that the outward migration of the bacteria is driven by a long-range chemorepellent. Whereas, as yet, no substance generating such a chemotactic movement of the bacteria has been identified experimentally, the fact that dendrites avoid each other is consistent with the existence of a chemorepellent. As was shown in Section 3., simulations of such models can indeed produce dendritic structures. However, the mechanisms for branching and the overall growth of dendrites are quite different in the model and in the experiments.

In particular, the models presented here, as well as most of those in the literature, introduce two 'states' of the bacteria: 'active' cells which diffuse and reproduce, and 'passive' ones that do neither move nor reproduce. As was shown in Section 3., a characteristic feature of these models during branch formation are 'hot spots' of active bacteria that are located at the tips of the branches, whereas the main parts of the branches consist exclusively of inactive bacteria. As a consequence, the elongation of the branches takes place by cell division in the 'hot spots'. The experimental observations yield a quite different picture. Although the tips of the dendrites can be described as 'hot spots', characterized by a higher population density of bacteria, which in addition move much faster than the average population, the doubling time of the bacteria under the conditions considered here is much longer than the typical time for swarming, indicating that the driving mechanism for the swarming is cell migration and not cell division. Moreover, although not as highly motile, the bacteria in the 'stems' are by no means inactive. Some cells at least appear to perform a random-walk type motion with a global drift towards the tips that may support tip motion since cutting the stem with an obstacle stops the swarming at the head of the dendrite (unpublished observations, Orsay).

These observations have major non-trivial implications for the formulation of new models, when considering the structure of the front of the swarm community. In the models presented here, the diffusion coefficient of the bacteria is always positive. In the 'hot spot', the balance between diffusion and cell division creates a propagating front solution that remains well localized. If the bacteria were to remain active, as experimentally observed, behind the tips, the diffusion process would lead to a spreading of the bacteria, and the gaps between the branches would disappear; only the transition to an inactive state, where bacteria neither move nor divide, maintains the stems intact. This conclusion remains valid even in models where the diffusivity of the bacteria depends on the local bacterial population density u and tends to zero when u tends to zero [20].

Consequently, a model must yield stable interfaces between the inside and the outside of the colony, even if all the bacteria are active. This necessarily requires some effect which leads to a 'cohesion' of the colony. In the Keller-Segel model, a short-range chemoattractant leads to the aggregation of the bacteria, but this model yields spot patterns, whereas stripes of active bacteria are unstable. This model therefore cannot describe stable branches. A class of models which can describe stable stationary and moving fronts is known from the physical description of phase transitions, the simplest example being the Cahn-Hilliard equation. In such models, two stable states (corresponding to the two thermodynamic phases in contact) are connected by a stable front solution of well-defined width. This front is maintained by the balance between a diffusion coef-

ficient which becomes negative in the front and terms containing higher-order spatial derivatives to prevent the solution from developing singularities. While such models have numerous potentially useful properties for the description of bacterial community expansion, it is difficult at the present stage to find a well-justified equivalent to the free energy functional that underlies their mathematical structure.

In summary, many fundamental aspects of the swarming process are not well reproduced by the models available at present. Furthermore, as we have discussed above, some experimental observations indicate that the structure of the models has to be profoundly modified. Thus, basing dendrite elongation on the exhaustion of nutrients by active cells at the swarm front, with non moving (non growing) cells behind, although these are two useful mathematical parameters, is untenable microbiologically, since nutrients are in excess on swarm plates and all cells should be growing. On the other hand, recent experimental evidence from studies in *B. subtilis* (Hamze et al submitted)has demonstrated the presence of distinctive cell types, swarmers and supporters, actively *moving* and *non-moving* respectively. In addition, preliminary mathematical analysis indicates that *B. subtilis* swarms may include cells growing at *different rates*. This would be a novel concept in the field of bacterial communities, but interestingly could explain some, so far, puzzling experimental observations. Future studies will be directed to testing this hypothesis, in addition to other experiments clearly needed to guide further model development. In particular, a much better understanding of the motion of a single bacterium and its interplay with the community is required. This necessitates further experiments focused on the analysis of the trajectories of single bacteria. In addition, a better understanding of the structure of the agar surface and its consequences for the local motility of the bacteria is desirable. Finally, models with several different population density fields of bacteria, reflecting distinct cell types, will certainly be required to account for the full complexity of the swarming process.

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