



## Simulating the migration and growth patterns of *Bacillus subtilis*

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### ABSTRACT

We introduce a cellular-automaton based model for the description of the migration and growth patterns of colonies of *Bacillus subtilis*. The two phases, associated with processes of migration and proliferation, are described by appropriate rules for the update of the automaton. We show that it is possible to reproduce the patterns present in the morphological diagram for *B. subtilis* and in particular the ring-like structure that the colonies adopt in the case of a not very hard medium and under nutrient abundance.

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### 1. Introduction

Pattern formation is ubiquitous in the living world. Patterns can be found in the spatial distribution of such simple organisms as bacteria or in complex cases such as the grazing patterns of sheep flocks or the rings observed in mushroom growth. The main question one asks in this case is how the simple rules that govern the individual give rise to the complicated structures observed as a consequence of the collective activity.

Modelling the collective behaviour which results in the emergence of patterns is a challenge in biological science. The difficulty is that biological modelling requires a very delicate balance. On one hand one must not produce an ad hoc model, where the rules are set so as to reproduce the observations without a deep understanding of the underlying mechanism. On the other hand the model should not be swamped by biological details which introduce too many unmanageable parameters and thus compromise the model's predictive power.

The object of the present paper is to study pattern formation in bacterial colonies. This is one of the rare domains in biological modelling where simple physical conditions dominate over biological ones. One can thus discuss the growth of bacterial colonies in physical terms and hope that the experience thus acquired will be useful in the understanding of more complex situations of pattern formation in biology. Bacteria are particularly versatile in their behaviour under adverse conditions. The strategies they develop in order to cooperate more efficiently lead to emerging patterns which become more and more complex as the external conditions become less favourable [29]. For example, *B. subtilis* forms a high variety of patterns, from scarce branches to concentric circles, when nutrients and agar concentrations are varied. A particularly convenient way to present these results is with the help of what is called the “morphological diagram”. In Fig. 1, we present this diagram drawn by I. Rafols based on detailed experiments conducted by Matsushita and collaborators [20] at Chuo University (Tokyo) [26] (note that the region C can have a slightly different shape in other publications [10,27,33]). The letters in the paragraph that follows correspond to the various parts of the morphological diagram. The two crucial parameters for the expansion of the *B. subtilis* colonies are the hardness of the medium (controlled by the agar concentration) and the availability of nutrient. When the medium is very hard and the nutrient is scarce the colony expands in the form of branches (A). The bacteria seek the scarce nutriment and follow its diffusion chemotactically. When the hardness of the

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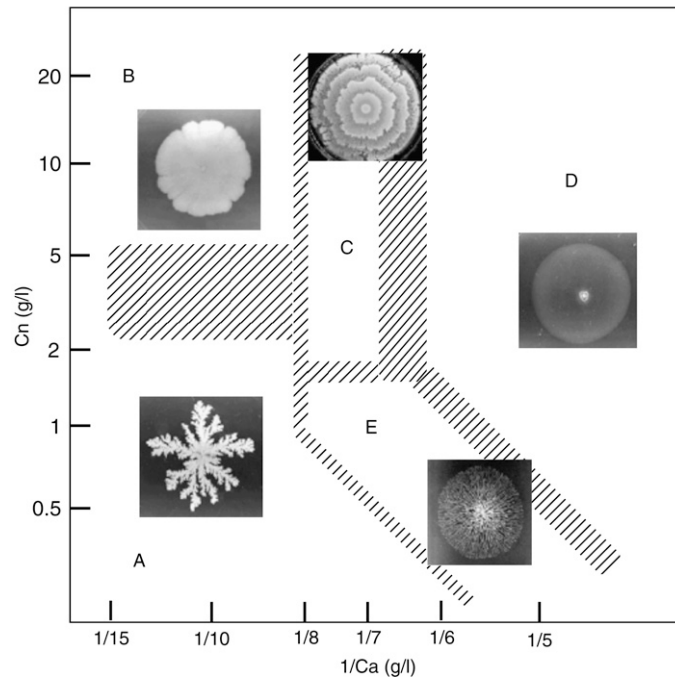


Fig. 1. Morphological diagram for *Bacillus subtilis*. Clichés are taken with permission from [26].

medium diminishes the motion of the bacterium fronts is facilitated. As a consequence, the number of branches increases but still the principle of the colony growing along paths persists (E). Increasing the nutrient concentration leads to a complete modification of the aspect of the colony. When the medium is soft, the colony expands as a disc of (almost) constant, low, density (D). The dominating process here is migration. In the opposite situation, of a hard medium, the colony stays compact (B). Migration is minimal and the expansion is mainly due to proliferation at the border. (The precise form of the interface has been the object of analyses and speculations, often making reference to Eden-like structures [20,31].) Finally an interesting intermediate region exists when the nutrient is in abundance and the medium is of moderate hardness: the growth of the colony leads to concentric rings of high bacterium density (C).

In order to construct a model which could describe the various patterns present in the morphological diagram it is useful to summarise the general principles underlying the mechanism of bacteria migration and proliferation. Two kind of cells seem to be present: active cells and less motile cells. The growth of a bacterial colony proceeds typically in two phases. During the phase known as “migration”, the colony expands rapidly thanks to the more motile cells. (In the case of a very hard medium, this phase is greatly inhibited and the colony expands essentially by division of the cells located at the border). In the phase known as “consolidation” or “proliferation” the outwards migration stops and the number of cells increases rapidly. The two phases alternate with clockwork regularity and it is this interplay of migration and proliferation that create concentric rings. A possible explanation of this two-phase mechanism may be sought in the optimal use of the nutrients in order to maximise the biomass of the colony. Detailed experiments have shown that nutrient diffusion does not play an important role in the process (as long as the nutrient is abundant) [28,35]. Also the characteristic migration and consolidation times do not depend crucially on the nutrient concentration. On the other hand the agar concentration, which conditions the hardness of the medium, does play an important role: migration time decreases and consolidation time increases with increasing hardness [28,35].

The concentric ring pattern is often the main challenge in modelling approaches. Most of the models are based on coupled reaction–diffusion equations, usually with some extra term added, with a nonlinear diffusion coefficient or hydrodynamical considerations [4,8,9,15–18,23] (for thorough reviews one can consult Refs. [5,19]). In Ref. [11] we have taken a minimalistic attitude and proposed an extremely simple model which, despite its simplicity, is able to reproduce the behaviour of concentric colonies of *B. subtilis* and *Proteus mirabilis* in a nutrient-rich medium.

If the nutrient availability is not a limiting factor for concentric rings, on a poor medium on the contrary, branches develop only through gradient of nutrients (nutrient chemotaxis), for *B. subtilis* as well as for *P. mirabilis* [10,24,27,26,33]. Therefore, modelling of the branches formation involves either coupled reaction–diffusion equations for bacteria and nutrient concentration [15,17,20], or a more discrete approach [6]. However, only a few of all these models describe the whole “morphological diagram” of *B. subtilis* [17,20,22]. The first one [20,22] is based on competition between nutrient diffusion and cell motion that does not correspond to experimental results, as explained above. The second one [17] assumes the existence of long and short bacteria with different cell density dependent diffusion coefficients.

In what follows we shall introduce a model based on a cellular automaton. We have already proposed cellular automaton models for the migration of tumour cells on different substrates [2,3]. Using the same basic tools, a geometrical background and automaton rules representing the interaction between the elementary objects (here, bacteria) we will show that it is possible to reproduce all the patterns observed in colonies of *B. subtilis*. In particular, our cellular automaton model does reproduce the concentric-ring pattern quite successfully.

While the present discrete-time, discrete-space model is fundamentally different from the continuous-time, continuous-space one, we introduced in Ref. [11], the two models share essential common features. Both are based on two different phases, proliferation and migration, characterised by specific times. In the continuous model one must swap the equations set between the phases while in the cellular automaton one implements different rules for each phase. (A more quantitative comparison would have necessitated the appropriate normalisation of the two models. In the case of the automaton, we have considered that it is simpler to work with dimensionless variables since the comparison with experiment is mainly qualitative, while in the continuous model the normalisation was chosen so as to allow quantitative comparisons with experimental data).

## 2. Model and results

### 2.1. The model

A dynamical model based on a cellular automaton is built upon two basic ingredients: the geometry and the evolution rules.

#### 2.1.1. The geometry

In previous publications of ours we have introduced a two-dimensional cellular automaton model for the description of the migration of tumour cells [2,3]. In those studies we have chosen a tessellation of regular polygons. The advantage of that choice was that its algorithmic definition was particularly simple. Still the symmetry imposed by the regular polygons is not a desirable feature. We were thus naturally led to a different choice for this study and the obvious one was a Voronoi tessellation [32]. Still, despite the underlying Delaunay triangulation, a Voronoi tessellation based on a random collection of points on the plane would have been inadequate leading to an unacceptable variation of the size of each cell. We should point out here that in the present study each site can accommodate a single bacterium. As a consequence a graphical representation of the occupied sites gives an idea of the distribution of the bacteria. It was thus of utmost importance that the surfaces of the elementary polygons do not vary much. The strategy we adopted was to start from the triangulation corresponding to a tessellation by squares and perturb the position of each site by a small random factor. The resulting Voronoi tessellation has polygons of comparable surface. Moreover, perturbing the square lattice has as a consequence that the mean number of neighbours of each cell has a value around 6 (rather than the strict 4 of the square lattice), a fact that greatly facilitates the kinetics. The notion of neighbourhood is pretty clear in this setting. Since each lattice element is a polygon its nearest neighbours (NN) consist of the polygons having a common side with it, its next-to-nearest neighbours being the polygons which are the nearest neighbours of the NN. In what follows, whenever we loosely refer to “neighbours” what we precisely mean are “nearest neighbours”.

The solution of the randomly perturbed lattice adopted here is not the only answer to the problem of the undesirable symmetry of the lattice. In Ref. [25] Nishiyama et al. have proposed a different solution by introducing variable thresholds for the speed of propagation of wave fronts on the lattice.

#### 2.1.2. The evolution rules

The evolution rules for the automaton have been largely inspired from the ones used in our previous model [2,3]. At each update of the automaton we define a random order of all bacteria and then we evolve them one after the other. We introduce two characteristic times, a proliferation one  $T_p$  and a migration one  $T_m$ . At each automaton cell we can have a bacterium or not. If a bacterium is present it can be in one of the three following states: the bacterium can migrate (state 1), the bacterium can proliferate (state 2) or the bacterium is inactive (state 3). At time zero a seed colony of bacteria is placed (at random) in the centre of the grid. We consider that first, bacteria migrate, and we have opted for seed colonies with a radius proportional to the migration time and an initial density proportional to the inverse of the migration time squared, in order to begin with always the same number of bacteria. At the end of this initial phase, all seed bacteria are set to state 2.

The proliferation time is split in two. During the first period  $T'_p$  the bacteria in state 2 generate bacteria in state 2 while in the second period  $T''_p$  they generate bacteria in state 1. Upon update each bacterium in state 2 places a new bacterium in all the free sites which lie next to it and outwards, i.e. in all the free sites situated at a larger radius than the one occupied by the bacterium. At the end of the proliferation time  $T_p$  the bacteria in state 2 become inactive (state 3). During the migration time those bacteria which are in state 1 start migrating. A bacterium may move only to a free site. When the position of a bacterium is to be updated a new position is chosen among its neighbours which are situated further from the centre. If this position is occupied, the bacterium does not move. We allow migrating cells to produce inactive cells with a probability equal to  $T_p/T_m$  at each time step. At the end of migration time  $T_m$  the bacteria change state to state 2, i.e. proliferating, with a probability proportional to  $T_p/T_m$ . In the case where  $T_p$  is small, the bacterium has a low probability to go back to state 2.

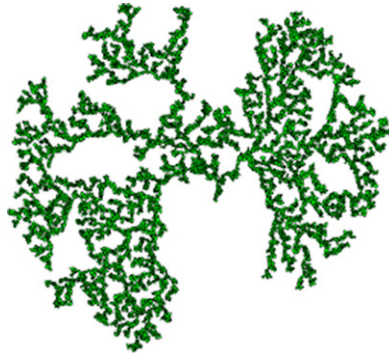


Fig. 2. Simulated pattern of bacteria distribution obtained with parameters:  $N = 8, T_p = 1, T_f = 4$ .

Finally, bacteria consume nutrients: at time zero, the available nutrient amount  $N$  is uniform. Only bacteria in state 2 consume nutrients. To mimic nutrient's diffusion, we assume that at each time step a bacterium eats  $n$  nutrient units on its site,  $n - 1$  on its nearest neighbouring sites and  $n - 2$  on the next-to-nearest neighbouring sites. Proliferating bacteria can only fill sites where the amount of nutrients is larger than  $n$  and bacteria on sites where the amount of nutrients is zero (or less) become inactive.

### 2.1.3. Time considerations

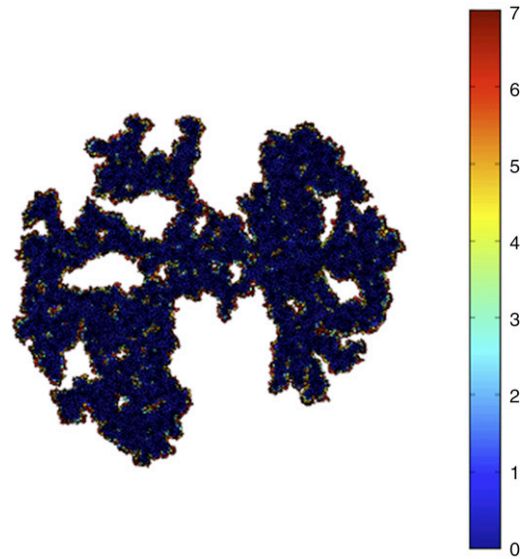
The total cycle time  $T_m + T_p$  is taken constant and can for simplicity be normalised to 1. (Calibrating time is quite straightforward if one wishes, as we did in Ref. [11], to compare quantitative results with experiment. In what follows we shall not attempt such a comparison but will limit ourselves to describing the morphology of the colony patterns). So the evolution is characterised by a single time parameter:  $T_p$ . As explained just above the proliferation time is split in two:  $T_p = T'_p + T''_p$ . For simplicity we choose  $T'_p/T''_p = T_p/T_m$ . (As a consequence we have  $T'_p/T_p \propto T_p$  and  $T''_p/T_p \propto T_m$ ).

We assume that the proliferation time  $T_p$  increases with the hardness of the medium, as it has been shown in experiments [35]. The rules are cyclic, i.e. at the end of a cycle (one proliferation phase and one migration phase), bacteria go back to state 2 and a new cycle starts. The number of cycles of “migration + consolidation” at the end of simulation is denoted by  $T_f$ . The computations were performed using MATLAB<sup>®</sup>7.6 on an iMac with 2 GHz Core 2 Duo processor, running OSX 10.4, with a lattice size of 250 by 250. The typical running time for the simulation was a few minutes. The graphical representation of each colony pattern was particularly time-consuming (but this is probably a defect of MATLAB<sup>®</sup>).

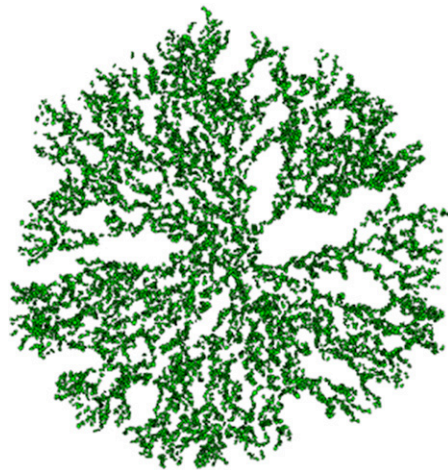
## 2.2. The results

Using the evolution rules presented in the previous section we have performed various simulations with parameters which are meant to reproduce the conditions of hardness and nutrient abundance of the experimental situation. The proliferation time is chosen to increase linearly with growing agar concentration, as:  $T_p = 8.3 \cdot 10^{-2} C_a - 0.25$  where  $C_a$  is in g/l and  $T_p$  is between 0 and 1 as explained above. This linear relation between  $T_p$  and  $C_a$  was inspired by the results of Ref. [35] and the precise numerical coefficients have been chosen so as to correspond to the experimental morphological diagram. For instance,  $C_a = 15$  g/l corresponds to a compact pattern (region B) and thus  $T_p = 1$ . On the other hand  $C_a = 3$  g/l corresponds to the limit case of a very diffuse pattern (far region D). Concerning the nutrient consumption, for simplicity,  $n$  is set to 3, i.e. each bacterium consumes at each time step 3 nutrient units on its site, 2 on the first closest neighbouring sites and 1 on the second closest neighbouring sites. The amount of nutrients per site,  $N$  is, taken equal to 8 in the nutrient-poor situation. For the nutrient-rich case a very large value (typically 500) is adopted. The parameters for which each pattern is obtained are given in the respective captions of the various figures.

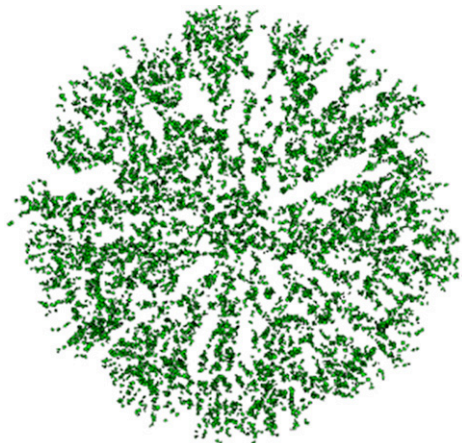
We first describe what happen when scanning a horizontal line in the morphological diagram, decreasing the concentration of agar of the medium progressively and keeping the initial nutrient constant to a small value. Fig. 2 represents a case corresponding to a hard medium and nutrient scarcity, that leads to branch-like structures. The tips of the branches are the only active part of the colony, since bacteria can only proliferate on sites with enough nutrients. In the inner part of the colony, nutrients are exhausted and the bacteria are inactive, cf. Fig. 3. The gradient from the intact outer region (in white) to the inner region depleted of nutrients is hardly visible on Fig. 3 since it extends over only three sites. A case of softer medium is represented in Fig. 4. In this case the migration of bacteria leads to the formation of multiple branches, since the chemotactical motion (due to the nutrient gradient) along paths is now greatly facilitated (just as in case E of the morphological diagram). Branches are well-defined. Moving further to a softer medium one observes ray-like branches in Fig. 5, stressing the “radiating-out” character of the chemotactical motion. Here, branches begin to fade away and are not as well defined as in the preceding case of Fig. 4. Decreasing the concentration of agar in the medium further leads to a diffuse pattern with nonzero density in the interior, cf. Fig. 7. In the limit case where  $T_p = 0$  the colony pattern becomes an



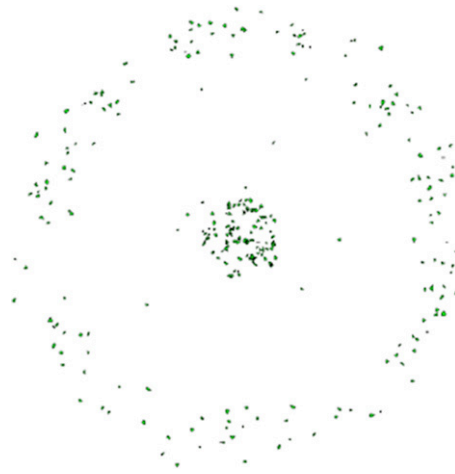
**Fig. 3.** Corresponding pattern of the nutrient distribution. The parameters are the same as in Fig. 2.



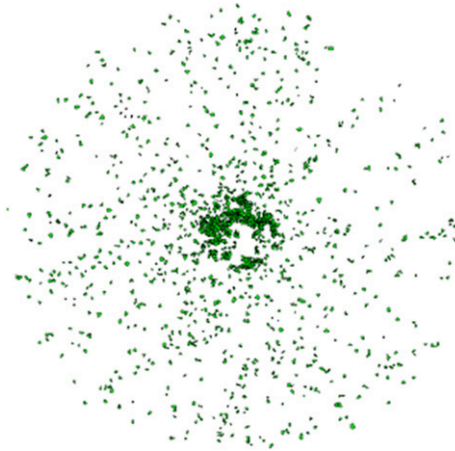
**Fig. 4.** Simulated pattern of bacteria distribution obtained with parameters:  $N = 8$ ,  $T_p = 0.4$ ,  $T_f = 6$ .



**Fig. 5.** Simulated pattern of bacteria distribution obtained with parameters:  $N = 8$ ,  $T_p = 0.25$ ,  $T_f = 6$ .



**Fig. 6.** Simulated pattern of bacteria distribution obtained with parameters:  $N = 8$ ,  $T_p = 0$ ,  $T_f = 6$ .



**Fig. 7.** Simulated pattern of bacteria distribution obtained with parameters:  $N = 8$ ,  $T_p = 0.01$ ,  $T_f = 6$ . The same pattern is obtained for  $N = 500$  (and all other parameters having the same values).

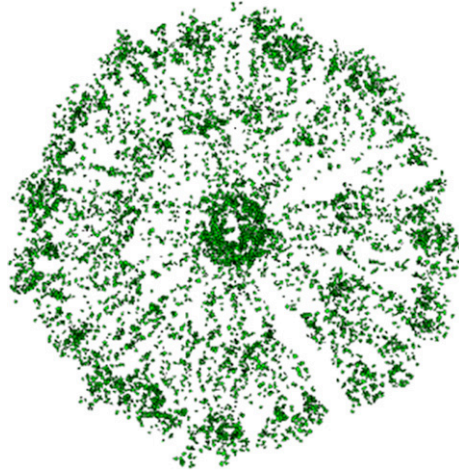
expanding ring with nothing inside, cf. Fig. 6. As bacteria do not proliferate at all in this case, the nutrient are not consumed at all outside the center.

Next we increase the amount of the nutrient somewhat, keeping a soft medium. The two cases (Figs. 6 and 7) remain the same for rich nutrients. If we keep the nutrient abundant and increase the medium hardness slightly we observe the appearance of ring-like structures, cf. Fig. 8. This concentric ring structure is manifest in Fig. 9: it exists for intermediate medium hardness (and nutrient abundance). In Fig. 10 we present another transitional case where we keep the medium nutrient-rich and increase its hardness somewhat. The ring structure is still discernible but the colony starts to fill-out because of the impeded migration. Fig. 11, finally, represents the case of a hard medium with nutrient abundance. In this case the parameters of the model correspond to absence of migration and the colony grows in size due only to proliferation. Thus by adjusting the parameters of the cellular automaton it is possible to obtain migration patterns of the colony which cover all the regions of the morphological diagram including the transitional regions.

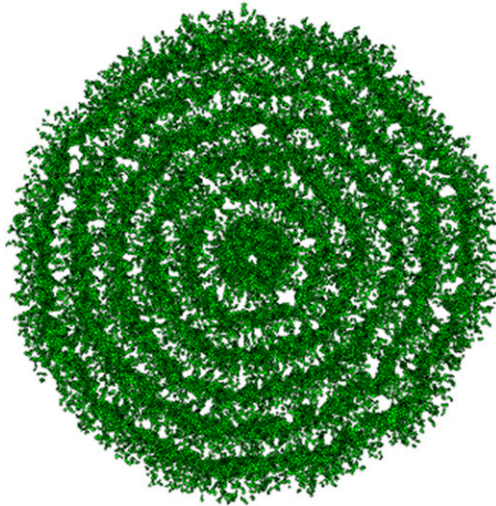
### 3. Discussion and outlook

In this paper we have introduced a cellular-automaton model for the description of the pattern observed during the growth (and migration) of colonies of *Bacillus subtilis*. We have shown that it is possible, with the appropriate choice of parameters to reproduce the totality of the morphological diagram of the colonies of this bacterium.

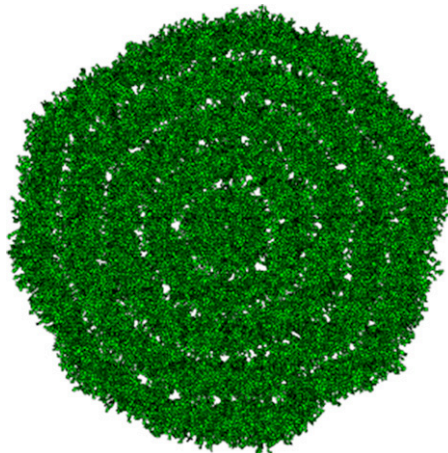
Most modelling approaches in this domain are based on (partial) differential equations. While, as we have shown in Ref. [11], it is possible to introduce extremely simple differential models which still manage to capture the essential dynamical behaviour of the bacterial colonies, no approach is simpler than that of the cellular automaton. One has just to introduce a geometry (typically a lattice on which the evolution takes place) and simple automaton update rules which



**Fig. 8.** Simulated pattern of bacteria distribution obtained with parameters:  $N = 500$ ,  $T_p = 0.15$ ,  $T_f = 6$ .



**Fig. 9.** Simulated pattern of bacteria distribution obtained with parameters:  $N = 500$ ,  $T_p = 0.4$ ,  $T_f = 6$ .



**Fig. 10.** Simulated pattern of bacteria distribution obtained with parameters:  $N = 500$ ,  $T_p = 0.7$ ,  $T_f = 6$ .

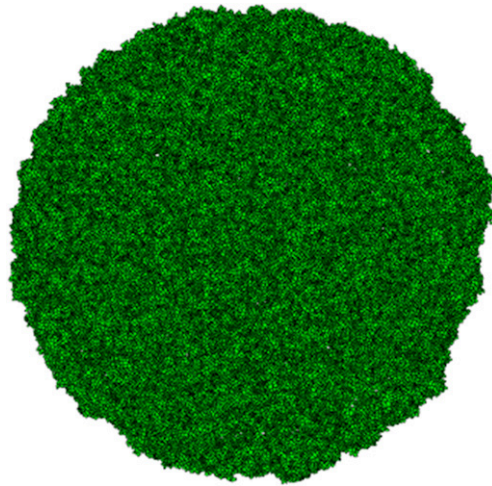


Fig. 11. Simulated pattern of bacteria distribution obtained with parameters:  $N = 500$ ,  $T_p = 1$ ,  $T_f = 6$ .

simulate the dynamics. Moreover in an approach such as ours (where each site of the automaton can be occupied by a single bacterium) the advantage of the automaton is that it offers an immediate visual information of the spatial distribution of the bacteria. Of course the automaton description is not without drawbacks.

First, the individual treatment of the elementary entities (here the bacteria) constrains the practical application of the model to the consideration of moderate populations. A consequence of this is the low density of the system and moreover (as explained in the previous section) the large variations of the local densities: when a site has just a few neighbours, a (dis)appearance of a single bacterium on a given site may result to a substantial change to the local density. Of course, we could say that one elementary unit does not correspond to one bacterium but is rather a mesoscopic entity that contains several bacteria (as, for instance, the “walkers” in Ref. [6] where each represents  $\approx 10^4$  bacteria). However, even if in this case the total number of bacteria is closer to a realistic one, the density would remain quantified, and the pattern would not be qualitatively different.

Second, the difficulty in working with cellular automata resides also in the extreme wealth of rules that one has at one’s disposal: choosing among all these possible rules requires particular care (as well as a dose of intuition). A consequence of this is that some rules may appear as ad hoc. It is thus necessary to discuss the biological relevance of the automaton rules.

### 3.1. Biological relevance of the automaton rules

The first point to be discussed concerns the fact that the migration and the proliferation phase duration  $T_m$  and  $T_p$  are fixed by an external clock. This could appear as an ad hoc rule; however it is motivated by biology: a fixed  $T_m$  could account for the finite lifespan of migrating cells. In the case of *P. mirabilis*, it has been shown that actively migrating cells, “swarmers”, have a finite lifespan (it is still unknown whether it is the same for all the swarmers or if there exists a distribution of lifespans). Swarmers are dividing cells which do not septate: they produce elongated and hyperflagellated cells which group in multicellular raft structures to increase migration efficiency. When they reach a maximum size, they break down into mononuclear cells [13,14] (for a review on the swarming process, see Ref. [12]). The fact that in our model migrating cells have a probability equal to  $T_p/T_m$  to produce inactive cells could account for the fact that active cells have a non zero probability to become inactive, during the migration phase. Several models have already taken into account this finite lifespan [4,9]. We assume that the mechanisms are the same for *B. subtilis* [35,30,37].

The definition of two periods inside the consolidation phase can be justified by the following experimental observation: when a colony of bacteria is inoculated on an agar surface, migrating cells appear only after a certain time lag, near the perimeter of the colony. Our first period (of duration  $T_p'$ ) corresponds to the production of non motile cells, whereas the second period (of duration  $T_p''$ ) corresponds to the production of active cells that will move in the next migration phase.

Another rule to be discussed is our assumption that at the end of migration time  $T_m$  the bacteria go back to state 2 with a probability proportional to  $T_p/T_m$ . If bacteria always go back to state 2 at the end of the migrating phase, rings disappear only in the extreme case where  $T_p = 0$ . The probability proportional to  $T_p/T_m$  ensures a smooth transition from the ring pattern to the diffuse one: as  $T_p$  decreases, the bacteria have fewer chances to go back to state 2 at the end of each cycle and thus have fewer chances to form a new ring. It should be possible to experimentally validate this assumption, by verifying if the concentration of remaining migrating cells at the end of the migration phase is positively correlated with  $T_p/T_m$ .

The next point is related to the fact that our bacteria proliferate and always migrate outwards: the new occupied site(s) must lie further from the center than the old one. For migrating cells, this rule seems natural, and there exists some experimental evidence for this [21]. For proliferating cells, this condition is necessary to create migrating cells in the second

period situated in the outer part of the colony (in experiments it is clear that migrating cells are located at the periphery of the colony). Finally, the fact that only cells in the proliferation phase consume nutrients is motivated by experimental observations. It is well established that active cell migration does not require exogenous nutrient sources [1,28,35] and it seems that the endogenous energy of the cells is sufficient to allow migration during a long time.

### 3.2. Discussion of the results

In the previous section we have seen that with realistic well-chosen rules, an automaton is able to qualitatively reproduce the whole morphological diagram of *B. subtilis*, cf. Fig. 1, even if, clearly, some of the patterns are better reproduced than others.

Fig. 2 obviously corresponds to region A in the experimental morphological diagram. One notices that our branches are thinner than the ones of the experiment. This is due to the fact that nutrients are rapidly exhausted in the inner part of the colony, and only branch tips stay active, cf. Fig. 3. Bacteria in the inner part of the colony thus stop proliferating very rapidly. This can be explained by the discrete structure of the model (experimentally, the inner part of the colony still continues to proliferate [28]). As in experiments, some branches inside the colony stop growing because other long branches around them exert screening effects by consuming all the nutrients. However, in our simulations, some branches with a lot of nutrients around them, stop growing without apparently any good reason. This is an artefact of the discrete structure of the automaton. When bacteria at the end of a branch tip are among the last to be updated, it may happen that nutrients in front of them are already exhausted. A larger amount of nutrients would solve this problem, but in this case, branches tend to fuse, contrary to what happens in experiments.

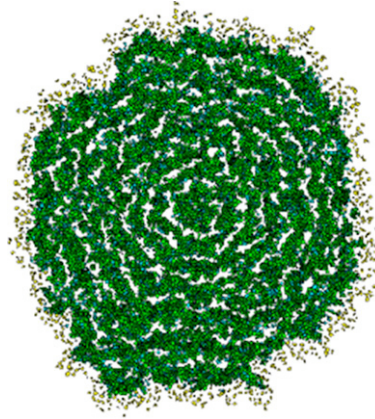
Fig. 4 in our model corresponds to region E, with a dense ray-like branching pattern. In our model, when the medium becomes softer, ray-like branches progressively fade thanks to the fact that when  $T_p$  decreases, the probability that migrating cells produce inactive cells, equal to  $T_p/T_m$ , falls to zero. In the limit case  $T_p = 0$ , we obtain an empty interior region in Fig. 6, that does not correspond to any region in the experimental morphological diagram. Again, this is due to the fact that the number of bacteria per site is 1 or 0 and thus the very-low density limit can be locally just 0. The experimental diffuse case (region D) corresponds rather to Fig. 7. What is interesting here is that our model provides a quite realistic (smooth) transition between regions E and D, which has been experimentally studied in Refs. [33,34].

In the case of rich nutrients, the qualitative patterns are quite well reproduced: the compact case, cf. Fig. 10 corresponds to region B and on a softer medium, Fig. 9 corresponds to region C. The fact that in our model migrating cells have a probability equal to  $T_p/T_m$  to produce inactive cells explains why there are a few bacteria in areas between successive rings: this corresponds to the experimental situation, where the density between rings is also not zero. Despite a very good qualitative agreement with the experimental pattern, our model does not account for the variation of the ring period with the concentration of agar that has been shown experimentally (for *B. subtilis* or *P. mirabilis*): the period of the rings increases when the concentration of agar decreases [28,35], even if the area of existence of the ring pattern in the *B. subtilis* morphological diagram is rather narrow. This is due to the fact that in our model the coarse graining of our automaton leads to too high a speed of propagation of the bacteria front due to proliferation compared with the one due to migration. So when the migration time increases, the period of the rings does not increase in parallel. In the case of a soft medium, rings disappear stochastically since at the end of migration time the bacteria go back to the proliferating state with a probability proportional to  $T_p/T_m$ . The hard medium case of Fig. 10 shows that the transition from the ring pattern to a compact pattern occurs not by a decrease of the ring period but by a filling up of the low density areas between terraces. We expect to address this difficulty through the introduction of a density-dependent model.

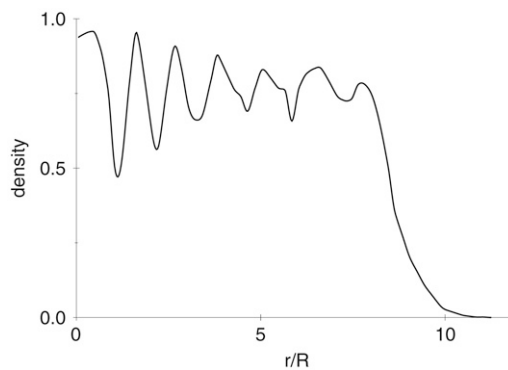
### 3.3. Towards an automaton with density thresholds

The strategy adopted in this paper was to control the proliferation and migration phases through the choice of characteristic times. However it would have been more natural to stay closer to what is happening in a realistic situation and consider the information available to the bacteria. Obviously each of them can sense what is happening in its immediate vicinity. Thus we expect the behaviour of the colony to be regulated (apart from the ubiquitous influence of the nutrient availability) by the density of the bacteria. Thus the thresholds for the switch from one behaviour to the other could be expressed in terms of the local density (depending strongly on the hardness of the environment). Indeed, it has been shown in experiments with *B. subtilis* and *P. mirabilis* that the lag phase time depends on the initial cell density, and that there is an upper density threshold where migration phase starts [28,35,30,37]. For *Proteus mirabilis* this can be explained by the fact that only swarmer cells in contact with other cells can form multicellular parallel arrays (“rafts”) that are capable of translocation over surfaces with high agar concentrations, by increased fluid retention [13,14,36]. In parallel, even if further investigations are needed to clearly confirm it, experiments strongly suggest the existence of a low density threshold that determines the end of the migration phase [7,30,37].

This is why it would be interesting to develop a new model where the migrating and the consolidation phases are entirely determined by density thresholds (cf. below) [35,30,37]. Despite a different detailed behaviour, we can reasonably assume that the physical principles are the same for *Bacillus subtilis* and *Proteus mirabilis*, a view supported also by the statement of Yamazaki et al.: “We expect that there exists a universal mechanism of the bacterial colony growth into concentric ring



**Fig. 12.** Simulated pattern of bacteria distribution obtained with the two density threshold model.  $N = 500$ ,  $T_f = 200$ .



**Fig. 13.** Density profile corresponding to the two density threshold model.

patterns irrespective of bacterial species" [37]. There already exist continuous models based on cell densities for *Proteus mirabilis* and for *Bacillus subtilis*, with thresholds [4,8,9,17] or not [20,22]. Our approach is to keep the automaton approach that has the advantage to involve a restricted number of parameters, once the correct rules have been defined.

Instead of proliferation time  $T_p$  we introduce a (high) density threshold: when the density of the bacteria exceeds this value locally, proliferation stops and the bacteria enter in a migration phase. The high density threshold has been set here to "all the neighbours are occupied". Unfortunately due to the discrete (and sparse) structure of our model it is not very easy to introduce a low-density threshold (which would control the migration phase). As a matter of fact as soon as migration starts the bacteria (in our model) are thinly spread and the number of neighbours in that case is either 1 or 0. A low-density criterion would necessitate taking mean values over a large region in order to be meaningful, and, by the same token, ceasing to be a local one. Still, surprisingly, a low-density threshold of 0 turns out to be sufficient in order to reproduce (coupled to the adequate high-density one) the appearance of concentric rings. In Fig. 12 we present a very preliminary result of this approach. One clearly distinguishes the ring structure by visually assessing the distribution of the bacteria. This structure becomes even more evident when one plots the density of the bacteria along the radius, cf. Fig. 13. Thus the density criterion manages to capture the concentric ring feature without any ad hoc assumptions. We intend to return to the exploration of this approach in some future work of ours.

Extending our model to other bacterial species should not present particular difficulties. As we have shown in Ref. [11], it is interesting to study in parallel species with different morphological diagrams. What is perhaps even more interesting is to make our model more "quantitative". The only way to achieve this is by being able to overcome the low density constraint, by allowing every site of the automaton lattice to be occupied by more than one bacterium. This would allow us to attempt comparisons with experimental data (although the visual assessment of the migration pattern might perhaps be less straightforward). The advantage of such a model of migration and proliferation involving a substantial number of individuals is that it can be applied to various domains of biological modelling. Malignant brain tumours, and especially glioblastomas, which are well-known to be invasive (as well as proliferating) are systems which would lend themselves to our approach, with the appropriate modifications of the automaton update rules so as to take into account the specificities of the tumoural migration.

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