Influence of Nutrient Limitation on Bacterial Patterns: Applying a new Bacterial Cellular Automaton Growth Model

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***Abstract*— The nutrient concentration greatly influences the formation of various colony patterns generated by bacterial populations. We consider a 2D cellular automaton growth model of bacterial colony in the case of nutrient limitation. The present cellular automaton simulates the growth process in order to obtain these patterns in the case of random inoculation. We show that numerical patterns are close to those experimentally observed in the literature.**

Keywords-Cellular automaton; bacterial colony; nutrient limitation.

I. INTRODUCTION

Bacteria predominantly live in surface-associated communities [1]. They develop at any interfaces that are suitable for microbial growth. Important examples where bacteria develop are teeth [2], waste water treatment [3], problem of biocorrosion [4]. It is well known that these biological systems are combination of several behaviors of interacting individuals. These interacting individuals are able to produce higher-level patterns especially in the case of in-plane expansion of colonies.

In the case of random inoculation, the collaboration of bacteria has been recently studied in the case of *Pseudomonas Aeruginosa* [5]. This study shows the existence of important links between patterns and the competition between growth and nutrient access. By varying the nutrient access, several patterns have been observed such as: dense, labyrinth, worm-like, spots or small and big holes (see Figure 1). The experimental setting consisted in cultivating a biofilm on glass coverslip submerged in inoculated liquid medium. This study investigated how evolutionary competition among individuals affects colony patterns. The main contribution was to provide a formal link between higher level patterning and the potential for evolutionary conflict in social systems. The "worm-like" configuration is obtained at the beginning of the experience when cells begin to colonize the surface. The nutrient competition between cells is very important due to a limited substrate. The colony growth is therefore limited and small colonies form the biofilm. If we increase the substrate concentration, nutrient competition is less important between bacteria, growth becomes heterogeneous in space, circular colonies deform due to fingering [6] [5] and leads to the "labyrinth" configuration. Conversely, if the nutrient concentration is saturated, all bacteria have a nutrient access, the nutrient competition is lower, the colony growth is fast until reaching the "dense configuration".

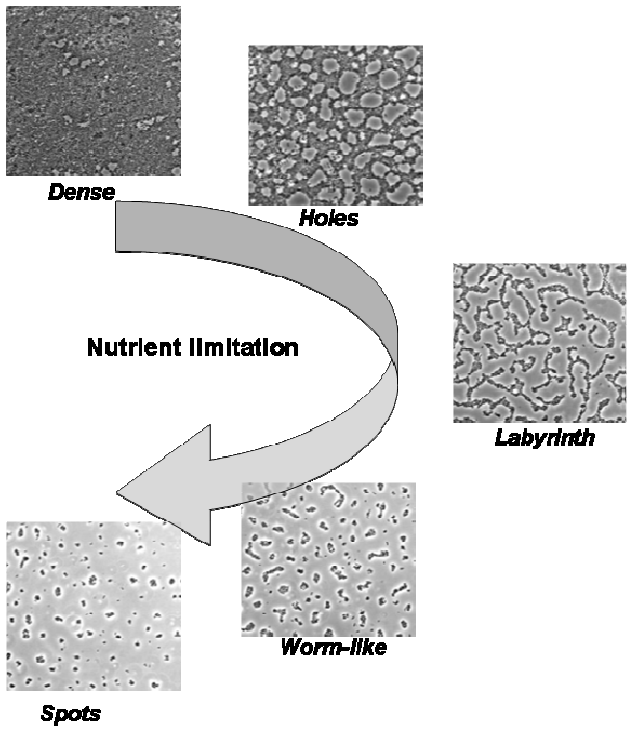

Figure 1. Different colony patterns depending on nutrient concentration [5].

Cellular automata are now commonly used in ecology research. This modeling approach is appealing because it represents directly the individuals and their behavior,

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making easier the link with scientific expertise about the ecosystem than in more abstract models. They show interesting spatio-temporal patterns that can be compared with observations. Several individual-based models have been used to model bacterial colony patterns in the case of in-plane expansion.

The aim of the present study is to include the competition phenomenon in the growth process and to highlight the advantages and the limitations of a such model. For this purpose, a simple cellular automaton based on the numerical and experimental observations is proposed in the first section by focusing on nutrient competition aspects. This model is then explored in the case of random inoculation and numerical patterns are compared to patterns experimentally observed in the literature. Results are analyzed so as to give new means about competition between growth and nutrient access and explanations on the emergence of higher-level patterns.

II. CELLULAR AUTOMATON OF BACTERIAL COLONY

Discrete modeling of bacterial dynamics has been developed using individual-based models or cellular automata [7]. They have been already used in several and various domains in order to simulate patterns and constitute an additional approach to the differential equation approach. Since 10 years, a lot of individual-based models of bacterial biofilms have been developed, mainly by the Delft team [7] [8] [9]. Individual-based model and cellular automaton have been developed in order to model bacterial patterns in the case of in-plane expansion. Here, we propose a very simple model of bacteria expansion that focuses on the growth process including a competition behavior with cooperation in order to obtain some of the patterns described in the introduction. The main originality lies in the fact that the nutrient dynamics is not modeled.

A. Spatial distribution of bacteria

In the cellular automaton growth model, each bacterium is only represented by its spatial coordinates (in 2 dimensions). Let *n* be the number of bacteria. The distribution of bacteria in the 2D space is given by the list $(x_i)_{1\le i\le n}$ of 2D positions.

B. Bacteria dynamics

Bacteria dynamics only includes a growth process. We assume that each bacterium cell has a constant probability *b* to produce a daughter cell during a time interval *dt*. Moreover, we had a competition function $C(d(x'))$ in order to take into account the competition behavior. For this purpose, we consider a bacterium *i*, located at *xi*, that gives birth to a new bacteria, located at x' such as to maximize the probability $B(x_i, x')$ as follows:

$$B(x_i, x') = b\omega_1(x' - x_i) \times C(d(x')) \quad (1)$$

This process represents the probability to a bacteria, located at x_i, to give birth to a new bacteria, located at x', that is dispersed following the kernel ω_1. This dispersion kernel allows us to model the cell spreading on a surface which is commonly observed in the literature [5] [10]. The second term $C(d(x'))$ represents a competition function. The aim is to propose a relevant competition function $C(d(x'))$ based on the numerical observations performed in Section 2. This competition function depends on the local bacterial density $d(x')$ of the new bacteria, defined as follows:

$$d(x') = \sum_{i=1}^{N} \omega_2(x_i - x') \quad (2)$$

where ω_2 represents a competition kernel. The ω_i -function is based on circular uniform kernels parameterized with σ_1 and σ_2 as follows:

$$\begin{cases} \omega_i(x'-x) = \dfrac{1}{(\pi\sigma_i)^2}, |x'-x| \le \sigma_i \\ \omega_i(x'-x) = 0, |x'-x| > \sigma_i \end{cases} \quad (3)$$

The function $d(x')$ takes into account the influence of other bacteria in the growth process. The function ω_2 enables us to model a nutrient competition due to a low diffusion coefficient through the value of σ_2. Indeed, if we consider a reaction-diffusion model, the distance between two bacteria affects the bacteria growth, especially in the case of a low diffusion coefficient of the nutrient. The influence of the nutrient diffusion coefficient process can be represented by the value of σ_2. In an experimental point of view, this competition process is observed for example in the DLA-like patterns [11]. The idea is to model this competition term with a simple function. This competition function $C(d(x'))$ has to have the following properties:

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$$\begin{cases} C(0) = 1 \\ \lim_{d(x')\to 1} C(d(x')) = 0 \end{cases} \quad (4)$$

Furthermore, the function has to have a parameter that enables us to tune the intensity of the competition due to the nutrient concentration. Different functions can be used such as exponential or polynomial We have chosen the following polynomial function:

$$C(d(x')) = [1 - d(x')]^n \quad (5)$$

where *n* enables us to control the intensity of the competition process. This function has been plotted on Figure 2.

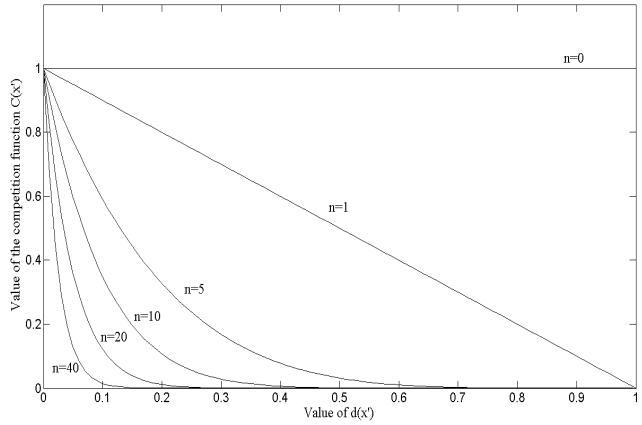

Figure 2. Value of the competition function $C(d(x'))$ following the number of bacteria in the local environment $d(x')$.

In the case of $n = 0$, the competition term $C(d(x'))$ is equal to 1, leading to no competition. It leads to have a classical growth process where the nutrient concentration is very important (limit case). For $n > 0$, the competition term $C(d(x'))$ tends toward 0 for a high bacterial density in the local environment of the potential new bacteria. In this case, the bacteria become "`non active"'. When there is no bacterium in the local environment of the potential new bacteria, the competition term $C(d(x'))$ is equal to 1, that is to say there is no competition. As explained above, *n* enables us to tune the intensity of the competition (see Figure 2). For high values of *n*, the competition is very important. It leads to slow down the growth of the bacterial population and to simulate bacterial behaviours observed in Figure 1.

Finally, the lower the value of the competition function $C(d(x'))$, the higher the competition, the lower the value of the probability $B(x_i, x')$. It leads that new bacteria has a higher probability to be located in a new place where there are few bacteria.

The cooperative aspect of bacterial biofilm has been described in [5] [12]. This cooperation is simply taken into account through the maximization of $B(x_i, x')$: bacteria mechanically push the new bacteria where the environment is the most favourable. It enables us to model the mechanical pressure with a preferential direction (where the competition is the lowest). Losses in biomass are not considered because we consider that this phenomenon can be neglected in this phase of growth. Some simulations have been done (not reported in this paper) and have shown that losses in biomass influence the density of bacteria and the dynamics but not the obtained patterns in a qualitative point of view. The aim here is to propose a new growth process (including competition behavior) and to show that we can obtain patterns observed in the literature with the proposed growth process.

C. Implementation

The current model depends on 4 variables: *b*, σ_1, σ_2 and *n*. A cellular automaton has been implemented using a 200x200 µm2 grid. The model was implemented in Matlab (7.2) for Windows with the following operations:

1. initialization of a population of *N* bacteria randomly located. The distribution of bacteria in the 2D space is given by the list $(x_i)_{1\le i\le n}$ of 2D positions. Go to step 2.1;

2. growth process:

2.1. initialization of index *i* (*i*=1). Go to step 2.2;

2.2. the value of $B(x_i, x')$ is calculated for all possible locations x' following the process defined above. Go to step 2.3;

2.3. the location x_m that maximizes the probability $B(x_i, x')$ is chosen. If there are several locations that maximize $B(x_i, x')$, the location x_m is randomly chosen. Go to step 2.4;

2.4. a random number α is chosen in the range [0,1]. If the value of α is inferior to the value of $B(x_i, x_m)$, go to step 2.5. Otherwise, go to step 2.6;

2.5. a bacteria located at x_m is added;

2.6. if $i < N$, advance bacterial index i and go to step 2.2. If $i = N$, go to step 3;

3. advance time and go to step (2) with the updated bacteria distribution.

III. RESULTS

In this section, we have computed the spatial patterns for different values of the parameters. However, we have decided to fix the value of σ_1 (σ_1=1µm). It seems to be unrealistic to have a higher value of σ_1. Moreover, if we increase this value, spatial structures tends to be uniform. We have chosen b = 1 bacteria.t^{-1}, here. Note that for low values of b, spatial structures don't change but execution times increase. In the following, we started simulations from an initial state that represented a uniform inoculation with individuals, placed at random locations. The initial density of individuals is equal to 1% of the domain. We have tested different values of n and σ_2 and highlighted their influence on the competition process: n = (1, 5, 15, 25) and σ_2 = (4, 6, 8) µm. Simulations have been stopped when the increase of individuals between two time steps is inferior to 0.1% in order to have a quasi-steady state. Results are plotted on Figure 3. Comments are:

- influence of n: n is directly linked to the competition between individuals. The n-parameter increases this competition and leads to a decrease of the number of individuals. When n=0 (not reported here) or 1, the competition is weak and the distribution of the bacteria is uniform. Then, when n increases, labyrinth appears and for high values of n we have worm-like configurations. These results are concordant with the observed patterns (see Figure 1): no competition leads to a dense and uniform configuration; important competition leads to worm-like patterns and the labyrinth pattern corresponds to the intermediate case;
- influence of σ_2 : σ_2 influences the competition distance. When the value of σ_2 is low, the competition between bacteria is not important. It leads to labyrinth with small voids. On the contrary, when the value of σ_2 is high, large voids are observed within the labyrinth;
- influence of the competition on the bacterial density ρ: we can see that configurations are directly linked to the final bacterial density. Indeed, the steady state depends on the competition between individuals and on the number of individuals, that is to say the bacterial density. When the competition is not important (n=5), bacteria can grow and we have a high steady bacterial density. On the contrary, when the competition is important, the growth is slowed down by the competition leading to a lower steady density. From a qualitative point of view, we have compared on Figure 7 the final density calculated from the simulated patterns and the density calculated from the experimental patterns. It clearly shows that we have a same qualitative correlation between densities and patterns;
- main models enable us to converge to a steady state using losses in biomass. The current model leads to a convergence of the bacterial density with the use of a competition term in the growth process. We can see that patterns are directly linked to the final bacterial density (see Figure 5).

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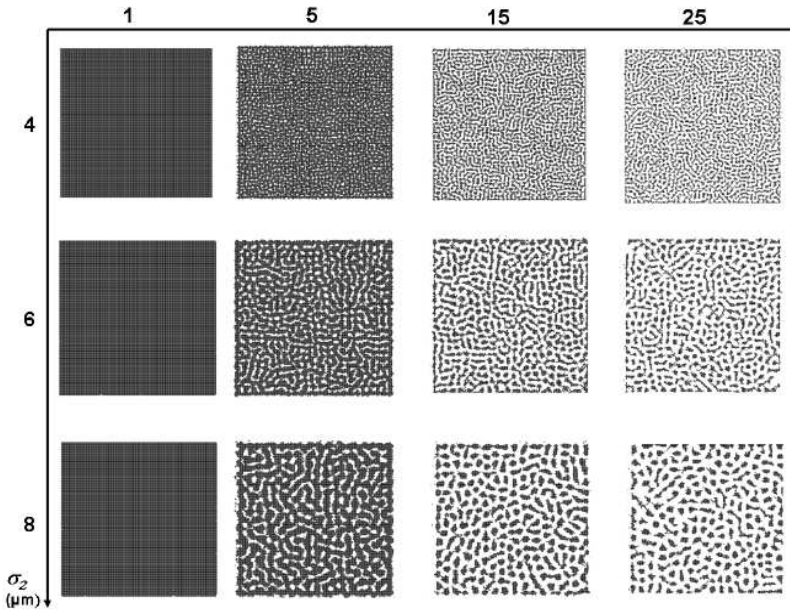

Figure 3. Colony patterns for different values of *n* and σ_2 .

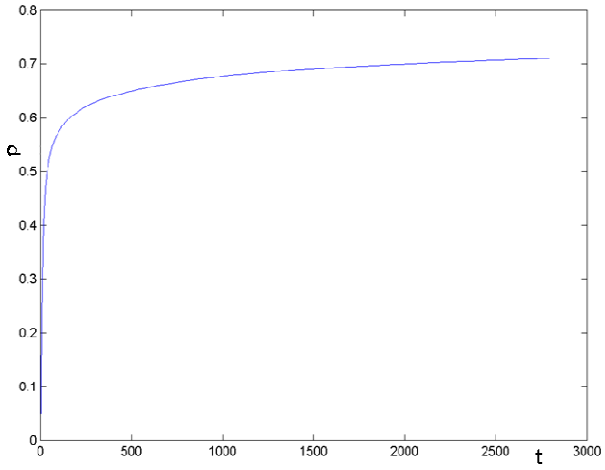

Figure 4. Evolution of the density ρ with respect to the time.

IV. CONCLUSION

Since twenty years ago, patterns of expansion produced by bacterial populations have experimentally been highlighted in the literature. Different models have been proposed in the literature, mainly based on differential equations, in order to simulate these patterns. A growth process including competition has been proposed in this paper that has been implemented in a cellular automaton. The competition aspect is taken into account by the calculation of a local bacterial density that is weighted by a polynomial function. Results have shown that this model enables us to obtain observed patterns in the case of random inoculations. This model leads to steady states with the use of the competition term in the growth process. Results have also shown that the obtained patterns are linked to the final bacterial density. Finally, this growth process (with a competition term) can be used in more complex models so as to take into account competition.

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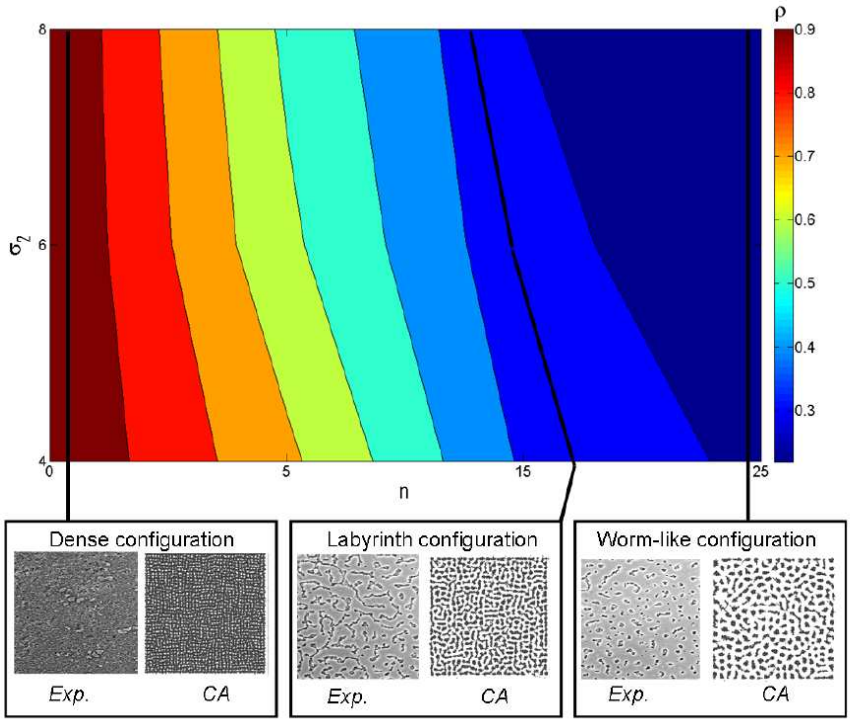

Figure 5. Steady state density ρ following *n* and σ_2: "`Exp"' corresponds to the pattern obtained experimentally in [5]; "`CA"' corresponds to the pattern obtained with the cellular automaton.

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