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# Simulating a Rock-Scissors-Paper Bacterial Game with a Discrete Cellular Automaton

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**Abstract.** This paper describes some of the results obtained after the design and implementation of a discrete cellular automata simulating the generation, degradation and diffusion of particles in a two dimensional grid where different colonies of bacteria coexist and interact. This lattice-based simulator use a random walk-based algorithm to diffuse particles in a 2D discrete lattice. As first results, we analyze and show the oscillatory dynamical behavior of 3 colonies of bacteria competing in a non-transitive relationship analogous to a Rock-Scissors-Paper game (Rock bacteria beats Scissors bacteria that beats Paper bacteria; and Paper beats Rock bacteria). The interaction and communication between bacteria is done with the quorum sensing process through the generation and diffusion of three small molecules called autoinducers. These are the first results obtained from the first version of a general simulator able to model some of the complex molecular information processing and rich communication processes in synthetic bacterial ecosystems.

**Keywords:** bacterial computing, cellular automata, lattice-based simulation, rock-scissors-paper game, quorum sensing, particle diffusion, autoinducers.

## 1 Introduction

This paper describes: (1) a lattice-based simulator used to model the diffusion of particles over a two dimension grid and (2) the results obtained modeling the cyclic dominance behavior of 3 colonies of bacteria communicating through quorum sensing signals. This simulator is used in BACTOCOM project [1]. The simulator is used to model the diffusion of the so called autoinducers (small molecules generated by bacteria when they sense they are in a high density). These autoinducers are the information carriers used by bacteria to take decisions by majority (by quorum) in a communication and decision making protocol called quorum sensing [6]. Biologists know the precise biological hardware used by bacteria to generate and to sense autoinducers. And synthetic biologists try to program artificial complex communication protocols in synthetic bacterial ecosystems. We try to model and to simulate natural computations made by bacteria with artificial/digital computations.

The design and study of engineered complex bacterial behavior has been a constant issue in synthetic biology. There are already synthetic bacterial colonies interacting through quorum sensing signals and competing in a predator-prey way [3], as well as colonies of bacteria forming bright complex patterns via quorum sensing [5] or competing in a rock-paper-scissors game [4]. Our purpose is to model, simulate and engineer sociobiological behaviors as cooperation or competition between bacteria. A simulator able to model and predict these complex behaviors would be an interesting aided design tool for microbiologists.

There exists many particle diffusion simulators, but we had some conditions to be followed. First of all, we should be working on a 2D Cellular Automata (CA) that would represent the Petri dish where bacteria will be living in. The reason behind the choice of working with a 2D CA is because our bacteria growth in a Petri dish with enough nutrients at the bottom. These bacteria growth in 2 dimensions. Third dimension (the formation of a biofilm) it's not necessary to model our bacteria environment. We also choose to work with discrete space and discrete time. All the events performed in the simulator should be executed in an asynchronous way to be more realistic.

There are some previous simulators of biofilm (colonies of bacteria in 3 dimensions) growth, like [12] which was one of the first bacterial simulators developed, or [13] and [14] which both of them are individual-based simulators, one using a discrete approach and the other one using a continuous space approach. Other simulators like [11] uses particle concentration to simulate the diffusion process or [9] which introduces a hybrid simulator combining two approaches: cellular automata and reaction-diffusion equations. Our simulator has been inspired from other previous simulators like BacMIST [10] and JCASim [7], [8]. BacMIST uses the idea of random walks to simulate the diffusion process and introduces the concept of "diffusivity" which allow the particles to be spread in the environment with more or less ease, depending on the nature of the particle or the viscosity of the media. JCASim introduces a discrete simulation of diffusion with the so called Block-Cellular-Automata (Block-CA). This diffusion algorithm will be explain in detail in the next section.

## 2 Description of the Simulator

In this section, the algorithms (emission, diffusion and degradation of autoinducers) inside the simulator will be explained.

The simulator is based on a 2D discrete cellular automata that represents the environment (a Petri dish) where the bacteria are placed. Each of the sites on the grid, can be filled with at most one bacteria and one autoinducer particle. In each time-step, among all the bacteria in the grid, one will be selected to perform an event in a probabilistic way (this is what it's called an iteration). This event could be reproduction, conjugation (transmission of DNA circular strands between a donor bacteria and a receiver bacteria) or autoinducer emission. Each event has its own conditions: reproduction can take place only if there is at least one empty site in the neighborhood, and conjugation takes place only if there is a receiver

and a donor in the vicinity. The general description of the simulation workflow is as follows.

*Quorum Sensing Simulator Workflow*

*Initialization:* Select the size of the grid and define initial values of the parameters (sigmoidal function rate, quorum sensing threshold, conjugation rate, diffusion rate autoinducer, decay rate autoinducer).

- **Step 0:** Input. Situate an initial colony of 3 bacteria on the grid (in this initial version of the simulator prototype the initial colony can only be formed by 3 different bacteria situated in the centre of the grid in a well-mixed manner or spatially separated at a short or large distance). These 3 bacteria emit 3 different autoinducers and form a Rock-Paper-Scissor bacterial game. Rock autoinducers repress light emission in Scissors bacteria, Scissors autoinducers repress light emission in Paper bacteria and Paper repress Rock light emission.
- **Step 1:** FOR ALL bacteria in the grid:
  - 1.1 Select an event randomly (reproduction or conjugation) and
  - 1.2. Calculate the number of Autoinducer (AI) particles in the vicinity of every bacteria and
  - 1.3 Depending on the value calculated in 1.2 and on the QS threshold parameter, decide the QS AI emission (emit or not an AI auto-inducer) and activate (or not) all other QS-dependent behaviors of the bacteria (in the prototype of the simulator only the light emission).
- **Step 2.** Increase time:  $t := t + 1$ .
- **Step 3.** FOR ALL AI particles: Apply decay function to decide which particles are degraded.
- **Step 4:** Diffuse all the AI particles
- **Step 5.** GO TO Step 1.

This QS simulator works in a synchronous way. All the bacteria in Step 1 must be selected before increasing time in step 2. All the AI particles must be degraded in Step 3 before applying diffusion in Step 4.

*Autoinducers Emission Process:* The process by which a bacterium  $x$  activates its autoinducers emission follows a sigmoid probabilistic function  $f(x, k) = 1/(1 + e^{-(K-m)/s})$ , where  $K$  is the number of autoinducers of the same specie in the neighborhood,  $m$  is a user customizable parameter (called Sigmoid function rate in the interface) and  $s$  is a fixed parameter which determines the function shape. By default,  $s$  has a value of 0.6 estimated by empirical test.

*Decay Process and degradation of AI particles:* All the particles in the environment follow an exponential decay probabilistic function  $f(t) = 1 - e^{-K*t}$ , being  $K$  the decay rate and  $t$  the variable time. As  $t$  increases the probability that a particle disappears tends to 1.

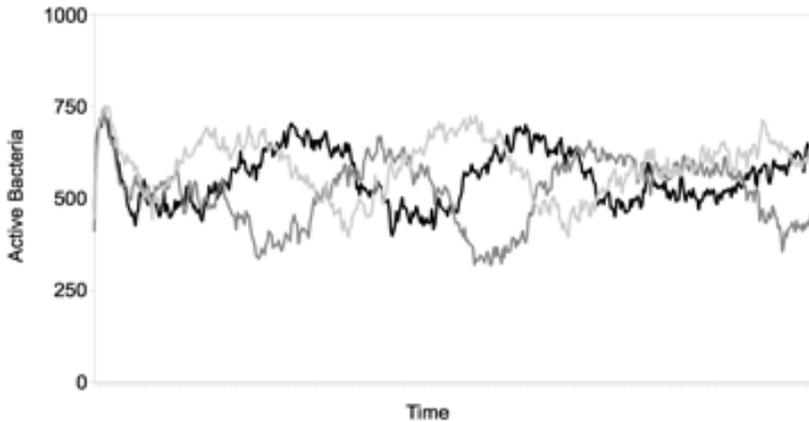
*Diffusion Process Algorithm:* We follow the approach called Block-Cellular-Automaton (Block-CA), which is an approach to simulate random walks in discrete cellular automata. This diffusion algorithm starts making a tessellation of the grid using square blocks with 9 cells. Then, the content of chosen pairs of cells inside those blocks are exchanged (or not) with a probability of  $\frac{1}{2}$  multiplied by the diffusivity rate of the media. This process is repeated 8 times with new pairs of adjacent cells selected in a new orientation (rotated clockwise).

### 3 Results

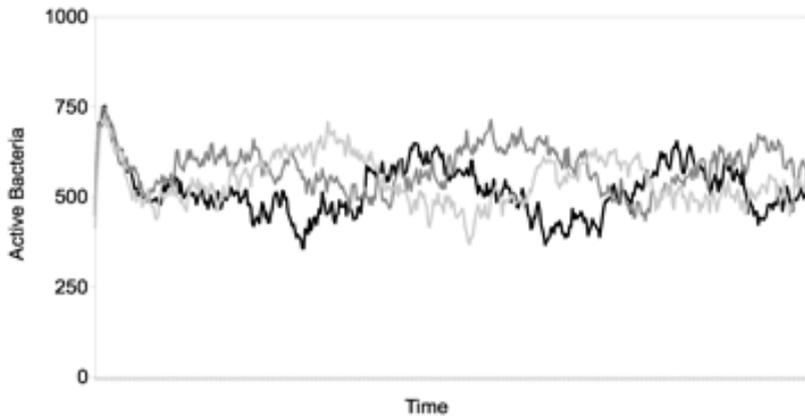
To test the correct behavior of the system and to see the effect of the diffusion of the autoinducer particles we run some simulations. We are interested in the dynamic behavior of interacting bacteria populations so we decided to simulate and replicate a Rock - Scissors - Paper (RSP) model. An example of this bacterial game is also described in the Southampton's work presented in the 2009 iGem contest [17]. The RSP model is a non-hierarchical competitive system performed by three different populations which usually compete for a resource. The competition is established by some simple rules: Rock crushes Scissors, Scissors cut Paper and Paper wraps Rock. In the Southampton's RSP model [17] they designed three types of an engineered bacteria which operates with different autoinducers. At the beginning of the experiment all the three bacteria are lighting and sending their own autoinducers. Using its own quorum sensing circuit, each population of bacteria will stop lighting and sending autoinducers if they detect a high concentration of their antagonist autoinducer. In other words, Scissors bacteria light emission will be stopped if they detect, via quorum sensing, a high concentration of Rock autoinducers, and so on. We wanted to see whether our simulator could or not replicate this cyclic dominance behavior. In the first simulation we made, all the bacteria were well mixed in the environment. The quorum sensing threshold that established if a bacteria becomes inactive was fixed to 0.3. That means, that there must be at least 3 particles of the corresponding population of autoinducers in its neighborhood to become activated. All the autoinducers in the simulation have the same decay rate (this establish an autoinducer half-life of 1386 iterations).

The next two figures show the number of active bacteria (bacteria emitting light) in each of the three populations (Fig. 1 and Fig. 2).

The difference between Fig. 1 and Fig. 2 is the diffusivity rate (propensity to diffuse). As one can observe in the figures, the oscillatory behavior is modified by the diffusivity rate. As the three populations are well mixed in the environment, they receive autoinducers from every population at the same time.

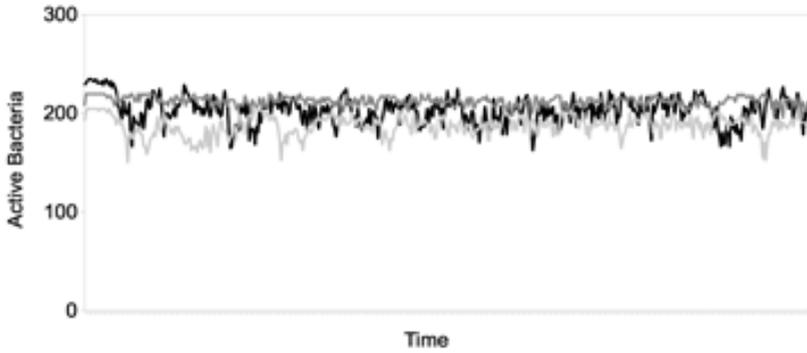


**Fig. 1.** Oscillations in the number of active bacteria following a Rock - Scissors - Paper cyclic dominance behavior. The diffusivity rate is 0.1 and the three bacteria are in a well mixed environment. A small diffusivity rate means that the movement of the autoinducer particles all along the grid is slow.

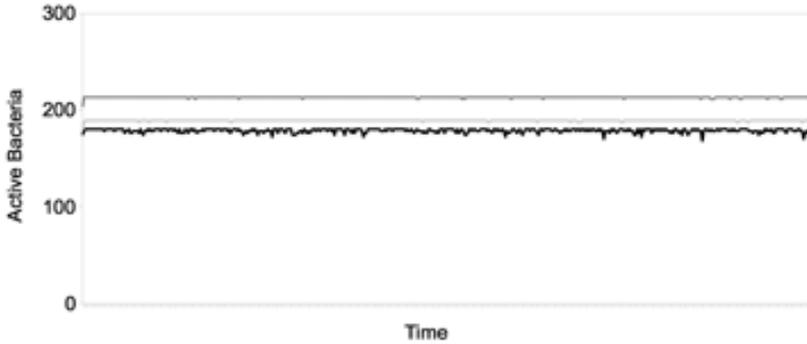


**Fig. 2.** Oscillations in the number of active bacteria following a Rock - Scissors - Paper cyclic dominance behavior. The diffusivity rate is 1 (autoinducer particles movement is faster). The system maintains the cyclic oscillatory behavior but with less amplitude in the oscillations.

In Fig. 3 and Fig. 4 the same simulation is performed but now the three bacteria colonies are not well mixed but spatially isolated one from the others. The RSP cyclic dominance is lost due to the distance and a low diffusivity rate of 0.1.



**Fig. 3.** The bacterial ecosystem is spatially separated and shows a soft oscillatory behavior but not Rock-Scissors-Paper cyclic oscillations. The bacteria are isolated and the diffusivity rate is 1.



**Fig. 4.** The bacterial ecosystem is spatially separated and shows almost no oscillations in the number of active bacteria. The bacteria are isolated and the diffusivity rate is 0.1.

## 4 Conclusions

This paper describes the results obtained after the design and implementation of a new cellular automata simulating particle diffusion on bacterial ecosystems. To achieve the main goals established, a random walk-based algorithm has been used to diffuse particles in a virtual 2 dimension environment. A particle decay and a bacteria degradation process has been implemented following an exponential function. Some others functionalities has been implemented, like bacteria autoinducers emission or behavior activation via quorum sensing which allows the bacteria to perform many different events as for example, light emission.

The next step we plan to develop with this simulator is to combine it with other simulator modules that our research group has been working on. With that improvement, we will be able to simulate and study more complex bacterial ecosystems and its sociobiological interactions [15].

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