Agent-based modeling of bacterial quorum sensing

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Theses of the PhD Dissertation

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1   Introduction

Unicellular organisms such as bacteria most often live in multi-species consortia. Living in a consortium may be advantageous for all participating species when symbiosis can help them to use environmental resources to attack host organisms more effectively. These populations can stably coexist; however species of the consortia are competing with each other for environmental resources – such as nutrients or space. This competition may lead to spatial segregation, or even to the extinction of some of the species.

The study of bacterial communities is an important research area where laboratory experiments, genomic-, and computer simulation studies have only recently started. The information that we can obtain by these approaches will certainly help us to deeply understand the mechanism of bacterial infections in plants, animals as well as in human diseases.

Members of complex bacterial communities can communicate and cooperate via the exchange of chemical signaling molecules and public goods. A well-known form of this mechanism is called quorum sensing (QS). Sensing the concentration of chemical compounds enables bacteria to monitor their own population density and decide whether the population is big enough for achieving a certain task ("quorum" means the minimum number of individuals necessary to take a decision). In many Gram-negative bacteria the QS mechanism is regulated by a gene pair – the *luxI* and *luxR* genes – that forms a positive feedback loop, usually called the "autoinduction circuit". Signal molecules are synthe-

sized by a LuxI-type protein, and above a critical concentration within the cell, the signal molecule binds a LuxR-type protein. This complex in turn acts on target genes that affect a variety of cellular processes including the up-regulation of the movement, metabolism or division of cells. The most important effect however is the production of the so called „public goods", for instance surfactants, rhamnolipids, digestive enzymes, that can make nutrients digestible which is a typical cooperative phenomenon. The role of the *luxI* and *luxR* genes can be studied conveniently using deletion mutants. Cells in which the *luxI* gene is deleted will not produce the signal, so they are termed "signal negative" or "SN". SN mutants can respond to signal molecules produced by other bacteria, by producing public goods. In other words, SN cells cannot communicate but can cooperate. On the other hand, cells in which the *luxR* gene is deleted cannot respond to the signal either by increased signal production or by producing public goods. These mutants are termed "signal blind" or "SB" since they neither communicate nor cooperate. It is important to realize that all deletion mutants have an energy advantage as compared to wild type cells, the advantage being greater for SB than for SN species.

Our group's research area is the study of stability in bacterial communities using computational models. Our long-term goal is to understand the principles underlying the stable coexistence of bacterial species and to explain as much as we can the laboratory experiments. In this project my role was to improve an existing agent-based model to simulate systems with more than one signal and model the results of laboratory experiments.

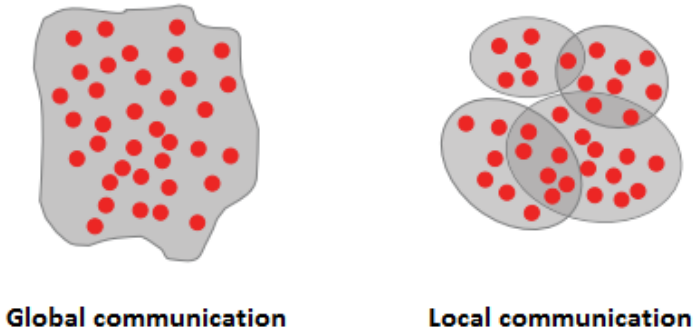**Global communication**   **Local communication**

Figure 1.1: Differences between global and local communication.

The simulations in this work suggest that consortia that can efficiently communicate and cooperate locally can easily emerge among bacteria capable of quorum sensing. Interestingly, a community that is stable at a certain set of parameters can be invariably destabilized if the movement of the agents i.e. the mixing of the community increases above some critical level. This phenomenon makes me believe that local communication and cooperation, as opposed to global interactions, are important for stabilizing communities and the same concept can provide an explanation for the apparent resistance of a colony against deleterious mutants. Differences of local and global communication are shown in Fig. 1.1.

2 Methods

Our goal was to model bacterial communication and cooperation, the so called quorum sensing.

We developed an agent-based model where bacteria are represented as agents and the spreading of chemical molecules (nutrient, signal and factor) in the environment is described by reaction-diffusion equations. We defined a discrete state model where agents can switch between the inner states according to the actual concentration of signal and factor molecules in the environment. This behavior results in the synchronization of bacterial inner states.

We developed two types of environments, the so-called open and closed models that are different in the description of the surface. The open model in our case means a longitudinal, cylindrical surface open on one end. The surface is divided into cells in which the concentration of the solutes (signal, factor and nutrient) levels is the same. The diffusion of these solutes happens between the cells. In the closed system the surface is toroidal, and the concentration of the nutrient, signal and public goods is constant throughout the entire surface.

We used Matlab programming language for the simulations. For larger simulation tasks when we needed higher efficiency we used a Linux cluster at ICGEB (International Centre of Genetic Engineering and Biotechnology), Trieste where we could run 20 simulations parallel that made it possible to speed up the execution of our simulations where we tested large parameter spaces.

3   New scientific results

Thesis 1.: Methodological developments.

Related publications of the author: [1, 2]

I developed novel methods in order to improve our agent-based model in such a way that allowed us to make large numbers of simulation experiments, and to process and visualize the results in an effective manner.

1.1. I developed a model where bacteria can communicate and cooperate via more than one chemical solute.

The original model of our group was built for simulating the growth of homogeneous bacterial colonies where bacterial agents were communicating with a single signal and cooperating with a single factor. We further developed this system to be able to handle more than one signal and factor. This made it possible to examine the competition of species that can "understand" each other.

1.2. I introduced visualization and numerical methods to evaluate results from large amount of simulations.

To examine the whole parameter space of multi-signal models we carried out a large number of simulations. To handle the results of these simulations first I introduced numerical methods that describe the spatial separation and stability of species and then I developed a visualization procedure based on 3D heat maps.
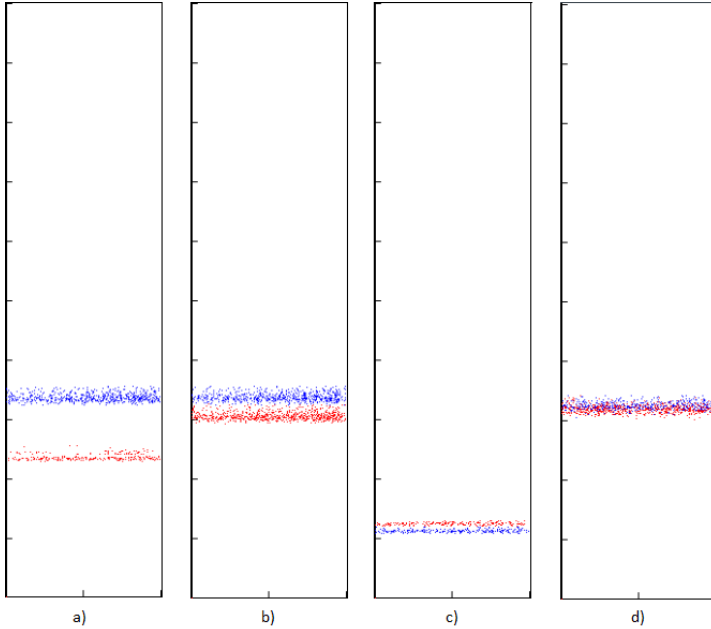
Figure 3.1: Equilibrium populations obtained with different initial parameters of LJ and WCA potentials .

1.3. I developed a method for the description of bacterial movement by physical potential fields.

In the original model system agents move randomly and the number of agents has an upper bound in each spatial cell. I have introduced a model where the movement of bacterial agents is described by physical potential fields. The attractive-repulsive interactions within species are described by Lennard-Jones (LJ) potentials, the repulsive and neutral interactions between different species are calculated with

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Weeks-Chandler-Andersen (WCA) potentials. However this system is more realistic than the previous one but the most important results (homogenous and segregating populations, see Fig. 3.1) are the same in both of them.

Thesis 2.: I showed that the competition of bacterial populations in open and closed systems leads to different outcomes that can explain the seemingly contradictory lab results reported in the experimental literature.

Related publication of the author: [3]

Quorum sensing can be conveniently studied using SN and SB type deletion mutants described in the introduction in detail. These mutations are rarely found in natural environments. In experimental, shaken cultures both mutants seem to outgrow the wild type species, however, on open surfaces such as agar plates, only the SN species can form a stable colony with WT, but SB cells collapse the mixed population – where WT species goes extinct. With agent-based simulations we showed that the difference is due to the open and closed nature of the systems. We concluded that a closed system can be easily saturated with signals and public goods (factors) that make quorum sensing unnecessary so in well-mixed cultures normally not viable species, such as SB, can proliferate. The four types of competition are shown in Fig. 3.2.
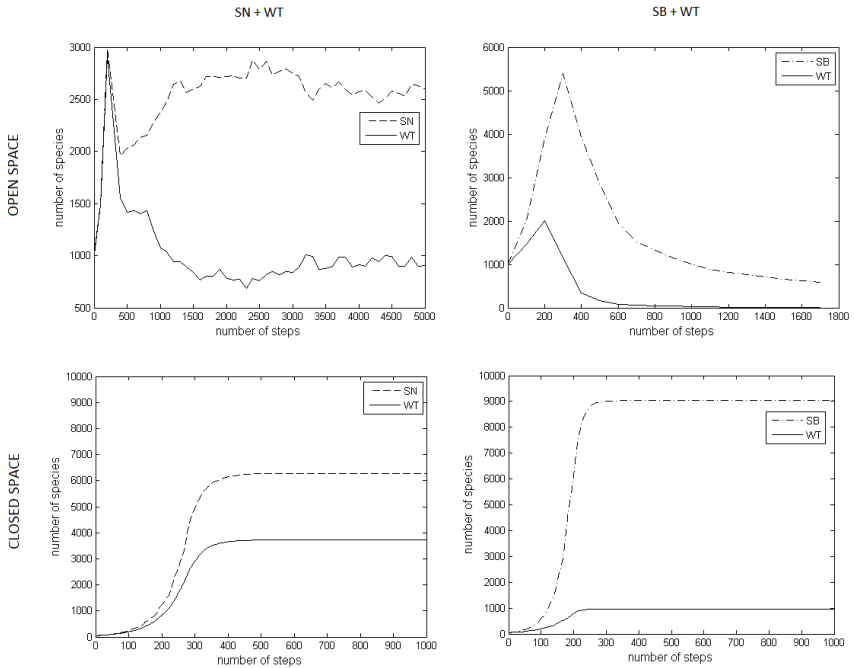
Figure 3.2: Competition of WT and mutant bacterial models in open (top) and closed (bottom) systems. The experiments were set up with an equal number of WT (solid line) and mutant (dashed line SN, dash-dotted lines SB) cells and the population size was plotted as a function of time.

Thesis 3.: I examined the competition of bacterial species that can share communicating and cooperating solutes resulting in a population where species can understand each other. I observed stable mixed populations can exist in a substantial
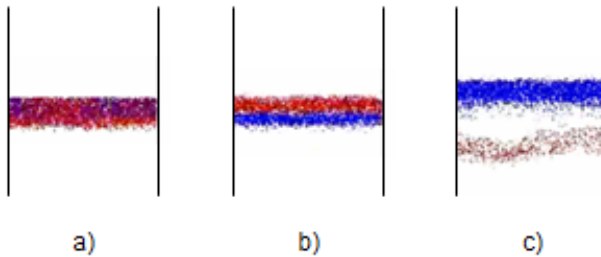
Figure 3.3: Observed patterns by competition of two species; a) mixed population, b) segregation, c) winning of one species.

part of the parameter space indicating that sharing of signals and public goods can facilitate the coexistence of species. Related publication of the author: [2]

An interesting phenomenon in bacterial colonies is that species are able to respond to signals released by other species and can utilize cooperative factors – like enzymes and siderophores – produced by each other. According to Gause's classical competitive exclusion principle only one of two competing species could stay alive. On the contrary, our simulations showed that communicating and cooperating species – i.e. species that can share signals and public goods to various extents – can form several types of stable communities like mixed or segregating colonies (Figure 3.3). Such stable communities exist in a significant part of the parameter space; however, in some of the cases we did observe competitive exclusion. Examples for the observed patterns are shown in Fig. 3.3 Results suggest that mutual communication and cooperation

can have a major role in the stability formation of bacterial colonies in nature.

Thesis 4.: I examined the behavior of binary communities where only one of the species can utilize signals and public goods of the other species. I found that unilateral eavesdropping always has a clear advantage over the eavesdropped species.

Related publication of the author: [2]

Many bacteria have signal sensing proteins (LuxR analogues) that are potentially able to sense external signals. Biochemical experiments show that these so-called solo LuxR proteins can detect signals produced by other species but it is hard to design experiments that prove the usefulness of this phenomenon. My results suggest however that unilateral sharing makes the "eavesdropper" always more viable than the eavesdropped species (Figure 3.4). This gives a potential explanation for the existence of solo *luxR* genes in nature since they allow a species to track and invade a population that produces the eavesdropped signals.

Thesis 5.: I demonstrated that by tuning the parameters of quorum sensing one can design situations wherein one of the species can parasite upon the other species without killing that.
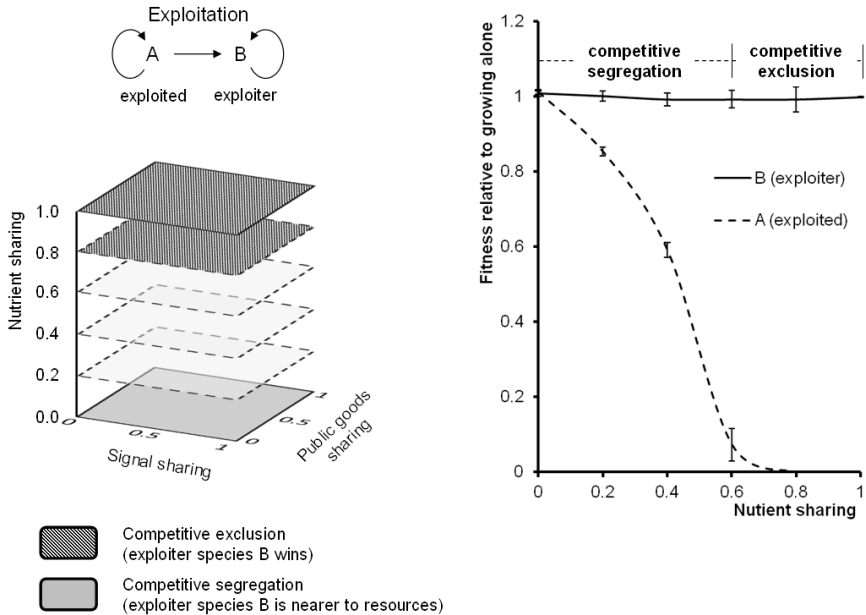
Figure 3.4: Species B exploits the QS system (signals, public goods) and nutrients of species A. Left: Regions of the parameter space represent either competitive exclusion or competitive segregation. Right: Fitness of the two species relative to growing alone, as a function of nutrient sharing.

A species is an efficient parasite or predator in nature if that can spread in a population without destroying it. Deletion mutants, especially the non-cooperating SB species are not optimal parasites because they easily collapse the population so that the wild type species goes extinct. By fine tuning the reaction thresholds of the QS systems we can

Table 1: Patterns observed by simulation of parasite species.[1]

Mutant	Alone	Competition[2]	Invasivity[3]
Parasite	small number	collapse	collapse
Parasite, signal+	small number	stable swarming	stable swarming
Parasite, factor+	small number	stable swarming	stable swarming
Parasite, 2. signal	small number	stable swarming	stable swarming

[1] "signal+" and "factor+" means species that react to higher solute concentrations, "2. signal" species have their own signal that causes to turn themselves off after reaching a certain population density.

[2] initial distribution: 50-50% of both populations

[3] initially 4 "parasite" and 1996 wild type bacterial agent

design parasite models that turn themselves off whenever the level of communication or cooperation in the prey population decreases below a critical level. Such parasite models will not collapse the host population. It is well known that quorum sensing systems in nature can have broad response specificities, which actually corresponds to the tuning phenomenon observed in our parasite models. So we argue that broad specificities exist in nature because they allow bacteria to form stable communities with other, related species.

4 Applications of the results

During my work I was using simulation models to investigate bacterial communication and different types of mutations. Our goal was – as it is usual in the field of modeling – to understand the described system more precisely. The model helped us to explain the behavior of bacteria in nature and the different patterns formed by the colonies we developed.

The results we obtained by the comparison of open and closed environments can help us to understand how mutant species that are not viable in nature can seem to be invasive in certain laboratory experiments.

Sharing communication solutes among species gives a possibility to describe how species in stable colonies can help each other or in what circumstances species preying on resources of one another can form a community. Results give a possible explanation of how having less specific quorum sensing system can be advantageous to bacterial species.

Furthermore, with the help of our model we can state some principles that do not use any bacteria-specific consideration therefore based on the same principles we can describe other systems or structures that are based on similar rules.

5 Acknowledgements

6   The author's publications

[1] **D. Bihary**, Á. Kerényi, Z. Gelencsér, S. Netotea, A. Kertész-Farkas, V. Venturi, and S. Pongor, "Simulation of communication and cooperation in multispecies bacterial communities with an agent based model," *Scalable Computing: Practice and Experience*, vol. Volume 13, no. 1, pp. 21–28, 2012.

[2] Á. Kerényi, **D. Bihary**, V. Venturi, and S. Pongor, "Stability of multispecies bacterial communities: signaling networks may stabilize microbiomes.," *PloS one*, vol. 8, p. e57947, Jan. 2013.

[3] **D. Bihary**, M. Tóth, Á. Kerényi, V. Venturi, and S. Pongor, "Modeling bacterial quorum sensing in open and closed environments: potential discrepancies between agar plate and culture flask experiments.," *Journal of molecular modeling*, vol. 20, p. 2248, July 2014.

[4] V. Venturi, A. Kerenyi, B. Reiz, **D. Bihary**, and S. Pongor, "Locality versus globality in bacterial signalling: can local communication stabilize bacterial communities?," *Biology Direct*, vol. 5, p. 30, 2010.