STUDY OF BACTERIAL COMMUNICATION WITH AN AGENT MODEL

Ph.D. Thesis

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1. Introduction

Microbes live in consortia with various size and composition, and its members use the resources of the environment together. These so called polimicrobial communities can be found everywhere on our Planet: in soil, at the bottom of the oceans and in extreme environments, such as heat springs or even in nuclear waste. They can live in symbiosis with higher organisms as well. These microbial consortia have an important place in the ecosystem of Earth because of their yield to biomass. They role in organic an inorganic circulation is also significant. Microbial communities can be made up by several hundreds or even thousands of species, which share different secreted materials amongst each other. Our knowledge is incomplete on the stability of these consortia and the mechanisms through they can tolerate environmental stress and the appearance of cheater mutants. These hypothetical questions have practical aspects, because the human microbiom, the nitrogen fixing bacteria of the rhizosphere, the bacterial-fungal consortia causing dental cavities, or the underwater biofilms causing corrosion of metallic surfaces are made up of microbial microbial consortia. Therefore explaining the stabilizing mechanisms of these communities has industrial and medical importance.

One of the most important microbiological discoveries of the past 30 years was the exploration of chemical communication between bacterial cells that changed our viewpoint on prokaryotes. Intercellular communication was mainly related to eukaryotes, but the chemical system and the complex mechanisms of microbial communication are obvious nowadays. For example several bacterial species can monitor the density of the population by producing and perceiving small molecules called *autoinducers*. This phenomenon is called *quorum sensing* (QS) which is coordinated gene expression depended upon cell density. Based

on this process the whole gene expression pattern of the population can change if the concentration of the autoinducer reaches a critical threshold. With this synchronized response the bacterial population can adapt and survive in the changed environment. Due to the expressed genes of QS the cells start producing extracellular materials, such as virulence factors, antibiotics, digestive enzymes, siderophores and surfactants. Furthermore, complex phenotypical changes can be initiated by QS such as *swarming motility*, what is the fast collective motion of the bacterial colony.

In the focus of my work were swarming motility and stability of multispecies consortia.

2. Aims of the study

The following goals were made up during my work:

1. Creation of a simplified, agent based hybrid model for simulating the swarming of different *P. aeruginosa* (wild type and QS null mutants) strains.

2. *In vitro* and *in silico* examination of the swarming behavior of *P. aeruginosa* in the presence and absence of exogenous signal molecules.

3. Studying the conditions of stable colony forming.

4. Development of the model for investigating the signal and cooperative factor sharing between species.

5. Simulating the co-swarming of species with different signal and factor sharing levels to explore the effect of communication and cooperation of bacterial strains on the stability and fitness of the population.

3. Methods

3.1. Software and hardware environment

The early versions of our hybrid model were implemented in Java and the later versions were written in MATLAB programming environment. During development the optimization of the source code was considered. The simulations were performed in parallel on a computer cluster of ICGEB in Trieste. The system was consisted of one frontend and 20 backend machines containing 2.2 GHz processors and 2 GB of RAM.

3.2. Bacterial strains used in the laboratory experiments

During the experiments the wild type and different kinds of QS deletion mutants of *P. aeruginosa* PUPA3 strain were used. Different types of mutants can be prepared based on the synthase (*lasI, rhlI*) and transcription regulation genes (*lasR, rhlR*) of the QS system. The mutants containing inactive synthase or regulation systems are called *Signal Negative* (SN, *ΔlasI/rhlI*) and *Signal Blind* (SB, *ΔlasR/rhlR*) mutants, respectively.

To investigate the phenomenon of swarming motility M8 agar medium containing 0.5% agar, 0.2% glucose and 0.05% glutamine was used. The medium was poured in Petri-dishes and after solidify bacterial suspension was inoculated at the center of the medium (0.5 μl). During incubation the agar plates were kept at 30°C.

3.3. The hybrid agent model

P. aeruginosa cells were modeled as agents performing random movements on the surface. They were in close contact with their environment by the produced signal and factor molecules and the nutrients. The members of the populations were made decisions based on

the local concentration of these chemical materials. Agents can exist in three different states based on the signal and factor concentrations: *solitary state* (basic level of signal production, metabolism and movement), *active state* (increased signal and factor production) and *swarming state* (increased signal and factor production and intense metabolism and motility). The agents can take up nutrient form the environment and they spend the energy on the production of extracellular materials, metabolic processes and movement. The rest of the energy is stored and if the level of the stored energy reaches a specific threshold, cell division is performed. If the nutrient runs out of form the environment, the agents will be inactivated after depleting the stored energy.

In our model the agar medium was implemented as a rectangular surface ("racetrack") discretized into squares and periodic boundary conditions were applied on the longitudinal sides.

The diffusible materials were described with reaction-diffusion equations and the solution was approximated with the Euler-method that is a conditionally stable explicit numerical method.

Different numerical values were defined for the easier evaluation of the simulations. One is the *relative fitness* that describes the efficiency of the stationer population correlated to a reference population. With the help of the *segregation index*, the final population can be easily characterized as mixed or segregated colony.

At the start of the simulation fixed number of agents in solitary state were placed near the start line. The program was iterative and in each cycle the agents were made a step with fixed length in random direction and they were producing materials and take up nutrition based on their inner state. In each iteration diffusion function was performed on the produced materials and the nutrients. The agents could change states

based on their local environment. Simulations were stopped if each of the agents were inactivated or if the main cycle reached the maximal simulation steps defined by the user.

4. Results

4.1. Construction of the hybrid agent model

We constructed an agent based hybrid model (described in Methods) in which the bacteria were represented as agents and the chemical environment (the signal and factor molecules produced by the cells and the nutrient) was interpreted with reaction diffusion equation. This model was suitable for simulation the QS controlled swarming motility of *P. aeruginosa* colony.

4.1.1. Examining swarming motility in the presence and absence of exogenous signal molecules

The *in vitro* swarming experiments of wild type and different kinds of QS deletion mutants of *P. aeruginosa* in the presence and absence of exogenous signal molecules were reproduced with our model system. In the presence of exogenous signal only the wild type strain was able to form swarming colonies, but when exogenous signal was added to the medium the SN colonies also presented swarming. SB mutants were unable to form swarming colonies in neither case.

4.1.2. Tracking of chemical signals

The signal following of SN mutants was demonstrated by creating an artificial track of signal on the simulation surface.

4.2. Local collapse of the colony in the presence of cheater mutants

The effect of cheater mutants on the colony was examined by our model and a hypothesis was created that could give an explanation for the local collapse that can prevent the spread of the cheater mutants in the colony. This phenomena was examined by the following experiments:

4.2.1. Co-swarming of wild type strains with different QS deletion mutants

Mixed wild type and QS deletion mutant strains were inoculated on agar plates and the following results were observed: "no-swarming", "swarming" and "collapsed" colonies. The WT and SN strains formed a stable mixed swarming colony, but experiments and simulations with WT with SB mutants were resulted collapsed communities. This can be explained by the reproductive advantage of the cheater mutant that can crowd out the cooperating WT cells from the population.

4.2.2. Population kinetics in vitro and in silico: swarming and collapse

The ratio of the different strains in the final population compared to the initial ratios was examined. Our results showed that the WT cells can form stable mixed colony with the cooperative SN mutants in which the ratio is nearly constant for the benefit of the mutant. Non-cooperative SB mutants were not formed swarming colonies with the wild type cells based on the hypothesis mentioned above. These results were observed in the simulations as well.

4.3. Examining *in silico* the cooperation and communication of communities made up by two species

The stability of the community and the fitness of the participant species at different signal, factor a nutrient sharing levels were examined by our agent model.

4.3.1. Competition without quorum sensing

Competing species lacking the QS system were unable to form mixed stable communities. At lower nutrient sharing stochastic segregation was observed, while at higher nutrient sharing the simulations resulted in stochastic exclusion.

4.3.2. Symmetric overlap: "sharing"

At higher nutrient sharing stable, mixed colonies with higher fitness (compared to colonies growing without competitor) were formed at specific regions of the parameter space. At other regions of the parameter space stochastic segregation was observed based on the level of nutrient sharing.

4.3.3. Asymmetric overlap: "exploitation"

In asymmetric cases one species can react to the signal and factor molecules of the other species and it can also use the nutrition of the competitor. During these simulations competitive exclusion and segregation were observed in which the exploiter species was favored.

5. Summary

The main goal of our work was to investigate the quorum sensing of different strains of *P. aeruginosa in silico* and *in vitro.* Therefore, an agent based hybrid model was constructed in which bacterial cells were represented as agents, endowed with individual decision-making capacities. In addition, the concentration changes of chemical materials in the environment were described with reaction-diffusion equations.

We examined the effect of cheater mutants that are not able to participate in quorum sensing activities on the colony. The laboratory experiments and the simulations both showed that *cheater mutants*, which do not participate in the production of public goods (i.e. the cooperation factors with high metabolic cost) will cause the *collapse of the community*, but the collapse is local i.e. it does not spread to other parts of a dendritic colony. The computational simulation implied that the colony was able to localize cheater mutants and prevent their invasion via the regulation of swarming motility and metabolism. Furthermore, it was established that the mutant to WT ratios in mixed populations shows characteristic values that are reached regardless of the initial ratios. It was shown by simulations that, in extreme cases, even a few cheater mutant cells were able to collapse a whole swarming community.

We also tried to answer the question how sharing QS signals and public goods affect the competition of species living in the same environment. To this purpose, we conducted simulations in which the competing agent populations shared their signals, public goods and nutrients to varying extents. It was found that sharing the signals and public goods lead to the formation of mixed and apparently stable two-species communities that persisted for several hundred generations. This phenomenon occurred in substantial parts of the parameter space. On the other hand, the phenomenon was never observed in systems without QS.

In the two-species colonies the competing agent populations were fully mixed i.e. co-localized, without patch formation. In some cases, the co-localized populations were growing faster alone i.e. in the absence of the other species. This phenomenon was observed when the competing populations were using partially different food sources. Using different nutrients can be considered as metabolic complementary that was observed in several natural microbial consortia. The opposite case was also observed during the simulations: if the competing populations were using the same nutrient then the simulations ended up with the exclusion one of the competing species. We concluded that sharing the public goods and utilizing different nutrients are essential for the formation of co-localized populations while signals could have key roles in recruiting species as mentioned earlier.

A species can have reproductive advantage if it can use the signals and public goods of the other species while the other one cannot do the same. This phenomenon is referred to as *asymmetric exploitation* that is beneficial for the exploiter in the entire parameter space. This simulation result made us conclude that a cell that can detect multiple signals with its LuxR receptor can have reproductive advantage, because it can exploit the signals of competing species. This can explain why aspecific LuxR receptors that can interact with several signal molecules are frequently observed in nature.

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