Studying of coordination in microbial communities via agent-based modelling

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Theses of PhD dissertation

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Budapest, 2018

1. Introduction

Bacteria represent the most widespread cellular life form on Earth. They populate nearly all habitats including the country-sized microbial mats on the seabed as well as the human microflora. It is a relatively new observation that bacteria are not solitary but social organisms that often live in large and diverse communities consisting of many thousand species. While solitary cells are only capable of competing for example for habitat and nutrients, community lifestyle requires more. It requires cooperation, coordination and synchronisation of cell behaviour. According to one of the basic hypotheses of our research group, it is cooperation that enables a community to solve complex tasks that individual cells cannot tackle. We use agent-based models wherein agents (cells) transfer information (e.g.: genes, chemical signals) to their neighbours via a simple response threshold-dependent mechanisms.

The first phenomenon I studied was horizontal gene transfer of antimicrobial resistance factors in microbial communities. I found that members of a community can get used to the antimicrobial factors (AM) produced by other community members, and such a microbiome is – somewhat surprisingly – stable against invading bacteria (such as pathogens).

The second part of my thesis is the study of a widespread phenomenon, the differential sensitivity of coexisting species to the same chemical signal. This phenomenon has been first described in the so called *quorum sensing* mechanism. *Quorum sensing* means that

cells in a colony can synchronise their behaviour via secreted, diffusible materials. My simulations showed that different signal sensitivities allow viable, but less fit cell lines to survive in a community. In other words, “self-restraint” or moderation can arise in microbiomes due to a simple tuning of thresholds in molecular switches.

2. Methods

I used agent-based computer models for studying both horizontal gene transfer (HGT) and quorum sensing (QS) thresholds. The agents are units with behaviour rules that are individually capable of environmental interactions and decision making. Such agents describe the bacterial cells in my models.

I developed the HGT model in collaboration with Attila Kertész-Farkas (ICGEB, Trieste) (figure 2.1.). The agents have a "genome" consisting of AM and AM resistance gene pairs that are randomly assigned at the beginning of the simulation. During the simulations the agents move randomly, divide and in each step scan their environment for antimicrobial factors (AMs). An agent dies if the concentration of foreign AMs – the ones it is not resistant to – is above a certain threshold in its vicinity. Survivors, on the other hand acquire a randomly chosen resistance gene from their neighbourhood (HGT). The simulation continues till cell death no longer occurs. This is the so called mature state of the community wherein the member species – usually much less than the starting number of species – are resistant to the AMs of each other.

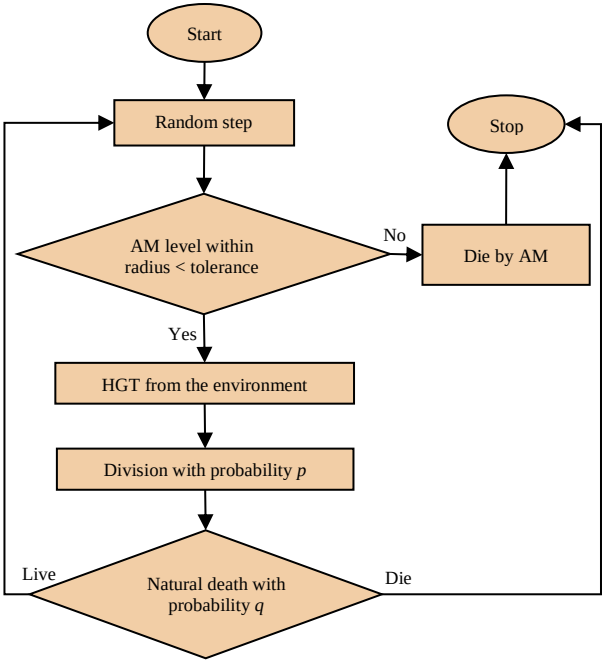

Figure 2.1: The life circle of agents in the HGT model

Previous members of our research group (Dóra Bihary, ITK, Ádám Kerényi, SZBK) developed an *in silico* modelling framework for QS (figure 2.2.). It is a so-called hybrid model, in which bacteria are represented via agents, while nutrients, signals and factors are described with reaction-diffusion equations. The agents of the model sense signal and factor concentrations and if these concentrations reach a certain level, agents react to them by changing their behaviour (state). If enough signal and factor are presented, the cells enter an

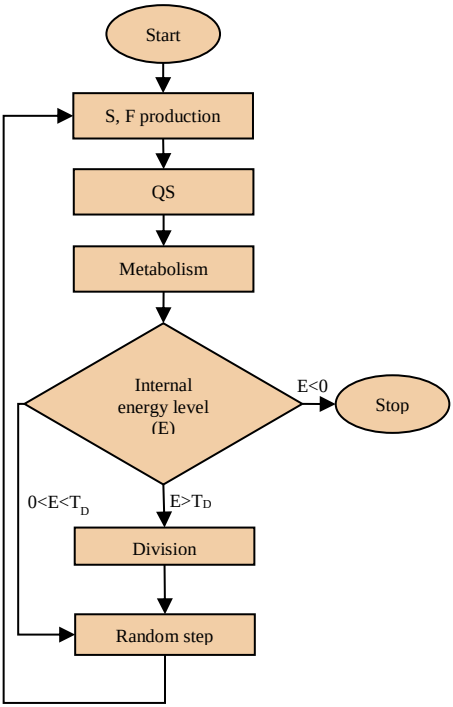

Figure 2.2: The life circle of agents in the QS model

active swarming state with faster movement an increased nutrient uptake. The model describes the growth of a colony branch in a surface that is infinite in one direction and having periodic boundary in the other (toroid). In each step, the cells consume some nutrient from the medium, produce and sense signals and factors, and decide about their state based on these parameters. If they reach the active state, they intensify their random movement, increase their nutrient uptake, and as a result, the community will spontaneously swarm forward in the medium. I studied the effects of different QS response thresholds in this model and introduced partial mutants (PM) with decreased signal and factor production as well as antibiotics mediated negative interactions.

Both computer models were implemented in Matlab programming language.

3. New scientific results

<u>Thesis 1:</u> I developed an agent-based computational model, and showed that horizontal gene transfer can facilitate the formation of stable, multispecies microbial communities. These communities are resistant to external invaders and they are also capable of displacing antibiotic-multiresistant opportunistic pathogens.

Related publications of the author: [J1], [C3], [C4]

I found that the members of a naïve (not mature) community can be displaced by a group of stronger monoclonal invader cells, while a mature, adapted microbiome can defend itself against such invaders, even though the invaders could outgrow each individual species of the resisting community (figure 3.1.A). This phenomenon can be simply explained by the fact that and invader arriving from the outside is not likely to be resistant to all AMs produced locally by the community. This explanation is in agreement with the biological observation that the gut flora, like a first defence line of the gastrointestinal tract, can deal with most of the microorganisms arriving via food intake.

Clostridium difficile (CD) infection is serious issue in healthcare. It is an antibiotics-multiresistant pathogen, so it cannot be eliminated successfully and permanently with antibiotics. However replacing the dysbiotic community with a healthy one (fecal transplantation) appeared to be an effective treatment. My simulation results support

these observations. A healthy, diverse microbiome has a broad metabolic capacity so it produces many AMs which can eradicate a pathogen. In other terms, a diverse microbial consortium matured via HGT of resistance genes can successfully outcompete invaders (figure 3.1.B).

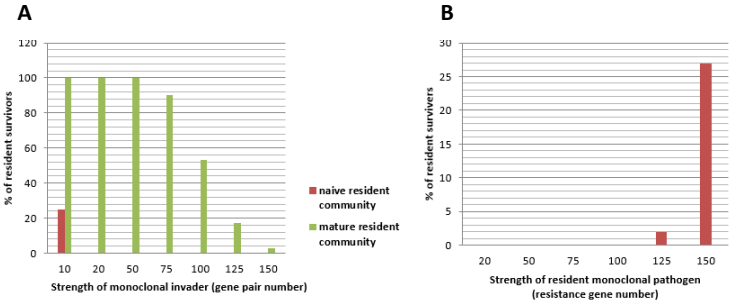

Figure 3.1: A: The response of mature and naïve communities to monoclonal invader species with varying strength. B: The interaction of mature communities and monoclonal multiresistant pathogens.

<u>Thesis 2a.:</u> I showed via agent-based modelling that different sensitivities to bacterial signals and cooperation factors enable the coexistence of species with different fitness values. I demonstrated that these different response thresholds facilitate division of labour between species by causing partial separation in their niche. This phenomenon could be interpreted as microbial "self-restraint" and could allow the survival of less fit species in a community.
Related publications of the author: [J2]

I performed simulations with two types of cells which differing only in their signal, factor or both sensitivity thresholds. In these cases the species with the lower threshold, i.e. the one with faster response to the chemical environment, always displaced the other one. In contrast, a stable coexistence between the two species became possible if the fast responding species had a generally lower fitness than the late responding one. For instance they had a lower division rate (figure 3.2.). This can be explained as follows: while in the first case the lower response thresholds represented a clear fitness advantage, in the second case this advantage was only partial. I demonstrated with these experiments that the differences in QS response thresholds can help to stabilise the coexistence of species in microbiomes. Simply put, a less fit species will not be eliminated from the community if it reacts to QS signals faster than the other community members.

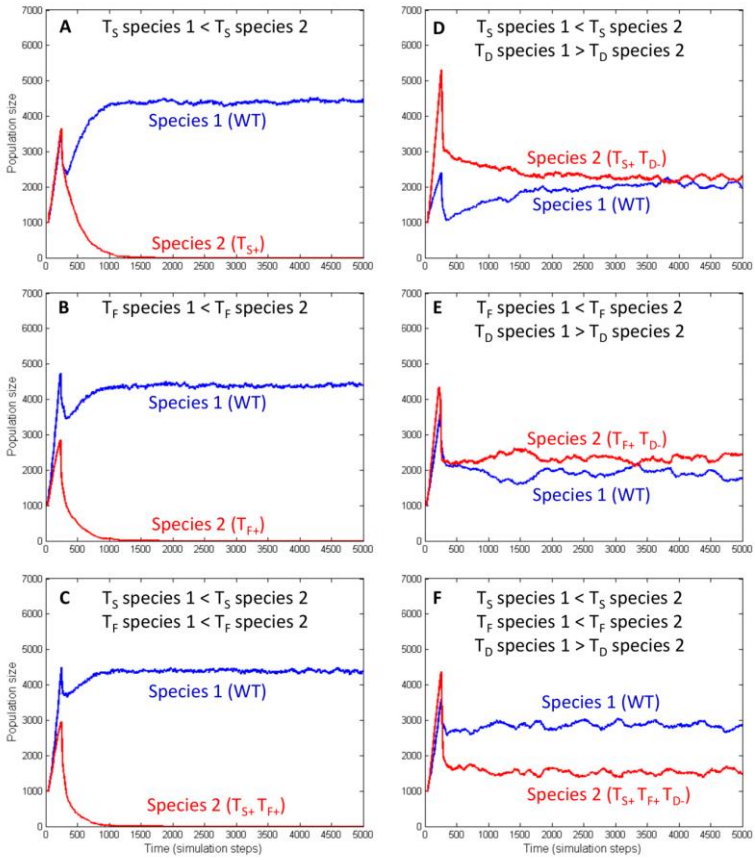

Figure 3.2: Competition between wild type cells (WT, blue) and cells with higher signal (T_S) (A,D), factor (T_F) (B,E), or signal and factor (C,F) thresholds (red) in cases of equal (A,B,C) or different (D,E,F) division thresholds (T_D).

<u>Thesis 2b.:</u> I used agent-based modelling to study the effects of external invader cells that invest less into cooperation and communication. I demonstrated that these cells can stably coexist with the wild type cells if they have higher response thresholds, i.e. if they apply self-restraint. This is a biologically relevant scenario since impaired communication or cooperation activity can easily arise by mutations.

Related publications of author: [J2], [C1]

PM (partial mutant) cells are WT cells with a mutation that makes them invest less into QS. PM mutants are capable to swarm alone, although they start swarming later and swarm slower compared to WT. However, when entering a WT community, they will make use of the public goods produced by the WT cells and as a result, they will grow faster. Figure 3.3. shows the interactions of partial mutant and wild type (WT) cells. If PM cells appear in a WT colony, even in small numbers, they will spread and the composition of the population will converge to a characteristic PM/WT ratio. Above this ratio, WT does not produce sufficient amount of public goods to support PM so PM would grow slower. Below this ratio, however, PM would grow faster than WT.

The metabolic differences also lead to a partial spatial separation within a mixed community. WT cells act as path-breakers in the forefront of the growing community. PM parasites on the other hand

lag behind, but usually constitute the majority of the mixed community. I demonstrated that such communities can form only if PM cells have higher response thresholds than WT, i.e. if they apply self-restraint in the sense that they allow PM cells to grow before they themselves switch to faster growth. These observations confirm the community level benefits of different QS response sensitivities in microbiomes.

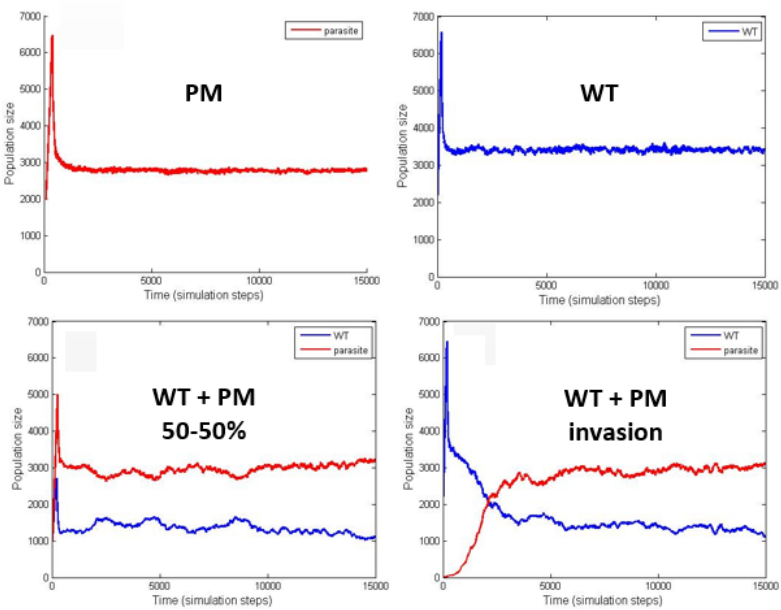

Figure 3.3: WT-65% partial mutant (red) cells with signal threshold 40 can swarm alone, they are invasive and can form stable coexistence with WT (blue) cells.

<u>Thesis 2c.:</u> I showed via agent-based modelling that stable coexistence is possible between antibiotics producing and antibiotic sensitive species, if their communication systems are compatible and the antibiotics producers have lower fitness under some circumstances than the sensitive species. Related publications of the author: [J2], [C2]

Antibiotics (ABs) were represented as materials secreted by the producer cells (ABP) that decreased the growth rate of AB sensitive cells (ABS) in our agent-based QS model. When the two species had equal metabolic and fitness parameters ABP cells always eliminated ABS cells. However, when the QS response thresholds of ABP cells were higher than the ABS cells, stable coexistence was observed. In other terms, ABS cells could grow faster before ABP cells turned on their antibiotic production. Similar to the previous cases, this phenomenon can be interpreted as "self-restraint" of the ABP cells. These results thus indicate that differential QS thresholds can stabilize coexistence between species even in the presence of AB production (i.e. negative, inhibiting interactions).

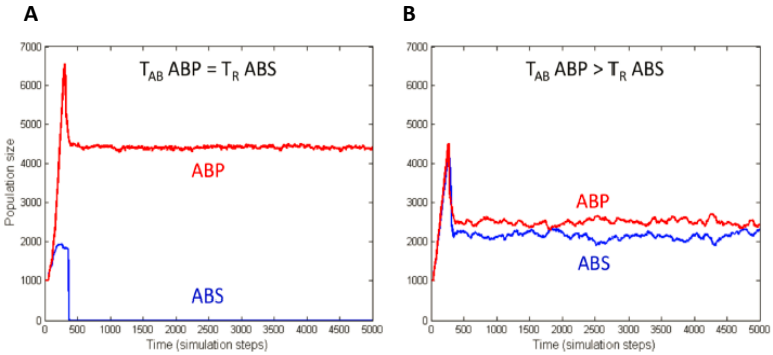

Figure 3.4: Interaction between AB sensitive (ABS, blue) and AB producer (ABP, red) cells. A: AB production starts immediately after the activation of the ABS population (T_{AB} ABP=T_R ABS). B: AB production starts with higher signal concentration (T_{AB} ABP>T_R ABS).

4. Applications of the results

I studied the communication and cooperation mechanisms between the members of microbial communities via agent-based modelling. I found that both horizontal gene transfer and differential QS responses can stabilize the coexistence of multispecies bacterial communities.

This theoretical work could help *in vitro* experiments through identifying factors and principles of bacterial interactions. My findings can contribute to validate experimental results and to select new targets that might be promising to study *in vitro* or *in vivo*. This will hopefully bring us closer to understand microbial interactions and controlling mechanisms in many fields of biotechnology industry and healthcare.

For instance, excessive use of antibiotics is a serious issue in current healthcare and it is widely considered as the main reason of the emergence of endemic multiresistant pathogens. They frequently appear in hospitals and pose a serious threat to patients with weakened immune system. These strains cannot be eliminated with antibiotics, so there is an urgent need to find new and effective countermeasures against them. My results indicate that disturbing the quorum sensing systems of pathogens by degrading or blocking the QS signal molecules or by initiating QS before the effective cell density is reached could be promising complementary therapies to antibiotics (for example in such cases as *Pseudomonas aeruginosa* infection of patients with cystic fibrosis). Such approaches can help to reduce

antibiotics usage, thus slowing the spread of resistant strains, and on the other hand they are also more specific, because they affect the pathogens only. In addition, such approaches may have fewer side effects, as they do not destroy the beneficial members of a micobiome that are necessary for its normal functions. My results also confirm the finding that AB resistant opportunistic pathogens (such as *Clostridium difficile*) can be efficiently treated with the introduction of a healthy bacterial community (such as fecal transplant or probiotics).

Biotechnology industry can also benefit from exploring the details of cellular communication and cooperation. For instance, engineering artificial microbial communities for specific roles may become possible using a more accurate knowledge of intercellular, interspecies and environmental interactions. These artificial communities could be useful alternatives to genetically modified cells, as they could perform more complex tasks and they could be more viable in various natural environments. The potential fields of use could be healthcare (e.g.: artificial gut flora), agriculture and food industry (e.g.: specific and precise setting of the soil microflora for crops), environmental protection (e.g.: engineering bacterial communities capable of cleaning waste water or degrading, processing contaminations). I hope that my results will contribute to the development of these fields and to the design of useful biotechnology applications.

5. Publications

[J1] **J. Juhász**, A. Kertész-Farkas, D. Szabó, and S. Pongor, "Emergence of collective territorial defense in bacterial communities: horizontal gene transfer can stabilize microbiomes.", *PLoS One*, vol. 9, no. 4, p. e95511, Jan. 2014.

[J2] **J. Juhász**, D. Bihary, A. Jády, S. Pongor, and B. Ligeti, "Differential signal sensitivities can contribute to the stability of multispecies bacterial communities", *Biol. Direct*, vol. 12, no. 1, p. 22, Dec. 2017.

[J3] B. Ligeti, R. Vera, **J. Juhász**, and S. Pongor, "CX, DPX, and PCW: Web Servers for the Visualization of Interior and Protruding Regions of Protein Structures in 3D and 1D", *Methods in molecular biology (Clifton, N.J.)*, vol. 1484, pp. 301–309, 2017.

[J4] D. Ábrahám, J. Fehér, G.L. Scuderi, D. Szabó, A. Dobolyi, M. Cservenák, **J. Juhász**, B. Ligeti, S. Pongor, M.C. Gomez-Cabrera, J. Vina, M. Higuchi, K. Suzuki, I. Boldogh, Zs. Radák, "Exercise and probiotics attenuate the development of Alzheimer's Disease in transgenic mice: role of microbiome", *Experimental Gerontology*, vol. 115, pp. 122–131, Jan. 2019.

[C1] **J. Juhász**, "Modelling moderate quorum sensing parasites in microbial communities**",** *PhD Proceedings Annual Issues of The Doctoral School Faculty of Information Technology and Bionics* 12: pp. 22-23., 2017.

[C2] **J. Juhász**, "Modelling the effects of internally produced antibiotics in multispecies bacterial communities", *PhD Proceedings Annual Issues of The Doctoral School Faculty of Information Technology and Bionics* 11: pp. 47-50., 2016.

[C3] **J. Juhász**, “Modelling horizontal gene transfer in bacterial communities”, *PhD Proceedings Annual Issues of The Doctoral School Faculty of Information Technology and Bionics* 2015/01, pp. 53-56., 2015.

[C4] **J. Juhász**, A. Jády, B. Ligeti, “Horizontal gene transfer can facilitate the formation of stable and diverse microbial communities: an *in silico* agent-based model”, *RECOMB 2018*, 21-24 Apr. 2018, Paris (poster)

[C5] T. Gaizer, R. Valaczkai, B. Pillér, D. Méry, M. Miski, D. Pesti, **J. Juhász**, I. Stefanini, B. Péterfia, A. Csikász-Nagy, „Role of cell-cell interactions in S. cerevisiae colony formation”, *Dynamics of biological systems: Modelling genetic, signalling and microbial networks*, 2-4 May 2018, Brussels (poster)

[J5] S. Pongor, **J. Juhász**, B. Ligeti, “Háború és béke a baktériumoknál”, *Természet Világa*, vol. 147, no. 5, pp. 208-211, May. 2016. (in hungarian)

[J6] S. Pongor, **J. Juhász**, B. Ligeti, “Valós és virtuális társadalmak a baktériumoknál”, *Élet és Tudomány*, vol. 71, no. 2, pp. 41-43., Jan. 2016. (in hungarian)