Communication and Stochastic Processes in Some Bacterial Populations: Significance for Membrane Computing

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Summary. Intercellular communication between bacterial cells belonging to the same population is well documented in Microbiology, sporulation and cannibalism in *B. Subtilis* and genetic competence and fratricide in *S. pneumoniae* being deeply studied in the last years. The investigation of individual cell behavior has revealed that populations of these bacteria sometimes bifurcate into phenotypically distinct, but genetically identical, subpopulations by random switching mechanisms. The probabilistic nature of the random switching mechanisms, the occurrence of some biochemical processes related to it at plasma membrane and the need to study the processes at the level of each individual cell make intercellular communication and stochastic processes very suitable to be modeled by P systems.

Motto: *But a system which has spherical symmetry, and whose state is changing because of chemical reactions and diffusion, will remain spherically symmetrical for ever.(...) It certainly cannot result in an organism such a horse, which is not spherically symmetrical.* [Turing, 1952]

1 Introduction

The concept of intercellular communication within a bacterial population belonging to the same species originates in the discovery of genetic competence in *Streptococcus pneumoniae* (1965) and of quorum sensing (1970) in *Vibrio* (Bassler and Losick, 2006). In the last decades, with special emphasis in the last years intercellular communication within a bacterial population started to be deeply documented in such a copious way that the scientific community started to introduce within their scientific language expressions such as "bacterially speaking", competence and "fratricide", "sporulation and cannibalism" (Bassler and Losick, 2006; Dubnau and Losick, 2006; Claverys and Havarstein, 2007) whereas few scientists put

forward and claim that bacterial communication includes assignment of contextual meaning and sentences (semantic/syntax functions) and conduction of "dialogue" – the fundamental aspects of linguistic communication (Ben Jacob et al., 2006, and citations herein), the same authors seeking for the foundation of cognition in bacteria (Ben Jacob et al., 2006). (It is to be noticed that for dialogue the authors used the commas, whereas for cognition they did not.)

In this short report we focus on some of these new research on intercellular communication within a bacterial population because:

- Communication and stochastic processes become more deeply known when they started to be studied at the level of individual bacterial cell, trend belonging to the so-called single cell microbiology (SCM) which opens a new vision on bacterial world (Brehm-Stecher and Johnson, 2004; Kearns and Losick, 2005; Claverys and Havarstein, 2007); furthermore it was put forward that P systems could become a specific tool to study single bacterial cells as each cell contain a relative small number of important signalling molecules whose behavior could be better described by a discrete systems than by a continuous one (Ardelean, 2006).
- SCM investigation by improved techniques has revealed that populations of certain bacteria sometimes bifurcate into phenotypically distinct, but genetically identical, subpopulations, bifurcation which is called bistability (Dubnau and Losick, 2006). The need to study the processes in the each individual cell was originally put forward by Turing (Turing, 1952) who wrote: "To find the rate of change due to chemical reactions only needs to know the concentration of all morphogens at that moment *in the one cell concerned*" (my underline), a suggestion largely ignored (forgotten?) for decades.
- Bistability in our opinion could be appropriately modeled by P systems because of their probabilistic nature of the processes occurring within plasma membrane (as well as in a bulk phase).
- Bistability is a random mechanism that switches on different genetic programmes within identical bacteria grown under the same conditions (Dubnau and Losick, 2006). This passage from homogeneity to heterogeneity, this bifurcation both at the level of biochemical reaction and at the level of cell population remembers the bifurcation of chemical reaction (starting from o homogenous medium) mathematically first demonstrated by Turing in his interdisciplinary scientific paper on chemical basis of morphogenesis (Turing, 1952). This type of bifurcation named by Prigogine "Turing bifurcation" (Prigogine, 1977) is one of the tools used by Prigogine (Nobel Prize 1977) to physically explain how biological life (an anti-entropic process) is physically possible in an Universe whose overall entropy is under increase.

2 Communication and Stochastic Processes in Some Bacterial Populations

The recent advent of techniques like flow cytometry and fluorescence microscopy that facilitate the investigation of individual cell behavior has revealed that populations of certain bacteria sometimes bifurcate into phenotypically distinct, but genetically identical, subpopulations by random switching mechanisms. This bifurcation of genetically identical bacterial populations, also called clonal populations exhibit (unimodal) variation in the expression of a given gene, due to random fluctuations in the rates of synthesis and degradation of the cognate gene product, which is referred to as 'noise' and we will employ this usage. Sometimes, the noise gives rise to another type of variation that is non-unimodal, meaning that the population bifurcates into subpopulations, phenotypic phenomenon known as 'bistability' (Dubnau and Losick, 2006). For example, when *Bacillus Subtilis* cells encounter conditions of nutrient deprivation or reach a critical cell density, the cell can choose between two type of bifurcation involving entirely different genetic programmes, according to culture conditions. They can fully induce motility and enter stationary growth, enter sporulation, which cumulates in the formation of an enduring spore or enter the state of competence, in which they are able to take up DNA from the environment for integration into their chromosome via homologous recombination. Both programmes, competence and sporulation, involve the formation of a bistable culture; about 20% of cells will become competent, or a maximum of 80% of the cells will initiate sporulation. The remaining 80% or 20% of the cells, respectively, simply enter stationary phase and, in the case of sporulation, are even killed by the sporulating cells, which secrete a specific toxin, to serve as a nutrient source (Gonzalez-Pastor et al., 2003). Thus, even though all cells encounter identical culture conditions, only a (relatively well-defined) subpopulation fully throws the switch towards the new mode of development. Nevertheless, the non-competent or non-sporulating cells can switch to a new developmental state at a later time (Graumann, 2006). It could be important from P systems the fact that bistability arise stochastically in populations of genetically identical cells, grown in homogeneous and theoretically identical environments (e.g. in liquid media in well-stirred flasks), the choice of which individual cells exhibit altered gene expression being random (Dubnau and Losick, 2006). So far, there are several examples communication and stochastic processes in some bacterial populations but for the sick of simplicity in this paper we will briefly focus only on two of them which involve bistability: sporulation and cannibalism in *B. Subtilis* and genetic competence and fratricide in *S. pneumoniae*.

2.1 Sporulation and cannibalism

When the nutrients are limited many types of bacteria including *B. subtilis* entry into sporulation, an elaborate developmental process that culminates in the formation of a specialized cell called spore or endospore. The spore is a dormant cell

type being able to resist environmental extremes: it is involved in the propagation in time and space not in the multiplication of the bacterial population to which it belongs. The master regulator for spore formation is Spo0A, a protein response regulator whose activity is governed by phosphorylation Spo0A is activated under conditions in which cells are limited for nutrients, but as demonstrated over a decade ago by flow cytometry some cells in a population of nutrient-limited cells activate the master regulator (Spo0A-ON cells) and some do not (Spo0A-OFF cells).

The results show that nutrient limitation is a prerequisite for entry into sporulation, but during nutrient limitation not all the bacterial cells starts the sporulation process because the activation of Spo0A is additionally subject to a bistable switch.

The scientists ask about the biological significance of subjecting entry into sporulation to bistability (Bassler and Losick, 2006; Claverys and Havarstein, 2007). One possible explanation comes from the fact that spore formation is an energy intensive process that becomes irreversible at an early stage. Thus, if the nutrient scarcity that triggers the activation of Spo0A in a population of cells proves to be fleeting, cells that have not entered the pathway to sporulate (Spo0A-OFF cells) will be able to rapidly resume growth when nutrients become available again. Studies of cells under conditions of high cell-population density reinforce the view that a mixed population of Spo0A-ON and Spo0A-OFF cells is a mechanism to cope with uncertainty in the future availability of nutrients. Furthermore, clonal colonies of *B. subtilis* cells are observed to exhibit a behavior referred to as cannibalism in which the Spo0A-ON cells in the population trigger the lysis of non-sporulating siblings (Spo0A-OFF cells) via the elaboration of a killing factor and a toxin (Gonzalez-Pastor et al., 2003) (Figure 1). Nutrients released from the non-sporulating siblings arrest or slow further progression into sporulation by the Spo0A-ON cells, impeding those cells from entering the irreversible phases of spore formation. Cannibalism is therefore a delaying tactic that helps the population to certify that lack of nutrients is not a fleeting condition. According to this view, the cost of fratricide is off set by the advantage of delaying commitment for as long as possible (Bassler and Losick, 2006; Claverys and Havarstein, 2007).

2.2 Competence and fratricide

The interplay between bacteria and antibiotics is very complex and very important for mankind both fundamentally and practically. It is universally accepted that the use of antibiotics will lead to antimicrobial resistance. Traditionally, the explanation to this phenomenon was based on : i) random mutation; ii) exchange of genetic information by horizontal gene transfer and iii) amplification by selective pressure. Subsequently, others mechanisms of antibiotic-induced antimicrobial resistance acquisition were proposed, based on the expected occurrence of bacterial transformation with DNA still present in antibiotic even after its purification (Woo et al., 2006) or on the fratricide behavior (Claverys and Havarstein, 2007). Bacteria

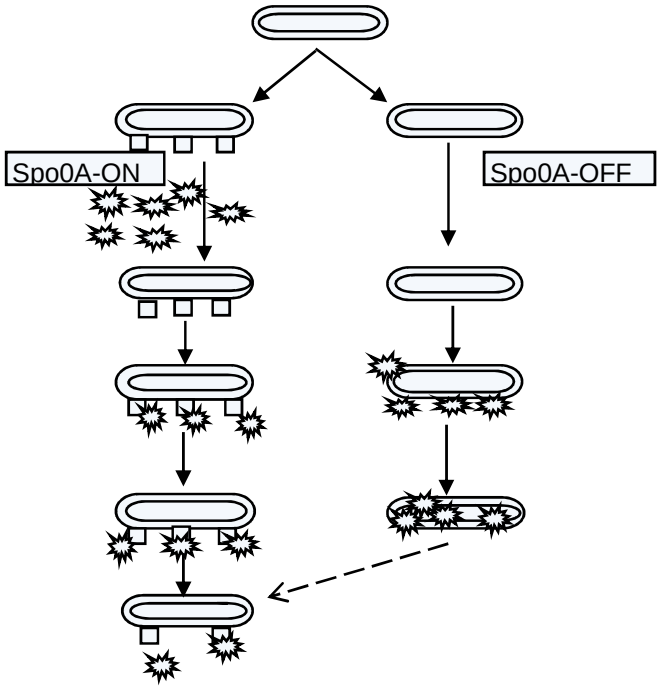

Fig. 1. Sporulation and canibalism in *Bacillus subtilis*. In the medium with nutrient limitation a clonal population of *Bacillus subtilis*, as an expression of bistability, bifurcate in two subpopulations: one in which the master regulator of sporulation is activated (Spo0A-ON) and the other one in which the master regulator of sporulation remains inactivated (Spo0A-OFF). The subpopulation entering the sporulation synthesize a toxin (killing factor - illustrated in the figure as a star) and the corresponding immunity device (illustrated in the figure as square located at the cell surface). The molecules of toxin destroy the nonsporulating cells whose chemical constituents become available (interrupted arrow in the figure) as nutrients for the cells in which the master regulator of sporulation is activated (Spo0A-ON) (modified after Claverys and Havarstein, 2007).

exchange genetic information either through the direct uptake of DNA (transformation), phage-mediated transduction, through inter-organism contact with DNA exchange (conjugation) or mobilization of DNA within organisms' genomes (transposition). Genetic transformation is possible when a cell has the ability to take up foreign DNA from the medium, ability which is named competence. Conjugation is one type of mechanism to transfer genes from one living bacterium to another living bacterium, the physical contact between the donor cell (called male type) and the recipient cell (called female type) being essential for gene transfer. Conjugation depends on the presence of certain plasmids, DNA closed molecules which are physically independent with respect to bacterial chromosome.

The ability of bacteria to sense some chemicals in the medium and to process this information is further illustrated by another bacterium *S. pneumoniae*. Transformation (the uptake and genomic integration of exogenous DNA) in *S. pneumoniae* can only occur when the bacteria are competent, which is a transitory state in bacteria. Although the competence state regulation is rather well understood, the signals that trigger it remain elusive. Recent evidence suggests that in *S. pneumoniae* competence is a stress response to environmental change (Prudhomme et al., 2006), who wondered whether antibiotic-induced stress might trigger competence. Out of the dozen or so antibiotics that they checked, six up-regulated the competence pathway when used at concentrations that killed approximately 50% of the bacteria. These antibiotics kill bacteria by either damaging DNA, inhibiting protein synthesis, or blocking DNA synthesis. The main conclusion is that the mechanism of action of a particular antibiotic cannot be used to predict its ability to induce competence further complicated the realities related to the generation of antibiotic-resistant bacteria; however the choose for clinical treatment of those antibiotics that do not promote genetic exchange may help to minimize future problems. These new findings (Prudhomme et al., 2006) which show that some bacteria become competent (able to take up foreign DNA from the external medium) as a response to the presence of an antibiotic in the external medium, further argue that bacteria posses a complex behavior. The molecular mechanism is not yet known but it could be speculated that it should involve signal transduction machinery which activity in other bacterial processes has already been modeled in the framework of P systems (Ardelean et al., 2006).

What it is really interesting is the fact that during the installation of competence, the competent cells synthesize a substance which is lethal for non-competent cells, which release in the growth medium their intracellular constituents, including nutrients and DNA. This type of killing is called fratricide and is a new type of bacterial behavior which further argue for the diversity of the interactions between bacterial cell and environment. The biological significance of fratricide related to environmental signal (e.g. antibiotic) induced competence is based on the hypothesis which invokes the provision of genetically diverse DNA molecules in the extra-cellular space to generate diversity by genetic transformation (Claverys and Havarstein, 2007). In *Enterococcus faecalis*, a Gram-positive species that commonly resides in the human intestine, it was discovered (Dunny et al. 1978) that gene transfer by conjugation can reach a very high frequency (10^2) compared with conjugative gene transfer in all other bacteria (frequency 10^4). It was shown that this significant difference is determined by the ability of some cells to synthesize and secrete in the extra-cellular medium a specific peptide. This peptide is called sex pheromone because it is involved in the attraction between donor and recipient bacterial cells, thus enhancing the frequency of conjugation. The pheromones are hydrophobic octa- or hepta-peptides and nowadays there are known more than four types, each pheromone being encoded by a gene located on the chromosome. All *Enterococcus faecalis* cells contain on their chromosome one type of gene for one type of pheromone but not all these gene are active. The pheromone gene

is active only when in that particular bacterial cell the corresponding plasmid is missing, because the plasmid contains a gene whose product inhibit pheromone synthesis. Each type of pheromone acts as a sex pheromone on bacterial cells which have a plasmid specific for that pheromone, plasmid encoding the resistance to a given antibiotic. To simplify the biological notations, the bacterial cell able to synthesize the sex pheromone A will attract cells belonging to the same species (population) which have the corresponding plasmid alpha; this last type of cells can not synthesize the sex pheromone A whereas the cells able to synthesize the sex pheromone A have not the plasmid alpha. The pheromone response is characterized by an induced aggregation of donor and recipient (plasmid-free) cells, which can lead to mating frequencies greater than 10^2 per donor cell within a few hours. In a liquid environment, pheromone induces donor cells to synthesize a plasmid-encoded "aggregation substance" (AS), a surface protein that binds to recipients and initiates the contact necessary for transfer of plasmid DNA. However, the pheromone induces the synthesis of several additional plasmid-encoded products necessary for DNA transfer by conjugation (Clewell, 1989; 2004). When a recipient strain acquires a copy of the plasmid there is a "shutdown" of the corresponding pheromone activity, as transconjugant themselves become donors. When a former recipient cell receive the plasmid alfa it stops the synthesis of A pheromone, but not the synthesis of other type of pheromones for which there are no corresponding plasmids in the cell. Thus, transconjugants (the former recipient cell), continue to secrete other different pheromones specific for donors carrying conjugative plasmids which confer resistance to other type of antibiotics. Gene transfer by conjugation is essential for those bacterial cells, devoid of a given plasmid carrying the gene for the resistance to a given (class) of antibiotics, to survive in media where antibiotics are present; this is the case of human body under clinical treatment, *Enterococcus faecalis* being one of the most common bacteria involved in nosocomial (hospital-acquired) infections and are notorious for being resistant to multiple antibiotics. The production of sex pheromones is another type of example of chemical communication between bacterial cells, enabling them to announce other cells that they are ready to receive foreign genetic material conferring them resistance to antibiotic 1, (especially?) when the recipient cells are in the presence of antibiotic 1 to which they are sensitive. Furthermore, the increase in the frequency of conjugation could be one possible explanation on those results concerning antibiotic-induced enterococcal expansion in the mouse intestine which correlates poorly with suppression of competing bacteria already present in the intestine as "normal" bacteria, suggesting that other factors favor the adherence and multiplication of *E. faecium* in the gastrointestinal tract of antibiotic-treated mammals (Woo et al., 2006). This biological phenomenon could probably be modeled by P systems with so- called query symbols in the string (E. Csuhaj-Varju and G. Vaszil, personal communication).

In my opinion, the recently discovered bistability in some bacterial populations is an example of biochemical bifurcation, which was first studied as a basic process in living cells more than half a century ago by Turing (Turing, 1952). This

bifurcation would deserve further mathematical approach. The probabilistic nature of bacterial bistability, the occurrence of some biochemical processes related to bistability at plasma membrane and the need to study the processes at the level of each individual cell make bistability very suitable to be modeled by P systems. The biologists involved in the study of intercellular communication and stochastic processes in some bacterial populations as it is illustrated by bistability could benefit from the collaboration with scientists working on P systems to start the mathematical modeling of these discrete biological processes and to understand what kind of biological experiments are still needed to further reach the full power of modeling and calculation of P systems.

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