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BITS AND BYTES

Cross- species communication in bacterial world

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Abstract Biofilms are the compact association of micro organisms and the communication processes in these biofilms are always a wonder. Electrical and chemical signaling mechanism are the key to understand the bacterial communication network. Quorum sensing so far has been able to explain the coordinated motion of bacteria through its chemical signaling mechanism. Bacteria residing within biofilm communities are trivial to communicate. But the recent observation in 2017 by Humphries et al. has revealed that the ion channels enabled electrical signaling mechanism can be as powerful as to attract the distant cells i.e., this signaling mechanism are capable of holding a long range behavior. As a result long range cross species communication in the bacterial world have been possible. This substantial outcome has brought this field into a new paradigm to investigate the complex co-existence of biofilm communities and distant cells with a possible scope of application in synthetic biology. In this present article, we briefly describe this new signaling mechanism and how it gives rise to a long range communication ability in bacterial communities.

Keywords Quorum sensing · Ion channels · Bacteria · Biofilms · Electrical signaling

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Introduction

Bacteria are the smallest living organism in nature which can communicate between themselves using electrical and chemical signaling. A small biomolecules are used in communication mechanism which are formally known as quorum sensing molecules or autoinducers and the coordinated biochemical procedure is known as Quorum Sensing (QS) (Williams et al. 2007; Shapiro,1998). This correlated regulation of behavior can include both intra and interspecies cell-cell signalling leading to sense and non-sense communication. QS is the key through which bacteria sense and respond to environmental changes via signal transduction events using signaling molecules in density dependent manner. Bacterium (like *P. aeruginosa*, *B. subtilis*, *V. fischeri* ect.) are secreted out of the cell and after attaining a certain threshold, they are sensed by other cells present in their vicinity (Rajput et al. 2016). When a threshold concentration of the molecules is achieved, a coordinated change in bacterial behavior is initiated. It further activates cascade of signaling events resulting in the activation of QS genes (Miller and Bassler 2001) (Gray et al., 1994) (Fuqua et al. 1994). This phenomenon was first observed by (Nealson et al. 1970) in two bioluminescent marine bacterial species: *Vibrio fischeri* and *Vibrio harveyi*. While some autoinducers are species specific, many bacteria also produce an universal autoinducer, known as AI-2, used across different bacterial species. AI-2 is synthesized from S-adenosyl methionine. The enzyme, which catalyzes the final step in this synthesis, is called LuxS. The gene for LuxS is found in many different bacteria, all of which make and respond to AI-2. This suggests that perhaps AI-2 allows bacteria to sense and react to not only members of their own species, but also to all other species that produces AI-2 (Majumdar et al. 2012; Miller and Bassler 2001). It has been observed that quorum sensing, as determinant of cell population density, is only one of the many different environmental signals which bacterial cell must integrate in order to determine their optimal survival strategy (Williams et al. 2007; Majumdar and Mondal 2016).

Bacterial biofilms (see Fig. 1) are organized communities which can accommodate millions densely packed cells within it. The coordinated motion within biofilm is the consequence of quorum sensing mechanism (the best characterized cell-to-cell signaling process so far) and recently realized ion channel mediated electrical signaling. It has been observed that such communities exhibit fascinating macroscopic spatial coordination. Thus, at this point it would be of greater interest to show if any of these signaling mechanism can extend their influence beyond the boundary of biofilms and can regulate the distant cell behavior. However, this possibility so far remained unexplored. In a recent investigation (Prindle et al. 2015), it has been reported that the metabolic oscillation of bacterial membrane is triggered by nutrient limitation. The oscillatory dynamics resulted from long-range metabolic co-dependence between cells in the interior and periphery of the biofilm.

Adherent communities of *Bacillus subtilis* form biofilms and grow in interval of cycles once the colony reaches threshold size of population. These cycles arise when the cells present in the biofilms rundown of glutamate due to consumption of high amount of amino acid by peripheral cells. Glutamate starvation in the interior cells reduces the production of ammonium ions, which is required by the peripheral cells. As a result, the cell growth diminishes drastically (Tolner et al. 1995; Beagle and Lockles 2015). Mathematical models on signaling explained to an extent the question raised on how following linked metabolic processes of cells within the biofilm community supports a distant communication. But, whether metabolic coordination among distant cells within the biofilm might also a form of electrochemical signaling, is still unforeseeable.

Attraction to biofilms through electrical signaling

The quorum sensing is the well known and most significant micro mechanism through which bacteria within biofilm coordinate their collective behavior. In past few decades, numerous experiments and extensive mathematical modelings have lead quorum sensing towards the best characterized cell-to-cell signaling mechanism. The recent advancements in the field of bacterial communication have shown quite interesting outcomes. Prindle et al. In 2015 has described a new cell – to – cell communication process based on ion channel- mediated electrical signaling. This study of bacterial ion channels has provided fundamental insights into the structural basis of neuronal signaling. They showed that ion channels conduct long-

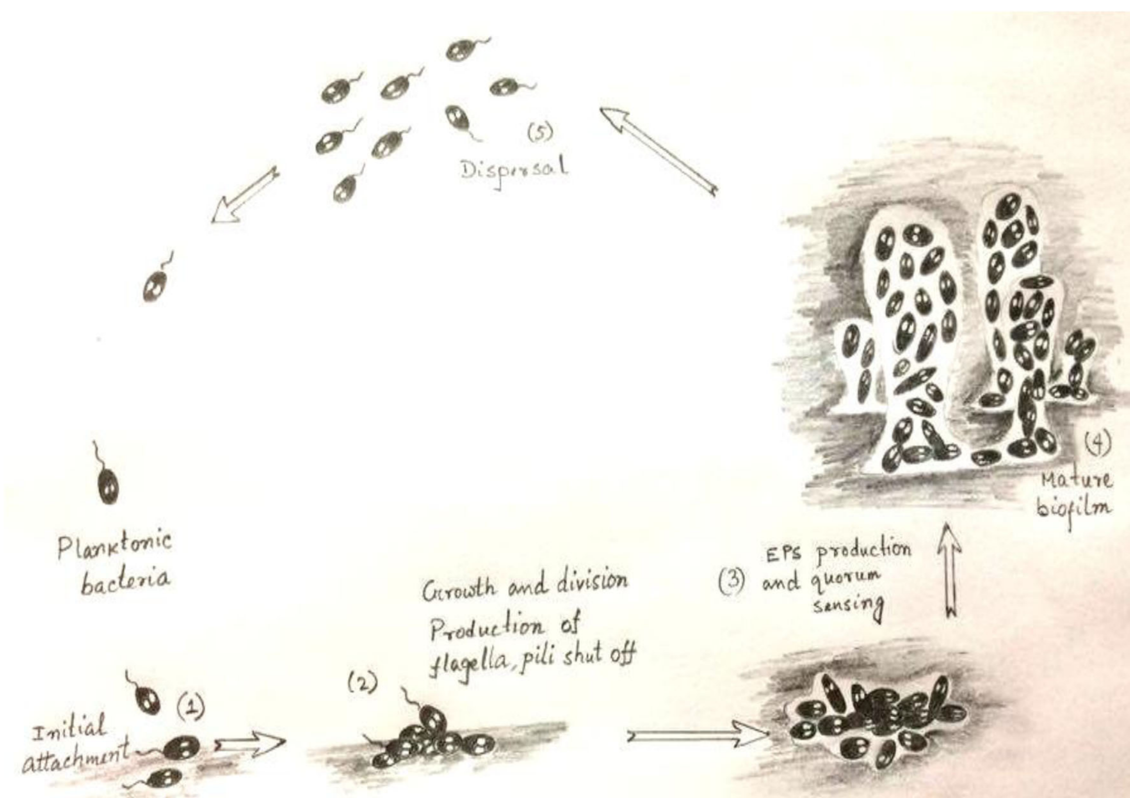

Fig. 1 Schematic diagram of quorum sensing and biofilm formations

range electrical signals within bacterial biofilm communities through spatially propagated waves of potassium. These waves form a positive feedback loop, in which a metabolic trigger induces release of intracellular potassium, which in turn depolarizes neighboring cells. This wave of depolarization coordinates metabolic states among cells in the interior and periphery of the biofilms. These findings raise the question of whether such extracellular signals could extend beyond the biofilm, resulting in long-range interactions that could affect distant bacteria that are not part of the biofilm. Jacqueline Humphries and his collaborators utilized a microfluidic approach to investigate this complex biological phenomenon experimentally and theoretically using a mathematical model developed by Hodgkin and Huxley (Hodgkin and Huxley 1952). They measured the interaction dynamics between a biofilm and motile cells in a large microfluidic chamber. A biofilm was produced in this chamber until it reached the size at which oscillations emerge, and then they introduced motile cells into the chamber and noticed that they were periodically attracted to the electrical oscillation. This approach revealed that the periodic increase in motile cell density at the biofilm edge accurately tracks the oscillation in the biofilm membrane potential. They observed no attraction of motile cells to biofilms that had not yet initiated electrical oscillations, that suggesting that electrical signaling plays an important role in motile cell attraction (Humphries et al. 2017).

Next, they studied the attraction of motile cells which was due to changes in extracellular potassium generated during biofilm oscillations using the microfluidic device. They demonstrated that changes in extracellular potassium gradients are sufficient to direct motile cell behavior. The role of the potassium ion channel in motile cell attraction is experimentally verified and it shows that the potassium ion channels in biofilm cells play very important role in generating the electrical signal that attracts motile cells. Moreover, it shows that attraction also depends on the membrane potential- mediated sensitivity of the motile cells to the potassium signals generated by the biofilm.

Mathematical Modeling

Now this experimental finding can be integrated in the framework of mathematical model. Basically, A.L. Hodgkin and A.F. Huxley first introduced this framework in the series of papers concerned with the flow of electric current through the surface membrane of a giant nerve fiber. Here, they utilized this electro-physiological model (Hodgkin and Huxley 1952) to predict changes in membrane potential in response to extracellular potassium. An agent based physical model has been incorporated in this electro-physiological model to simulate the motility of individual cells. Cells were modeled as spherocylinders that moved according to Newton's law under the forces and torques caused by their own motility and contacts with other cells. Each cell was equipped with a system of ordinary differential equations coupling its electrophysiological state with its motility. These modeling approach shows that an oscillation source (biofilm) of extracellular potassium can periodically attract motile cells by changing their membrane potential. This model is providing the opportunity to independently validate modeling predictions through additional motile cell measurements. However, these results further validated the mathematical model and allow us to build a coherent framework to interpret experimental observation (Humphries et al. 2017; Liu et al. 2015; Prindle et al. 2015).

Distinct bacterial species communication

The membrane potential plays a general role in bacterial motility. Thus a new hypothesis is that the process of attraction based on inducing changes in membrane potential could apply to other bacterium as well. To answer this hypothesis, they studied the interaction of *P. aeruginosa* (quorum sensing, Gram- negative bacteria) cells with the pre-existing *B. subtilis* (quorum sensing, Gram- positive bacteria) biofilm. Experiment shows that motile *P. aeruginosa* cells are also become periodically attracted to the *B. subtilis* biofilm during electrical oscillations. It has been observed that a variation in the period of electrical signaling within the biofilm is directly matched by the period of *P. aeruginosa* attraction to the biofilm edge. This indicates that the mechanism of electrically mediated attraction is not limited to *B. subtilis* cells and thus enables cross- species interaction (Humphries et al. 2017).

The long range signaling mediated through potassium ions can generate a rapid response in cell motility, because it does not require biochemical synthesis or complex signaling networks. This work also suggests a new paradigm for the long range cross-species signaling that is generic in nature, where specific receptor or signaling pathways are not required. The result of this work raises many interesting questions regarding the effect of the newly invented signaling process over the quorum sensing bacterium in the complex co-existence of the biofilm communities and surrounding cells (Humphries et al. 2017).

Discussion and future scope

This work quite efficiently demonstrate the importance of membrane potential in bacterial motility. This ion-channel mediated signaling procedure is blessed with rapid response in cell motility of even physically distant cells due to its independence on complex signaling network. Thus as a result of distant species signaling, bacteria from a different species are attracted and become incorporated into a pre-existing biofilm. Thus the complex coexistence of biofilm with its surrounding cross species network has to raise many intriguing aspects of signaling mechanism in the mysterious micro-organism world.

Although the chemical forms of cell-to-cell communication i.e., quorum sensing is well recognized now a days, this work opens up a new direction on the possibility to couple chemical signaling with the ion channel mediated electrical signaling. In 2015, a study by Prindle et al. have already suggested that to coordinate metabolism within biofilm, bacteria use potassium ion-channel-mediated electrical signaling mechanism. The chemical signaling spontaneously turns off while this generic electrical signaling is on its way for cross species communication. As a result chemical signaling or the quorum sensing halts within the biofilm through the electrical signaling mechanism. Thus the effect of electrical signaling on the chemical signaling is the next chapter to explore in future.

The regulation of bacterial gene expression through quorum sensing mechanism is well known. Thus the electrical communication between distant cells can have an impact on the bacterial gene expression. Apart from that, the effect of environmental noise on this efficient electrical signaling can promote diverse effects in bacterial communities.

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