EFFECT OF CHEMICAL SIGNALING ON THE SWIMMING BEHAVIOR OF *Vibrio fischeri*

Sandeep S. Suravajhala* and Prabhu R. Nott

Department of Chemical Engineering, Indian Institute of Science, Bangalore, 560012, India

Summary Bacterial motility is affected by autoinducers. The reduction in motility is temporary and occurs only when cells sense a quorum in their environment. This behavior is attributed to the cross-talk that occurs between the quorum sensing circuit and the motility circuit.

Bacteria are social creatures and talk to each other by releasing and detecting chemical signals. As in higher organisms, the information supplied by these chemical signals is critical for synchronizing activities of large group of cells. Quorum sensing is a term used for the ability of bacteria to detect local cell density with the help of these chemical signals, also called autoinducers. It has been discovered that the formation of biofilms, bioluminescence and motility are under the control of quorum sensing. The present study tries to understand the effect of autoinducer on the swimming motility of *Vibrio fischeri*, a marine bacterium, which is highly motile and possesses luminescent properties. The motivation in carrying out this study lies in the fact that motility is one of the important factors, which causes bacteria to become virulent invaders in humans and other animals causing various diseases. Bacteria are also responsible for food poisoning and formation of thick biofilms, which causes fouling of surfaces of ships and submarine structures.

Bassler et al.[1] had shown that cell density-dependent expression of luminescence in *Vibrio harveyi* is regulated by the concentration of an extracellular autoinducer in the culture medium. Fuqua et al.[2] had called this phenomenon quorum sensing and proved that it is responsible for the collective behavior of bacteria. But they did not consider the effect of motility in the description of collective behavior. On the other hand, recent studies[3, 4] have investigated the collective behavior of swimming microorganisms mediated purely by physical forces in the liquid. But these studies did not consider chemical communication present among bacterial cells. Therefore, a study that combines motility and chemical signaling is necessary to give a complete picture of interactions present in bacterial cells.

Here, we describe the swimming behavior of *V. fischeri*, affected by the external addition of the autoinducer. We propose that the signaling circuit affects the motility circuit, which in turn affects other coordinated activities of the bacterium.

MATERIALS AND METHODS

Bacteria

Wild-type *V. fischeri* strain (provided by Dr. Santosh Dubey, Goa University) was obtained from the shores of the Arabian Sea. The strain was found to be highly motile and luminescent.

Growth media and conditions

Marine broth was used as the growth media containing the following ingredients per litre-peptone(5 g), yeast extract(1g), NaCl(15 g), $MgSO_4.7H_2O$(1.5 g), KCl(0.38 g), glycerol(3 ml), double-distilled water(500 ml) and artificial sea-water(500 ml). Artificial sea-water was prepared by adding various salts in required concentrations. *V. fischeri* cells were inoculated from glycerol stock in 10 ml of the media and incubated with shaking at 28°C and 130 rpm.

Preparation of cell suspensions

The culture was grown to an absorbance of 1 (600 nm) and the cells were suspended in the growth media to a density of $8x10^8$ cells/ml. The cells were highly luminescent at this point.

Autoinducer

V. fischeri autoinducer, N-3-oxo-hexanoyl-L-homoserine lactone was obtained from Sigma Aldrich (Bangalore, India).

---

*Corresponding author. Email: srinivasa@chemeng.iisc.ernet.in

Live-cell imaging

The cell-suspension was further diluted to $1x10^2$ cells/ µl. These cells were taken as a 1 µl drop on a glass plate and autoinducer at concentrations of 25, 50, 75 and100 nM was added to the cells. Imaging was done by using Olympus BX51W1 upright microscope with a camera attached to it.

RESULTS AND ANALYSIS

Live-cell imaging revealed that the cells, which were highly motile instantly slowed down upon the addition of the 50 nM and higher concentrations of the autoinducer. They further slowed down and completely lost their motility as time lapsed by 3 min. This behavior could be because of the sudden shock that the bacterium had experienced upon coming into contact with the autoinducer. We hypothesize that there could be a signaling pathway connecting the autoinducer molecule and the flagellar motor proteins, which sets in as soon as a cell comes in to contact with the autoinducer. This pathway might not allow sodium ions (protons for non-marine bacteria), which are responsible for the movement of the motor, to flow into the cell. Cells regained their lost motility when the culture drop was diluted with the growth medium. It could be because the signaling pathway breaks and the sodium ions freely flow into the cell rotating the motor, as the autoinducer concentration is not high enough to establish a connection with the motor proteins. No change in motility was observed when the autoinducer at a concentration of 25 nM was added to the cells. This behavior follows the explanation give above. It should be noted that the cells were taken for imaging only after they had sensed the quorum, during which, they were highly luminescent. Addition of high concentration of autoinducer to the cells, which were taken before they had detected the quorum, did not affect the motility in any way. Long term effects of autoinducer on the motility of *V. fischeri* need to be studied, as this microorganism forms biofilms, and motility plays an important role under such circumstances.

We propose to establish a connection between the quorum circuit and the motility circuit. This connection would envelop the explanation given for the effect of the autoinducer on motility. We hypothesize that the complex, which controls the transcription of the light-regulating operon affects the motility circuit. It might direct the gene that is responsible for the motility of cell, to further direct the autoinducer to establish a signaling pathway with the motor proteins. This mechanism would be active only when the cell-density is high enough to sense a quorum. Consequently, motility decreases when the cells start luminescing. Therefore, we say that there is a cross-talk between the quorum and the motility circuits. We hope to prove our hypotheses by conducting appropriate studies on the organism and also bring out a quantitative analysis of our studies. The solution to this problem might reveal some of the aspects of bacterial behavior in general and virulence, biofilm formation, etc., in particular.

CONCLUSIONS

Quorum sensing in *V. fischeri* affects motility by means of an exchange of information between the quorum sensing circuit and motility circuit. Decrease in motility of the cells is a short-term effect of the autoinducer. This behavior, we propose, is the result of an establishment of the pathway between the autoinducer and motor protein. It should be seen whether this behavior follows a particular pattern in long time, when the cells are continuously exposed to the autoinducer.

References

1. Bassler BL, Wright M, Showalter RE, Silverman MR, 1993, Intercellular signalling in *Vibrio harveyi*: sequence and function of genes regulating expression of luminescence. Molecular Microbiology, Volume 9, Issue 4, 773-786.

2. W. Caliborne Fuqua, Stephen C. Winans and E. Peter Greenberg, 1994, Quorum Sensing in Bacteria: the LuxR-LuxI Family of Cell Density-Responsive Transcriptional Regulators. Journal of Bacteriology, Volume 176, No. 2, 269-275.

3. Vishwajeet Mehandia and Prabhu R. Nott, 2008, The Collective dynamics of self-propelled particles, Journal of Fluid Mechanics, Vol. 595, pp. 239-264.

4. Donald L. Koch and Ganesh Subramanian, 2011, Collective Hydrodynamics of Swimming Microorganisms: Living Fluids, Annu. Rev. Fluid Mech, 43:637-59.