MULTISCALE MODELING OF *PSEUDOMONAS AERUGINOSA* SWARMING

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Summary Many bacteria including *P. aeruginosa* are able to spread rapidly over surfaces by the process of swarming. Swarm motility of *P. aeruginosa* transpires as cells use their single polar flagellum as a rear propulsion engine. *P. aeruginosa* swarming has also been shown to be greatly influenced via the production of rhamnolipid, a bio-surfactant which acts to lower the local surface tension of the liquid layer and ease spreading over surfaces. Rhamnolipid synthesis is only initiated in the presence of a threshold, or quorum, of cells. We developed a three-dimensional (3D) multiscale modeling environment to gain insight of bacteria swarming.

INTRODUCTION

Swarming is a mode of bacterial motility where cells act not as individuals but as coordinated groups to move across surfaces. Regulation of swarming is controlled by complex and various multiscale events. While swarming, motion of a bacterial community may depend upon the gene expression of individual cells, the sensing of chemical signals present in a hydrating environment, and the physical surface characteristics influencing the swarming bacteria. Some swarming bacteria are also aided by production of a surfactant that lowers surface tension of the liquid film to improve bacterial motility. In *P. aeruginosa* swarming, tendril patterns at the colony edge are usually observed in experiments (see Figure 1(left)). Rhamnolipid as a product of quorum sensing works as a wetting agent extracting liquid from substrate and as a bio-surfactant lowering surface tension to ease liquid spreading and bacteria swarming. Surface tension gradient which drives fingering pattern formation of a liquid film has been observed and studied before in many physical systems [2]. The goal of this paper is to study how this mechanism evolved and optimized as a result of evolution, in a biological system of swarming bacteria in order to facilitate efficient swarming.

MATERIALS AND METHODS

Experimental Method

Bacterial Strains. Swarming was examined for *Pseudomonas aeruginosa* ATCC strain 15692 harboring miniTn7*gfpmut2* for fluorescence.

Swarming Assays. Swarming was studied using plate assays of and FAB medium with 12 mM glucose containing soft (0.45%) noble agar. Swarm assay plates were inoculated with log-phase cells (OD600~0.7). Rhamnolipid upon these plates was imaged by quantifying fluorescence of stain Nile Red, a known lipid stain. These rhamnolipid indicator plates included a 1 in 100 dilution of filter-sterile stock containing 1 mg mL^{-1} Nile Red (MP Biomedicals) dissolved in 85% propylene glycol (prepared the day of use to limit photoinactivation). Plates were cured and incubated in the dark.

Imaging Techniques. Fluorescence images of swarm plates were acquired using a Carestream Multispectral FX (MSFX) imaging station (Carestream Health, Woodbridge, CT). Excitation and emission wavelengths for GFP and Nile Red were 480/535nm and 540/600nm, respectively. Images were further processed using Nikon Elements software.

Multiscale Computational Model

Below we briefly describe individual submodels and their integration into a multiscale computational model of *P. aeruginosa* swarming.

Off-lattice Cell-based Submodel. Each *P. aeruginosa* cell is represented by three connected nodes. We did not consider cell alignment since *P. aeruginosa* is less understood compared to Myxobacteria [1] and it is not known if *P. aeruginosa* aligns during swarming. Cell growth is simplified to depend upon the local nutrient concentration; an individual cell consumes nutrients and stores energy within the cell. Once the stored energy meets a threshold value, this cell divides into two daughter cells. The cell movement is controlled by the local environment. If the liquid depth is greater than $10\mu m$ (about 10 times of the cell width), cells swim with liquid in addition to random movement; otherwise, cells just move randomly.

Quorum Sensing Process and Nutrient Uptake Submodel. A simplified quorum sensing model is employed for each cell. We only include one signal molecule, which corresponds to AHL of *P. aeruginosa*. This signal activates the synthesis of rhamnolipid. If the concentration of rhamnolipid is greater than a given threshold, liquid is extracted from

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the substrate. In our model, the cells have two stages controlled by threshold levels of quorum sensing chemical concentration: 1) the solitary state, and 2) the activated state. Macroscopic nutrient level, production and propagation of quorum sensing signals and rhamnolipid are modeled using convection-diffusion-reaction equations describing evolution of these fields. The model does not include chemotactic effects: cells do not sense the nutrient concentrations or gradients, and/or AHL signals, and they do not orient their movements as a function of these gradients. Rather, they simply switch their state in a threshold-dependent manner.

Thin Film Submodel. We employ the following thin viscous fluid flow equation obtained by a lubrication approximation of the Navier-Stokes equations [2] to describe the liquid layer: $h_t = -\nabla \cdot \left(h \left(\frac{\gamma_m}{3\mu} h^2 \nabla\nabla^2 h + \frac{h}{2\mu} \nabla\gamma \right) \right) + Eh.$ The vertically averaged fluid velocity is $U = \frac{\gamma_m}{3\mu} h^2 \nabla\nabla^2 h + \frac{h}{2\mu} \nabla\gamma$, where h is the thickness of the liquid coating layer on a planar substrate, U is the vertically averaged fluid velocity, μ is the dynamic viscosity ($\mu = \rho\nu$); γ_m is the minimal surface tension at maximum packing. E describes extraction of liquid by the bacteria. Surface tension γ depends on the rhamnolipid concentration Γ. We use the constitutive law used in [2] to describe dependence of the surface tension on the rhamnolipid concentration. Viscosity γ depends on the levels of volumetric density of cells.

Coupling of the Off-lattice Submodel and Continuum Submodels. The off-lattice grid is superimposed on and aligned with the partial differential equation (PDE) grid and can be viewed as the refinement of the PDE grid. Each simulation time step consists of a substep of evolution of states of the PDEs followed by a substep to solve cell-based off-lattice model. During the substep of the cell-based off-lattice model, cells move to a new location, consume nutrients, divide and produce quorum sensing signals, which modify the local nutrient and quorum sensing fields.

Parallel Implementation. We utilize the Newton-Krylov subspace iteration method implemented in KINSOL package to solve system of nonlinear equations resulted from discretizing thin film equation. Due to high nonlinearity and stability issues related to solving thin film equation, small time steps of the order $O(10^{-5})$ are used. Current simulation code has been parallelized on the MPI-based Linux cluster at the University of Notre Dame and a linear speed-up is achieved.

RESULTS AND DISCUSSION

Figure 1(left) shows the experimental snapshots of *P. aeruginosa* wild-type swarming on 0.45% agar+glucose over one day. The wild-type strain swarms fast and expands to cover the whole plate in about two Days. Green fluorescent images were pseudo-colored using Blue-Green-Fire intensity settings. And simulation results (Figure 1(right)) show cell density distribution of primary and secondary tendrils at t=7hrs, similar to the ones obtained in experiment.

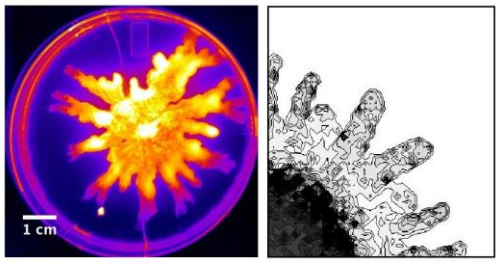

Figure 1. Experimental snapshot (left) and simulation results (right).

Simulation results qualitatively agree with the experimental observations, indicating that our model captures the biological phenomena of *P. aeruginosa* swarming. The fractal tendril formation at the colony edge helps the cells to spread in the radial direction and occupy nutrient in a fast way. The bacteria produce rhamnolipid in response to the quorum sensing. Then rhamnolipid extracts liquid from the agar as well as decreases the surface tension of the liquid. Rhamnolipid plays an important role in bacteria swarming in two ways: high level of rhamnolipid concentration results in extraction of liquid from substrate and the spreading of thin liquid film helps *P. aeruginosa* swarm by carrying bacteria cells forward and allowing them to move on their own using flagella; as a bio-surfactant, rhamnolipid lowers local surface tension, and thus eases liquid spreading and cell movement. Simulations demonstrated that combination of these mechanisms could result in formation of tendrils at the edge of a swarm which have been earlier observed in experiments.

References

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