Osmotic growth of *Vibrio cholerae* biofilms

Howard A. Stone[1], Ned S. Wingreen[2], Bonnie B. Bassler[3], and Jing Yan[4]

***Short Abstract* —Biofilms are surface-attached communities of bacteria encased in an extracellular matrix. Much is known about the matrix components and their regulatory processes. However, largely unexplored is how the material properties of the matrix contribute to biofilm growth. Here, using *Vibrio cholerae* as our model organism, we show that matrix production establishes an osmotic pressure difference between the biofilm and the external environment. This difference promotes biofilm expansion on nutritious surfaces, enables matrix-producing cells to outcompete cheaters, and controls the growth of submerged biofilms. Our findings have broad implications for other biofilm-forming species, as the principles of osmotic swelling are universal.**

***Keywords* — Biofilms, Osmotic pressure, Hydrogel, Polymer physics, *Vibrio cholerae*.**

I. Introduction

BACTERIA survive over a remarkable range of osmotic pressures. Adaptation to extremes in osmolarity depends on active and passive mechanisms that maintain constant osmotic pressure differentials between individual cells and the environment. However, how bacteria respond collectively to osmotic pressure changes is not clear, particularly in spatially-structured communities such as biofilms [1,2] – surface-attached bacterial collectives embedded in a secreted polymeric matrix [3].

Major components of the typical matrix are extracellular polysaccharides (EPS), which function in conjunction with accessory proteins. Intensive research has focused on defining the functions of matrix components and the regulatory mechanisms driving matrix production [4]. Much less well studied is the physical nature and material properties of matrix networks. In biofilms, the high local concentration of polymer molecules surrounding the cells necessarily produces an osmotic pressure difference between the matrix and the external environment. It is not clear if or how such osmotic pressure gradients influence the mechanical properties of biofilms or the growth and fitness of the bacteria residing in them.

[1]Department of Mechanical and Aerospace Engineering, NJ, USA. E-mail: hastone@princeton.edu

[2]Department of Molecular Biology, Princeton University, NJ, USA. E-mail: wingreen@princeton.edu

[3]Department of Molecular Biology, Princeton University, NJ, USA. E-mail: bbassler@princeton.edu

[4]Department of Mechanical and Aerospace Engineering, Princeton University, NJ, USA, E-mail: jingyan@princeton.edu

II. Key Results

In this presentation, using the model biofilm-producer *Vibrio cholerae* [5], we systematically studied the effects of osmotic pressure on different aspects of biofilm development. In particular, we found that to a comparable extent, both passive physical swelling of the colony matrix and enhanced nutrient uptake leading to increased cell doubling contribute to *V. cholerae* colony biofilm expansion, and both processes are only possible if the cells produce EPS, which functions as an extracellular osmolyte. The osmotic pressure driven colony expansion also gives rise to characteristic colony morphologies, characterized by large and periodic radial ridges. The expansion process is modulated by the accessory proteins in the *cholerae* biofilms, which either increases cell-to-cell adhesion or cell-to-substrate adhesion. We further demonstrated that, despite the possibility of being exploited by the non-producer cells, matrix producing cells use the physical process of osmotic-pressure-driven swelling of the EPS network to exclude and outcompete cheater cells. With regards to biofilms submerged in water, we demonstrated that the osmotic-pressure-driven polymer expansion controls the biofilm architecture and expansion process.

III. Conclusion

We have demonstrated here the importance of osmotic pressure generated by the hydrogel-like *V. cholerae* EPS matrix to the growth and surface coverage of *V. cholerae* biofilms residing at air-solid interfaces and submerged in liquids. The physical principles underlying the behavior of this biological hydrogel should be applicable to other biofilm matrices. Our results, furthermore, underscore the importance of understanding the physical forces that together with biochemical and genetic features drive biofilm ecology.

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