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Biofilms: Microbial Cities Wherein Flow Shapes Competition

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The phenotypic diversity in biofilms allows bacteria to adapt to changing environmental conditions. Stochastic gene expression and structural differentiation are believed to confer phenotypic diversity. However, two recent publications demonstrate how hydrodynamic flow and substrate topography can also alter the competitive outcomes of different bacterial phenotypes, increasing biofilm phenotypic diversity.

Biofilms are microorganisms embedded within a self-secreted matrix to form robust communities where competitive and cooperative interactions take place. Biofilms often have increased defenses to antimicrobial attack and cause chronic infections. This is often attributed to the exuded matrix that protects the cells by concealing them from host immune responses, enhances horizontal gene transfer, alters virulence and increases metabolic cooperativity. The ability of biofilm bacteria to exist in a wide range of physiological states is also considered a major contributor to biofilm robustness because it provides insurance against changing environmental conditions and stressors.

Biofilm bacteria share extracellular polymeric substances (EPS) that build the matrix, secreted enzymes, and useful metabolites such as iron scavenging siderophores as a form of cooperativity. These pooled resources secreted by the bacteria are commonly termed as 'public goods'. As such, they are vulnerable to exploitation by 'cheaters', i.e., mutants that no longer contribute, but still benefit from these resources. Clearly, mechanisms that prevent the proliferation of cheaters must exist, and several have been hypothesized and identified. For example, theoretical modeling shows bacterial strains that produce EPS can push cells of their own lineage out into nutrient rich conditions and are better at invasion and adhesion than non-EPS producers [1]. Indeed, *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* EPS-producing strains can form floating biofilms (pellicles) to provide their kin with greater access to oxygen [2, 3]. However, in the case of *P. fluorescens*, when excess EPS-producing variants accumulate in the pellicle, the pellicle becomes too heavy and collapses. Constitutively EPS-producing *Vibrio cholerae* mutants greatly outcompete non-EPS producing mutants in biofilms due to their superior adhesive capabilities. This increased adhesion, however, can also impair dispersal and re-colonization of new niches [4]. In order to understand the evolution of microbial cooperation, it is important to identify the factors that maintain the balance between different variants (producers, over-producers and cheaters).

The cost, diffusion and durability of the public good, and cell diffusion are examples of such factors. In the case of *P. aeruginosa*, growth differences between wild-type EPS producers and non-EPS producers can be small [2, 5]. The EPS itself is of low diffusivity due to its high molecular weight and polymeric nature, and lessens cell diffusion by trapping them within it. It can also be physically attached to producer cells [6] and thus helps producers to 'privatize' EPS production, making the phenotype very competitive and less prone to cheating.

Given that public good and cell diffusion are recognized as important factors, the biofilm flow landscape may affect the balance between producers and cheaters, and therefore strain variation. However, most biofilm studies are performed on uniform substrates where cells are exposed to simple laminar flow, when in reality biofilms in nature grow in complex heterogeneous environments such as porous aquifers, filters, living tissue and soil. Heterogeneous environments are rich in ecological niches that would facilitate the evolution of variants that are

better adapted to these niches. In addition, *P. aeruginosa* PA14 biofilms using Pel as the dominant EPS form streamers under complex flow regimes and non-uniform substrates [7], which could provide alternative niches to the flat cell layers and differentiated microcolonies that form under uniform flow and substrate regimes.

Recently, Nadell *et al.* have shown that selection of EPS-producing strains in *P. aeruginosa* biofilms can be altered according to flow regime [8]. Under uniform flow and substrates using planar chambers, wild-type PA14 increases in abundance relative to a $\Delta pelA$ mutant in the biofilms, irrespective of the inoculation ratio. Instead, an abundance of $\Delta pelA$ cells relative to the wild-type cells resulted in the effluent, indicating that they were removed from the substratum or biofilm over time. However, a shift in competitive balance between EPS-producers resulted when grown in chambers featuring a heterogeneous micropillar distribution and size, with flow rates reflecting those of a porous soil environment. Specifically, a negative frequency-dependent selection for Pel production was observed in 3-day-old biofilms when the inoculum ratio of wild-type to $\Delta pelA$ was greater than 3:2. This was attributed to the wild-type obstructing the flow to certain regions, particularly by forming streamers between the micropillars. This allowed $\Delta pelA$ to accumulate in the regions protected from shear flow, and thus stably coexist with the wild-type.

In another recent study by Coyte *et al.*, *Escherichia coli* strains that lacked the RpoS factor, and thus were slower growing, stably co-existed with the wild-type on porous substrates [9]. Here, rapidly growing strains developed thick biofilms that reduced channel space, thereby diverting nutrient flow to channels with thinner biofilms formed by slow growing strains. This may be applicable to EPS mutants as well, as they form thinner biofilms.

In conclusion, the studies by Nadell *et al.* [8] and Coyte *et al.* [9] highlight the importance of hydrodynamic shear and substrate geometry in determining biofilm structure and function, as they affect cell detachment and transport of nutrients, as well as generate more niches, impacting on the phenotypic diversity of microbial communities. How different public goods affect biofilm mechanical and structural properties to alter the response to hydrodynamic shear, substrate geometry and niche generation requires further exploration. For example, the Psl polysaccharide in *P. aeruginosa* densely crosslinks and makes the biofilm elastic, which enhances surface-attached biofilms and microcolony formation, but reduces spreading and streamer formation, which is supported by the Pel polysaccharide [10]. Such studies can unravel the complex interplay between external environmental factors and biofilm internal properties involved in niche generation and microbial diversity, thus further enhancing our understanding of this fundamental microbial lifestyle.

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