# The interplay between environmental flow and extracellular matrix production determines lineage segregation during bacterial surface colonization.

Ricardo Martínez-García[1], Carey D. Nadell[2] and Juan A. Bonachela[3]

[1] Department of Ecology and Evolutionary Biology, Princeton University, Princeton, NJ 08544, USA
[2] Max Planck Institute for Terrestrial Microbiology, Marburg 35043 , Germany
[3] Department of Mathematics and Statistics, University of Strathclyde, Glasgow, Scotland, UK

Contrarily to the traditional view of bacteria as single drifting organisms that inhabit liquid environments, they often form dense conglomerates attached to surfaces, termed biofilms. Within a biofilm, cells are embedded in a matrix of extracellular polymeric substances (EPS) that binds bacteria among them and to the surface, thus magnifying cell-cell interactions and the complexities of their life cycle [1, 2].

As a consequence of reinforced adhesiveness, cells become more resistant to shear forces induced by flows at the liquid-solid interface, which increases their resources exploitation rate but reduces their chances of being dispersed and colonize new environments [3]. Therefore, EPS production and the subsequent biofilm formation not only influence the spatial structure of the bacterial colony (Fig. 1), but it can also determine its survival in different environmental conditions, for example in the presence of flows.

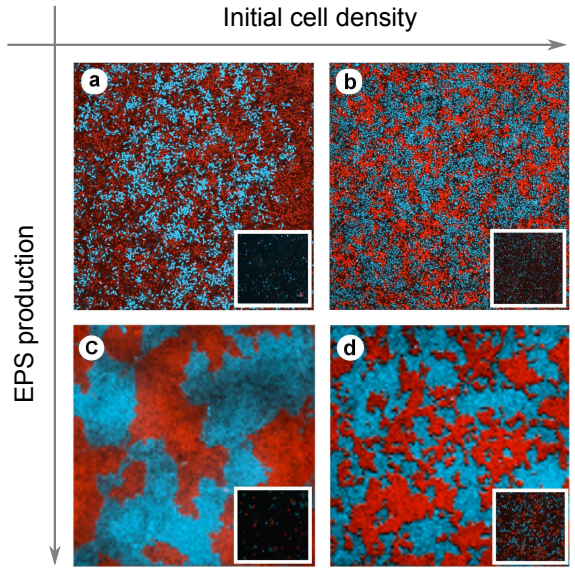


Figure 1: Experimental colonization patterns for nonadhesive EPS-nonproducers (top) and adhesive EPS-producers (bottom). Initial cell density, whose spatial distribution is shown in the insets, increases from left to right.

Surprisingly, the influence that an environmental flow may have on bacterial spatial organization has received little attention in the literature. Here, we aim to fill this gap by studying experimental and theoretically the surface colonization patterns of a population of the bacterium *Vibrio cholerae* growing in microfluidic devices from different initial densities. Our results suggest that adhesiveness plays a key role in the surface colonization pattern, with flow playing an important role even under controlled environments such as the microfluidic chamber (Fig. 2). We use spatial correlation functions to quantify the differences between the emergent two lineage segregation patterns (each lineage is represented by a different color in the pattern, see Fig. 1), as well as to determine with numerical models the dependence of the competitive ability of the cells on both adhesiveness and strength of the flow in the chamber.

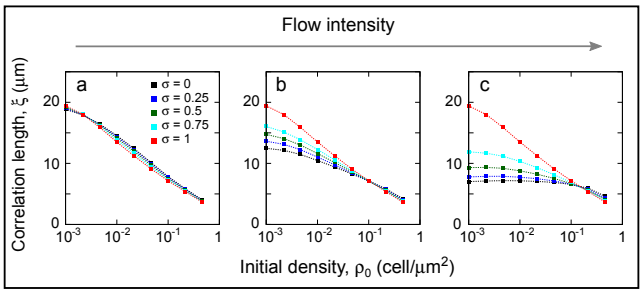


Figure 2: Effect of cell adhesiveness, $\sigma$, and flow intensity on the mean correlation distance of simulated patterns. The role of the density of founder cells is shown in the horizontal axis of each panel.

Finally, we discuss the evolutionary implications of matrix formation for the colonization strategies of founding cells, with a main focus in public good production. Our results emphasize the importance of considering both flow and adhesiveness when estimating the virulence of pathogens such as *V. cholerae*, which colonize environments that, like the human gut, are intrinsically affected by flows of different strengths. More importantly, the generality of our model and the iniquitousness of biofilms and flows like the ones described here facilitate the extrapolation of our theory to other organisms.

[1] C.D. Nadell, K. Drescher, K. Foster, "Spatial structure, cooperation and competition in biofilms", *Nature Rev. Microbiology*, **14**(9), 589-600, 2016.

[2] J.K. Teschler *et al.*, "Living in the matrix: assembly and control of *Vibrio cholerae* biofilms", *Nature Rev. Microbiology*, **13**(5), 255-68, 2015.

[3] C.D. Nadell, B. Bassler, "A fitness trade-off between local competition and dispersal in *Vibrio cholerae* biofilms", *PNAS*, **108**(34), 14181-85, 2011.