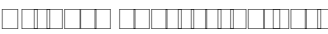


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Abstract

We present data of spatial organizations arising from competition and cooperation in biofilms and provide a computational model for its interpretation. Using a simple assay that varies nutrients available to cells we observe that  biofilms form Turing patterns with length scales in the tens of micrometers. Using a spatial model that combines kinetics of microbial growth with mechanics of spreading we show that physical interaction and nutrient competition drive evolutionary competition among cells and explain the patterns. We combine this with social evolution theory to show that selection can favor energetically costly spreading strategies that disrupt the patterns but also leave biofilms vulnerable to antimicrobials.

Introduction

Biofilms are central to microbial life and have implications in many human activities (Kolter and Greenberg 2006). In particular, biofilms are involved in persistent infections (Costerton, Stewart et al. 1999) including opportunistic lung infection of cystic fibrosis patients (Singh, Schaefer et al. 2000). However, the evolution of bacterial biofilms remains unclear. If biofilm formation requires the production of shared resources that carry a cost in reproductive success of individual bacteria then it is open to exploitation by selfish individuals (West, Griffin et al. 2006; Xavier and Foster 2007). Here, we ask what are the selective forces that maintain biofilms in the face of selection for rapid growth?

Experimental observations

Biofilms of the opportunistic human pathogen *Pseudomonas aeruginosa* were incubated for 24 h in still liquid in the absence of hydrodynamic influences. At the end of this period, a dense band of biofilm is visible to the naked eye close to the air-water interface. Microscopic observation reveals structural patterns located on upper and lower edges of the band. The sparseness in patterns increases for locations away from optimal locations, displaying regular shapes over hundreds of micrometers that shift through hole- (figure 1), labyrinth- and spot-patterns.

Mathematical model

We investigated whether the patterns can be the product of self-organized morphogenesis induced by nutrient limitation. For this, we used a mathematical model of horizontal spreading. The model considers a source of nutrients with a constant

concentration located above the surface. Nutrient transport occurs by diffusion, driven by consumption in the biofilm. Lateral nutrient gradients are computed using mass-balance equations with rates set by simple Monod kinetics. Cell growth causes microbial spreading into unoccupied areas of the solid surface, maintaining cell density constant (figure 2).

Our model reveals that scale-dependent social interactions between bacteria produce the patterns observed in *P. aeruginosa* biofilms. Nutrient consumption is a long-range negative interaction that contradicts spreading, a short-range positive interaction from neighbor-pushing towards nutrient rich regions.

In these settings, competition at the biofilm level can lead strains to evolve surface spreading abilities that leave the biofilm more susceptible to antimicrobials.

Conclusions

Many traits essential for bacterial pathogenicity are based on cooperation, such as the production of shared products (Griffin, West et al. 2004). Since cooperation may be exploited by cheaters, virulence is expected to decrease where conflict occurs. This explains the counter-intuitive observations that multiple-strain infections often favor the less virulent strain and suggests that the link between pathogenicity and cooperation may be exploited in novel infection control strategies (Foster 2005). Our data shows that biofilm sociality is best described as scale-dependent, rather than strictly competitive or cooperative and points out new directions for biofilm control.

References

- Costerton, J. W., P. S. Stewart, et al. (1999). "Bacterial biofilms: a common cause of persistent infections." Science **284**: 1318-1322.
- Foster, K. R. (2005). "Biomedicine. Hamiltonian medicine: why the social lives of pathogens matter." Science **308**(5726): 1269-70.
- Griffin, A. S., S. A. West, et al. (2004). "Cooperation and competition in pathogenic bacteria." Nature **430**(7003): 1024-1027.
- Kolter, R. and E. P. Greenberg (2006). "Microbial sciences: the superficial life of microbes." Nature **441**(7091): 300-2.
- Singh, P. K., A. L. Schaefer, et al. (2000). "Quorum-sensing signals indicate that cystic fibrosis lungs are infected with bacterial biofilms." Nature **407**(6805): 762-764.
- West, S. A., A. S. Griffin, et al. (2006). "Social evolution theory for microorganisms." Nat Rev Microbiol **4**(8): 597-607.
- Xavier, J. B. and K. R. Foster (2007). "Cooperation and conflict in microbial biofilms." Proc Natl Acad Sci U S A **104**(3): 876-81.

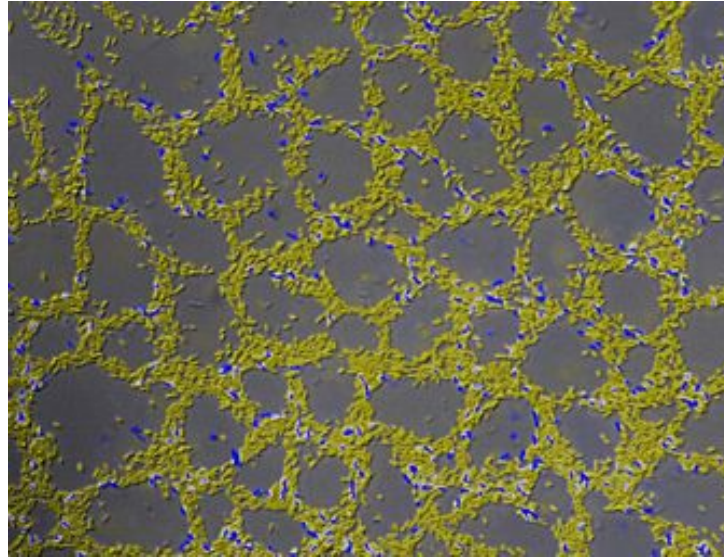


Figure 1 – Hole-patterns in self organized patchiness in Biofilms of *P. aeruginosa*.

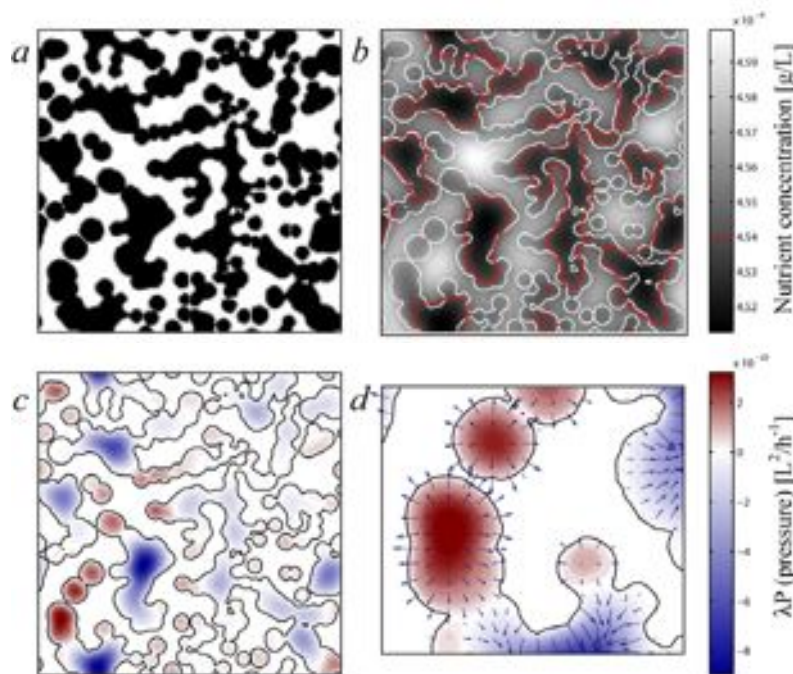


Figure 2 – Details on the 2-D simulation of biofilm spreading on a flat surface. Nutrients diffuse into the system from a source located above the surface. Microbial growth (**a**, biomass represented in black over a white background) causes horizontal gradients in nutrient concentration (**b**). The growth rate is a function of local nutrients, and biofilm grows only when concentration is high enough to overcome maintenance requirements (the red isoconcentration line in **b** represents limit nutrient value where maintenance requirements match). The cumulative effects of growth in a patch of biofilm generate pressure (**c**) which causes the biofilm front to advance. The velocity at which the microbial front advances (represented by the arrows in the close-up **d**) is determined from the gradient of the pressure.