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PHAGE MOBILITY IS A CORE DETERMINANT OF PHAGE–BACTERIA COEXISTENCE IN BIOFILMS

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ABSTRACT

Many bacteria are adapted for attaching to surfaces and for building complex communities, termed biofilms. The biofilm mode of life is predominant in bacterial ecology. So too is the exposure of bacteria to ubiquitous viral pathogens, termed bacteriophages. Although biofilm–phage encounters are likely to be common in nature, little is known about how phages might interact with biofilm-dwelling bacteria. It is also unclear how the ecological dynamics of phages and their hosts depend on the biological and physical properties of the biofilm environment. To make headway in this area, we develop a biofilm simulation framework that captures key mechanistic features of biofilm growth and phage infection.

Using these simulations, we find that the equilibrium state of interaction between biofilms and phages is governed largely by nutrient availability to biofilms, infection likelihood per host encounter and the ability of phages to diffuse through biofilm populations. Interactions between the biofilm matrix and phage particles are thus likely to be of fundamental importance, controlling the extent to which bacteria and phages can coexist in natural contexts. Our results open avenues to new questions of host–parasite coevolution and horizontal gene transfer in spatially structured biofilm contexts.

1.0 INTRODUCTION

Bacteriophages, the viral parasites of bacteria, are predominant agents of bacterial death and horizontal gene transfer in nature. Their ecological importance and relative ease of culture in the laboratory have made bacteria [6] and their phages a centerpiece of classical and recent studies of molecular genetics and host–parasite interaction. This is a venerable literature with many landmark discoveries, most of which have focused on liquid culture conditions [5]. In addition to living in the planktonic phase, many microbes are adapted for interacting with surfaces, attaching to them and forming multicellular communities.

These communities, termed biofilms, are characteristically embedded in an extracellular matrix of proteins, DNA and sugar polymers that have a large role in how the community interacts with the surrounding environment [11].

There is currently only a limited understanding of the mechanisms responsible for this observed variation in outcome, and there has been little exploration of how phage infections spread within living biofilms on the length scales of bacterial cells. Biofilms [10], even when derived from a single clone, are heterogeneous in space and time. The extracellular matrix can immobilize a large fraction of cells, constraining their movement and the mass transport of soluble nutrients and wastes. Population spatial structure, in turn, has a fundamental impact on intraspecific and interspecific interaction patterns. Theory predicts qualitative changes in population dynamics when host–parasite contact rate is not a simple linear function of host and parasite abundance[12], which is almost certainly the case for phages and biofilm-dwelling bacteria under spatial constraint [7].

2.0 LITERATURE REVIEW

Abedon S. (2017) Many bacteria are adapted for attaching to surfaces and for building complex communities, termed biofilms. The biofilm mode of life is predominant in bacterial ecology. So too is the exposure of bacteria to ubiquitous viral pathogens, termed bacteriophages. Although biofilm–phage encounters are likely to be common in nature, little is known about how phages might interact with biofilm-dwelling bacteria. **Rohan (2008)** Using these simulations, we find that the equilibrium state of interaction between biofilms and phages is governed largely by nutrient availability to biofilms, infection likelihood per host encounter and the ability of phages to diffuse through biofilm populations. **Chassis R. (2012)** Biofilms present complex assemblies of micro-organisms attached to surfaces. They are dynamic structures in which various metabolic activities and interactions between the component cells occur. When phage comes in contact with biofilms, further interactions occur dependent on the susceptibility of the biofilm bacteria to phage and to the availability of receptor sites. **Poper T. (2015)** The canonical bacteriophage is obligately lytic: the virus infects a bacterium and hijacks cell functions to produce large numbers of new viruses which burst from the cell. These viruses are well-studied, but there exist a wide range of coexisting virus lifestyles that are less understood. Viral steady state density maximization leads to coexistence of temperate and chronic viruses, explaining the presence of multiple viral strategies in natural environments.

3.0 METHODOLOGY

Bacteria and cultures

The strains used include E. coli NDM-1 (ATCC BAA-2452), P. aeruginosa PA01 (ATCC 15692), Bacillus subtilis 168 (ATCC 23857), and Shewanella oneidensis MR-1 (ATCC 700550). E. coli NDM-1 represents an enteric pathogenic bacterium[16] with multidrug resistance.25 P. aeruginosa is an opportunistic pathogen commonly active in biofilm formation.26 B. subtilis and S. oneidensis are relatively benign bacteria widely distributed in the environment.27,28 These bacteria were initially cultured in tryptic soy broth (TSB) [8] medium overnight and then transferred to modified M63 medium [2.4 g of KH2PO4, 5.6 g of K2HPO4, 1.6 g of (NH4)2SO4, 0.3 mg FeSO4 per liter water supplemented with 1 mM of MgSO4, 0.2% glucose and 0.5% casamino Acids [15] for biofilm formation in microtiter plates (Grace Bio-Labs, Bend, OR) and in a Center for Disease Control (CDC) biofilm reactor (Bioscience, Bozeman, MT) with polypropylene coupons. Total viable bacteria were counted by plate assay using Difco standard bacterial count agar (BD, Sparks, MD) and expressed as colony-forming-units (CFU) [14].

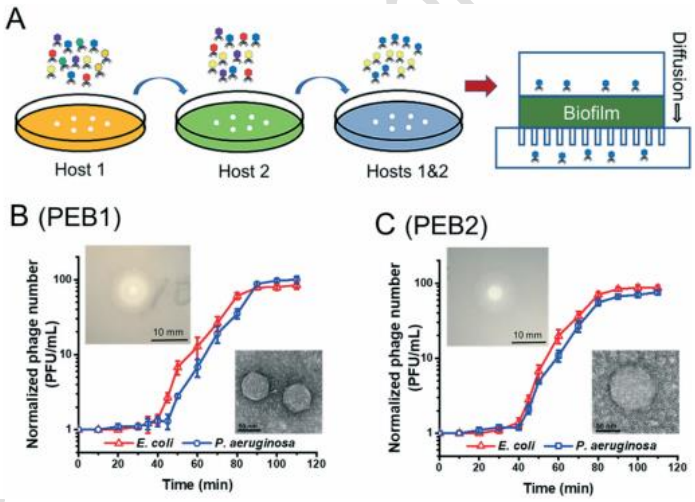


Figure Isolation and screening of phages with high biofilm disruption capability. (A) Schematic illustration of sequential multiple-host phage isolation approach followed by phage biofilm diffusion assay. (B) The plaque of isolated phage PEB1 on DLP and its morphology under TEM. (C) The plaque of isolated phage PEB2 on DLP and its morphology under TEM.

Phage isolation, screening and characterization

SM buffer was used for phage harvest, storage and dilution. The double-layer plaque (DLP) assay was adopted for phage enumeration as plaque-forming-units (PFU) [9]. Polyvalent phages

infecting both E. coli and P. aeruginosa [7] were isolated from wastewater using a sequential multiple-host approach.

4.0 RESULTS

The primary features distinguishing biofilm populations from planktonic populations are spatial constraint and heterogeneity in the distribution of solutes and cellular physiological state, which includes growth rate, and – we hypothesize – phage infection. Our aim here is to identify how these features qualitatively influence bacteria-phage population dynamics in biofilms. We omit the possibility of co-evolution, i.e., we do not consider the origin and maintenance of phage resistance among bacteria, or mutations that alter phage host-range. This simplification was made in order to focus clearly on the mechanisms and impacts of limited movement (of growth-limiting nutrients, bacteria, and phages) on bacteria-phage interaction. The foundation established in this way will be a starting point for understanding the broader problem of eco-evolutionary interplay between phages and their hosts in biofilms.

We began by exploring the different possible outcomes of phage infection in biofilms as a function of phage infectivity, before moving on to a more systematic study of phage transport, phage infection, and bacterial growth rates.

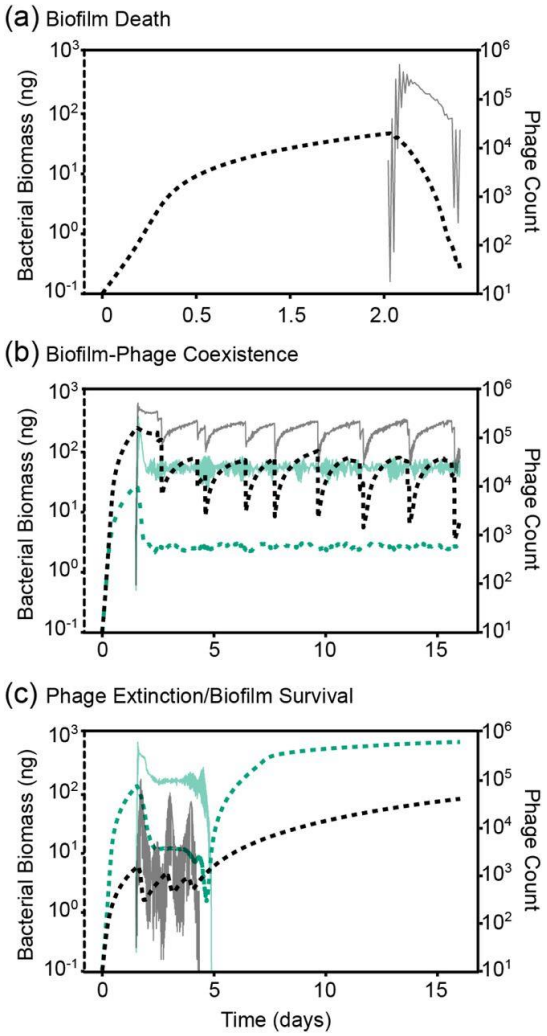


Figure Population dynamics of biofilm-dwelling bacteria and phages for several example cases.

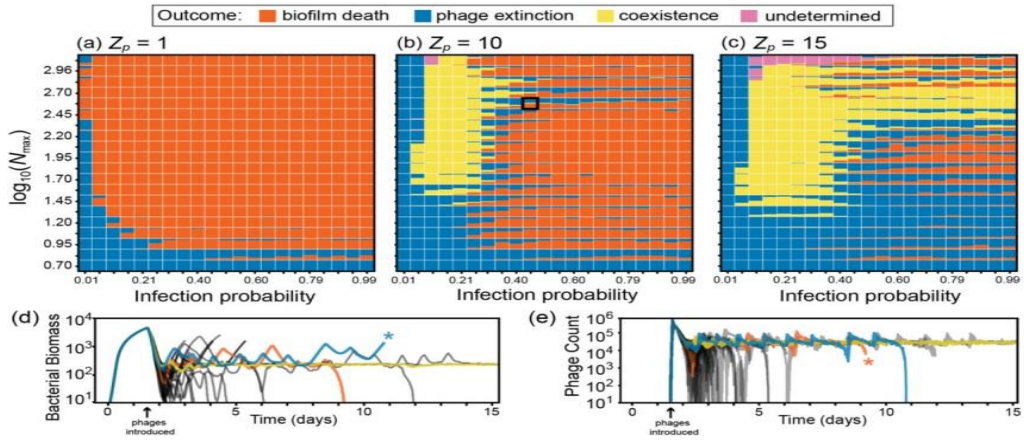
(a) Stable states of bacteria and phages in biofilms

Intuitively, the population dynamics of bacteria and lytic phages should depend on the relative strength of bacterial growth and bacterial removal, including erosion and cell death caused by phage infection. We studied the behavior of the simulations by varying the relative magnitude of bacterial growth versus phage proliferation. In this manner, we could observe three broad stable state classes in the bacteria/phage population dynamics. We summarize these classes here before proceeding to a more systematic characterization of the simulation parameter space.

(i) Biofilm death

If phage infection and proliferation sufficiently out-pace bacterial growth, then the bacterial population eventually declines to zero as it is consumed by phages and erosion. Phage infections

progressed in a relatively homogeneous wave, if host biofilms were flat (Supplementary Video SV1). For biofilms with uneven surface topography, phage infections proceeded tangentially to the biofilm surface and "pinched off" areas of bacterial biomass, which were then sloughed away after losing their connection to the remainder of the biofilm (Supplementary Video SV2). This sloughing process eventually eliminated the bacterial population from the surface.



Graph Steady states of biofilm-phage population dynamics as a function of nutrient availability, phage infection rate, and phage impedance.

(ii) Coexistence

In some instances, both bacteria and phages remained present for the entire simulation run time. We found that coexistence could occur in different ways, most commonly with rounded biofilm clusters that were maintained by a balance of bacterial growth and death on their periphery (Supplementary Video SV3). When phage infection rate and nutrient availability were high, biofilms entered cycles in which tower structures were pinched off from the rest of the population by phage propagation, and from the remaining biofilm, new tower structures re-grew and were again partially removed by phages. We confirmed the stability of these coexistence outcomes by running simulations for extended periods of time, varying initial conditions and the timing of phage exposure to ensure that host and phage population sizes either approached constant values or entrained in oscillation regimes (see below).

(iii) Phage extinction

We observed many cases in which phages either failed to establish a spreading infection, or declined to extinction after briefly propagating in the biofilm. This occurred when phage infection probability was low, but also, less intuitively, when nutrient availability and thus bacterial growth were very low, irrespective of infection probability. Visual inspection of the

simulations showed that when biofilms were sparse and slow-growing, newly released phages were more likely to be swept away into the liquid phase than to encounter new host cells to infect (Supplementary Video SV5). At a conservative maximum bacterial growth rate, biofilms were not able to out-grow a phage infection. However, if bacterial growth was increased beyond this conservative maximum, we found that biofilms could effectively expel phage infections by shedding phages into the liquid phase above them (Supplementary Video SV6). This result, and those described above, heavily depended on the ability of phages to diffuse through the biofilms, to which we turn our attention in the following section.

(b) Governing parameters of phage spread in biofilms

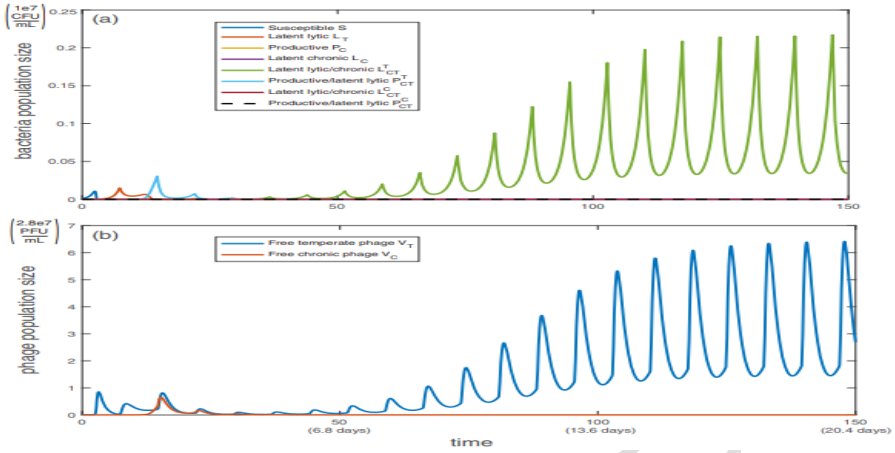
Many processes can contribute to the balance of bacterial growth and phage propagation in a biofilm. To probe our simulation framework systematically, we used our pilot simulations to choose control parameters with strong influence on the outcome of phage-host population dynamics. We then performed sweeps of parameter space to build up a general picture of how the population dynamics of the biofilm-phage system depends on underlying features of phages, host bacteria, and biofilm spatial structure.

We isolated three key parameters with major effects on how phage infections spread through biofilms. The first of these is environmental nutrient concentration, N_{max}, an important ecological factor that heavily influences biofilm growth and architectur. Importantly, varying N_{max} not only changes the overall growth rate but also the emergent biofilm spatial structure. When nutrients are sparse, for example, biofilms grow with tower-like projections and high variance in surface height, whereas when nutrients are abundant, biofilms tend to grow with smooth fronts and low variance in surface height.

Pharmacological implications with antibiotic resistance

The main concern when treating an infection with antibiotics is the size of the bacterial population. Therefore, we investigate the total bacterial population under a range of antibiotic dosing frequencies. We compute the average total bacterial population over the first 300 bacterial reproductive cycles (40.8 days), and we find that both antibiotics and temperate phage are critical to controlling the infection and work synergistically even when bacteria are antibiotic resistant. We define infection control to be an average bacterial population less than 10% of carrying capacity (i.e., 1-log decrease in bacterial levels compared with placebo). If only chronic phage is present in the system (see Fig. in the supplemental material), effective antibiotics are required to

control the infection. If all bacteria are sensitive to antibiotics, the presence of chronic phage controls the infection slightly better than if there are no chronic phages due to the cost of production during productive infection.



Graph Simulation of population dynamics with no antibiotic resistance: bacterial population (a) and free phage population (b)

If only temperate phage are present in the system, infection is controlled even when bacteria are resistant. In fact, the efficacy of temperate phage alone is similar to the efficacy of antibiotics alone. With both effective antibiotics and temperate phage, the number of antibiotic doses required to keep the infection under control is cut in half compared with antibiotics alone or temperate phage alone. If both phages are present in the system, infection control is marginally better than if only temperate phage are present. These results demonstrate the synergy between temperate phage and antibiotics even in resistant populations. No deliberate combination therapy may be needed to treat these infections because temperate phage are commonly found in natural populations of P. aeruginosa bacteria (53).

6.0 CONCLUSION

The complete eradication of both single and dual species biofilms was not achieved. Indeed, communities of bacteriophage and bacteria have been shown to be remarkably stable. Horne (1970) reported the coexistence of phage T4 and E. coli for periods as long as 52 weeks. Much of the coexistence of phage and bacteria has been attributed to the continual appearance of phage-resistance mutants, and phage mutants unable to overcome bacterial resistance. Although the emergence of resistance was not evident in this study, the bacteriophage and bacterial biofilms were only incubated for a maximum of 24 h. Given longer periods of incubation, resistance of the bacteria to the bacteriophage would undoubtedly appear. Of more importance to biofilm

communities, brought about through growth on the walls of culture vessels, might be important in stabilizing bacteria-phage interactions. Nutritional limitation is also known to influence stability. Within a biofilm environment, both spatial heterogeneity and nutritional limitations are common occurrences. These theories suggest growth as a biofilm would increase the stability of bacteria-phage interactions

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