Altruists vs. Free Riders: How Vibrio Cholerae Solves the Public Goods Dilemma

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Abstract

Many bacteria live in biofilms: a surface-bound cluster of bacteria that is densely populated, held together by extracellular substances secreted by the community members. Biofilm's role is twofold: keeping other bacteria out, and keeping the nutrients they feed on in. The success of biofilm producing bacteria is dependent on properties of their biofilm with respect to their environment. To analyze this, we use the model organism *Vibrio cholerae*, which receives most of its sustenance by producing extracellular enzymes (chitinases) which break down chitin in the environment into absorbable nutrients. These secreted factors form a public goods dilemma—competing bacterial strains that don't produce chitinase (non-producers) can freeload off of *V. cholerae*, which produces chitinase at an energy cost (producers). This is where biofilm comes in: a thicker biofilm translates to a lower diffusion rate of nutrients. The fluid flow rate over the biofilm affects the dynamics between producers and non-producers since higher flow rate translates into a higher decay rate as nutrients are washed away. Using an agent based model of *V. cholerae* and other non-producing bacteria, we show that the success of producers depends on the interaction between the biofilm thickness and fluid flow rate, and the dynamics of this relationship is dependent on the nutrients present in their environment. Our results provide insight into the optimal conditions for *V. cholerae* to flourish, and under what conditions they will be outcompeted by non-producing populations. Future research may build off of these findings to investigate how we can engineer conditions that are less favorable for *V. cholerae* and other bacterial biofilms in medical applications.

Introduction

Bacterial systems play a crucial role in many widespread diseases and biotechnological applications. Increasing our understanding of bacterial systems is important for improving our treatment of many diseases and the efficiency of bacteria-based protein factories. A recurring property in bacterial systems is the formation of biofilm, a collection of bacterial colonies adhered to a surface through the production of extracellular polymeric substance (EPS). Biofilm plays a critical part in many fatal diseases such as cystic fibrosis, and causes huge problems in current applications of *in vivo* medical devices. In this paper, we will be

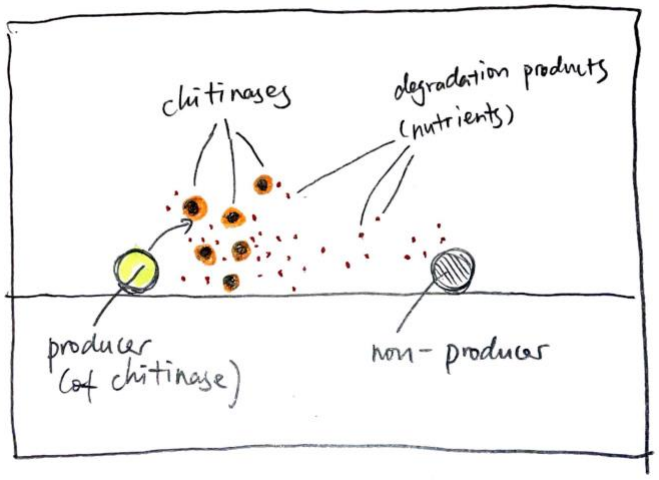


Figure 1: Illustration of Vibrio Cholerae on chitin surface. Cooperative cells produce chitinase enzymes, which digest the chitin into ingestible degradation products (nutrients). Non-cooperating cells do not produce chitinase but share in the absorption of degradation products.

investigating the formation of bacterial biofilm from a perspective of game theory, using an agent-based model of the model organism *Vibrio cholerae* interacting with other bacteria.

The primary food source of *V. cholerae* is chitin, however, they can only ingest the degradation products of chitin which are produced by an enzyme called chitinase (**Fig. 1**). *V. Cholerae* produce chitinase at an energy cost to itself. However the chitin degradation products—which we will simply call nutrients—are able to be consumed by other bacteria, regardless of whether or not they produce chitinase themselves. We will call these producers of chitinase *producers.* In contrast, there are strains of *V. cholerae* that do not produce chitinase, yet still consume the nutrients. We shall call these strains, as well as other bacterial species, *non-producers.* We see that the cost of producing chitinase is a private cost, but the benefits of chitinase – nutrients, is a public benefit. Thus, non-producers can easily exploit producers. We see this experimentally in *Solutions to the public goods dilemma in bacterial biofilms* by Drescher et al, where wild type producing strains are outcompeted by nonproducers due to the energy cost of producing chitinase. In contrast, when wild type producer *V. cholerae* are competing against nonproducers in environments where they don't produce chitinase, or are competing against strains producing inactive chitinases, we observe that competition is again neutral. This dilemma, where producers produce public goods at a private cost, is termed the *public goods dilemma*.

To investigate the public goods dilemma in bacteria, we wish to understand how cooperating, enzyme-producing bacteria can thrive in an environment with defecting, non-producing bacterial populations. For producers, there are two primary solutions to this dilemma that are conducive to cooperation. First, *V. cholerae* are able to produce thick biofilms that would decrease nutrient diffusion, thus limiting the spread of nutrients to non-producers. Thus, thicker biofilm facilitates the success of cooperative strategies. Second, a fast fluid flow rate in the environment would increase nutrient decay, washing away the degradation products before a sufficient amount of the goods could reach non-producers (Drescher 2014). This means that fast fluid flow would benefit the producer cells. We hypothesize that the interaction between these two variables will lead to an optimal condition for producer success with high flow rate and high thickness. Separately, we also hope to investigate how the harshness of the environment would impact strategy domination in *V. cholerae*. We define a harsh environment as one without environmental nutrients, and a hospitable environment the opposite. Environmental nutrients are simply absorbable nutrients that are present in the environment without the presence of chitinase. We hypothesize that a harsher environment will decrease the needed biofilm thickness and fluid flow rate, as it places greater stress on non-producers to survive.

With these hypotheses in mind, we want to study the specific conditions where the cooperate strategy dominates. In this project, we will use an agent-based simulation to model a population of chitinase-producing *V. cholerae* cells and non-producing "cheater" cells. They will interact and compete with each other via the production of chitinase and the consumption of nutrients. We will vary biofilm thickness, fluid flow rate, and harshness of the environment, and observe the conditions (in terms of these variables) in which producers dominate non-producers.

Methods

To investigate the effects of biofilm thickness, fluid flow rate, and environment harshness on the dynamics between cooperating bacteria and cheating bacteria, we implemented an agent-based simulation using netlogo version 6.2.0 (http://ccl.northwestern.edu/netlogo/).

Model Description

In nature, bacterial biofilms have a 3D structure composed of a 2D surface it adheres to, with a vertical dimension composed of bacterial extracellular secretions and other macromolecules. For the purposes of our simulations, we simplified the environment into a top-down 2D plane where the vertical properties of the biofilm were compressed into scalar parameters of the environment that we could change. Thus our simulation runs on a 2D grid of patches that model biofilm thickness, fluid flow rate, and environment harshness using parameters diffusion rate, decay rate, and environmental nutrients per patch respectively. The environment patches will also each have a nutrient count, which represents how the nutrient concentration at that patch. Every tick, each patch will get an amount of nutrients equal to the environmental nutrients + the amount produced by chitinase on the patch. These will diffuse to nearby patches according to our diffusion parameter and decay according to our decay parameter. These are governed by the following equations:

diffusion rate = 1 - (Biofilm-Thickness * 0.1)
decay rate = Flow-Rate * 0.05

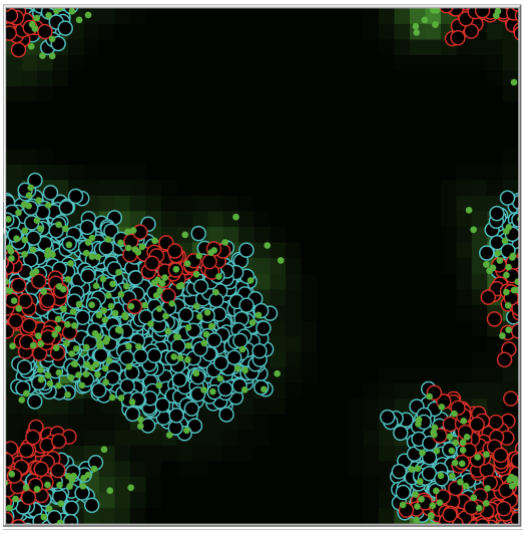


Figure 2: A screenshot of a sample run of a simulation after 500 ticks with default parameters (with 3 biofilm thickness and 4 flow rate). Producers are shown in blue, non-producers in red, chitinases in green, and nutrients as the green coloration of patches (black patches have none).

With our environment set up, we begin creating our agents to model our bacterial populations. We display cooperating producing bacteria as blue circles and cheating non-producing bacteria as red circles (see **Fig. 2**). These agents have energy, hunger, and grid position properties, but are immobile and stay in the same spot after being 'born'. Although bacteria can move, they become essentially immobile when they form biofilm, so we chose to ignore motion to simplify our model. These agents will gain energy from consuming nutrients every tick if there are nutrients on their patch. They will also lose energy every tick according to a metabolic rate parameter. Once a bacteria reaches an energy threshold parameter, they will reproduce by creating a child cell of the same strategy in a nearby radius. The hunger level governs how often producers will produce chitinase and increases when agents perform actions or don't consume nutrients. Hunger will decrease whenever the agent consumes nutrients. Although both types of agents have these properties, only producers use this property since non-producers cannot

produce chitinase. In our simulation, chitinase are modeled as a third type of agent and have no properties. However, they move according to brownian motion with their walk distance governed by the diffusion rate and their lifetime governed by the decay rate.

Having introduced our environment and agents, we initialize the simulation by placing 100 agents randomly throughout the grid. We also chose 30% of them to start off as cooperating, producing bacteria, although these numbers are arbitrary and can be changed with little effect on our simulations.

These two types of agents — producers and non-producers — interact and compete against each other indirectly through the production and consumption of nutrients, as well as reproduction and death rates. When agents run out of energy, they die. When there are more agents on a patch than the carrying capacity of 5, agents are randomly chosen to die until the carrying capacity is reached again. Both types of agents consume nutrients to gain energy and reproduce. Thus, the consumption of nutrients directly contributes to the fitness of the agents. However, the making of chitinase comes at an energy cost. Thus producers suffer a loss in fitness to create nutrients in the environment. We observe from the payoff matrix in **Fig 3** that this interaction between producers and non-producers matches the signature of a public goods dilemma and is suggestive of a prisoner's dilemma payoff matrix. Non-producers have a distinct advantage over producers in a setting with many producers and high nutrient density. However, the non-producer strategy is not pareto-optimal since agents would obviously be better off if everyone was a producer. Indeed, if all the population was mostly non-producers, they quickly would die off since there would be too few nutrients produced.

	Other cooperate	Others defect
Cooperate (Produce)	+ nutrients - cost	- cost
Defect (Don't produce)	+ nutrients	

Figure 3: A payoff matrix representing the interaction between a cell and surrounding cells. When the cell produces chitinase, it incurs an energy cost, which decreases its fitness. When surrounding cells produce chitinase, the cell is able to absorb nutrients, which increases its fitness.

Simulation Experiments

We conducted 20 model runs co-varying biofilm thickness and fluid flow rate in a harsh environment with no environmental nutrients. We varied biofilm thickness from 0 to 10 in increments of 1, while we varied flow rate from 1 to 10 in increments of 1. We used default parameters for these runs (see **Table 1**). We then conducted another 20 model runs under the same conditions, except this time we made the environment a hospitable environment. We set the environmental nutrients to be 0.3 instead of 0.0, which means that every tick, every patch gets 0.3 nutrients in addition to any that it receives from chitinases or diffusion. These represent nutrients that bacteria can consume that are not chitin-based.

For each model run, we ran simulations for 3000 ticks for each of the flow rate – biofilm thickness pairs. and counted the number of ticks in which producers dominated — i.e., when the number of producers was larger than the number of non-producers. We then averaged the number of ticks over the 20 model runs to find the average success of producers.

We also stopped the simulation when non-producers died out. Because our mutation rate is 0, non-producers cannot re-enter the simulation. If producers die out, then non-producers will soon die out too since they cannot nutrients for themselves.

Parameter	Default Value	Description
Initial bacteria	100	Bacteria at start of simulation, initialized at random positions on the plane.
Starting proportion of producers	0.3	Starting ratio of producers to non-producers. When initialized, bacteria have a random chance to be producer or non-producer, as determined by this ratio.
Energy threshold for reproduction	75	The energy level required for a bacterium to reproduce. If the bacterium's energy level is above this, they reproduce and decrease their energy level by an amount equal to this threshold - 20. They also increase their hunger level by 1, to simulate the cost of reproduction.
Hunger threshold for chitinase production	10	The hunger level required for a bacterium to produce chitinase (if the bacterium is a producer). If the bacterium's hunger level is above this, they produce 1 chitinase and decrease their energy level by the cost of chitinase production. They also increase their hunger level by 1, to simulate the cost of making chitinase. Furthermore, each tick that a bacterium doesn't eat, their hunger level is increased by this amount.
Nutrients from chitinase	5	Each tick, chitinases deposits this much nutrients to their current patch.

Cost of chitinase production	10	Producer's energy levels are decreased by this amount when they produce chitinase.
Metabolism cost	5	Each tick, all bacteria's energy level decreases by this amount.
Energy from nutrients	10	When a bacterium's patch holds more than 1 nutrient, they consume 1 nutrient and gain this much energy. This also decreases their hunger by 1.
Initial energy	50	The amount of energy an initial bacterium has. Bacteria produced through reproduction have 20 energy to start. Initial bacteria have more energy, because our simulation begins with cells in the middle of their life span, rather than cells which were just born. Novel cells should only appear if their environment supports them, so we don't begin the simulation with novel cells.
Mutation chance	0	Each time a bacterium reproduces, their spawn mutates into the other kind (producer ⟺ non-producer) with this probability.
Environmental nutrients	Varies, either 0 or 0.3	Each tick, a patch's nutrient amount increases by this number. 0 environmental nutrients is a harsh environment, while 0.3 is a hospitable environment.
Biofilm thickness	Varies from 0 to 10	This affects the diffusion of chitinase and nutrients. The diffusion number, derived from this, is (1 - (Biofilm Thickness * 0.1)). Each tick, chitinases pick a random direction and move a distance equal to the diffusion number (a patch is length 1). Each tick, a proportion (equal to the diffusion number) of a patch's nutrients are shared equally among its neighbors.

Flow rate	Varies from 1 to 10	This affects the decay of chitinase and nutrients. The decay number, derived from this, is (Flow rate * 0.05). Each tick, chitinases have a chance to die equal to the decay number. Each, a ratio (equal to the decay number) of a patch's nutrients are deleted. This occurs after diffusion.

Table 1: This table contains the parameters and their values that we used in our experiments. Each row represents a parameter and includes the value we set it to as well as a description of what that parameter's role is in the simulation.

Results

We tested our hypotheses by varying biofilm thickness and fluid flow rate in two different environments: the harsh environment and the hospitable environment. For independent variables biofilm thickness and flow rate, we use the range [0.0 to 1.0] and [0.1 to 1.0] respectively. This varies diffusion from [1 to 0] and decay from [0 to 0.5] respectively. The scenario with no flow rate was ignored, as a simulation with no decay of nutrients or enzymes is both unrealistic and leads to massive computations.

Domination of the Cooperative Strategy in Harsh Environments

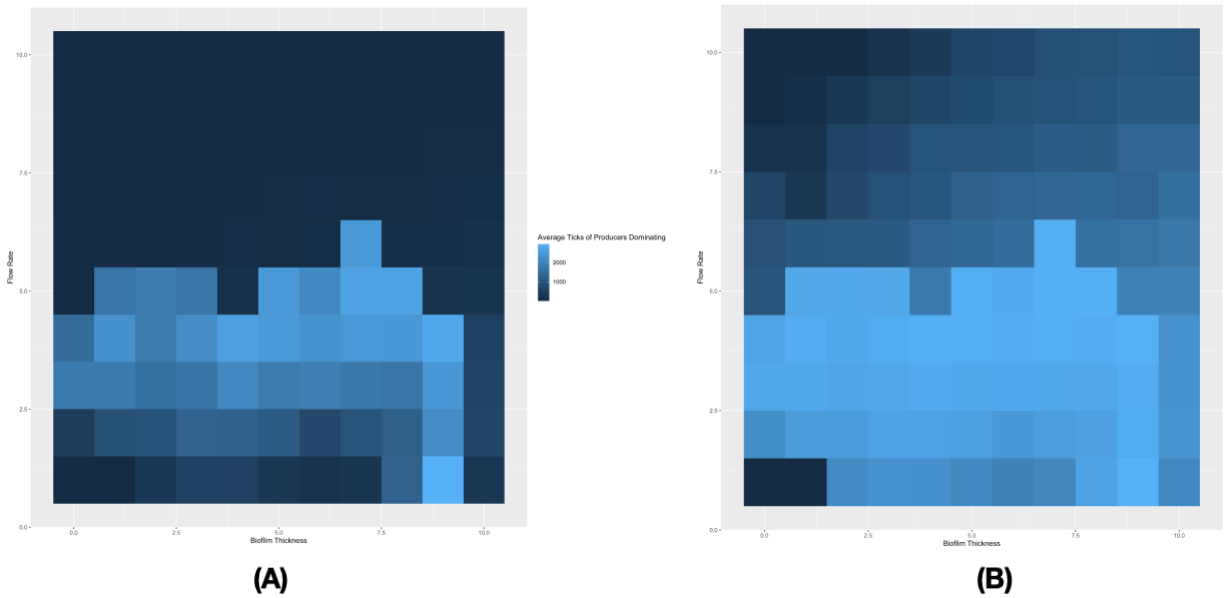


Figure 4: Heat maps showing the average number of ticks where producers dominate in a harsh environment. Both figures have biofilm thickness on the x-axis and flow rate on the y-axis. (A) is scaled normally between 0 ticks and 3000 ticks while (B) is scaled logarithmically.

In this harsh environment, there are no environmental nutrients. While producers are still able to provide for themselves by producing chitinase, non-producers have to completely rely on producers to survive. We observe that under harsh conditions, producers were most successful at low to medium flow rates (1-5), irrespective of biofilm thickness (**Fig. 4**). It is worth noting that producers do not dominate, even in an environment favorable to them, when the flow rate is above 5.

Domination of the Cooperative Strategy in Hospitable Environments

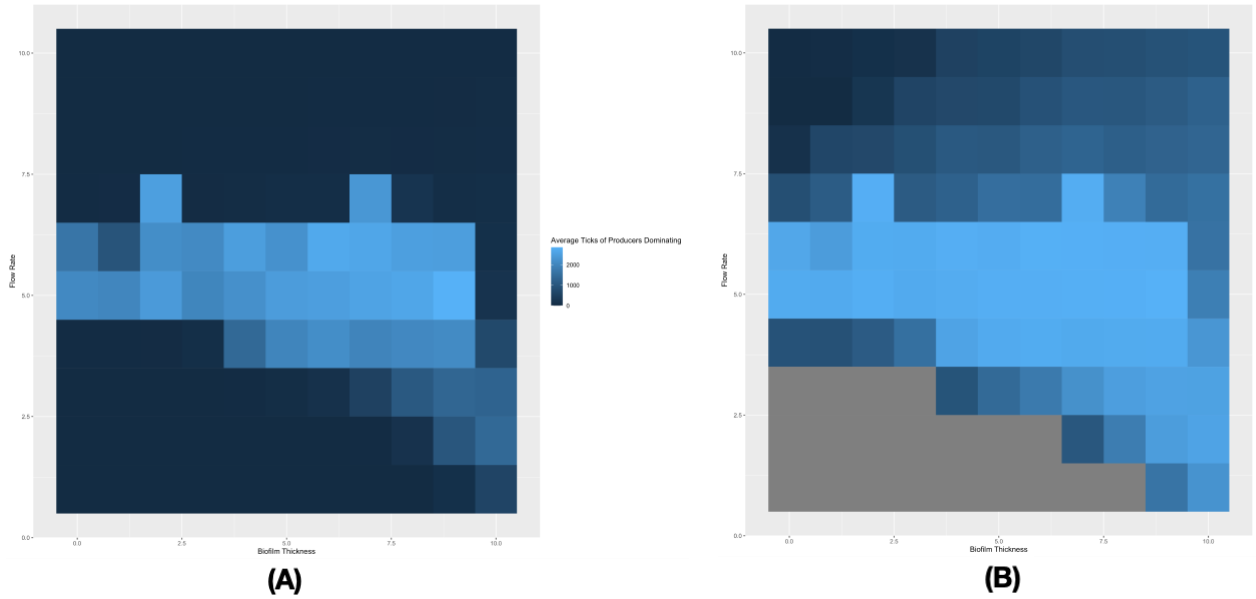


Figure 5: Heat maps showing the average number of ticks where producers dominate in a hospitable environment. Environmental nutrients was set to 0.3 without changing other parameters. Both figures have biofilm thickness on the x-axis and flow rate on the y-axis. (A) is scaled normally between 0 ticks and 3000 ticks while (B) is scaled logarithmically.

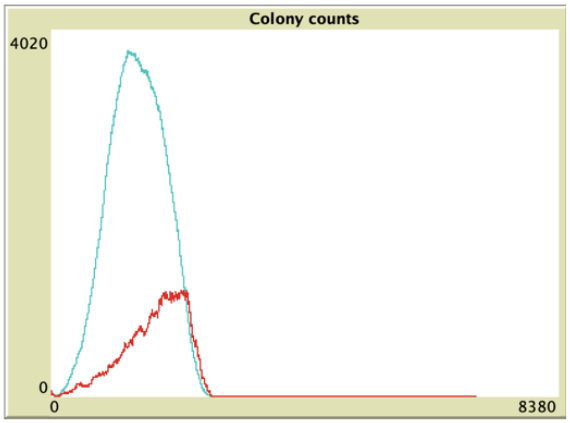
Hospitable environments have environmental nutrients freely available for both producers and non-producers, although the rate at which environmental nutrients is added to each patch is not enough for non-producers to survive independently of producers.

We observe in **Fig. 5** that under hospitable conditions, producers were most successful at medium flow rates irrespective of biofilm thickness. However, at low flow rates, they seem to only be successful when there is high biofilm thickness.

Interesting dynamics

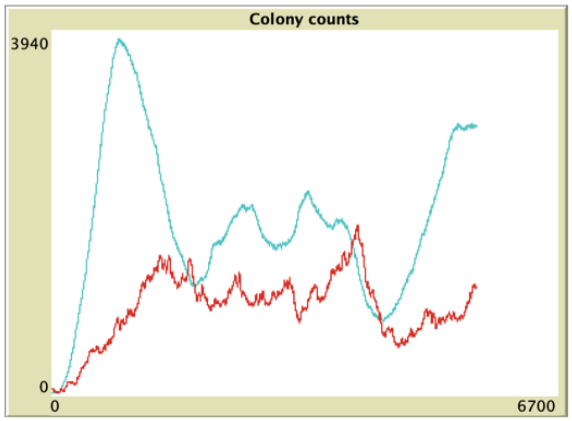
What the heat map results above do not display is what the populations of bacteria look like overtime. Below in **Fig. 6** are several examples of different systems which may arise based on the flow rate and biofilm thickness.

(A) Medium flow rate, low thickness



(B) Low flow rate, variable thickness

(C) Medium flow rate, medium thickness



(D) Medium flow rate, high thickness

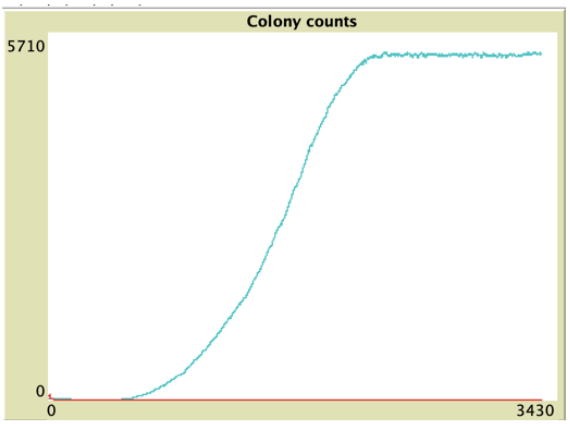


Figure 6: Systems that arose under hospitable conditions with varying flow rate and biofilm. (A) shows a simulation with medium flow rate and low thickness, where producers and non-producers rise together then die off. (B) shows a simulation with low flow rate and varying thickness where non-producers dominate producers. (C) shows a simulation with medium flow rate and medium thickness that has cyclic behavior. (D) shows a simulation with optimal parameters: medium flow rate and high thickness where non-producers die off and producers dominate.

In systems with a **medium flow rate** (ideal for producers), and a **low biofilm thickness**, producers outcompete non-producers at first, then drive themselves (and as a result, non-producers) to extinction (**Fig. 6a**). In these cases, the producers explode in population to start, but as non-producers are able to invade the producer colonies, producers die off quickly as they lose the support of their community and are outcompeted by non-producers. This rapid decline in producer population causes non-producers to completely dominate, driving both of the populations to extinction.

In systems with a **low flow rate**, non-producers tend to establish dominance (**Fig. 6b**). In these cases, non-producers are seen almost shepherding the producers, forming perimeters around their colonies and feeding off of their nutrients. Over many generations, the producers slowly migrate towards open space, while the non-producers overtake the old region that producers used to be in.

In systems with a **medium flow rate** (ideal for producers), and a **medium biofilm thickness**, the producers and non-producers form a predator-prey relationship (**Fig. 6c**). When there is an abundance of producers, it's very easy for non-producers to take advantage of the free nutrients and spread out in the population. However, as non-producers become more prevalent in the environment, they outcompete producers so the producer population starts dying off. However, this means there's less nutrients for non-producers to consume, so then they start dying off. The low level of non-producers then allows producers to regain temporary dominance as they grow without competition. However, this eventually leads to non-producers spreading in the population again, leading to a cycle.

In systems with a **medium flow rate** (ideal for producers) and **high biofilm thickness**, the producers fully outcompete the non-producers, allowing them to exist at their carrying capacity (**Fig. 6d**). These scenarios are very stable, and any introduction of competing bacteria is outcompeted almost instantaneously.

Discussion

In our results, we saw that for the harsh environment, producers were successful at a medium flow rate and high biofilm thickness. This aligns with previous findings that thicker biofilm would help solve the public goods dilemma. Our findings for the flow rate also makes sense, since a low flow rate would allow nutrients to be exploited by non-producers, but a high flow rate would wash everything away before the producing cell itself could consume it. We saw a similar trend in the hospitable environment, but everything was shifted slightly upwards, creating a smaller band where producers were successful. We interpret this to mean that harsh environments are more conducive to cooperation compared to more hospitable environments. This is likely because the harsh environment places stress on the non-producers and forces them to rely more heavily on producers. This aligns with prior research that the evolution of bacterial cooperation is closely related to nutrient density (Travisano 2000).

Knowing how bacteria react to different flow rates and biofilm thickness might be useful for medical applications. For example, if a patient has an infection, medical personnel may be able to alter their body's environment (through drugs, medical devices, etc.) in order to cause bacteria to compete with each other to extinction. The ideal flow rate for producers is limited (2-5 harsh, 5-7 hospitable)—indicating that if we can control the flow rate of the environment, we may be able to limit the success of producers. Notably, in hospitable environments, producers

completely died off with low flow rates. Hospitable environments are likely more comparable to a human body, where nutrients are present for other purposes, such as being produced by the body itself.

Furthermore, if we had the means to affect the thickness of biofilm, it may be possible to create an environment similar to the one presented in **Fig. 6c,** where the bacteria drive each other to extinction, and any introduction of new bacteria would be unlikely to survive, due to no chitinase production in the environment. Keeping infectious bacteria from reaching a state similar to **Fig. 6d** is also a priority, where producers completely dominate, and spread until reaching their carrying capacity.

Future research may look into the effects of antibacterial drugs on these biofilm systems. In these cases, the drugs can act as disturbances to the system which kill off a large majority of cells but leave some alive. In systems affected by periodic disturbances, research has shown that producing cells thrive in environments with a medium frequency of disturbances. Low frequencies give non-producers enough time to dominate whereas high frequencies keep cell density too low for producers to truly thrive (Brockhurst 2010). Future work may look into the interaction between periodic disturbances and biofilm properties as well as environmental harshness. In doing so, we can better understand bacterial biofilm systems and their interactions with medical treatments, as well as how we can use that knowledge to design more effective antibacterial treatments.

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