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The Evolution of Bacteriocin Production in Bacterial Biofilms

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abstract: Bacteriocin production is a spiteful behavior of bacteria that is central to the competitive dynamics of many human pathogens. Social evolution predicts that bacteriocin production is favored when bacteriocin-producing cells are mixed at intermediate frequency with their competitors and when competitive neighborhoods are localized. Both predictions are supported by biofilm experiments. However, the means by which physical and biological processes interact to produce conditions that favor the evolution of bacteriocin production remain to be investigated. Here we fill this gap using analytical and computational approaches. We identify and collapse key parameters into a single number, the critical bacteriocin range, that measures the threshold distance from a focal bacteriocin-producing cell within which its fitness is higher than that of a sensitive cell. We develop an agent-based model to test our predictions and confirm that bacteriocin production is most favored when relatedness is intermediate and competition is local. We then use invasion analysis to determine evolutionarily stable strategies for bacteriocin production. Finally, we perform long-term evolutionary simulations to analyze how the critical bacteriocin range and genetic lineage segregation affect biodiversity in multistrain biofilms. We find that biodiversity is maintained in highly segregated biofilms for a wide array of critical bacteriocin ranges. However, under conditions of high nutrient penetration leading to well-mixed biofilms, biodiversity rapidly decreases and becomes sensitive to the critical bacteriocin range.

Keywords: social evolution, spite, agent-based modeling, bacteriocins, microbiome, biodiversity.

Introduction

Bacteriocin production, a trait by which bacteria secrete toxic substances to suppress the growth of competitors, has long fascinated the scientific community. Though metabolically expensive, bacteriocin secretion is a very common behavior among known bacteria and is thus likely to be a signature of environmental factors and selective forces that are common to many bacterial species (Kerr 2006). From the perspective of social evolution theory, bacteriocin secretion also represents a rare example of spiteful behavior that is expensive for the producer and harmful to others (Hurst 1991; Gardner et al. 2004; Foster 2005; West et al. 2006, 2007; West and Gardner 2010). What conditions lead bacterial strains to evolve highly costly behaviors that are harmful to others? This question has motivated a diverse series of experimental (Adams et al. 1979; Chao and Levin 1981; Levin 1988; Tait and Sutherland 2002; Inglis et al. 2009; Be'er et al. 2010; Majeed et al. 2010) and theoretical studies (Frank 1994; Durrett and Levin 1997; Iwasa et al. 1998; Riley and Gordon 1999; Nakamaru and Iwasa 2000; Kerr et al. 2002; Czaran and Hoekstra 2003; Gardner et al. 2004; Szabo et al. 2007; Prado and Kerr 2008).

Social evolution theory has traditionally focused on cooperative behaviors, which are costly to the actor and beneficial to the recipient. Such traits are predicted to evolve under a condition known as Hamilton's Rule, $rb > c$, where c is the fitness cost of performing cooperative behavior, b is the fitness benefit conferred to the recipient, and r is relatedness, or the coefficient of correlation of recipient genotype on actor genotype (Hamilton 1964*a*, 1964*b*, 1970). Fixing b and c, cooperation is more likely to evolve when r is high, namely, when cooperative benefits are preferentially distributed to other cooperative individuals relative to the total frequency of cooperative individuals in the competitive neighborhood. Hamilton immediately recognized that his rule might also apply to spiteful behaviors, which are in some ways the inverse of cooperation. For spiteful traits, recipients are harmed; that is, b is negative. Spite is therefore predicted to evolve when r is also negative, which is satisfied when the harmful effects of the spiteful behavior are preferentially directed toward competitors that do not share the same genotype.

Gardner and colleagues refined the theoretical foundation describing the evolution of spite in general and of bacteriocin production in particular (Gardner and West 2004; Gardner et al. 2004). Without assuming any partic-

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ular toxin production cost function, they demonstrated that bacteriocin production is most strongly favored at intermediate relatedness. Their conclusion, which was confirmed in a recent experimental study (Inglis et al. 2009), rests on the fact that the net benefit for a lineage of bacteriocin producers is largest when lineages are spatially mixed and competition for resources is localized, such that suppressing the growth of nearby competitors becomes an effective strategy. The analysis of Gardner et al. (2004) was also supported by experiments in which bacteriocin-secreting cells were competed against nonproducing cells in surface-bound bacterial communities, or biofilms (Tait and Sutherland 2002). One major difference between theory and experiment arose here, however: spatially structured biofilm environments allowed the coexistence of both bacteriocin-producing and bacteriocin-sensitive strains, which was not observed under planktonic or well-mixed conditions.

Understanding the evolution of bacteriocin production in biofilms is important, as biofilm formation plays a major part in bacterial life. For example, it is estimated that the vast majority of human bacterial infections involve biofilms (Costerton et al. 1999). The spatial structuring inherent to these closely packed communities can dramatically influence the evolution of bacterial social interactions (Nadell et al. 2009). While the predictions of social evolution theory (Gardner et al. 2004) agree qualitatively with experimental biofilm observations (Tait and Sutherland 2002), we do not understand the mechanistic bridge between abstract theory and lab experiment. How do the physical and biological processes involved in biofilm formation influence relatedness and the size of competitive neighborhoods, such that bacteriocin production is advantageous?

Here, we analyze the evolution of bacteriocin production in biofilms using several modeling approaches. We apply mathematical analysis to identify the threshold length-scale within which a focal bacteriocin-producing cell achieves greater fitness than a sensitive cell. The concept of length-scale is widely used in the physical sciences to describe the order of magnitude of a system. In this study, our derived length-scale groups together the physical (e.g., bacteriocin diffusivity) and biochemical (e.g., rate of bacteriocin synthesis) properties of the system that ultimately set the spatial scale at which bacteriocin secretion confers a competitive advantage. By determining the critical bacteriocin range in this way, we simplify the total evolutionary analysis of bacteriocin production by collecting important parameters into one compound parameter.

We then use individual-based simulations to test the hypothesis that the critical bacteriocin range controls the evolution of bacteriocin production in bacterial biofilms. Individual-based modeling, which uses multiple agents governed by a set of rules that mimic the behavior of real microbes, is a powerful approach for studying the evolution of microbial communities in the environment (Hellweger and Bucci 2009). The spatial and evolutionary dynamics of the population emerge from the interactions among individuals. Using these simulations we carry out in silico competition between bacteriocin-producing and -sensitive strains. By initiating biofilm growth with different ratios of the two strains or by changing environmental conditions that lead to variation in lineage segregation, we confirm that in dual-strain biofilms, bacteriocin production is most strongly favored when relatedness is intermediate and competition is predominantly local. Finally, we carry out long-term evolutionary simulations to analyze the influence of bacteriocin-mediated competition and genetic lineage segregation in the maintenance of bacteriocin diversity in dual- and multistrain biofilms.

Results and Discussion

Gardner et al. (Gardner and West 2004; Gardner et al. 2004) find two critical factors influencing the evolution of bacteriocin production: (1) relatedness, which measures the association between genotypes of cells affected by bacteriocins and cells producing bacteriocins, and (2) the scale of competition, that is, the length-scale on which cells compete with each other for limited growth substrate. Here, we develop a mechanistic model of bacteriocin evolution in biofilms to provide an explanation for their findings in relation to the spatially structured environments that bacteria often occupy.

In addition to relatedness and the scale of competition for nutrients, we hypothesized that the length-scale at which bacteriocins exert an influence on competitors would be important for the evolution of bacteriocin production. To determine the physical and biological parameters that set the critical bacteriocin range in biofilms, we use the model of bacteriocin kinetics introduced by Wilkinson (2002). Bacteriocin producers secrete toxin at a rate proportional to their growth. Bacteriocin-sensitive cells, in turn, are killed at a rate proportional to bacteriocin concentration (T, for "toxin"). As previously described (Nadell et al. 2010), the growth of both bacteriocin-producing and bacteriocin-sensitive bacteria is also a function of a growth-limiting nutrient (N):

$$\frac{dP}{dt} = (1 - f)\mu_{\max}\frac{N}{N + K_N}P, \tag{1}$$

$$\frac{dS}{dt} = \left(\mu_{\max}\frac{N}{N + K_N} - k_T T\right)S, \tag{2}$$

where $\mu_{\max}$ (1/h) is the maximum specific growth rate, N

(g *N*/L) is the nutrient concentration, K_N (g N/L) is the half saturation constant for nutrient, *P* and *S* (g X/L) are, respectively, the concentrations of bacteriocin-producing and bacteriocin-sensitive cells, *f* is the fraction of substrate invested in bacteriocin production rather than biomass production, and k_T (L/g T/h) is the killing rate per unit mass of bacteriocin.

The solute species, toxin and nutrient, are governed by reaction-diffusion:

$$\frac{dT}{dt} = \alpha f \mu_{max} \frac{N}{N + K_N} P + D_T \nabla^2 T, \quad (3)$$

$$\frac{dN}{dt} = -\frac{1}{Y} \mu_{max} \frac{N}{N + K_N} (P + S) + D_N \nabla^2 N, \quad (4)$$

where α (g T/g X) is the stoichiometric coefficient of bacteriocin production, *T* (g T/L) is the bacteriocin concentration, *Y* (g X/g N) is the yield of biomass production, and D_T and D_N are respectively the diffusivities of toxin and nutrient ($\mu m^2\ h^{-1}$). The stoichiometric table for this model (table A1) and a table explaining our choices for parameter values (table A2) are available online.

We carried out a dimensional analysis of this model (app. A, available online) and derived the critical bacteriocin range (L_{bac}):

$$L_{bac} = \frac{\alpha k_T < m >}{2\pi D_T}, \quad (5)$$

where $<m>$ (g X/L) is the average biomass of a bacteriocin-producing cell. The critical bacteriocin range, L_{bac}, combines physical and biological parameters to represent the threshold distance within which the cost of bacteriocin production is offset by the killing of sensitive cells in the surrounding area. Smaller values of L_{bac} indicate more localized bacteriocin-mediated killing, and vice versa. Below we demonstrate that bacteriocin-mediated killing is a function of L_{bac} but largely independent of the specific values of the parameters that define it.

In Silico Competitions Using Agent-Based Modeling

We performed in silico competitions between bacteriocin-producing and bacteriocin-sensitive strains to analyze how the critical bacteriocin range (L_{bac}) influences the evolution of bacteriocin production in biofilms (fig. A1, available online). We used a well-established individual-based simulation framework, which mechanistically describes diffusion of solutes and cell growth (Xavier and Foster 2007; Nadell et al. 2008, 2010). Bacterial cells are modeled as circular agents growing on a flat surface. Cell growth is a function of local microenvironment conditions experienced by individual cells, as described in equations (1) and (2). Each cell grows until it reaches a maximum radius, at which point it divides to produce two daughter cells. Cells also move passively when pushed by neighboring cells as they grow and divide. The spatial concentrations of solutes—bacteriocin and nutrient—are updated at each iteration by solving reaction-diffusion equations. We initiated our simulations by inoculating the bacteriocin-producing and -sensitive strains on the solid substratum at varying initial ratios, depending on the aim of the particular simulation. For this first set of simulations, we allow the biofilm to grow until a predefined height of 150 μm, which is a typical size for bacterial biofilms and within a range (50–250 μm) often used in biofilm modeling (Picioreanu et al. 2000).

Bacteriocin Production Is Favored as the Critical Bacteriocin Range Increases

We first evaluated the effect of L_{bac} on competition between a bacteriocin producer and a sensitive strain. In order to focus on the effect of L_{bac}, we assumed high nutrient penetration (*N*), which leads to well-mixed biofilms with relatively smooth advancing fronts (Nadell et al. 2010). We carried out competitions in which the two strains were inoculated at a 1 : 1 ratio, and we measured the outcome competition by calculating the change in frequency of the producer strain ($\Delta P/P_0$), defined as

$$\frac{\Delta P}{P_0} = \frac{P_f - P_0}{P_0}, \quad (6)$$

where P_0 and P_f are, respectively, the initial and final proportions of bacteriocin producers in the biofilm. Normalizing by P_0 effectively measures the fold change in producer frequency and allows for clearer comparison among experiments with different initial sensitive-to-producer ratios (see next section). We performed competitions for several values of L_{bac}, which we varied by changing either D_T, k_T, α, or $<m>$ (fig. 1). As one might expect, these simulations showed that the advantage of bacteriocin production increases with the critical bacteriocin range (fig. 1; video 1, video 2, and video 3, available online). As before (Xavier and Foster 2007; Nadell et al. 2008, 2010), we carried out replicate runs by altering the seed for the simulation framework's random number generator. The results were robust, with very little variation among replicates (fig. 1).

Next, we evaluated the effect of L_{bac} on the competitive advantage or disadvantage of different levels of investment into bacteriocin production. We carried out a range of simulations varying L_{bac} and *f*, calculating the fold change in bacteriocin-producer frequency at the end of each replicate (fig. 2). For values of L_{bac} lower than 0.002 μm, we

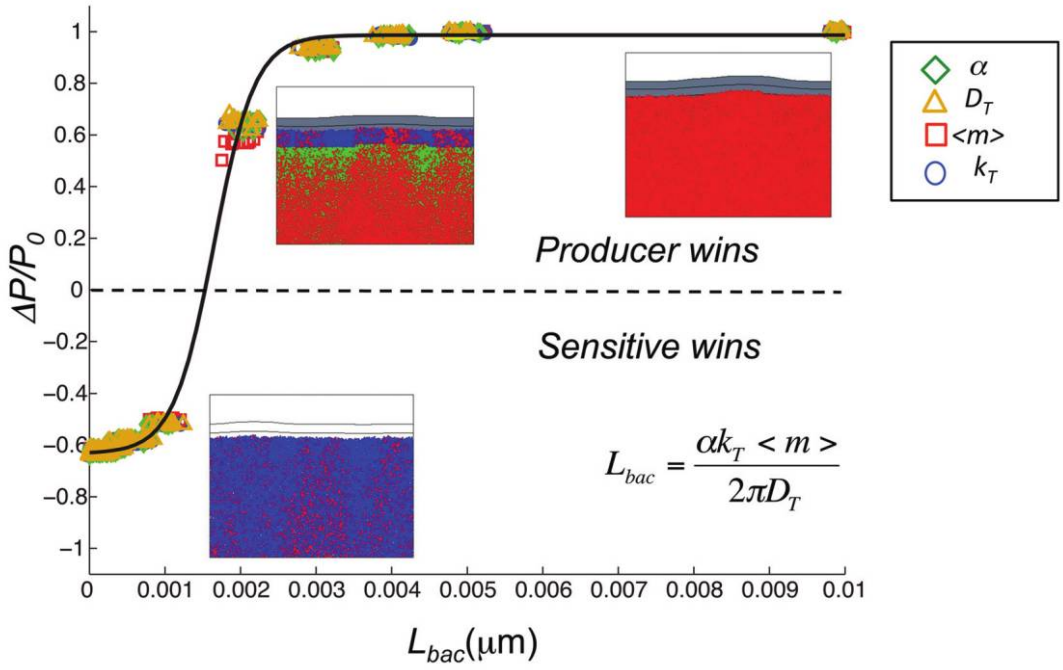

Figure 1: Effect of the critical bacteriocin range (L_{bac}) on selection for bacteriocin production. The fold change in bacteriocin-producer frequency ($\Delta P/P_0$) is plotted as a function of L_{bac} for competition between bacteriocin producers and bacteriocin-sensitive strains in biofilms with high nutrient penetration. Results are for simulations with a bacteriocin production investment value (f) of 0.3. Simulations were run varying one parameter defining L_{bac} at a time and keeping the others constant: k_T (blue circles), D_T (yellow triangles), α (green diamonds), and $<m>$ (red squares). For each parameter, the simulations were repeated 11 times with different initialization of the random number generator in order to estimate the robustness of our system's analysis. Also, for ease of visualization, the data are randomly positioned around their respective L_{bac} values, and a sigmoid expression (black line) was fitted to the data. Images are model snapshots for L_{bac} values of (1) 0.000, (2) 0.002, and (3) 0.005 μm. In inset panels, blue circles represent bacteriocin-sensitive cells, green circles represent bacteriocin-sensitive cells experiencing local bacteriocin concentrations such that their growth rate is lower that the one of the neighboring bacteriocin producers, and red circles represent bacteriocin-producing cells.

observed no increase in bacteriocin-producer frequency for any level of investment in production. On the other hand, as L_{bac} increases above 0.002 μm, bacteriocin production becomes favorable, but only up to a point. Allocating more than 70%–80% of resources to bacteriocin production becomes too costly and results in a decrease in bacteriocin producers' frequency for all L_{bac} values.

Bacteriocin Production Is Favored at Intermediate Relatedness

After confirming the central role of the critical bacteriocin range, we analyzed the effect of relatedness on selection for or against bacteriocin production. As was done in existing experiments (Inglis et al. 2009), we changed relatedness in our individual-based simulations by inoculating the biofilm with different initial ratios of the two competing strains (fig. 3*A*). We conducted the simulations at $L_{bac} = 0.002$ μm, which we found to be the minimum value at which bacteriocin production can be advantageous (fig. 2). Our simulations showed that bacteriocin production is most advantageous at intermediate initial frequencies of the bacteriocin-producing strain. This result supports existing theoretical and experimental studies (Gardner and West 2004; Gardner et al. 2004; Inglis et al. 2009). Interestingly, our simulations show that the advantage of bacteriocin production vanishes for extreme values of investment (f less than 0.1 or more than 0.7), suggesting that both intermediate relatedness and intermediate investment are important for the competitive advantage afforded to bacteriocin producers. Also consistent with previous experimental and theoretical results (Inglis et al. 2009), selection for bacteriocin production is not symmetric with respect to relatedness but rather biased toward higher relatedness values. Starting at higher frequency allows producers to flood the biofilm with bacteriocin early during biofilm growth, a highly effective strategy (see video 4, available online).

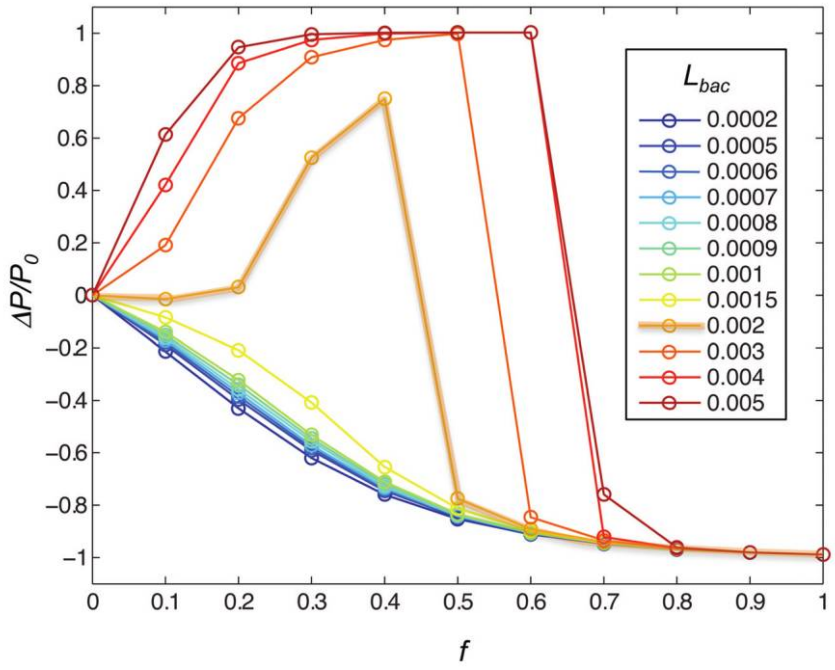

Figure 2: Selection for bacteriocin production as a function of investment in bacteriocin production (f). Results are from simulated competitions between a bacteriocin-producing and a bacteriocin-sensitive strain inoculated at a 1 : 1 ratio in a smooth biofilm characterized by high nutrient penetration. Different colors correspond to simulations performed for different L_{bac} values (0.0002–0.005 μm). The thick orange line corresponds to the L_{bac} value chosen for the subsequent analysis.

Bacteriocin Production Is Favored When Genetic Lineages Are Mixed and Competition Is Localized

After confirming selection for bacteriocin production is strongest at intermediate frequencies of producing cells, we analyzed the effects of nutrient penetration and genetic lineage segregation on competition between bacteriocin-producing and -sensitive strains. Previous work has shown that the penetration of a growth-limiting nutrient strongly influences segregation between genotypes in biofilms and, consequently, the evolution of cooperation (Nadell et al. 2010). The same work defined a dimensionless compound parameter δ to quantify nutrient penetration:

$$\delta = \sqrt{\frac{N_{\text{bulk}} D_{\text{N}} Y}{\mu_{\max} \rho h^2}}, \tag{7}$$

where N_{bulk} (g N/L) is the nutrient concentration in the liquid bulk, D_{N} (μm^2/h) is the nutrient diffusivity, ρ (g X/L) is the bacterial density, and h (μm) is the thickness of the diffusion boundary layer. High values of δ translate to deep nutrient penetration into the biofilm, producing smooth biofilms with thorough cell lineage mixing. Conversely, low values of δ translate to decreased nutrient penetration and lead to genetic segregation as well as highly structured biofilms with towerlike surface projections (Nadell et al. 2010). Notably, δ also dictates the scale of competition for growth substrate in biofilms. When δ is high, biofilms are largely saturated with nutrients, and substrate consumption by a focal cell only reduces nutrient availability to its immediate neighbors. When δ is low, on the other hand, nutrient access is more limited within biofilms, and substrate consumption by a focal cell reduces nutrient availability for other cells well beyond its immediate vicinity. In other words, δ is inversely proportional to the scale of competition for nutrients.

In agreement with the social evolution literature, selection for bacteriocin producers was maximized at high δ values (small scale of nutrient competition) and for an intermediate investment value (f) equal to 0.4 (figs. 3*B*, 4*A*; video 5, available online). For low δ values (large scale of nutrient competition), the bacteriocin-sensitive cells outgrow producers in the initial instants of biofilm formation and build fingerlike protrusions that limit their competitors' access to growth substrate (fig. 4*B*; video 6, available online). Similar to our earlier observations (fig. 3*A*), as the system is tuned away from an intermediate bacteriocin investment value of 0.4, bacteriocin production is less favored.

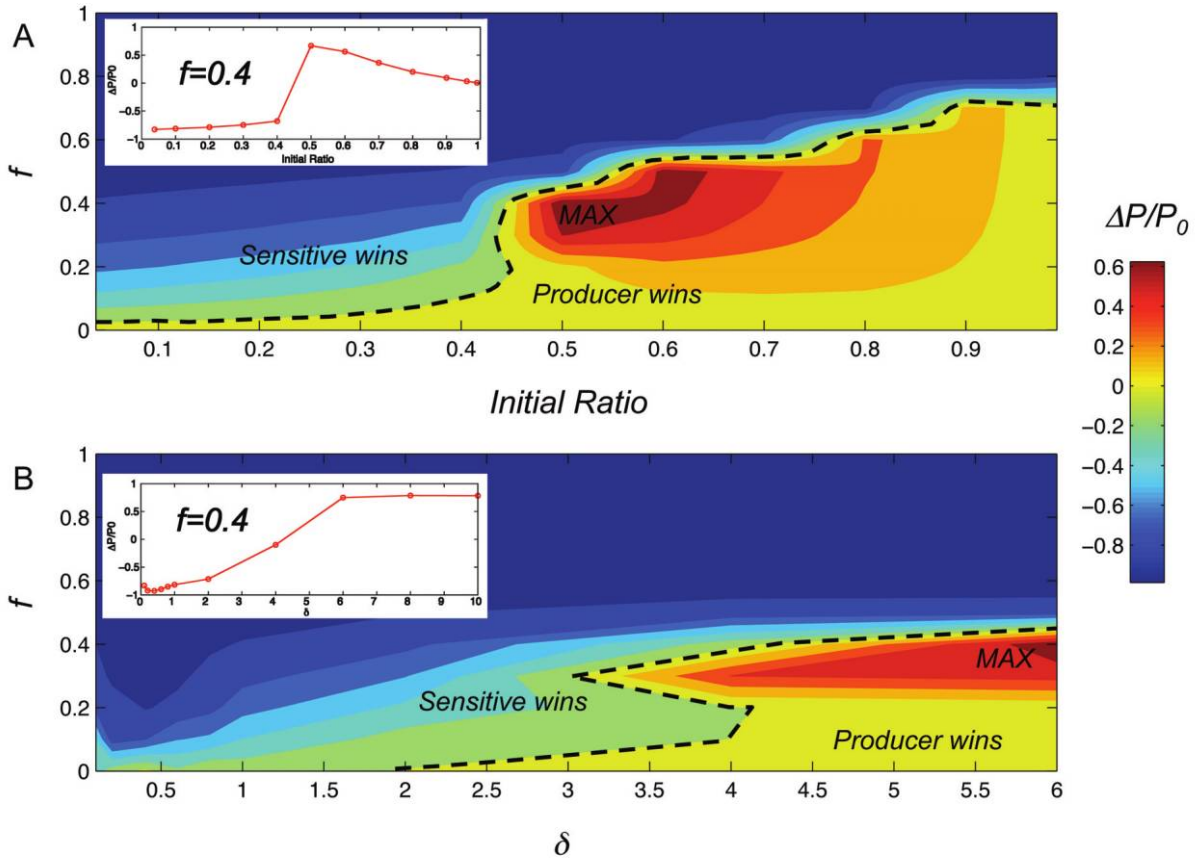

Figure 3: Effects of relatedness and cell lineage segregation on selection for bacteriocin production in dual-strain biofilms. *A*, Selection for bacteriocin production as a function of producer initial frequency for different investments in toxin production (*f*). Simulations were run by inoculating the producer and sensitive strains at different initial proportions. *B*, Selection for bacteriocin production as a function of δ for different investment in bacteriocin production. In this particular case, high δ values (>6) correspond to intermediate relatedness (local mixing ratio of 1 : 1 ratio) and more localized competition, while lowering δ corresponds to increasing relatedness and more globalized competition. Results shown are for an L_{bac} value of 0.002 μm (see text). Simulations were run until a maximum biofilm height (150 μm) was achieved.

Evolutionarily Stable Strategies

So far, our results illustrate how physical and biological processes affect the scale of bacteriocin-mediated competition within biofilms and, consequently, the competitive advantage gained by bacteriocin producers. Next, we conducted simulations to determine the optimal level of bacteriocin investment that prevents rare mutants from invading, that is, the evolutionarily stable strategy (ESS; Maynard Smith and Price 1973; Otto and Day 2007). The ESS was investigated by competing two producer populations (each sensitive to the other's toxin) that differed in bacteriocin investment by a small quantity Δf (Xavier and Foster 2007; Nadell et al. 2008, 2010). The simulations were seeded with the two producer strains at a 1 : 1 ratio, and simulations were stopped again when biofilms reached 150 μm. For consistency with the simulations shown above, we used $L_{bac} = 0.002$ μm.

The results from the ESS calculation are presented in figure A2, available online. Consistent with our earlier conclusions (fig. 3*B*), bacteriocin production is favored for decreasing genetic segregation and more localized competition for nutrients (increasing δ) and maximized when cells are locally assorted at a 1 : 1 ratio. The predicted optimal investment in bacteriocin production was lower than that observed in previous sections (0.2 rather than 0.4), suggesting that the benefit of investing into bacteriocin production is lower when competing with other producers than with bacteriocin-sensitive cells.

We expect that interesting dynamics could arise if a bacteriocin-resistant (but nonproducing) strain were added to the system. This could conceivably occur via mutation in a bacteriocin-sensitive strain that confers resistance or through mutation in a bacteriocin-producing strain that disrupts bacteriocin production while leaving its resistance intact. The presence of a sensitive strain could lead to complex "rock-paper-scissor" dynamics. Such systems have already been successfully studied (Durrett and Levin 1997; Kerr et al. 2002; Kirkup and Riley 2004) and are beyond the context of this work.

Coexistence in Dual-Strain Biofilms

In well-mixed environments, competition between bacteriocin-sensitive and bacteriocin-producing cells always leads to the extinction of one of the two competing strains, depending on initial conditions (Riley and Gordon 1999). However, competition in spatially structured environments such as agar plates (Frank 1994; Durrett and Levin 1997) and biofilms (Tait and Sutherland 2002) can lead to coexistence of producers and nonproducers. In order to investigate the coexistence of multiple strains producing different bacteriocins, we extended our model to perform long-term simulations of mixed biofilms. We included two new processes essential for the realistic modeling of biofilm dynamics over longer periods: (1) endogenous biomass decay and (2) biofilm detachment (Xavier et al. 2005*b*). Our extended model assumed a first order biomass decay rate, and that biofilm detachment increases with the square of the distance from the solid substratum (Xavier et al. 2004, 2005*a*). For each simulation we quantified biodiversity in the biofilm using Shannon's index (S_H):

$$S_H = -\sum_{i=1}^{N} p_i \log_2 p_i. \quad (8)$$

Shannon's index has been used previously to quantify biodiversity in microbial consortia (Turnbaugh et al. 2009). Term p_i is the proportion of the ith population in the system. In our first set of simulations, $N = 2$. Below, we will use the same index to quantify biodiversity in multistrain biofilms ($N = 10$).

The results of our long-term, dual-strain biofilm competition simulations (sensitive vs. producer) are presented in figure 5. Biodiversity is strongly influenced by nutrient penetration (parameter δ; fig. 5*A*). For δ lower than 0.1, the biofilm forms thin fingerlike protrusions that are mostly monoclonal (fig. 5*C1*). As δ increases, protrusions become less defined and local mixing among the two strains increases (fig. 5*C2*, 5*C3*). This leads to a reduction in biodiversity, which is ultimately lost when nutrients fully penetrate and the biofilm is completely mixed (fig. 5*C3*).

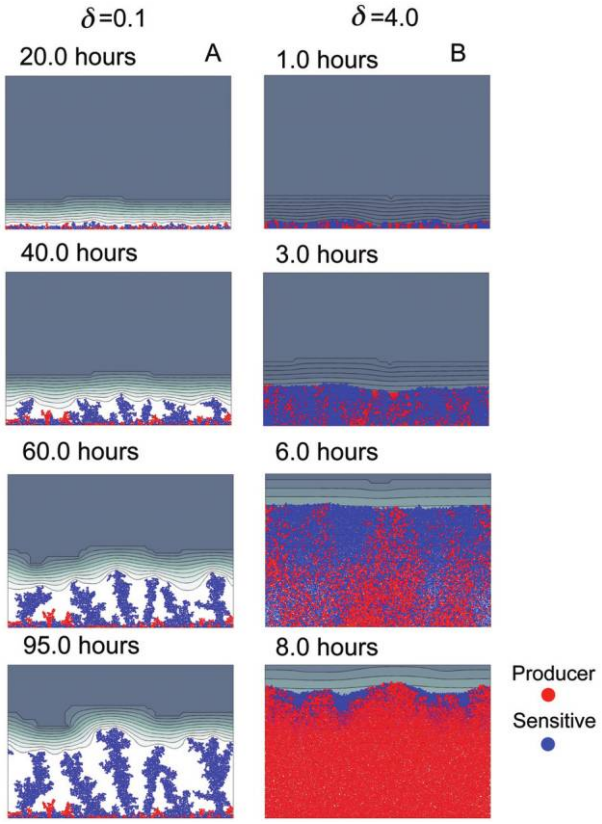

Figure 4: Effect of genetic segregation on bacteriocin-mediated competition. *A*, High segregation (high relatedness, large scale of competition) and formation of single-strain towerlike protrusions. Bacteriocin-sensitive cells (blue) outgrow bacteriocin-producing cells (red) in the early stages of competition and prevent bacteriocin producers from accessing nutrients. *B*, Low segregation (intermediate relatedness, small scale of competition) and formation of smooth biofilm. Due to higher nutrient penetration, bacteriocin-producing cells are able to flood the biofilm with the secreted toxin and dominate the population. The isocontour lines represent gradients of nutrient concentration in the biofilms.

Under this latter condition, one strain or the other goes to fixation, and the outcome of competition appears to be strongly dependent on L_{bac} (fig. 5*B*). The model, therefore, predicts that biodiversity is strongly related to environmental factors influencing biofilm morphology, such as nutrient penetration. In agreement with laboratory observations (Tait and Sutherland 2002), our simulations show that biofilm heterogeneity with respect to lineage segregation and surface structure tends to favor the coexistence of bacteriocin producers and sensitive strains.

It is interesting to note that introducing biomass decay and biofilm erosion changes the evolutionary dynamics of bacteriocin production, which is evident from a comparison between the results of this section and those reported

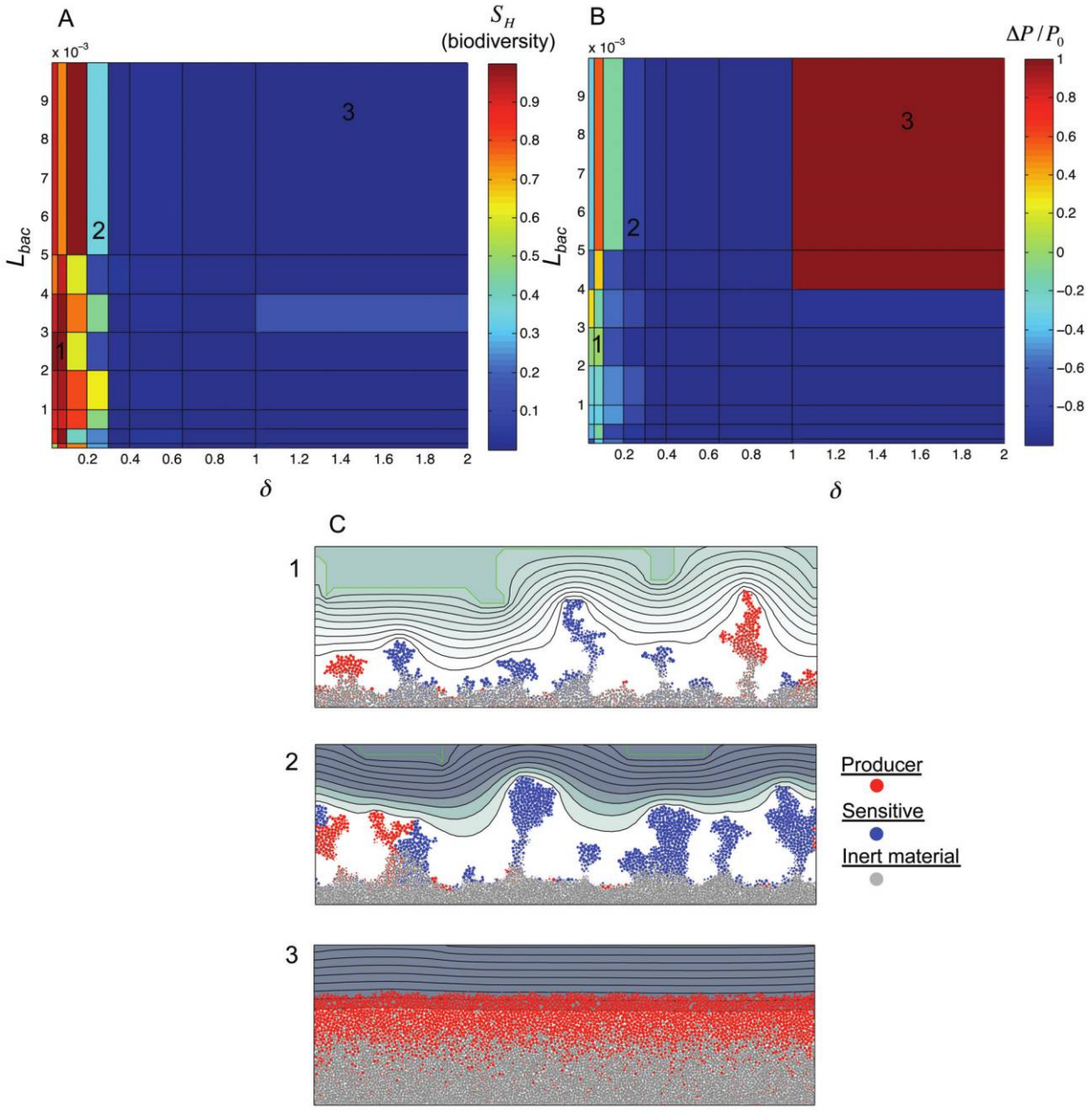

Figure 5: Results from long-term simulations in dual (bacteriocin-producer vs. bacteriocin-sensitive) strain biofilms. The δ-L_{bac} parameter space was analyzed for regions of high bacteriocin-producer and bacteriocin-sensitive strain coexistence. Biodiversity was calculated using the Shannon (S_H) index (see main text). Biodiversity (coexistence) is predicted to be strongly dependent on genetic segregation and biofilm morphology. *A*, Biodiversity as a function of δ and L_{bac}. *B*, Mean change in producer frequency as a function of δ and L_{bac}. *C*, Representative frames depicting different biofilm structures, which are largely a function of δ: (*C1*) single-strain towerlike protrusions, (*C2*) a large dual-strain protrusion (at left), and (*C3*) a completely smooth biofilm. Bacteriocin-producing cells are red, bacteriocin-sensitive cells are blue, and dead/inert biomass is gray. The isocontour lines represent the gradients of nutrient concentration in the biofilms.

in the sections above. In simulations that do not implement biomass decay or erosion, bacteriocin production is negatively selected when nutrient penetration is low and positively selected when nutrient penetration is high. We expect this pattern to apply most strongly when biofilms are relatively short-lived. On longer timescales, however, when biomass decay and biofilm erosion must be explicitly considered, bacteriocin production may persist when nutrient penetration is low, and its evolutionary dynamics are sensitive to initial conditions when nutrient penetration is high.

Biodiversity in Multistrain Biofilms

We extended our simulations to include multiple strains of bacteriocin producers and investigated the conditions governing biodiversity with respect to bacteriocin production. We considered two slightly different scenarios. First, nine bacteriocin producers and one bacteriocin-sensitive strain were inoculated at equal initial frequencies. Each producer strain allocated the same amount of resources ($f = 0.1$) to bacteriocin production and was immune to its own toxin but sensitive to all eight of the other producers' bacteriocins. In the second scenario, we omitted the sensitive strain, and 10 bacteriocin producer populations were competed with each other. As before, each strain allocated an equal amount of resources to bacteriocin production ($f = 0.1$), and was immune only to its own toxin. Since all strains had the same competitive features, this second scenario served to evaluate the effect of stochasticity (genetic drift) on the decay of bacteriocin biodiversity.

Our simulations were run until their global strain compositions appeared to reach a steady state. We then assessed the population composition by determining each strain's frequency with time (fig. A3*A*, available online) and measuring Shannon's index (S_H; fig. A3*B*). In all simulations, biodiversity decreased from its initial value, albeit at different rates. In order to compare simulations carried out with different values of δ and L_{bac}, we quantified the rate of biodiversity loss by calculating the biodiversity half-life ($\tau_{1/2}$), which estimates the time it takes for biodiversity to decay down to half of its initial value. This was done by fitting the rate of exponential decay from a biodiversity time series (fig. A3*C*) using a least-square fitting method (Michel et al. 2011).

We obtained curves to determine biodiversity half-life versus δ for different values of L_{bac} and for both competition scenarios. In accordance with simulations in which only one bacteriocin producer and one sensitive strain competed (fig. 5), the multistrain competitions illustrate two important results (fig. 6*A*,). First, biodiversity remains higher in spatially structured, genetically segregated biofilms (i.e., low δ for low nutrient penetration; fig. 6*C1*, 6*C3*, 6*C5*). Biodiversity is rapidly lost in simulations carried out at higher nutrient penetration (high δ; fig. 6*C2*, 6*C4*). Second, if δ is fixed, biodiversity remains higher for smaller critical bacteriocin ranges (lower L_{bac}; fig. 6*C3* cf. 6*C5*). Higher L_{bac} produces longer-range interactions among competing strains, increasing the probability of driving some more quickly to extinction. This is evident from the concentrations of toxin accumulated in the biofilm (higher in fig. 6*C5* cf. fig. 6*C3*. Higher L_{bac} produces longer-range interactions among competing strains, increasing the probability of strain extinction).

It is, however, important to note that the mechanism responsible for decreasing biodiversity is different in the two scenarios analyzed. When a sensitive, nonproducing strain is included with bacteriocin producers, the model predicts that the sensitive strain goes to fixation. The increase in "warfare" among bacteriocin-producing strains resulting from increased cell lineage mixing (high δ) and increased bacteriocin killing efficacy (higher L_{bac}) decreases the average fitness of all producing strains. They are thus more easily outcompeted by a sensitive strain investing all of its resources into growth than a single bacteriocin producer with greater strength in numbers (fig. 6*C2*). When only bacteriocin-producing strains are present, on the other hand, the loss of biodiversity is purely stochastic. At the beginning of biofilm growth, chance effects lead a few strains to increase in frequency. These strains have an inherent advantage, as their secreted toxins accumulate to higher concentrations, and ultimately one strain can drive out all the others (fig. 6*C5*). The dynamics of this system may thus be described as genetic drift with positive feedback. The rate at which this stochastic loss of biodiversity occurs is an increasing function of both the scale of bacteriocin-mediated killing (L_{bac}) and nutrient penetration (fig. 6*B*).

Conclusions

Bacteriocin production is an important feature of bacterial life and a key process in the ecology of multistrain biofilms (Tait and Sutherland 2002). Our study aims to bridge general social evolution theory and specific experimental findings in biofilms by analyzing how physical and biological processes combine to drive the evolution of bacteriocin production in spatially structured biofilms. With generality in mind, we chose to implement a simple kinetic model of bacteriocin production and its action (Wilkinson 2002). The model omits processes that can regulate bacteriocin production but may be restricted to specific bacterial species, such as bacteriocin signaling (Gillor et al. 2008*b*), cross-induction mechanisms (Majeed et al. 2010), or quorum-sensing regulation (Nadell et al. 2008).

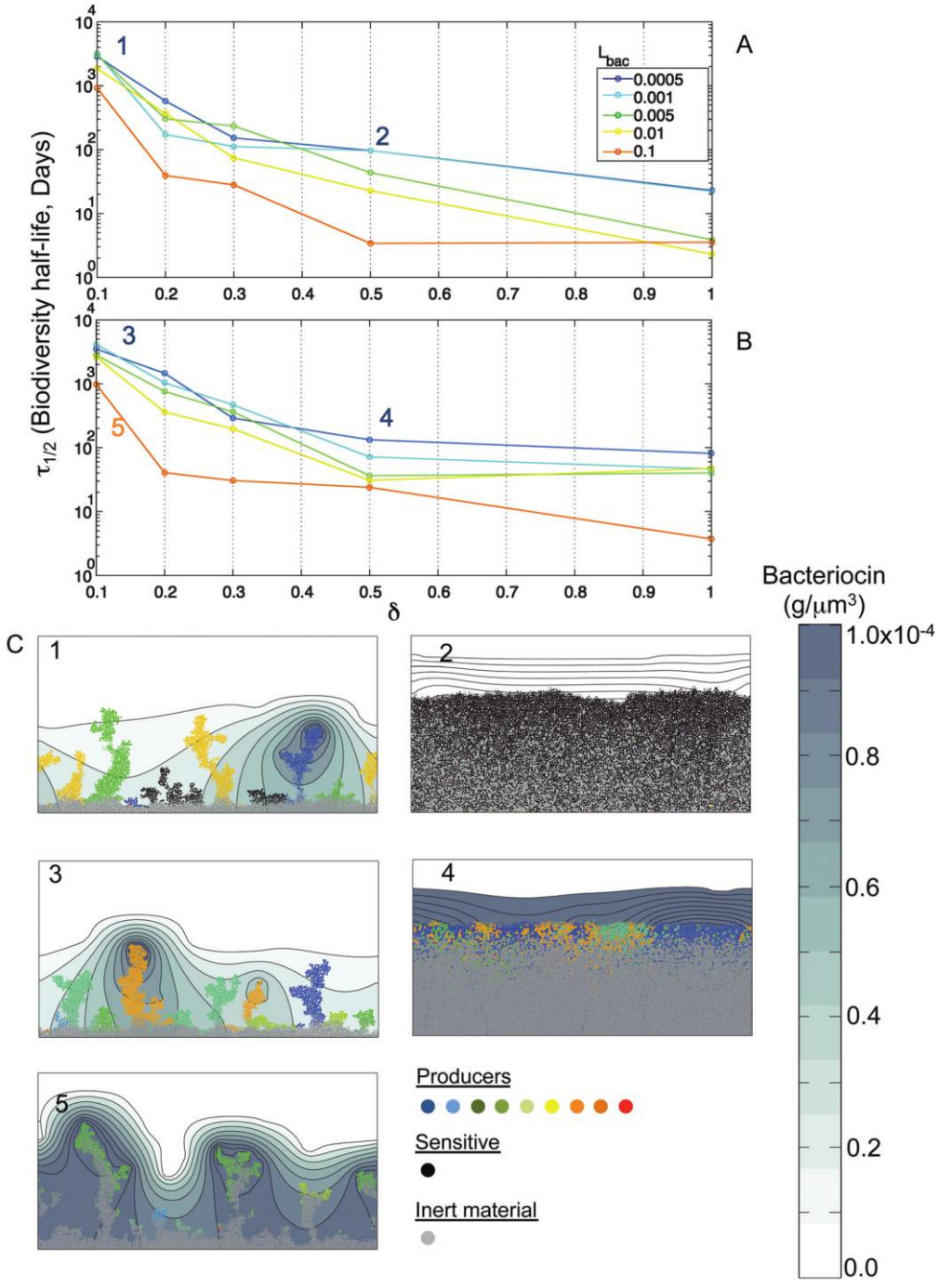

Figure 6: Bacteriocin biodiversity is maintained longer in structured biofilms. *A*, Bacteriocin biodiversity half-life for biofilms with one sensitive strain and nine bacteriocin-producing strains. *B*, Bacteriocin biodiversity half-life for biofilms with 10 bacteriocin-producing strains; 95% confidence intervals were smaller than the point markers and were therefore omitted. *C*, Representative frames from biofilm simulations containing (*C1*) nine bacteriocin producers and one bacteriocin-sensitive strain, with $\delta = 0.1$ and $L_{bac} = 0.0005$, and (*C2*) nine bacteriocin producers and one bacteriocin-sensitive strain, with $\delta = 10.5$ and $L_{bac} = 0.0005$. *C3*, Ten bacteriocin-producing strains, with $\delta = 0.1$ and $L_{bac} = 0.0005$. *C4*, Ten bacteriocin-producing strains, with $\delta = 0.5$ and $L_{bac} = 0.0005$. *C5*, Ten bacteriocin-producing strains, with $\delta = 0.1$ and $L_{bac} = 0.1$.

By analyzing a simple model we derive a length-scale of bacteriocin-mediated killing, L_{bac}, a single compound parameter that describes the combined effects of bacteriocin production, transport, and toxicity to a recipient. Parameter L_{bac} sets the critical spatial range around a focal bacteriocin-producing cell within which its fitness is higher than that of a recipient sensitive cell. Similarly, by mechanistically modeling biofilm nutrient penetration, set by another compound parameter δ, we describe how the scale of competition for limited nutrients is affected by other physical and biological properties. Building upon previous social evolution models (Frank 1998; Gardner and West 2004; Gardner et al. 2004), we decouple the effects of these two processes and show that both the critical bacteriocin range and the scale of competition are important drivers governing the evolution of bacteriocin production in biofilms. The critical bacteriocin range is particularly important under conditions of high nutrient penetration and localized competition for limited growth substrates. We also show, in agreement with previous laboratory findings (Tait and Sutherland 2002), that conditions of nutrient limitation that lead to the formation of monoclonal towerlike protrusions are essential to the maintenance of biodiversity with respect to bacteriocin production (fig. 6*C*).

Scientists are increasingly interested in social interactions among microbes in biofilm communities and their implications for human health (Nadell et al. 2009). For example, bacteriocin-mediated competition between commensal and pathogenic *Streptococci* species in the oral cavity has been intensively studied and recognized to affect the development of oral disease (Kreth et al. 2008). Spatially explicit theoretical models such as those discussed in this article are important for developing new conceptual approaches to tuning such competitive bacterial environments, and enhancing the success of beneficial species could become an important strategy to treat biofilm-related pathogenic infections (Nadell et al. 2009; Xavier 2011).

The human gut microbiota is an especially promising target for study, as it is a spatially structured multispecies consortium that plays an important role in resisting infection by enteropathogenic bacteria (Stecher and Hardt 2008; Neish 2009; Ubeda et al. 2010). Many examples show that bacteriocins play a major role in gut microbial ecology (Gillor et al. 2008*a*). *Ruminococcus gnavus*, a common commensal, produces the antibacterial rumococcin, which is effective against several pathogenic Clostridia (Dabard et al. 2001). The probiotic *Lactobacillus salivarius* UC118 protects against the pathogen *Listeria monocytogenes* through bacteriocin secretion (Corr et al. 2007). Recent advances in metagenomics are leading a generation of new studies into gut microbiota and its implications for human health and disease (Marchesi and Shanahan 2007). These advances must be accompanied by mechanistic knowledge of the processes governing species composition dynamics and function. Our study sheds new light onto the ecology of microbiomes by unveiling the mechanisms driving the evolution of bacteriocin-mediated competition.

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