Killing as means of promoting biodiversity

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Abstract

Bacteriocins are usually viewed as the effective weapons of bacterial killers. However, killing competitors with bacteriocins may be not only a means of eliminating other strains, but also a crucial unappreciated mechanism promoting bacterial diversity. In the present short review, we summarize recent empirical and theoretical studies examining the role bacteriocins that may play in driving and maintaining diversity among microbes. We conclude by highlighting limitations of current models and suggest directions for future studies.

Introduction

A recent study of the microbiomes of 242 healthy individuals found 1221 bacterial species living in or on the human body [1]. Extensive differences were found in the composition of bacterial flora across 18 distinct human body sites, and no species were found to colonize all 18 sites. Some body sites, e.g. the vagina, showed low Alpha diversity and high Beta diversity, because species richness was low, but there were significant differences in species composition across study participants. In contrast, other sites, such as saliva, showed the opposite pattern, with high levels of species diversity and significant microbial overlap between individuals. No matter how microbial diversity is quantified or partitioned in this or other similar studies [2–5], the human body possesses a bewildering richness of bacteria. Scientists are beginning to understand the crucial role that these bacteria play in regulating human health [6,7]. However, we still lack a basic understanding of the factors giving rise to bacterial diversity. How can so many species live together? What determines their spatial and temporal composition? Why are some environments highly homogeneous across individuals, whereas others are highly heterogeneous? How do the ecological interactions between species influence their composition and spatial organization?

Richness from killing

Recent theoretical and experimental work suggests that bacteriocins might play a key role in answering these questions. Bacteriocins are small heat-stable proteins [8] and peptides [9] that kill or inhibit the growth of taxonomically closely related bacteria [10]. Unlike antibiotics, bacteriocins usually have a narrow target range [11]. Furthermore, producing strains are immune to the toxins they produce, as are other strains sharing mechanisms of immunity.

Key words: bacteriocin, biodiversity, evolution, quorum sensing, signalling.
Abbreviations used: ODE, ordinary differential equation; RPS, rock–paper–scissors; QS, quorum sensing.
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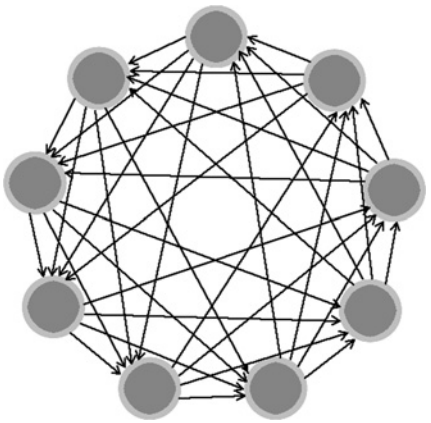
How is it possible that factors that permit one cell to kill another can increase diversity? In the simplest ecological scenario containing two genotypes, one a toxin producer and the other a sensitive strain, it has been shown that it cannot. One strain always wins, although which strain wins depends on the environment in which the strains interact as well as on their relative frequencies [12,13]. When the killer strain is rare in a mass-action environment such as a shaken flask, it loses because these cells pay a cost to produce their toxin, yet benefit only minimally from its production and secretion. This happens for two reasons. First, diffusion instantaneously carries the toxin away from the rare producing cell. Secondly, the toxin's concentration is rapidly diluted, thereby rendering it ineffective. When the killer strain becomes common, the concentration of toxin builds sufficiently to ensure its effectiveness and to allow the benefits of toxin production to exceed its costs. Here, the benefits of production are shared among producing strains, and now the killers win. However, it remains unclear how the killer strain becomes common enough to 'win' to begin with. Chao and Levin [14] classically showed that spatial structure provides a partial answer to this question. Under these conditions, the toxin producer takes over the sensitive strain even when it is initially extremely rare. This is because the toxin secreted by an individual cell remains in close proximity to this cell owing to the diffusion limitations of a spatial environment. This cell is thus able to kill its immediate neighbours and create a buffer zone in which it has sole access to resources. Regardless of the environment, the outcome is a community of one genotype; that is, bacteriocin production in this simple scenario reduces diversity.

Add a very small amount of realism and complexity, however, and the situation changes. In a slightly more elaborated scenario, where strains immune to bacteriocins are added to the community of toxin-producing and -sensitive strains, coexistence of three strains is now possible [12]. A sensitive strain outcompetes the immune strain, because it does not pay the cost of expressing immunity, the immune strain outcompetes the toxin producer because it does not pay the cost of expressing the toxin and is resistant to the toxin, and the toxin producer eliminates the sensitive strain. This

Figure 1 | **Interaction networks leading to coexistence**
Arrows indicate killing. Each strain kills half of the total number of strains and is killed by the other half. All can coexist in this scenario.



scenario can be mapped to the children's game of RPS (rock–paper–scissors), a game with no single winning strategy. One can extend this easily to multiplayer games, e.g. a five-strategy game, and have five strains coexisting. Each strain produces its own type of toxin, which can kill two other strains in the population and to which two strains are immune. This game can grow infinitely large (Figure 1) as long as the participant strains adhere to certain strict, if unrealistic, rules. Specifically, each strain should produce exactly one toxin that should be different from any other toxin in the game, and each toxin should kill exactly $(n-1)/2$ strains (when n is odd), or $n/2$ (when n is even) participant strains, where n is the total number of strains. Although these conditions are unlikely to be realized under natural conditions, this example demonstrates how toxin production can be an important factor maintaining diversity and how infinitely large groups of bacteria could coexist via this process.

Some existing models of coexistence and bacteriocins

Several models, using different approaches and assumptions, have been developed to understand microbial coexistence as a function of bacterial killing. We outline some of these below, mainly to highlight their different approaches (while not intending to be comprehensive), and conclusions. Most models begin with the two-member community outlined above, and obtain results consistent with experiments. They then go on to explore stability and coexistence in the more complex scenarios expected of natural bacterial communities.

Durrett and Levin [15] compared ODE (ordinary differential equation) models and spatial models in order to understand the effects of bacteriocins on bacterial population dynamics. In the case of two strains, the ODE and spatial models both predicted that one strain would exclude the other. In the ODE model, the established strain (either one) could never be invaded. This left unanswered the question of how toxin producers would arise in a population of sensitive microbes. The spatial model allows invasiveness, but the winner is strongly influenced by the model parameters, such as the respective 'death' and 'birth' rates of the two strains. In the case of three strains, a killer, a sensitive and a 'cheater' that produces less toxin and has a higher 'birth' rate, the spatial model showed that coexistence could be achieved, whereas the ODE always predicted a single winner: either the 'cheater' or the sensitive strain.

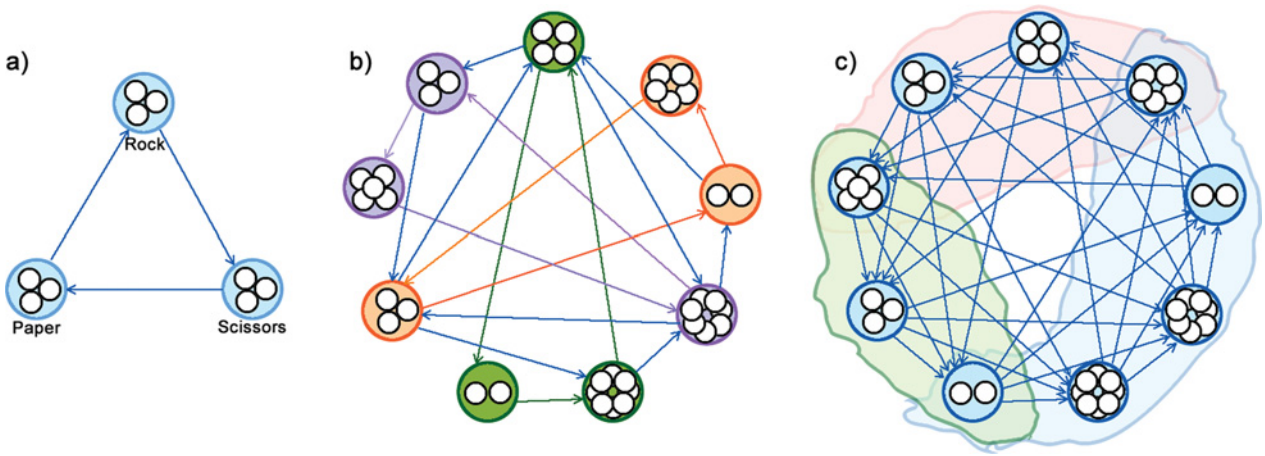
Nakamaru and Iwasa [16] built a spatial model with three strains whose competitive relationships adhered to RPS conditions. Space in this model is divided into patches and each patch is dominated by a different strain. Boundaries between patches can move and form travelling waves; when travelling waves collide, strains can outcompete each other. In contrast with the spatial model of Durrett and Levin [15], this model determined that coexistence could not take place, because the sensitive strain is likely to take over the whole space. This occurs because interactions occur one at a time when two waves meet, and from these interactions there is only one winner. When this winner meets another wave, another winner is determined. One by one, waves are eliminated. In the spatial model of three strains of Durrett and Levin [15], the three strains interact simultaneously, thus each strain gains and loses some members at every time point.

Discrete models such as agent-based models have also proved to be an attractive modelling approach to study bacteriocin dynamics, in part because these are more easily translated into experimental studies. As described in [17], "individual-based models [also called agent-based models] are population or ecosystem models that do not state or prescribe any properties of the population they model. Rather, they describe all the actions of the organisms and their interactions with the environment and each other. The population structure and dynamics emerges from this". Time in individual-based models is discrete and in each time step each individual performs a certain set of actions. Individuals generally share the food resources and the same physical space. Using this approach, Czárán and Hoekstra [18] investigated the coexistence of two strains, one killer and one sensitive. They defined a common habitat for the sensitive and toxic strains, consisting of discrete patches with unlimited food resources. The patches randomly go extinct and are repopulated, with equal migration rates for both strains. By design, the sensitive strain grows faster than the toxic strain; however, if they share the same patch, the toxic strain will outcompete the sensitive one due to its toxicity. With this approach they concluded that sensitive strains are likely to go extinct locally, but they can persist by embracing a nomadic lifestyle. That is, coexistence is locally prevented but possible globally.

Proceeding from the approach of Kerr et al. [12], Reichenbach et al. [19] analysed the diversity of bacterial communities in a spatial environment where strains were capable of different levels of mobility. At low mobility rates, strains arrange themselves in fascinating patterns. As the mobility rate grows, so does the size of the pattern, until the pattern outgrows the matrix, leaving just one surviving strain.

Figure 2 | Theoretical mechanisms of coexistence potentially explaining Alpha and Beta diversity

Arrows represent killing between groups, and no arrows represent mutual immunity between groups. (**A**) Model presented in [20]: mutually immune strains form units that play the RPS game. (**B**) The model could be expanded, similar to the game presented in Figure 1. Mutually immune strains form unit that play the RPS game. A set of units can form an 'alliance' (alliances have different colours). Alliances could represent the Alpha diversity. They can interact with each other. (**C**) Alliances need not be mutually exclusive in terms of consisting units, as long as they respect the overall rules of interactions.



All strains have an equal chance of becoming the unique survivor.

Adding further complexity by including more strains and toxins, Szabó et al. [20] developed a spatial model with nine strains that can produce up to two toxins. In this model, subgroups of three strains each organized themselves in 'units' containing mutually immune species (see Figure 2A). At a higher level, these three units, each of them acting like a single entity, play the RPS game against one another. This model could grow infinitely large by adding more strains and toxins, allowing an infinitely large number of strains to coexist (Figures 2B and 2C) in the following manner: a variable number of strains could group themselves into 'units' containing mutually immune species and then the units could play a game similar to the one described in Figure 1.

Depending on the model assumptions and structure, killing by bacteriocins can either decrease or increase diversity. As yet, it remains unclear which sets of assumptions are most biologically meaningful. Consequently, testing these model assumptions experimentally remains a crucial next step.

The importance of evolution

With notable exceptions, few of the current bacteriocin models consider how the evolution of component strains influences the dynamics of bacterial coexistence. Given the propensity of bacteria to rapidly diversify due to mutation and recombination, this is a key limitation. In an important effort to integrate evolutionary change and bacteriocin dynamics, Czárán and Hoekstra [21] developed a spatial model using a cellular automation approach where each strain can produce or be immune to up to 14 toxins. Strains mutate between states of production and immunity to evolve new phenotypes. In the most interesting outcome, coexistence between strains was extensive. At equilibrium, the model predicted more than 1000 coexisting strains; however, the structure of these strains was highly dynamic on the route towards this equilibrium. Strains at the beginning of the simulations evolved rapidly to produce multiple toxins. Gradually, strains gained immunity to all of the toxins produced, which thus rendered toxin production a costly trait with limited utility. At the quasi-equilibrium, strains produced few toxins each, but retained immunity to most. Czárán and Hoekstra termed this state hyper-immunity, a prediction with some support in colicin communities (22% of the *Escherichia coli* strains in [22] were immune to all colicins tested). The crucial addition of evolution in this model allowed the transitions between community properties, from multi-toxicity to hyper-immunity to be observed.

More recently, Kerr [13] modelled a situation where the metabolic burden of the immune strains can evolve. In the case where the immune strain entirely occupies one environment, if a mutant with lower metabolic burden arises, then it easily eliminates all the other bacteria. Thus the population evolves towards the minimum possible cost of resistance. However, in an environment with toxin-sensitive, -resistant and -producing strains, surprisingly, the resistant strain does not evolve towards this minimum cost. This does not happen because it is not in the evolutionary 'interest' of the immune strain to achieve the minimum metabolic burden: a mutant with a low metabolic burden will replace the immune population and it will increase the rate at which it replaces the toxin-producing strain. Once the toxin-producing strain becomes extinct, the sensitive strain will have no natural competitor and will easily eliminate the immune strain. By retaining a moderate cost of resistance, and, in turn, ensuring

the longer-term persistence of the killer, the immune strain thereby permits its own persistence. Thus 'the enemy of my enemy is my friend' applies in this case.

Nahum et al. [23] explored the evolution of the resistant strain in a non-transitive form of the RPS game. Non-transitivity ensures that the growth of each of the three strains is controlled through a negative-feedback loop. If, for example, the resistant strain starts to grow more quickly, then it replaces the toxin producer at a higher rate, allowing the sensitive strain to grow faster, which in turn replaces the resistant strain more rapidly. In a series of analyses and experiments, these authors showed that the resistant strain evolves towards an apparently altruistic behaviour, reducing its fitness on a local level (which might appear to be disadvantageous), but, in fact, guarantees its longer persistence owing to the negative feedback from rapid growth. This is the result of a very general fascinating feature of RPS games: it is not the fastest reproducing strain that is the most abundant in a spatial RPS game, but the one that is the successor (defeater) of the fast reproducing one [24].

Interactions between cells

In all of the previous models, each strain or cell behaved independently of the other. However, this assumption of bacterial behaviour can be violated in several ways that modify the role that bacterocins may have on diversity. Bacteria can potentially respond to one another in order to autonomously regulate their bacteriocin production. Alternatively, they may regulate bacteriocin production socially, in order to produce co-ordinated responses at the population level.

In an important recent study, Majeed et al. [25] tested the interactions between two similar *E. coli* strains, each of them producing one type of toxin. What is unique here, compared with similar scenarios used previously, is that the two toxins are cross-inducing. That is, toxin production by strain A depends on its detecting the toxin of strain B. In an unstructured environment, one of the strains eliminated the other. By contrast, in a structured environment, the two strains ended up in a 'frozen' spatial pattern and occupied an equal share of the Petri dish. In simulations of these experiments, where strains cannot cross-induce each other, the strain with higher reproductive rate wins. However, when cross-induction occurs, the strain with higher reproduction cannot invade because the production of its toxin triggers the production of the other toxin. Thus cross-induction may be a defence mechanism that promotes coexistence.

Although bacteriocin secretion in Gram-negative bacteria is induced by the SOS response, in Gram-positive bacteria such as *Streptococcus pneumoniae*, bacteriocins are induced via a quorum-dependent signalling system. QS (quorum sensing) is a process enabling bacteria to co-ordinate group decisions or behaviour [26]. For this, they use small signalling molecules, including peptides that are secreted outside the cell and accumulate in the extracellular medium. When the concentration of the signalling molecule reaches a certain threshold, a population-wide response ensues. For bacteriocins, QS can induce toxin secretion or the induction of immunity, or specify the time point or density during growth when toxins are released [10]. Czárán and Hoekstra [27] proved in a theoretical model that QS could regulate bacteriocin production, but only in the case where there is no associated cost for expressing the signalling peptide. Nevertheless, QS is absent from the other models that we have discussed above and this may be a very important omission, as QS has the potential to strongly modify the interactions between individual cells and genotypic clusters. Although the precise consequences of these dynamics remain unclear, it is certain that adding this mechanistic reality will significantly increase the strategy set bacteria employ in their antagonistic interactions with one another.

Looking forward

The idea that killing by bacteriocins could facilitate the origin and maintenance of biodiversity is still very much in its infancy. In order to move this provocative area forward, the next generation of theoretical models must overcome several limitations, e.g. the mechanisms of toxin production (constitutive, induced and cross-induced), as well as the induction of immunity and the evolution of (costly) resistance. This will be needed not only to understand their effects on the evolution of biodiversity, but also to understand the feedback between patterns of diversity and the mechanisms that regulate the bacteriocins themselves. Most important, however, is the need for an expanded experimental approach that begins to test the predictions of models, both in the short term and over the longer term where evolutionary changes are allowed to occur. The landmark study of Kerr et al. [12] paved the way for the experimental work that must follow. It also, however, serves as a cautionary tale. Although this study provided a clear demonstration of how coexistence via an RPS competitive series could occur, this coexistence was short-lived. A mere 1 week after the three coexisting strains were established, a new resistant strain evolved from the sensitive strain that then went on to win. It may not be immediately obvious how to build this reality into new models and experiments, but it is surely worth considering how to do so.

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