The ecological role of bacteriocins in bacterial competition

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Bacteriocins are an abundant class of antimicrobial molecules that appear to mediate population dynamics within species. The bacteriocins of *Escherichia coli* have served as a model for exploring the ecological role of these potent toxins. Studies suggest that colicins provide a competitive edge in nutrient-poor environments and that there might be a trade-off between the costs and benefits of colicin production.

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Bacteriocins are one of the most abundant and diverse classes of antimicrobial molecules, having been detected in all major lineages of Eubacteria and in Archaebacteria[1–3]. They are potent, often highly specific toxins that are usually produced during stressful conditions and result in the rapid elimination of neighboring cells that are not immune or resistant to their effect[1,4]. Given their often narrow range of activity, it has been proposed that the primary role of bacteriocins is to mediate intraspecific, or population-level, interactions[5].

One class of bacteriocins, the colicins produced by *Escherichia coli*, has been the focus of numerous ecological studies[6–10]. Mathematical models and empirical studies suggest that colicins play a key role in mediating *E. coli* population dynamics[5]. In this review, we describe what these studies reveal about the interactions between colicin producers and non-producers in *E. coli* populations.

Colicins as potent antimicrobials

Under conditions of stress, such as nutrient depletion or overcrowding, a small proportion of colicin-producer cells in a population are induced to produce colicin. Induction results in the rapid release of colicin into the environment, generally through lysis of the producing cell. Colicins bind to specific cell surface receptors and are transported into the cell. Having gained access, they kill the cell by one of three primary mechanisms: forming channels in the cytoplasmic membrane, degrading cellular DNA or inhibiting protein synthesis[1,4,11].

Colicin-producer cells also synthesize an immunity protein that provides protection against their own colicin. The immunity protein is encoded in the same gene cluster as the colicin protein and is constitutively expressed (Fig. 1). It recognizes its own colicin, generally binds to the carboxy-terminal end of the colicin and inhibits cell killing. Resistance to colicin killing can evolve through alterations in the cell surface receptors that colicins use to gain access to the cell (true resistance)[1,9,12] or through changes in the transport mechanisms that colicins use to cross the cell membrane (tolerance)[1,9,11]. Only immune or resistant cells survive under conditions of colicin production.

The mechanisms of colicin recognition and transport result in the very narrow target range of colicins; they generally kill only *E. coli* and its close enteric relatives. In fact, a recent survey of colicin resistance across several members of the Enterobacteriaceae suggests that colicins mainly act intraspecifically; levels of colicin resistance among other enteric bacteria are as high or higher than levels detected within *E. coli*[9].

Colicin production and resistance in natural populations

Numerous surveys of colicin production reveal that, on average, 30% of natural populations of *E. coli* are composed of producer strains (see Box 1 for an example of one such survey)[5,13]. Over 25 colicin types have been characterized[4]. Each of the populations studied differs with respect to the particular combination of colicins it possesses, and, in general, there is one dominant colicin and several rare colicins present (Fig. 2a)[5,14]. Most cells in the population are resistant to one or more of the colicins produced, with an average of 70% being resistant to any one colicin and 30% being resistant to all colicins produced in the

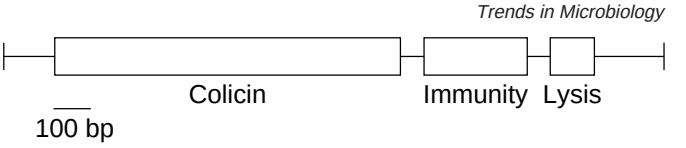

Fig. 1. Colicins are encoded in colicin gene clusters. Generally, a gene cluster comprises three tightly linked genes: a colicin gene (which encodes the colicin protein that functions in killing), an immunity gene (which encodes an immunity protein that functions in conferring specific immunity to that colicin) and a lysis gene (which encodes a lysis protein that functions in releasing the colicin). Colicin gene clusters are encoded on plasmid replicons in *Escherichia coli*. Colicins A, D, E1–E9, K, 10, 5 and N are located on small plasmids (~6 kb in size). Colicins B, Ia, Ib, L and M are located on large plasmids (100 kb).

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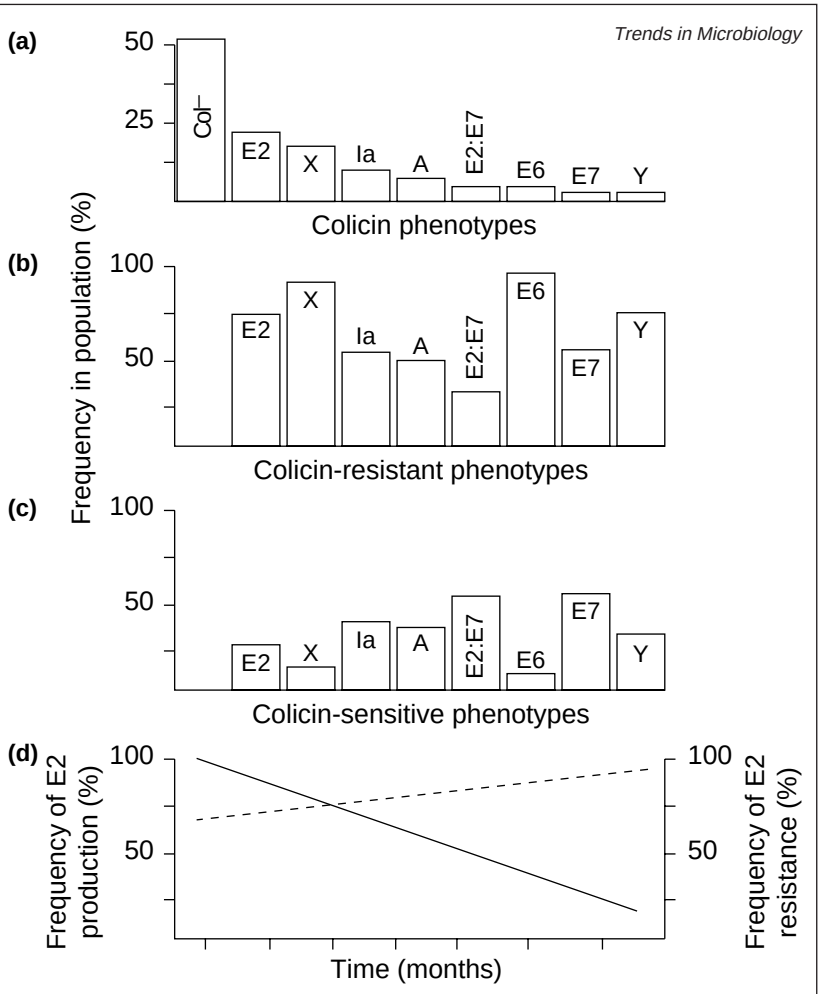

Fig. 2. A survey of colicin production and resistance in *Escherichia coli*. Over 400 *E. coli* were isolated from two populations of feral mice in Australia over a period of seven months[14]. The isolates were scored for colicin production and resistance. **(a)** Colicin production is abundant. Numerous colicin types were detected (with colicin E2 being the dominant producer type), and just under 50% of the strains were producers. Col$^-$ represents non-producer strains. **(b)** The majority of isolates are resistant to most co-occurring colicins. **(c)** A small proportion of the population is sensitive to co-occurring colicins. **(d)** Over time, the levels of colicin E2 production (unbroken line) decreased, whereas the levels of E2 resistance (broken line) rose.

population (Fig. 2b)[9,12]. The relatively few remaining cells are colicin sensitive (Fig. 2c). The one available survey of colicin distribution over time suggests that there might be a constant flux in the relative frequencies of producer, resistant and sensitive phenotypes in natural populations (Fig. 2d)[14].

Any description of colicin dynamics must therefore account for the consistent presence of colicin-producer, colicin-resistant and colicin-sensitive cells in natural populations and, possibly, a continuous flux in the frequencies of these three phenotypes. In addition, such a description must explain the high diversity of colicin types among different populations.

Mathematical studies of colicin dynamics

Early theoretical work involved the modeling of colicin invasion as a mass action process[6,7]. In these studies, the invasion dynamics of colicin-producing and colicin-sensitive strains were shown to be frequency dependent, largely because of the costs associated with colicin synthesis[6,7]. In liquid culture, when the initial frequency of the colicin producer is low, too little colicin is produced to kill enough sensitive cells and thereby offset the cost of colicin production. Similarly, a sensitive strain will only invade a colicin-producing population at high frequencies. If colicin-producer frequencies are high, the levels of toxin present prohibit sensitive cells from invading[6,7]. Furthermore, in liquid culture, the long-term coexistence of sensitive and producer strains cannot be achieved[6,7]; depending upon initial frequencies, either the producer or the sensitive strains predominate.

More recent mathematical models have incorporated structure in the environment and spatial heterogeneity in resource abundance, as would arise if cells were growing on a solid surface, such as an agar plate[7,8]. In these models, the coexistence of producer and sensitive strains is possible[7]. Sensitive strains persist in poor habitats where the rate of resource competition is high (relative to the cost of colicin production), whereas producer strains persist in rich habitats where resource competition is low[7]. In this more complex model, the relative abundance of sensitive and producer cells is determined by initial abundance, levels of competition, migration and diffusion of toxin. These models demonstrate that it is possible to achieve a dynamic equilibrium between bacteriocin-producer and -sensitive strains, as is observed in natural populations.

Another model incorporates a resistant class, albeit indirectly, as a 'cheater' strain that has lower levels of colicin production, and therefore less costly production, but maintains colicin immunity[8]. A resistant cell is, in some respects, like the cheater cells; i.e. it is not killed by the colicin. In this model, it is possible to achieve the coexistence of producer, sensitive and cheater populations in spatially structured environments.

Empirical studies of colicin dynamics

Serial-transfer culture experiments have provided additional insight into the dynamics of colicin invasion[10]. Serial transfer involves the continuous propagation of cultures through daily transfer of a sample of the population into fresh liquid media. The frequency of colicin-producer and -sensitive strains can be easily monitored in such an experiment. It is also possible to quantify critical features of the invasion process, such as levels of colicin production, lysis rates and growth rates of the competing strains, under the same culture conditions.

The behavior of a mathematical model developed to mimic the serial transfer experimental protocol,

together with independently estimated values for the critical parameters in the model, adequately predicts the results of the serial transfer invasion experiments[10]. In particular, these experiments suggest that the most important parameter in predicting the rate of colicin invasion is the number of colicin molecules released per cell. Colicinogenic strains producing high titers of colicin tend to invade more rapidly than those producing low titers, and the time before invasion occurs is frequency dependent, with more rapid invasion obtained with higher initial colicin-producer frequencies. What is most surprising is that the models and experiments both suggest that, even though there can be large differences in growth rate, lysis rate and the amount of colicin produced per cell among colicin producers, these differences translate into relatively small differences in invasion dynamics. In other words, although there is a significant advantage gained from colicin production, the magnitude of this advantage appears to differ little among colicin types. This suggests that factors other than colicin production might determine which colicin producer type will invade a particular population.

The modeling and empirical studies provide answers to several questions. The three phenotypes – colicin production, colicin resistance and colicin sensitivity – can be maintained in populations with subdivision and access to a patchy distribution of resources. Such heterogeneity will be the norm in the majority of habitats in which *E. coli* is found. Furthermore, these results suggest that the benefit of colicin production in areas of lower nutrient availability will offset the cost of production.

A conceptual hypothesis for colicin-mediated dynamics

Imagine a population of bacteria that initially consists of only colicin-sensitive cells. Theoretical and empirical studies show that, if a colicin-producing cell migrates into this population, there is a very broad range of conditions (for example, initial frequency, growth rate and lysis rate) under which it will succeed in rapidly displacing the sensitive resident[6–8,10,15]. The population will quickly be dominated by the producer.

However, mutants resistant to the colicin will quickly arise in the sensitive population[9,12]. These resistant cells will increase in frequency at the expense of the producer. The speed with which resistant strains invade will depend upon the relative growth rates of the producer and resistant strains. Growth rate differences between resistant and producer types result from the general costs of colicin synthesis and carrying a colicin plasmid (costs to producers) and the cost of alterations in cell surface receptors or translocation systems (cost to resistant cells) (M. Feldgarden and M.A. Riley, unpublished). The resistant cells will soon displace the producer and dominate the population. However, relative to a sensitive cell, there is a significant cost to resistance[9,12]. Thus, sensitive revertants appear quickly and eventually displace the resistant strains.

Box 1. The evolution of colicin diversity

Colicins have been the focus of numerous evolutionary investigations, including DNA and protein sequence comparisons, experimental studies of invasion dynamics and competitive abilities, and mathematical modeling. The results of these studies suggest that the current diversity of colicins is the product of recombination and natural selection.

The nuclease colicins comprise a closely related lineage of toxins. Their diversification appears to result from the action of strong, positive, diversifying selection acting on immunity function. Novel nuclease colicin immunities evolve as a result of one, or a few, point mutations, which confer a broadened immunity. These rare variants have a competitive advantage and are maintained in the population. Subsequently, one, or a few, mutations in the immunity binding domain of the colicin protein result in a 'super killer' colicin that is immune to self and ancestor, but to which its ancestor is not immune. This super killer is rapidly fixed in the population until a further round of diversification results in yet another novel immunity function. Over time, this results in the accumulation of nuclease colicins that are closely related except for high levels of divergence in the immunity region of the colicin gene cluster.

In contrast, the pore-former colicins represent a highly divergent class of proteins that, despite sharing a common killing function, show limited apparent common ancestry. The process of pore-former diversification is the result of numerous recombination events that serve to shuffle functional domains among the pore-formers, creating ever increasing numbers of novel colicins. This process is illustrated in Fig. I, with a comparison of levels of DNA and protein sequence similarity between the chimeric pore-former colicin B and those pore-formers implicated in previous recombination events with colicin B (including colicins D, E1 and A).

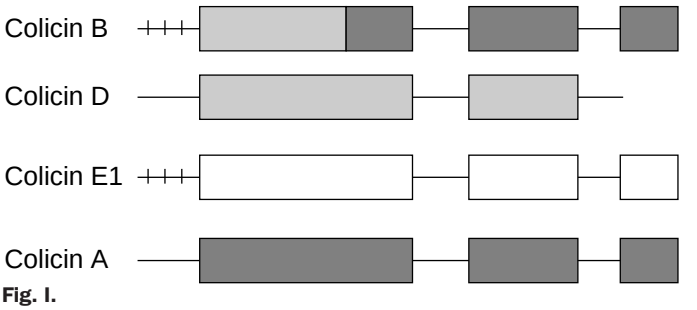

Fig. I.

Common gray tints denote regions that have potentially recombined and thus show high levels of sequence similarity. The result of this frequent recombination is a class of mosaic proteins that share short stretches of DNA with several different pore-formers (as shown for colicin B). Given the high frequencies with which pore-former colicins are found in nature, it is tempting to speculate that these novel recombinants also experience some form of positive selection.

The processes of displacing the producer population by a resistant population and displacing the resistant population by a sensitive population will occur more slowly than will displacement of the sensitive population by a producer population. In the first two cases, replacement of one population by the other will occur at a rate that is dependent only on the difference in relative growth rate between the two strains. In contrast, when the producer strain invades the sensitive population, once a critical frequency of producer cells is achieved, the production

Questions for future research

- Do most populations undergo a continual flux between colicin production, colicin sensitivity and colicin resistance?
- Do colicin production and colicin resistance confer a significant cost compared with colicin sensitivity?
- Do all colicins have similar competitive abilities?
- Do colicins serve primarily in intraspecific rather than interspecific competition?

of toxin rapidly eliminates the sensitive population. Thus, this phase of the dynamic is probably relatively short.

This simple scenario suggests the predictable sequential replacement of sensitive cells by producer cells and producer cells by resistant cells. However, replacement of the dominant population will not necessarily proceed in such an orderly fashion. For example, the resistant population might be replaced by a different colicin producer to which it is not resistant rather than by a sensitive population. As different classes of colicins use different cell surface receptors and translocation systems, it is possible that a cell resistant to one class of colicins will not be resistant to another class of colicins that recognize a different receptor[1,9,12]. Similarly, a dominant producer population could be replaced by a novel producer (which can kill the resident producer) rather than by a resistant cell. Invasion studies have revealed that novel colicins can easily evolve and displace the resident producer population (Box 1)[5,15,16]. Studies of the molecular evolution of colicins suggest that high levels of colicin diversity can be rapidly generated, providing a constant influx of novel colicin types into a population[5].

In its simplest form, our hypothesis describes the sequential replacement of one cell type by another; a result that would be predicted to occur under liquid culture conditions. However, the mathematical models incorporating a structured environment predict the coexistence of producer, resistant and sensitive cell types. One model suggests that the relative frequency of cell types may fluctuate temporally[8].

Our description of colicin-mediated population dynamics captures several features observed in natural populations of *E. coli*. First, the hypothesis suggests that a combination of producer, resistant and sensitive cells will be present in every population. Indeed, in every case in which all three phenotypes have been screened, all three have been detected[4,9,13,14]. Second, the hypothesis predicts that colicin production and resistance will be the dominant phenotypes, as the displacement of sensitive populations is predicted to occur rapidly. Results of resistance surveys report that $<15\%$ of any population is sensitive to colicins[9,14]. Third, a continual flux in the abundance of producer, resistant and sensitive types in natural populations is expected. Only one study has examined changes in frequency of these three phenotypes over time (Fig. 2d)[14] and reports a 30% decline in the frequency of production of the most common colicin during a seven-month period. Over the same time period, resistance to this colicin increased. Sequential surveys of the same host population over time are needed to assess this prediction.

Populations of bacteria harbor different colicins

Results from empirical work and mathematical modeling suggest that there is little difference in competitive ability between colicin-producer types. Although different producer types show substantial differences in the number of colicin molecules produced per cell, different levels of cell lysis and slightly different growth rates, they invade sensitive populations at similar rates[10]. These observations suggest that the colicin composition of a population might be determined by factors other than between-producer competition.

Each population studied to date has its own unique colicin composition, which probably results from several factors[5]. The migration patterns of colicin producers between populations will determine the pool of potential producer types that compete for access to niches within a population. Populations will almost certainly differ in the pool of migrants to which they are exposed.

Several other factors might also be involved in determining the relative abundance of the colicin types within a population. Several colicin plasmids have been shown to be effective in protecting cells against phage attack[17]. For example, colicin Ib encodes a phage defense system[18]. Phage densities in the environment can thus favor one colicin producer over another. Similarly, at least one colicin plasmid (colicin V) has been implicated in virulence determination[19]. Not surprisingly, this colicin plasmid is detected at high frequencies in sick humans and other animals[20,21]. In this case, the presence of aerobactin, a putative virulence factor, rather than colicin production, might be the selected trait[19]. Thus, although it has been clearly shown that colicin production is a potent determinant of invasion success, in certain populations other factors might determine the particular producer types that 'win the war'.

Conclusions

Colicin production has long been assumed to play a role in mediating intraspecific interactions in *E. coli*. Mathematical models suggest that colicin producers can invade a population under a wide range of environmental conditions. Furthermore, at equilibrium, producer, sensitive and resistant cells are predicted to co-occur. Recent studies of the invasion dynamics of colicins under laboratory conditions provide confirmation of these predictions and suggest that there might be a constant flux of sensitive, producer and resistant cell types in natural populations. By combining empirical and mathematical modeling approaches, we have arrived at a fairly simple hypothesis of the pattern of intraspecific interaction that might occur in natural populations of *E. coli*. This hypothesis accurately describes the distributions and relative frequencies of producer, sensitive and resistant cells that have been observed in natural populations of *E. coli*.

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The planetary crucible

Bacterial Biogeochemistry: The Ecophysiology of Mineral Cycling (2nd edn)
by T. Fenchel, G.M. King and T.H. Blackburn

Academic Press, 1998.
$64.95 hbk (viii + 307 pages)
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If Gaia is a useful metaphor for planetary biogeochemistry, then most of her complex physiology can be found in the microbial world. The lungs that respire planetary gases, the circulatory and metabolic mechanisms that transform energy and nutrients, and the organs that process and purify catabolic end products are all largely attributable to the diverse activities of Earth's microbiota. Simply put, bacteria are the master chemists and biochemists of our planet. *Bacterial Biogeochemistry: The Ecophysiology of Mineral Cycling* explores, in some detail, bacterially mediated chemical cycling of energy and matter in Nature.

Bacterial Biogeochemistry: The Ecophysiology of Mineral Cycling is conceptually the second edition of the Fenchel and Blackburn book, *Bacteria and Mineral Cycling*[1]. New discoveries that have accumulated over the intervening 20 years have been incorporated, and the new text focuses on the effects of bacterial activities on Earth's chemical environment. The intention of this book is not to provide a detailed review of specific bacterial species or their particular physiological properties. Rather, the authors take an ecosystem perspective on bacterial activities that mediate major biogeochemical cycles of matter and energy. In most cases, they seem to achieve their goal of presenting a broad overview of biogeochemical cycling in different environmental contexts, illustrating major points with specific examples. Microbial involvement in the carbon, nitrogen and sulfur cycles, mass balance of energy, and matter input and export are the central themes of each habitat-specific chapter.

The introductory chapters cover general aspects of bacterial metabolism, energetics, community structure and mineral cycles. Subsequent chapters focus on the critical microorganisms, processes and cycles in specific environments; the chapters on the water column, soils, aquatic sediments and mats are especially useful. The qualitative and quantitative differences between each environment-specific chapter are a good indication of the status of each research area; for some habitats (especially aquatic sediments), there is detailed understanding of specific details of biogeochemical cycling and the microorganisms involved, whereas in other environments (for example, soils), it is a much more difficult task to present specific details in a general context. A few of the chapters (for example, 'Extreme environments' and 'Origins and evolution') are, because of their focus, necessarily speculative.

Bacterial Biogeochemistry: The Ecophysiology of Mineral Cycling is informative and enjoyable to read. However, in some places, the authors make claims that are not well qualified and sometimes unreasonably opinionated, and some of the coverage is not as complete nor accurate as the primary literature might allow. In addition, some parts of the book focus too much on the specific areas of interest of the authors. On the whole, however, *Bacterial Biogeochemistry: The Ecophysiology of Mineral Cycling* is authoritative, comprehensive in scope and