Chapter 2
Bacteriocin-Mediated Competitive Interactions of Bacterial Populations and Communities

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Abstract Explaining the coexistence of competing species is a major challenge in community ecology. In bacterial systems, competition is often driven by the production of bacteriocins; narrow spectrum proteinaceous toxins that serve to kill closely related species providing the producer better access to limited resources. Bacteriocin producers have been shown to competitively exclude sensitive, non-producing strains. However, the interaction dynamics between bacteriocin producers, each lethal to its competitor, are largely unknown. Several recent studies have revealed some of the complexity of these interactions, employing a suite of in vitro, in vivo, and in silico bacterial model systems. This chapter describes the current state of knowledge regarding the population and community ecology of this potent family of toxins.

Introduction

Bacteria engage in a never-ending arms race in which they compete for limited resources and niche space. The outcome of this intense interaction is the evolution of a diverse and powerful arsenal of biological weapons. Most species of bacteria produce one, and usually many more, of these potent biocontrol agents, including classical antibiotics, lytic agents, lysozymes, and bacteriocins (Cascales et al. 2007). The microbial weapons of choice, as assessed by the frequency with which they are encountered in natural populations of bacteria and in their diversity of forms, are the bacteriocins.

Bacteriocins are loosely defined as biologically active protein moieties with a bacteriocidal mode of action (Tagg et al. 1976; James et al. 1991). Two main features distinguish the majority of bacteriocins from classical antibiotics: bacteriocins are

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ribosomally synthesized and have a relatively narrow killing spectrum (Riley and Wertz 2002). Indeed, bacteriocins are often only toxic to bacteria closely related to the producing strain. The bacteriocin family includes a diversity of proteins in terms of size, microbial target, mode of action, release, and immunity mechanisms and can be divided into two main groups: those produced by Gram-negative and Gram-positive bacteria (Gordon et al. 2006; Heng et al. 2007).

Their production occurs across all major groups of Eubacteria and the Archaebacteria (Webster 1991). Within a species, tens or even hundreds of different kinds of bacteriocins are produced (James et al. 2002; Riley and Gordon 1992). Colicins, bacteriocins produced by *Escherichia coli*, are found in 30–50% of the strains isolated from human hosts and are often referred to as virulence factors (Riley and Gordon 1992). Much higher levels of bacteriocin production have been found in some Gram-negative bacteria, such as *Pseudomonas aeruginosa*, in which >90% produce bacteriocins (Michel-Briand and Baysse 2002).

Despite high levels of bacteriocin diversity, these proteins share many general characteristics (James et al. 2002; De Vuyst et al. 1994). They are generally high-molecular weight protein antibiotics that kill closely related strains or species. The bacteriocin gains entry into the target cell by recognizing specific cell surface receptors and then kills the cell by forming ion-permeable channels in the cytoplasmic membrane, by nonspecific degradation of cellular DNA, by inhibition of protein synthesis through the specific cleavage of 16s rRNA, or by cell lysis.

Without question, bacteriocins serve some function in microbial communities. This statement follows from the detection of bacteriocin production in all surveyed lineages of prokaryotes. Equally compelling is the inference of strong positive selection acting on some bacteriocins (Tan and Riley 1996; Riley 1998). Finally, there is a well-developed body of theory and empirical data that details the potential role of bacteriocins which play in maintaining microbial diversity at the population and community levels (Chao and Levin 1981; Frank 1994; Gordon and Riley 1999; Czárán et al. 2002; Kerr et al. 2002). Such empirical observations and theoretical results argue that these toxins play a critical role in mediating microbial interactions. What remains in question is what, precisely, that ecological role is. Bacteriocins may serve as anticompetitors enabling the invasion of a strain or a species into an established microbial population or community. They may also play a defensive role and act to prohibit the invasion of other strains or species into an occupied niche or limit the advance of neighboring cells. An additional role has recently been proposed for bacteriocins produced by Gram-positive bacteria, that of regulating quorum sensing (Miller and Bassler 2001). This chapter describes the current state of knowledge regarding the population and community ecology of this potent family of toxins.

Colicins: The Model Bacteriocin

The most extensively studied bacteriocins are the colicins, which are produced by *E. coli* (Pugsley 1985; Pugsley and Oudega 1987; James et al. 2002; Cascales et al. 2007). Colicins were first identified almost 100 years ago as a heat-labile product

present in cultures of *E. coli* V and toxic to *E. coli* S. They were given the name of colicin to identify the producing species (Gratia 1925). Fredericq demonstrated that colicins were proteins and that they had a limited range of activity due to the presence or absence of specific receptors on the surface of sensitive cells (Fredericq and Levine 1947).

Colicins are archetypical of a large subfamily of bacteriocins found primarily in the family *Enterobacteriaceae*. One of the defining features of colicin-like toxins is that they are composed of three functional domains: a central binding domain that recognizes and adheres to specific receptor sites on the surfaces of target cells, an amino-terminal translocation domain responsible for entry into the cell, and a carboxy-terminal killing domain that actually kills the cell. Colicins kill target cells through a variety of mechanisms. Nomura showed that colicins E1 and K inhibit macromolecular synthesis without the arrest of respiration, colicin E2 causes DNA breakdown, and colicin E3 stops protein synthesis (Nomura and Witten 1967). In each case, he showed that the lethal action is reversed by treatment with trypsin. Since his pioneering work, colicins were shown to kill their targets by either membrane permeabilization or nucleic acid degradation (Braun et al. 1994; Riley and Wertz 2002; Smarda and Smajs 1998). Colicins are classified according to the nature of the killing domain. The nuclease group includes colicins that degrade DNA, rRNA, or tRNA. The pore former colicins kill by the formation of voltage-gated channels in the cytoplasmic membrane. The third group contains colicins that affect the peptidoglycan cell wall. Colicin operons typically contain three genes: the toxin-encoding gene; an immunity gene, whose product specifically binds to and confers protection against the encoded toxin; and a lysis gene, whose product contributes to the release of toxin into the environment.

Recent surveys of *E. coli*, *Salmonella enterica*, *Hafnia alvei*, *Citrobacter freundii*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, and *Enterobacter cloacae* reveal levels of bacteriocin production ranging from 3 to 26% of environmental isolates (Gordon 2006; Riley 2002). Colicins are found in 30–50% of the strains isolated from human hosts (Riley and Gordon 1992). Much higher levels of bacteriocin production have been found in some Gram-negative bacteria, such as *P. aeruginosa*, in which >90% of both environmental and clinical isolates produce bacteriocins (Michel-Briand and Baysse 2002).

Until recently, little was known about the phylogenetic breadth over which bacteriocins kill. To produce such an estimate, we took advantage of a recently determined molecular phylogeny of enteric bacteria (Wertz et al., 2003). The frequency of killing within each taxon for each bacteriocin was mapped onto the enteric phylogeny (Fig. 2.1) (Riley et al. 2003). Not surprising, these data reveal that bacteriocins usually kill members of their own species. However, a surprisingly high level of interspecific killing was observed, with almost half of the bacteriocins killing in more than one taxon. Further, the relationship between killing ability and phylogenetic distance is nonlinear. In other words, some bacteriocin producers kill distantly related bacteria but not their closest relatives. This nonlinear relationship is seen in Fig. 2.1, in which 18 of 36 columns indicate killing outside of the producer strains own species (off the diagonal). The observation of a broad killing range for numerous enteric bacteriocins requires that the ecological role proposed for bacteriocins be reconsidered. It may well be that they serve a broader, community

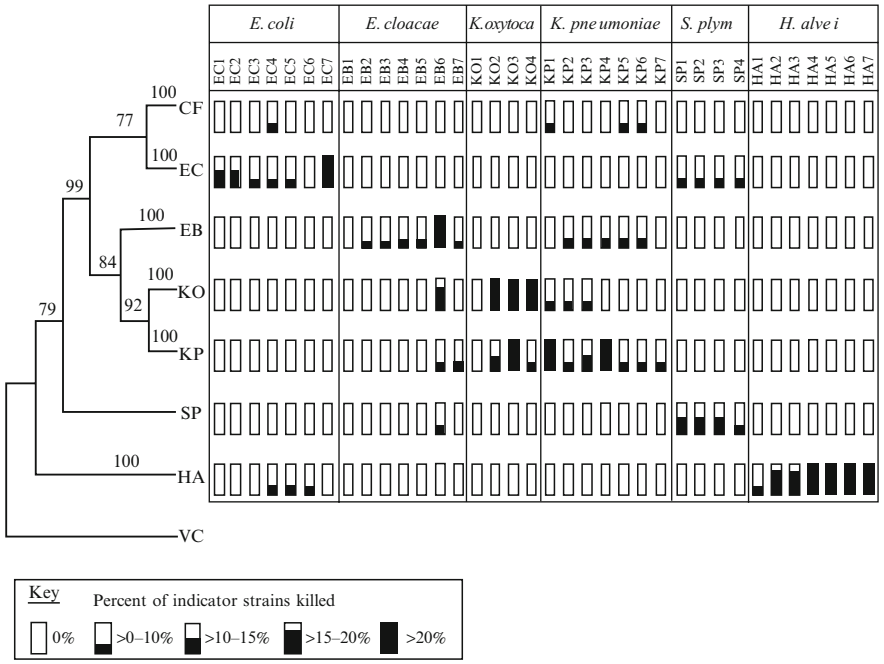

Fig. 2.1 Enteric bacteriocin phylogenetic killing range. The frequency of bacteriocin killing within each of the seven enteric taxa is mapped onto a composite molecular phylogeny of enteric bacteria (adopted from Wertz and Riley 2003). The bacteriocins assayed for killing breadth are indicated across the *top* (EC = *Escherichia coli*, CF = *Citrobacter freundii*, KO = *Klebsiella oxytoca*, KP = *Klebsiella pneumoniae*, EB = *Enteroterobacter cloacae*, HA = *Hafnia alvei*, SP = *Serratia plymuthica*, VC = *Vibrio cholera*). Each column provides the frequency of killing for each bacteriocin assayed against 40 indicator strains for each taxa in the molecular phylogeny

level role than has been envisioned to date. This finding complements recent theoretical work, which suggests that bacteriocins (and other microbial defense systems) may be responsible for maintaining much of the extraordinary diversity of microbes observed in nature (Czárán et al. 2002; Kerr et al. 2002).

The Ecological Role of Colicins

Despite the pervasive role of toxin production in the microbial world, little is known about the ecology of this form of competition. Previous theoretical and empirical studies have suggested that toxin production serves as a strategy to obtain access to nutrients (Chao and Levin 1981; Ivanovska and Hardwick 2005; Riley and Gordon 1992). However, a more recent study testing competitive interactions between toxin producers and sensitive yeast strains under low and high nutrient conditions concluded that toxin producers only out-compete sensitive cells in high

nutrient environments (Wloch-Salamon et al. 2008). This observation supports the theoretical prediction that toxin production has evolved to occur as a competitive strategy under conditions of abundant resources (Frank 1994). Both studies suggest that toxin production may be more important in the invasion of niches, than in obtaining nutrients (Brown and Taddei 2009).

One of the more compelling models of toxin-mediated bacterial competition employs *E. coli* and their colicins. Some of the earliest ecological studies were inconclusive and sometimes even contradictory (Ikari et al. 1969). More recently, however, a more robust theoretical and empirical base has been established defining the conditions that favor maintenance of toxin-producing bacteria in both population and community settings. Chao and Levin (1981) showed that the conditions for invasion of a colicin-producing strain are much broader in a spatially structured environment (i.e., one that restricts the movement of strains and nutrients), than in an unstructured one (i.e., where mass action prevails). In an unstructured environment with mass action, a small population of producers cannot invade an established population of sensitive cells (Durrett and Levin 1997). This failure occurs because producers pay a price for toxin production (energetic costs of plasmid carriage and lethality of production), while the benefits (the resources made available by killing sensitive organisms) are distributed at random. In a physically structured environment, such as on the surface of an agar plate, the strains grow as separate colonies and toxin diffuses out from a colony of producers, killing sensitive neighbors (Kerr et al. 2002). The access to resources that would have otherwise been consumed by the sensitive cells, as well as the nutrients from the killed individuals, is made available to the producing colony owing to its proximity. Therefore, killers can increase in frequency even when initially rare.

Several modeling efforts have incorporated additional biological reality. Two such efforts introduced a third species, one that is resistant to the toxin but cannot itself produce the toxin (Nakamaru and Iwasa 2000). Resistance can be conferred through mutations in either the binding site or the translocation machinery required for a bacteriocin to enter the target cell. Acquisition of an immunity gene will also confer resistance to its cognate bacteriocin. It is assumed that there is a cost to resistance and that this cost is less than the cost of toxin production borne by the killer strain (Riley and Wertz 2002). Owing to this third member, pair-wise interactions among the strains have the nontransitive structure of the childhood game of rock–scissors–paper (Karolyi et al. 2005; Kerr et al. 2002). The producer strain beats the sensitive strain, owing to the toxin's effects on the latter. The sensitive strain beats the resistant strain because only the latter suffers the cost of resistance. And the resistant strain wins against the producer because the latter bears the higher cost of toxin production and release while the former pays only the cost of resistance.

Kerr et al. (2002) investigated this colicin-mediated nontransitive interaction. Their study revealed that in environments where interactions and dispersal are not solely local, the resistant strain overtook the community during the course of the experiment. In contrast, in a structured environment, this game permits a quasi-stable global equilibrium, one in which all three strains can persist with nearly constant global abundance (Laird and Schamp 2008; Neumann and Schuster 2007).

A third environment, mixed plate, which is intermediate between structured and unstructured, revealed that growth on a surface is not the key factor, as resistance overtook the other strains on this plate also. Additional complexity was incorporated by the addition of a second producing strain. In one such study, two producing strains were grown in competition in a static environment, on plates. In this study, each colicin was able to induce its counterpart's production. The static plate experiment showed that in a structured environment that allow only local interactions coexistence results in absolute spatial isolation of the two strains. These results support the prediction that balanced chasing in a spatially structured, nonhierarchical community may result in the maintenance of diversity.

Surveys of colicin production in natural populations suggest that natural populations of *E. coli* partially match the predictions of these ecological models: *E. coli* producer strains are found at frequencies ranging from 10 to 50% (Riley and Gordon 1992; Gordon and Riley 1999; Gordon and O'Brien 2006; Barnes et al. 2007). Resistant strains are even more abundant, being found at frequencies of 50–90%. In fact, most strains are resistant to all co-segregating colicins. Finally, there is a small population of sensitive cells. The simultaneous presence of both colicin-producing and -resistant strains raises an interesting paradox. How can we explain the persistence of colicin-producing strains when their putative targets are relatively rare? The models predict this distribution of phenotypes results from frequent horizontal transfer of resistance and the significant cost associated with colicin production (Barnes et al. 2007). In other words, if a strain can gain resistance and lose production, they will over time – just as was observed in *E. coli* isolated from field mouse population over a period of 3 months (Gordon et al. 1998).

Some of the most recent investigations into colicin ecology and evolution have examined how the presence of a colicin impacts levels and types of gene expression. The first such study (Vreizen et al. 2009) followed the population dynamics of a colicin-producing strain of *E. coli* exposed to serial transfer conditions, with no competitors, over 253 generations. To this end, an in vitro serial transfer experiment was conducted in which a colicinogenic strain and its nonproducing ancestor were evolved for 253 generations. As was previously observed in a similar experiment carried out in vitro, the strain evolved a reduction in killing activity and a corresponding increase in fitness, relative to the ancestral strain. This result is not surprising, given that colicin production is quite costly due to (1) the cost of replication and maintenance of the colicin plasmid (Feldgarden et al., 1995), (2) the cost of colicin production, and (3) the occurrence of autolysis of the producing cells (Braun et al. 1994). When colicins are produced in an environment with no competitors, as is the case in this serial transfer experiment, it is reasonable to expect that the costs of colicin production will outweigh its benefits. We speculate that the lack of competitive interactions results in strong selection for a decrease or elimination of colicin production. In the in vitro study described above, molecular characterization of the evolved strains revealed no changes in the DNA sequence of the evolved colicin plasmids. However, a set of chromosomally encoded loci experienced changes in gene expression that were positively correlated with the reduction in killing. Further studies are required to more accurately identify the precise molecular changes that result in the observed reduction in killing.

Perhaps these future studies will also reveal why selection results in changes in gene expression, rather than simple elimination of the plasmid itself.

Experimental and theoretical work on the ecology of bacteriocin-mediated allelopathy highlights the importance of cell–cell interactions and spatial structure in mediating the outcome of competition. One recent study explored this hypothesis using two *E. coli* colicin producers (colicins E2 and E9), which are sometimes found together in the same host (Gordon et al. 1998). This study demonstrated that each colicin has the ability to induce its counterpart's production (Fig. 2.2). Over 50 years ago, it was found that colicin induction could be enhanced by the addition of mutagenic agents, such as mitomycin C and UV light (Herschman and Helinski 1967). Induction by DNA damaging agents was later linked to the SOS motifs, conserved in all the promoter regions of colicins (Gillor et al. 2008). Interestingly, transcriptional response of an *E. coli* strain to damage induced by a DNA degrading colicin showed strong induction of the LexA-regulated SOS system (Walker et al. 2004). These data suggest that the colicin mutual induction presented in this study results from the DNase toxins induction of the SOS response that in turns control colicin production.

The next step in these studies involved the introduction of two colicin-producing strains in a structured environment that allow only local interactions, such coexistence results in absolute spatial isolation of the two strains. These results support the prediction that balanced chasing in a spatially structured, nonhierarchical community may result in the maintenance of diversity. This was tested in vivo using a murine model by allowed pairs of co-caged mice, each carrying a single enteric

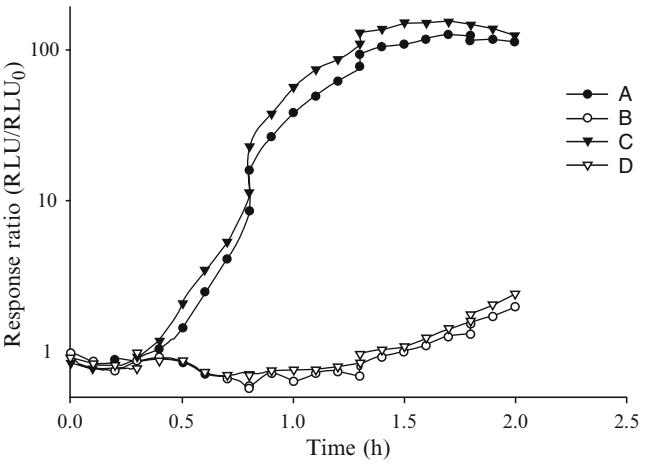

Fig. 2.2 Mutual colicin induction. The proteins of isogenic strains carrying colicin E2 or E7 plasmids and a colicin-free control strain were crudely extracted and used to induce reporter strains carrying *ce2a* and *ce7a* promoters (colicin E2 and E7 promoters) fused to *Photorhabdus luminescence luxCDABE* reporter operon. Colicin E2 crude protein extract was used to induce pDEW-E7 reporter vector (A; *filled circle*), while colicin E7 extract was used to induce pDEW-E2 reporter vector (B; *filled triangle*); the colicin-free strain was tested with the pDEW-E7 (C; *open circle*), and pDEW-E2 (D; *open triangle*) reporter vectors

strain producing one of the tested colicins, to interact freely and monitored their colon enteric residents for almost 4 months. Unlike previous reports in which colicin producers, nonproducers, and resistant strains competitively replaced one another (Kirkup and Riley 2004); in the current setting, no such invasion was observed. Throughout the experiment, the colicin-producing strains persisted, each in its host's colon, excluding the invasion of the competing producer population. Finally, in an attempt to generalize these observations, simulations were undertaken with competing bacteriocin-producing populations assuming mutual induction. The mathematical model further supported the empirical results, demonstrating that spatially distributed, cross-inducing producer populations can mutually exclude one another and thus maintain a steady-state coexistence (Fig. 2.3).

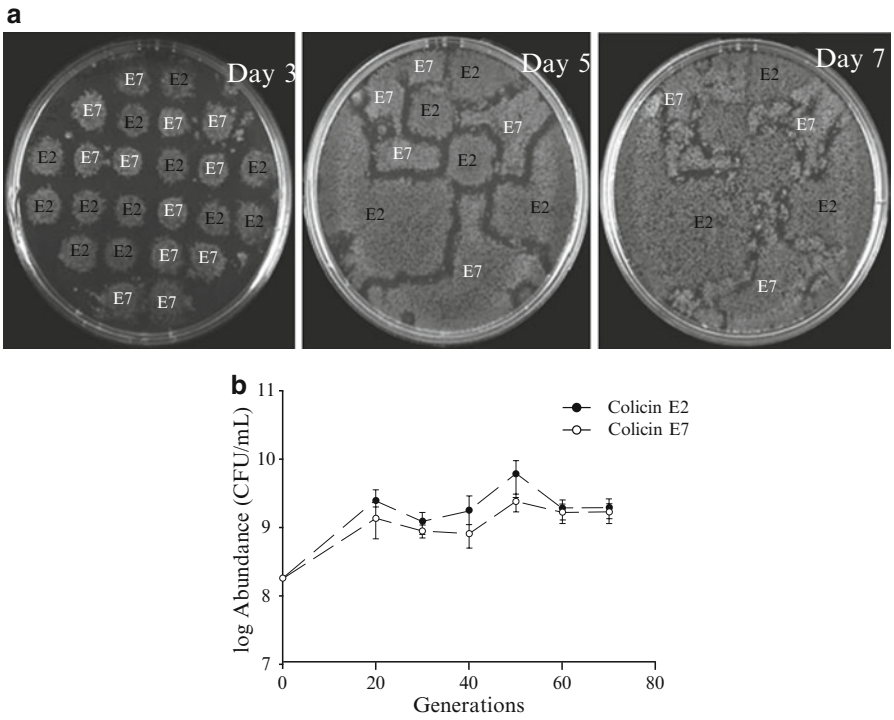

Fig. 2.3 Community dynamics in a structured environment. We initiated a static plate environment by randomly depositing 24 droplets from pure culture of *E. coli* strain BZB1011 carrying a plasmid encoding either colicin E2 or E7. The changing spatial pattern of the community is photographed over time (**a**) showing the spread of the strains droplets (day 3) to lawns bordered by clearing zone (day 5) that was later colonized by strains resistant to both colicins (day 7). Upon analysis of the cells concentration (**b**), the abundance of the *E. coli* harboring colicin E2 (*filled circles*) and E7 (*open circles*) encoding plasmids was shown to remain invariable throughout the experiment. Data points are the mean of two impendent experiments each performed in duplicate, the *bars* represent the standard deviation of the average cell concentration. The data points depicted on the *X*-axis are separated by 24 h and approximately ten bacteria generations

Such studies suggest that cross-induction in structured environments controls the invasion of susceptible bacteriocin producers as the established community acts to increase the local concentration of the toxin to a lethal dose in order to prevent invasion. The outcome of such an interaction pattern on the relative cost and benefit of investment in allelopathy has strong implications on an evolutionary scale. It has been shown that when toxin producers are locally scarce, they are unable to generate sufficient toxins to compensate for the cost of production (Chao and Levin 1981; Gardner et al. 2004). In contrast, if toxin producers are induced by their competitors/invaders, then bacteriocin production confers a fitness advantage, as the gain from a given investment in killing is maximized, being directed against an evident adversary. This will favor the regulation of bacteriocin that is readily susceptible to induction by a competing toxin producer, which should, in turn, become widespread, and thereby maintain and even enhance bacteriocin diversity.

The Colicin Mouse Model

A variety of mouse models have been employed to provide a more realistic evaluation of the role of bacteriocins in mediating bacterial strain dynamics. Although none of these models are able to control precisely the bacterial communities in mice or measure the impact of host responses, they do provide a clear indication of population dynamics of strains that have differing abilities to produce or resist colicins. One of the very first such studies demonstrated that bacteriocin production improves the establishment success of the producing strains in the mouse colon (McCormick et al. 1989). In this case, a pair of *E. coli* strains, isogenic save for the production of microcin V, were introduced into the large intestine of streptomycin-treated mice. When the two strains were fed together, the microcin-deficient strain was eliminated from the large intestine.

Additional realism was incorporated into such studies with the introduction of the same three strains employed by Kerr et al. (2002) (producer, sensitive, and resistant), which were monitored after introduction into a mouse colon (Kirkup and Riley 2004). These experiments revealed exactly the same nontransitive interactions described in the in vitro studies of Kerr et al. (2002). When a mouse harbored a sensitive strain, an introduced colicin-producing strain was able to invade. When a colicin-producing strain was resident, an introduced R strain was able to invade. In both experimental systems, the nontransitive nature of colicin-mediated dynamics was further revealed (Kirkup and Riley 2004).

Further mouse-based studies of colicin ecology focused solely on the colicin-producing strains (Majeed et al., 2011). One such study monitored the evolution of killing phenotype of a colicin-producing strain maintained in a mouse colon. Prior to introduction of the colicin-producing strains in the mice, the native Gram-negative bacterial community was eliminated with antibiotic application. Thus, there was a little or no competition with close relatives in this experimental design. The producing strains evolved significantly reduced killing activity over the 4 months of sampling.

More recent studies employed the same mouse model and incorporated additional colicin diversity into the experimental design (Gillor et al. 2009). Mice were inoculated with a single enteric strain producing one colicin. The mice were then paired in cages and allowed to interact freely, while their colon-based enteric residents were monitored for almost 4 months. Unlike previous reports in which colicin producers, nonproducers, and resistant strains competitively replaced one another (Kirkup and Riley 2004), in the current setting, no such invasion was observed (Fig. 2.4). Throughout the experiment, the colicin-producing strains persisted, each in its host's colon, excluding the invasion of the competing producer population.

These results may be subject to spatial dispersal; it has been previously suggested that limited dispersal favors toxin producers, while high dispersal, although still beneficial to toxin producers, is nevertheless insufficient to compensate for the cost of carrying toxin-encoding agents (Cascales et al. 2007; Wloch-Salamon et al. 2008; Inglis et al. 2009). Enterobactereaceae adhere to the colon epithelial cells, forming a stable biofilm (Everett et al. 2004), in other words, a structured environment in which cell–cell interactions are localized. Thus, in an unstructured environment at high dispersal, the interactions between bacteriocin producers may increase, and due to mutual induction, lead to elevated toxin production and release. As colicin production is costly and release is lethal to the producing cell, the competitive advantage of the producer's population will consequently be reduced. However, in a structured environment at low dispersal, competitive interaction will be localized and only a small percentage of the producer's population will be induced to fend off invading cells.

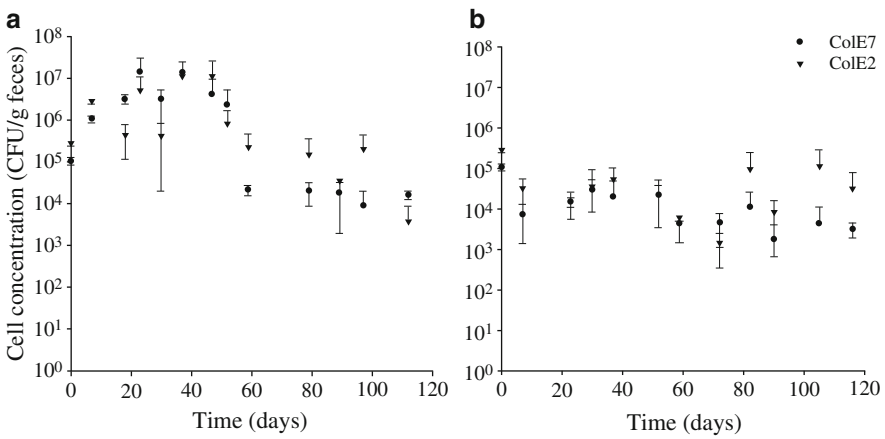

Fig. 2.4 Effect of competition on bacterial population size in mice. Bacterial density (CFU per gram fecal matter) monitored over time in mice in control (**a**) or experimental (**b**) cages. The control cages hosted mice harboring either *E. coli* strain BZB1011 bearing pDEW-E2 or mice harboring *E. coli* strain BZB1011 bearing pDEW-E7. The experimental cages contained one mouse established with the *E. coli* strain BZB1011 bearing pDEW-E2 and one mouse with *E. coli* strain BZB1011 bearing pDEW-E7. Each point represents the mean CFU/g feces averaged for strains bearing pDEW-E2 (*filled triangle*) or pDEW-E7 (*filled circle*) recovered from the mice. *Bars* represent the standard error for each point

In this final in vivo study, the competitive interactions between two populations of colicin producers in the mouse colon were resolved in mutual exclusion. This result suggests that the established biofilm of each of the producing cells could successfully prevent the invasion of cells producing a different colicin, both competing in a structured environment at low dispersal. It is assumed that the interaction between the populations was localized such that only a small part of the established population was induced by the invaders, just enough to prevent their advance. The mathematical model demonstrating mutual exclusion to be a robust result when bacteriocin producers interact locally, cross-inducing one another, further supported this observation.

These experimental and theoretical studies of the ecology of bacteriocin-mediated allelopathy highlight the importance of cell–cell interactions and spatial structure in mediating the outcome of competition. The data suggest that cross-induction in structured environments controls the invasion of susceptible bacteriocin producers as the established community acts to increase the local concentration of the toxin to a lethal dose in order to prevent invasion. The outcome of such an interaction pattern on the relative cost and benefit of investment in allelopathy has strong implications on an evolutionary scale. It has been shown that when toxin producers are locally scarce, they are unable to generate sufficient toxins to compensate for the cost of production (Chao and Levin 1981; Gardner et al. 2004). In contrast, if toxin producers are induced by their competitors/invaders, then bacteriocin production confers a fitness advantage, as the gain from a given investment in killing is maximized, being directed against an evident adversary. This will favor the regulation of bacteriocin that is readily susceptible to induction by a competing toxin producer, which should, in turn, become widespread, and thereby maintain and even enhance bacteriocin diversity.

Conclusions

There has been a virtual explosion in studies of the probiotic and antibiotic use of bacteriocins (Breukink and de Kruijff 1999; Audisio et al. 2005; Hillman et al. 2000; Aroutcheva et al. 2001; Avonts and De Vuyst 2001; Brashears et al. 2003; Gillor et al. 2004; Joerger 2003; Aslim and Kilic 2006; Corr et al. 2007; Diez-Gonzalez 2007; Falagas et al. 2007). As the potential health benefits of bacteriocins are being realized, it is critical to maintain a strong link between the ecological and applied studies. We learned far too late the cost (in terms of human morbidity and mortality) of our overuse and misuse of classical antibiotics. This failure to understand how we were creating ideal conditions for the rapid evolution and spread of antibiotic resistance has, unfortunately, resulted in one of the most significant human health challenges, the rise of human pathogens able to resist many of our most powerful antibiotic drugs. We are in a far better position to employ our growing understanding of bacteriocin ecology to develop sound and resilient methods with which to apply the power of bacteriocins for humans needs. As this review clearly

illustrates, the studies of bacteriocin ecology and evolution have kept pace with, and in some cases advanced far beyond, our more limited forays into their medical applications. Clearly, we have far to go in developing a generalized model of bacteriocin ecology. With each new finding, we learn how complex are the roles these toxins serve in bacterial populations and communities. Even more challenging, it is almost certainly the case that such roles will differ for different species and even for the same species experiencing different environmental pressures.

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