Annu. Rev. Microbiol. 2002. 56:117–37
doi: 10.1146/annurev.micro.56.012302.161024

First published online as a Review in Advance on April 8, 2002

BACTERIOCINS: Evolution, Ecology, and Application

Margaret A. Riley and John E. Wertz
Department of Ecology and Evolutionary Biology, Yale University, New Haven, Connecticut 06511; e-mail: margaret.riley@yale.edu; john.wertz@yale.edu

Key Words colicin, resistance, diversity, nisin

■ **Abstract** Microbes produce an extraordinary array of microbial defense systems. These include classical antibiotics, metabolic by-products, lytic agents, numerous types of protein exotoxins, and bacteriocins. The abundance and diversity of this potent arsenal of weapons are clear. Less clear are their evolutionary origins and the role they play in mediating microbial interactions. The goal of this review is to explore what we know about the evolution and ecology of the most abundant and diverse family of microbial defense systems: the bacteriocins. We summarize current knowledge of how such extraordinary protein diversity arose and is maintained in microbial populations and what role these toxins play in mediating microbial population-level and community-level dynamics. In the latter half of this review we focus on the potential role bacteriocins may play in addressing human health concerns and the current role they serve in food preservation.

CONTENTS

INTRODUCTION

What Are Microbial Defense Systems?

Microbes produce an extraordinary array of microbial defense systems. These include broad-spectrum classical antibiotics so critical to human health concerns, metabolic by-products such as the lactic acids produced by lactobacilli, lytic agents such as lysozymes found in many foods, numerous types of protein exotoxins, and bacteriocins, which are loosely defined as biologically active protein moieties with a bacteriocidal mode of action (41, 90). This biological arsenal is striking not only in its diversity but in its natural abundance. For instance lactic acid production is a defining trait of lactic acid bacteria (36). Bacteriocins are found in almost every bacterial species examined to date, and within a species tens or even hundreds of different kinds of bacteriocins are produced (41, 72). Halobacteria universally produce their own version of bacteriocins, the halocins (95). Streptomycetes commonly produce broad-spectrum antibiotics (79). It is clear that microbes invest considerable energy into the production and elaboration of antimicrobial mechanisms. Less clear is how such diversity arose and what roles these biological weapons serve in microbial communities.

One family of microbial defense systems, the bacteriocins, has served as a model for exploring evolutionary and ecological questions. In this review, current knowledge of how the extraordinary range of bacteriocin diversity arose and is maintained in microbial populations is assessed, and the role these toxins play in mediating microbial dynamics is discussed. Fascination with bacteriocins is not restricted to the evolutionary and ecologically minded; in the latter half of this review our attention focuses on the potential application of these toxins to address human health concerns and the current and growing use of bacteriocins to aid in food preservation.

BACTERIOCINS: THE MICROBIAL WEAPON OF CHOICE

Bacteriocins differ from traditional antibiotics in one critical way: They have a relatively narrow killing spectrum and are only toxic to bacteria closely related to the producing strain. These toxins have been found in all major lineages of Bacteria and, more recently, have been described as universally produced by some members of the Archaea (95). According to Klaenhammer, 99% of all bacteria may make at least one bacteriocin and the only reason we haven't isolated more is that few researchers have looked for them (47, 67).

Bacteriocins of Gram-Negative Bacteria

The bacteriocin family includes a diversity of proteins in terms of size, microbial targets, modes of action, and immunity mechanisms. The most extensively studied,

the colicins produced by *Escherichia coli*, share certain key characteristics (3, 6, 13, 30, 40, 48, 64). Colicin gene clusters are encoded on plasmids and are composed of a colicin gene, which encodes the toxin; an immunity gene, which encodes a protein conferring specific immunity to the producer cell by binding to and inactivating the toxin protein; and a lysis gene, which encodes a protein involved in colicin release through lysis of the producer cell. Colicin production is mediated by the SOS regulon and is therefore principally produced under times of stress. Toxin production is lethal for the producing cell and any neighboring cells recognized by that colicin. A receptor domain in the colicin protein that binds a specific cell surface receptor determines target recognition. This mode of targeting results in the relatively narrow phylogenetic killing range often cited for bacteriocins. The killing functions range from pore formation in the cell membrane to nuclease activity against DNA, rRNA, and tRNA targets. Colicins, indeed all bacteriocins produced by gram-negative bacteria, are large proteins. Pore-forming colicins range in size from 449 to 629 amino acids. Nuclease bacteriocins have an even broader size range, from 178 to 777 amino acids.

Although colicins are representative of gram-negative bacteriocins, there are intriguing differences found within this subgroup of the bacteriocin family. *E. coli* encodes its colicins exclusively on plasmid replicons (65). The nuclease pyocins of *Pseudomonas aeruginosa*, which show sequence similarity to colicins and other, as yet uncharacterized, bacteriocins are found exclusively on the chromosome (81). Another close relative to the colicin family, the bacteriocins of *Serratia marcesens*, are found on both plasmids and chromosomes (20, 26, 32).

Many bacteriocins isolated from gram-negative bacteria appear to have been created by recombination between existing bacteriocins (6, 51, 68, 76). Such frequent recombination is facilitated by the domain structure of bacteriocin proteins. In colicins, the central domain comprises about 50% of the protein and is involved in the recognition of specific cell surface receptors. The N-terminal domain ($\sim$25% of the protein) is responsible for translocation of the protein into the target cell. The remainder of the protein houses the killing domain and the immunity region, which is a short sequence involved in immunity protein binding. Although the pyocins produced by *P. aeruginosa* share a similar domain structure, the order of the translocation and receptor recognition domains are switched (80). As we explore further below, the conserved domain configuration of these toxins is responsible for much of the bacteriocin diversity we find in nature.

Bacteriocins of Gram-Positive Bacteria

Bacteriocins of gram-positive bacteria are as abundant and even more diverse as those found in gram-negative bacteria (39, 90). They differ from gram-negative bacteriocins in two fundamental ways. First, bacteriocin production is not necessarily the lethal event it is for gram-negative bacteria. This critical difference is due to the transport mechanisms gram-positive bacteria encode to release bacteriocin toxin. Some have evolved a bacteriocin-specific transport system, whereas others employ the *sec*-dependent export pathway. In addition, the gram-positive bacteria

have evolved bacteriocin-specific regulation, whereas bacteriocins of gram-negative bacteria rely solely on host regulatory networks.

The lactic acid bacteria (LAB) are particularly prolific in bacteriocin production. Klaenhammer distinguishes three classes of LAB bacteriocins (47). Class I bacteriocins are the lantibiotics, so named because they are post-translationally modified to contain amino acids such as lanthionine and B-methyllanthionine, and several dehydrated amino acids (33). Lantibiotics are further divided into two subgroups, A and B, based on structural features and their mode of killing (43). Type A lantibiotics kill the target cell by depolarizing the cytoplasmic membrane (2, 84). They are larger than type B lantibiotics and range in size from 21 to 38 amino acids. Nisin, the archetypal and best-studied gram-positive bacteriocin, is a type A lantibiotic (31). The type B lantibiotics have a more globular secondary structure and are smaller than type A, with none exceeding 19 amino acids in length. Type B lantibiotics function through enzyme inhibition. One example is mersacidin, which interferes with cell wall biosynthesis (7).

Class II LAB bacteriocins are also small, ranging in size from 30 to 60 amino acids, and are heat-stable, nonlanthionine-containing peptides (43). They are organized into subgroups: Class IIa is the largest group and its members are distinguished by a conserved amino-terminal sequence (YGNGVXaaC) and a shared activity against *Listeria*. Like type A lantibiotics, class IIa bacteriocins act through the formation of pores in the cytoplasmic membrane. Examples include pediocin AcH (4), sakacin A (83), and leucocin A (35). Class IIb bacteriocins such as lacticin F (58) and lactococcin G (60) form pores composed of two different proteins in the membrane of their target cells. A third subgroup (IIc) has been proposed, which consists of bacteriocins that are *sec*-dependent (such as acidocin B) (52). Class III bacteriocins are large heat-labile proteins such as helveticins J and V (42, 98) and lactacin B (1). An additional proposed class (VI) requires lipid or carbohydrate moieties for activity. Little is known about the structure and function of this proposed class. Examples include leuconocin S (8) and lactocin 27 (96).

Gram-positive bacteriocins in general and lantibiotics in particular require many more genes for their production than do gram-negative bacteriocins. The nisin gene cluster includes genes for the prepeptide (*nisA*), enzymes for modifying amino acids (*nisB*, *nisC*), cleavage of the leader peptide (*nisP*), secretion (*nisT*), immunity (*nisI*, *nisFEG*), and regulation of expression (*nisR*, *nisK*) (9, 21, 22, 44, 50, 66, 97). These gene clusters are most often encoded on plasmids but are occasionally found on the chromosome. Several gram-positive bacteriocins, including nisin, are located on transposons (18).

The conventional wisdom about the killing range of gram-positive bacteriocins is that they are restricted to killing other gram-positive bacteria. The range of killing can vary significantly, from relatively narrow as in the case of lactococcins A, B, and M, which have been found to kill only *Lactococcus* (77), to extraordinarily broad. For instance, some type A lantibiotics such as nisin A and mutacin B-Ny266 have been shown to kill a wide range of organisms including *Actinomyces*, *Bacillus*, *Clostridium*, *Corynebacterium*, *Enterococcus*, *Gardnerella*, *Lactococcus*, *Listeria*, *Micrococcus*, *Mycobacterium*, *Propionibacterium*, *Streptococcus*, and

Staphylococcus (57). Contrary to conventional wisdom, these particular bacteriocins are also active against a number of medically important gram-negative bacteria including *Campylobacter*, *Haemophilus*, *Helicobacter*, and *Neisseria* (57).

Production of bacteriocins in gram-positive bacteria is generally associated with the shift from log phase to stationary phase. Nisin production begins during mid-log phase and increases to a maximum as the cells enter stationary phase (9). The regulation of expression is not cell cycle–dependent per se, but rather culture density–dependent. It has been demonstrated that nisin A acts as a protein pheromone in regulating its own expression, which is controlled by a two-component signal transduction system typical of many quorum-sensing systems. The genes involved are *nisR* (the response regulator) and *nisK* (the sensor kinase) (16). Nisin transcription can be induced by the addition of nisin to the culture medium with the level of induction directly related to the level of nisin added (49, 50).

Bacteriocins of Archaea

The Archaea produce their own distinct family of bacteriocin-like antimicrobials, known as archaeocins. The only characterized member is the halocin family produced by halobacteria, and few halocins have been described in detail (11, 75, 85). The first halocin discovered, S8, is a short hydrophobic peptide of 36 amino acids, which is processed from a much larger pro-protein of 34 kD (63). Halocin S8 is encoded on a megaplasmid and is extremely hardy; it can be desalted, boiled, subjected to organic solvents, and stored at 4°C for extended periods without losing activity. Expression is growth stage–dependent. Although basal levels are present in low concentrations during exponential growth, there is an explosive ninefold increase in production during the transition to stationary phase (85). The mechanism of halocin action has been established only for halocin H6 (a Na+/H+ antiporter inhibitor), and the immunity mechanism is unknown (94).

Archaeocins are produced as the cells enter stationary phase. When resources are limited, producing cells lyse sensitive cells and enrich the nutrient content of the local environment. As stable proteins, they may remain in the environment long enough to reduce competition during subsequent phases of nutrient flux. The stability of halocins may help explain why there is so little species diversity in the hypersaline environments frequented by halobacteria (85).

As is clear from this brief survey of bacteriocin diversity and distribution, this heterogeneous family of toxins is united only by the shared features of being protein-based toxins that are relatively narrow in killing spectrum and often extremely hardy and stable. What makes these the weapons of choice in the microbial world remains an intriguing question.

EVOLUTION OF BACTERIOCIN DIVERSITY

Colicins as a Model for Evolutionary Studies

The colicins and other enteric bacteriocins, such as klebicins, remain the only bacteriocins for which detailed evolutionary investigations have been undertaken.

Among the colicins, there are two main evolutionary lineages, which also distinguish the two primary modes of killing: pore formation and nuclease activity (70). Studies that include DNA and protein sequence comparisons (6, 68), surveys of DNA sequence polymorphism in natural isolates (62, 74, 92), experimental evolution (28, 91), and mathematical modeling (28) have revealed two primary modes of colicin evolution (93).

The Role of Diversifying Recombination in Colicin Evolution

The more abundant pore-former colicins are generated by domain shuffling, which is mediated by recombination (6, 93). All characterized pore-former colicin proteins share one or more regions with high levels of sequence similarity to other pore-former colicins (Figure 1). This patchwork of shared and divergent sequences suggests frequent recombination. The location of the different patches frequently corresponds to the different functional domains of the proteins. The most recent illustration of the power of diversifying recombination is seen in the first published klebicin sequence (Figure 2), which is a nuclease klebicin that shares sequence similarity with both colicin A–like pore former and pyocin S1–like nuclease sequences (73). Such domain-based shuffling between bacteriocins is responsible for much of the variability observed among gram-negative bacteriocins.

The influence of diversifying recombination is not limited to the closely related bacteriocins of enteric bacteria. As mentioned above, the S pyocins of *P. aeruginosa* are the result of recombination between several pore-former and nuclease colicins with other, as yet uncharacterized, bacteriocins (81, 82). Even altering the domain structure of the protein, as seen for pyocins that have switched the receptor

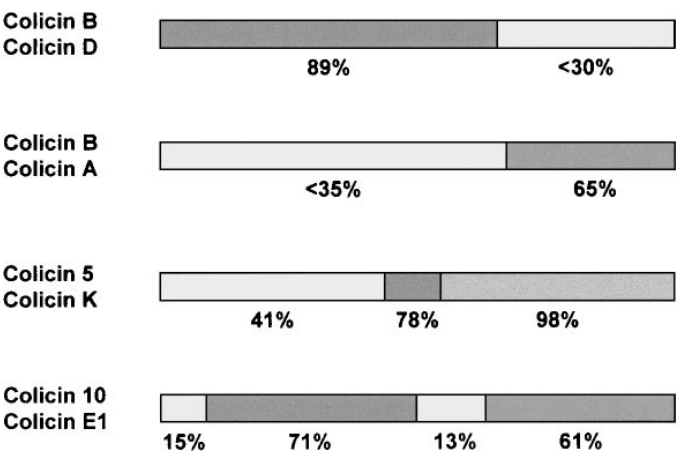

Figure 1 Pairwise comparisons of pore-forming colicin protein sequences. Values below each comparison indicate the percent sequence identity for the region indicated. Colicin proteins are not drawn to scale.

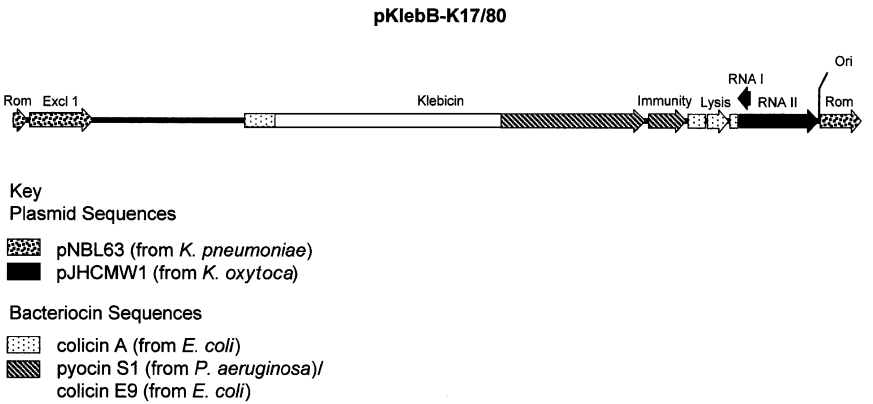

Figure 2 Patterns of sequence similarity in klebicins suggest recombination. The chimeric nature of the pKlebB plasmid sequence is indicated by alternate shadings. The key notes regions of sequence similarity with other bacteriocin gene clusters and plasmids. pKlebB illustrates a pattern typical of other bacteriocin-encoding plasmids where sequences encoding plasmid functions are similar to sequences found in other plasmids segregating in the host species, whereas those sequences composing and flanking the bacteriocin gene cluster show similarity to bacteriocin sequences from other species.

recognition and translocation domains relative to the order found in colicins, has not limited the influence of diversifying recombination.

The Role of Diversifying Selection in Colicin Evolution

An alternative mode of evolution is responsible for the current diversity of nuclease colicins. These colicins, which include both RNase-and DNase-killing functions, share a recent common ancestry. Their DNA sequences are quite similar, ranging from 50% to 97% sequence identity. However, many pairs of nuclease colicins have elevated levels of divergence in the immunity region (Figure 3). To explain this pattern of divergence, Riley and collaborators have proposed a two-step process of mutation and selection (68, 69, 93).

The diversifying selection hypothesis posits the action of strong positive selection acting on mutations that generate novel immunity and killing functions (Figure 4). The first event in this process is the occurrence of a mutation in the immunity gene resulting in a broadened immunity function. The resulting producer cell is now immune to the ancestral version of the colicin as well as having gained immunity to some number of similar colicins. This broadened immunity function increases the fitness of the producer strain in populations where multiple colicins are found, which is the case in all *E. coli* populations sampled to date (29, 72). A second mutation, this time in the colicin gene, is paired with the immunity mutation.

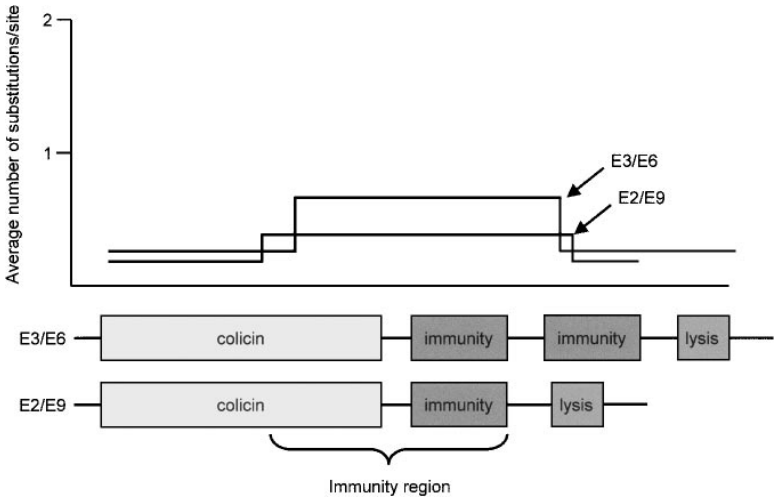

Figure 3 The graph indicates the average number of total nucleotide substitutions between pairs of nuclease-type colicin gene clusters (colicin pairs E2/E9 and E3/E6). Most of the divergence between colicins occurs in the immunity region of the gene cluster (composed of the immunity gene and the immunity-binding region of the colicin gene).

This pair of mutations produces a novel colicin that is no longer recognized by the ancestral immunity protein. Thus, the possessor of the novel colicin will rapidly displace (by killing) the ancestral, formerly abundant bacteriocin-producing strain. This evolved colicin will ultimately be replaced by yet another novel colicin as the cycle repeats itself. This process results in a family of closely related proteins that have diverged most extensively in the region involved in immunity binding and killing function, as seen for nuclease colicins (69).

Recently, the DNA sequence of a new pore-former colicin, Y, was determined (71). Colicin Y is a close relative of colicin U, another pore-former colicin isolated from a different continent over 20 years earlier (86). This pair of colicins has a pattern of DNA substitution identical to that observed among the nuclease colicins with an elevated level of substitution in the immunity region. This observation suggests that the process of diversifying selection is not restricted to nuclease colicins. Further, several E2 colicins isolated from Australia suggest that diversifying recombination is not restricted to pore-former colicins (92). Half of the E2 producers carry the characterized E2 plasmid. The other half carry a recombinant plasmid with sequences derived from colicin E7 and the characterized E2 plasmid. These isolated observations suggest that it is not the case that pore formers diversify only by means of recombination and nuclease colicins by diversifying selection. The evolutionary process is more complex than the proposed simple dichotomy suggests.

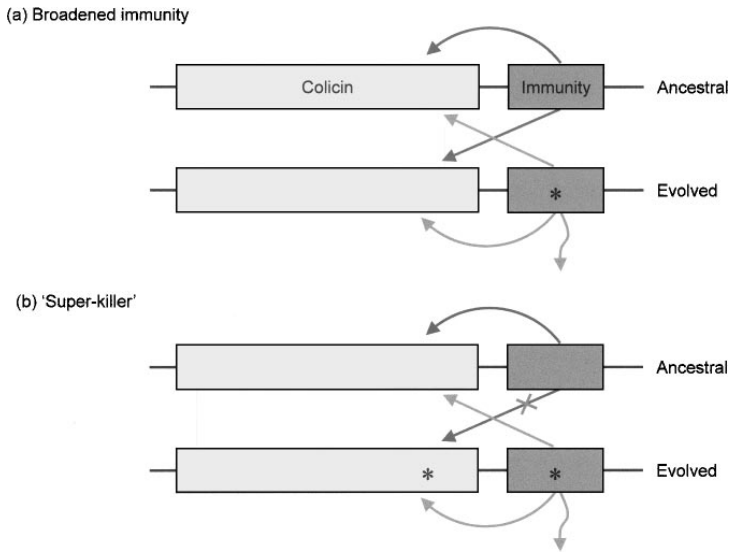

Figure 4 The hypothesis of diversifying selection invokes two steps in the generation of a novel immunity function. (*a*) A point mutation in the immunity gene generates a broadened immunity function (noted with an *asterisk*). The strain with this colicin gene cluster is immune to itself, to its ancestor, and to other closely related colicins (noted with *gray arrows*). The ancestral colicin is immune to itself and to the evolved colicin (noted with *black arrows*). (*b*) A paired mutation occurs in the immunity-binding portion of the evolved colicin gene that generates a "super-killer" (noted with a second *asterisk*). The evolved strain is still immune to itself, its ancestor, and other colicins. However, the ancestral strain is now no longer immune to the evolved strain (noted with an *X*).

A Two-Step Process of Colicin Evolution

Riley has developed a model of colicin diversification that involves two phases (70). When rare, as is currently the case for most nuclease colicins, the occurrence of point mutations that alter immunity function may be the primary mode for generating novel bacteriocin phenotypes. Novel immunity and killing functions are rapidly selected since they allow a cell to avoid being killed by other bacteriocins or allow cells carrying them to displace their ancestors. These novel bacteriocins are then maintained until a new immunity or killing function emerges. When colicins are abundant, as is the case for many pore-former colicins, domain swapping may become a more frequent mode of diversification. This "switch" in evolutionary mechanism is due simply to the requirement for a set of bacteriocins to be abundant enough to serve as templates for recombination. Once abundant, recombination can more rapidly generate additional diversity.

We have only just begun to tap into the diversity of enteric bacteriocins. However, recent work suggests that similar evolutionary mechanisms may play a role in the diversification of other enteric bacteriocins. Sequence comparisons reveal that in several cases, enteric bacteriocins are chimeras of known gram-negative bacteriocins (73; M.A. Riley, C.M. Goldstone & J.E. Wertz, unpublished information.) For other enteric bacteriocins, the action of diversifying selection has been proposed (M.A. Riley, C.M. Goldstone & J.E. Wertz, unpublished information). Finally, some new enteric bacteriocins have no similarity with those characterized previously. A particularly interesting example of this latter observation is the recently described Colicin Js (87). This plasmid-borne bacteriocin has a typical colicin gene cluster composition, with toxin, immunity, and lysis genes. However, the organization of the gene cluster is unique in that the lysis gene is transcribed 5′ to the toxin gene. The genes themselves show no similarity to any known bacteriocin genes, and the encoded toxin is 94 amino acids, which is smaller than any other described colicin.

Bacteriocin-encoding plasmids, such as pColJs (which encodes colicin Js) and pKlebB (which encodes klebicin B), demonstrate another aspect of bacteriocin evolution (73, 87). These bacteriocin plasmids are chimeras with a plasmid "backbone" comprising replication and maintenance sequences typical of plasmids found in the bacteriocins' host species. In the case of pKlebB isolated from *Klebsiella pneumoniae*, the plasmid contains sequences similar to pNBL63 (102) and pJHCMW1 (17), isolated from *K. oxytoca* and *K. pneumoniae* respectively, encoding plasmid maintenance functions. The sequence surrounding and comprising part of the klebicin B gene cluster shares similarity with colicin A and E9, originally isolated from *E. coli* (73). In the case of pColJs, the plasmid backbone is virtually identical to ColE1, whereas the DNA flanking the colicin Js gene cluster shows high similarity to pPCP1 from *Yersinia pestis* (37). The colicin Js gene cluster itself has a significantly lower G + C content (33.6%) than the rest of the plasmid (52.9%), indicating that it originated from yet a third source (87), perhaps even outside of the *Enterobacteriaceae*. This type of recombination, although not altering the bacteriocin genes proper, results in an increased host range. As we continue to explore bacteriocin diversity, our model of bacteriocin evolution will almost certainly become more elaborate and complex.

ECOLOGICAL ROLE OF BACTERIOCINS

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 enteric bacteriocins. Such observations argue that these toxins play a critical role in mediating microbial population or community interactions. What remains in question is what, precisely, that role is.

Bacteriocins may serve as anti-competitors enabling the invasion of a strain into an established microbial 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 gram-positive bacteriocins, in which they mediate quorum sensing (55). It is likely that whatever roles bacteriocins play, these roles change as components of the environment, both biotic and abiotic, change.

Theoretical and Experimental Studies of Bacteriocin Ecology

Early experimental studies on the ecological role of bacteriocins were inconclusive and contradictory (14, 24, 27, 34, 38, 45, 101). More recently a theoretical and empirical base has been established that has defined the conditions that favor maintenance of toxin-producing bacteria in both population and community settings. Almost exclusively, these studies have modeled the action of colicins. Chao & Levin showed that the conditions for invasion of a colicin-producer strain were much broader in a spatially structured environment than in an unstructured one (10). In an unstructured environment with mass-action, a small population of producers cannot invade an established population of sensitive cells. This failure occurs because the producers pay a price for toxin production—the energetic costs of plasmid carriage and lethality of production—but the benefits, the resources made available by killing sensitive organisms, are distributed at random. Moreover, when producers are rare, the reduction in growth rate experienced by the sensitive strain (owing to extra deaths) is smaller than the reduction felt by the producer (owing to its costs), and the producer population therefore becomes extinct. In a physically structured environment, such as on the surface of an agar plate, the strains grow as separate colonies. Toxin diffuses out from a colony of producers, thus killing sensitive neighbors. The resources made available accrue disproportionately to the producing colony owing to its proximity, and therefore killers can increase in frequency even when initially rare.

The Rock-Paper-Scissors Model

Recent 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 (15, 46). 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. The authors in both studies reasonably assume there is a cost to resistance and that this cost is less than the cost of toxin production borne by the killer strain (25). Owing to this third member, pairwise interactions among the strains have the nontransitive structure of the childhood game of rock-paper-scissors (Table 1) (53). 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. In an unstructured environment,

TABLE 1 Chemical warfare among microbes as a non-transitive, three-way game similar to the "rock-paper-scissors" game

Strain below	Wins against	Loses against
Killer	Sensitive	Resistant
Sensitive	Resistant	Killer
Resistant	Killer	Sensitive

this game allows periodic cycles, in which all three types coexist indefinitely but each with fluctuating abundance. 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 (15).

Further effects of evolution were incorporated into the Czárán et al. model by allowing as many as 14 distinct systems of toxin production, sensitivity, and resistance, along with the genetic processes of mutation and recombination that can alter these traits and their associations (15). The permutations of these systems permit the existence of several million different strains. From this additional complexity emerges two distinct quasi-equilibrium conditions, the "frozen" and "hyper-immunity" states. In the frozen state, all the toxins are maintained globally, but most colonies are single-toxin producers. That is, each colony produces one toxin to which it is also immune. By contrast, in the hyper-immunity state, many colonies produce no toxin, many others make one, still others produce several toxins, but only a few produce most of the available toxins. Resistance shows a different distribution, with all of the colonies being resistant to most or all of the toxins. Which of these two outcomes is obtained depends upon initial conditions. If the evolving system begins with the entire population sensitive to all toxins, then the frozen state results. The hyper-immunity state is reached if the system starts with enough diversity that most colonies already have multiple killer and resistance traits.

Numerous surveys of colicin production in natural populations suggest that populations of *E. coli* may closely match predictions of the Czárán model (29, 72). In *E. coli*, producer strains are found in frequencies ranging from 10% to 50%. Resistant strains are even more abundant and are found at frequencies from 50% to 98%. In fact, most strains are resistant to all co-segregating colicins. Finally, there is a small population of sensitive cells. Figure 5 provides a summary of phenotype distributions in a population of *E. coli* isolated from wild field mice in Australia (29). The Czárán model predicts this distribution of phenotypes results from frequent horizontal transfer of resistance, and the significant cost to colicin production (15). In other words, if a strain can gain resistance and lose production, it will over time—just as was observed in the *E. coli* isolated from the field mouse population over the course of a summer (29).

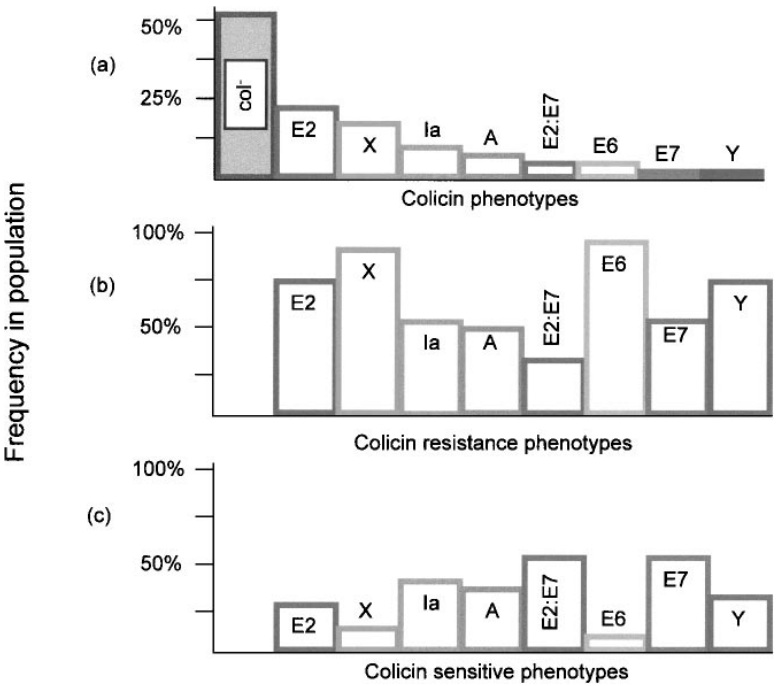

Figure 5 A survey of colicin production and resistance in *E. coli*. Over 400 strains were isolated from two populations of feral mice in Australia over a period of seven months. The isolates were scored for colicin production and resistance. (*a*) Colicin production is abundant with just under 50% of the strains producing eight distinct colicin types. Col^- represents nonproducer 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.

The Killing Breadth of Bacteriocins

We assume bacteriocins play a role in mediating within-species (or population-level) dynamics. This assumption is based upon the narrow killing range exhibited by most bacteriocins. However, recent work calls this assumption into question. Bacteriocins from natural isolates of several species of enteric bacteria were assayed for their killing effect against a large set of nonproducers isolated from the same sources (M.A. Riley, C.M. Goldstone, J.E. Wertz & D. Gordon, unpublished information). Figure 6 reveals that contrary to expectations killing breadth varies significantly for different bacteriocins. Some are clearly most effective at killing within the producer strains own species. Others kill more broadly or kill quite specifically isolates of a different species. This diversity of killing breadth argues that bacteriocins, contrary to prior suggestions, play an equally compelling role in mediating both population-level and community-level interactions. A more

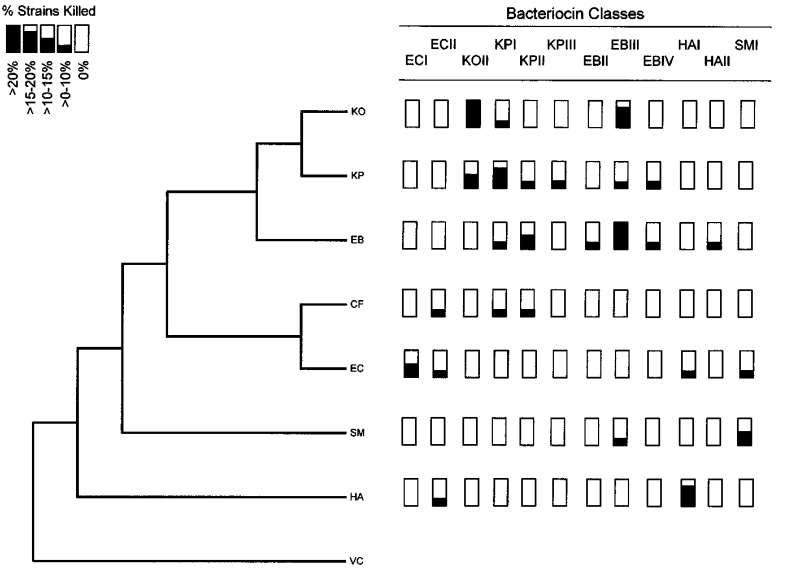

Figure 6 Phylogenetic breadth of bacteriocin killing. The killing spectrum of each class of bacteriocins was cross-referenced with a phylogenetic tree of the enteric species they were screened against. Heights of the black boxes are proportional to the percentage of strains sensitive to each class of bacteriocin. Bacteriocins were screened against 40 natural isolates from each enteric species. The molecular phylogeny of a subset of enteric bacteria is based on a composite of five housekeeping genes (*gapA, groEL, gyrA, ompA, pgi*) and 16s ribosomal sequences. The tree is rooted using *Vibrio cholerae* as an outgroup. KO, *Klebsiella oxytoca*; KP, *Klebsiella pneumoniae*; EB, *Enterobacter cloacae*; CF, *Citrobacter freundii*; EC, *E. coli*; SM, *Serratia marcescens*; HA, *Hafnia alvei*; VC, *Vibrio cholerae*.

thorough understanding of how bacteriocins function awaits the development of a more biologically realistic experimental approach. Prior studies have considered how producer, sensitive, and resistant strains within the same species interact. If the goal is to understand the role these toxins play in nature, our experiments must incorporate more complex microbial communities and environments.

BACTERIOCIN APPLICATIONS

Bacteriocins and Human Health

The rapid rise and spread of multi-resistant bacterial pathogens have forced the consideration of alternative methods of combating infection (59, 78). One of the limitations of using broad-spectrum antibiotics is that they kill almost any bacterial species not specifically resistant to the drug. Given such a broad killing spectrum, these antibiotics are used frequently, which results in an intensive selection pressure

for the evolution of antibiotic resistance in both pathogen and commensal bacteria (100). Once resistance appears, it is simply a matter of time and the intensity of human-mediated selection before human pathogens will acquire resistance (54).

Current solutions to this dilemma involve developing a more rationale approach to antibiotic use, which involves curtailing the prescription of drugs for anything other than bacterial infections, cycling through different drugs over a shorter time frame, and educating the public about the necessity of taking an entire course of antibiotics (54, 89). Bacteriocins provide an alternative solution. With their relatively narrow spectrum of killing activity, they can be considered "designer drugs," which target specific bacterial pathogens. Given the diversity of bacteriocins produced in nature, it is a relatively simple task to find bacteriocins active against specific human pathogens (R.L. Dorit & M.A. Riley, unpublished information). The development and use of such narrow-spectrum antimicrobials not only increases the number of drugs on the pharmaceutical shelf but, more importantly, extends their shelf life. This latter feature emerges because with a designer drug approach, each antibiotic is used infrequently, which results in a reduction in the intensity of selection for resistance. From an ecological and evolutionary perspective, the use of narrow-spectrum antimicrobials to address the current threat posed by multi-resistance bacterial pathogens makes quite a bit of sense. It leads to a reduction in the collateral killing of nonpathogen species, i.e., commensal species, which in turn leads to a decrease in nosocomial infection levels. It also results in a reduction in the intensity of selection for antibiotic resistance. With so few species of bacteria killed by each designer drug, antibiotic resistance resulting from antibiotic use will evolve and spread more slowly.

Bacteriocins and Food Preservation

The only bacteriocins currently employed in food preservation are those produced by LAB used in the production of fermented foods (56). Because LAB have been used for centuries to ferment foods, they enjoy GRAS (generally regarded as safe) status by the U.S. Food and Drug Administration (FDA). This permits their use in fermented foods without additional regulatory approval (56).

Nisin was the first bacteriocin to be isolated and approved for use in foods, specifically to prevent the outgrowth of *Clostridium botulinum* spores in cheese spreads in England (12). By 1988, the FDA had approved its use as a biopreservative for a narrow range of foods, including pasteurized egg products. Today, nisin is accepted as a safe food preservative by over 45 countries, and it is the most widely used commercial bacteriocin and it remains the only bacteriocin that may be added to U.S. foods.

Over the past decade the recurrence of listeriosis outbreaks, combined with the natural resistance of the causative agent, *Listeria monocytogenes*, to traditional food preservation methods such as its ability to grow at near-freezing temperatures has focused the attention of bacteriocin researchers on this organism (61). This attention has resulted in the isolation of a large number of class IIa bacteriocins, all of which are highly active against *L. monocytogenes* [recently reviewed in (23)].

The next wave of development of bacteriocins as food preservatives is at hand. Bacteriocins have been discovered in cured meats, milk and cheese, spoiled salad dressing, and soybean paste. Luchansky and colleagues have developed a gelatin form of pediocin, a class IIa bacteriocin made by lactic acid–producing bacteria, that protects hot dogs from *Listeria* contamination (67). His team has also added a strain of pediocin-producing bacteria to sausage and found a reduction of *Listeria* numbers to be fewer than one ten-thousandth the original number in untreated sausage. Equally compelling, active pediocin was found in the sausage after two months of refrigeration. At the University of Melbourne in Australia, Barrie Davidson has been targeting *Listeria* with piscicolin, a bacteriocin from yet another lactic acid–producing bacterium (67). Piscicolin has already been patented and it will soon be ready for use in meat products and as a rinse for salad greens or chicken parts (67).

A natural concern about using bacteriocins for the preservation of food is the selection of resistant strains. Studies in LAB have shown that resistance carries a significant fitness cost, with resistant strains having a slower growth rate than their sensitive ancestor (19). Treatment with a combination of bacteriocins, for instance nisin and a class IIa bacteriocin, would theoretically reduce the incidence of resistance (5, 99). There is currently conflicting evidence as to whether resistance to one class of LAB bacteriocins can result in cross-resistance to another class (5, 88).

CONCLUSIONS AND FUTURE DIRECTIONS

Bacteriocins represent one of the best-studied microbial defense systems. Although we are still in the earliest stages of exploring their evolutionary relationships and ecological roles, it is clear from their abundance and diversity that they are the microbial weapons of choice. Sorting out why they are such a successful family of toxins will require a substantial commitment to future research. Answering this question will require a substantial effort to more fully characterize the diversity of bacteriocin proteins, their modes of targeting and killing, the gene clusters that encode them, and the mechanisms of bacteriocin gene regulation. In addition, we require more sophisticated ecological models (both empirical and theoretical) to aid in our growing sense of the diverse roles the toxins play in mediating microbial dynamics and maintaining microbial diversity. The impact of such studies is not solely academic. The potential for bacteriocins to serve as alternatives to classical antibiotics in treating bacterial infections is real, and the application of bacteriocins in food preservation is exploding. The future roles bacteriocins may serve is limited only by our imagination.

ACKNOWLEDGMENTS

We thank Carla Goldstone for her help in preparing this review and acknowledge financial support from the NIH GM 58433 and the Rockefeller Foundation.

The *Annual Review of Microbiology* is online at http://micro.annualreviews.org

LITERATURE CITED

1. Barefoot SF, Klaenhammer TR. 1984. Purification and characterization of the *Lactobacillus acidophilus* bacteriocin lactacin-B. *Antimicrob. Agents Chemother.* 26:328–34
2. Belkum MJ, Hayema BJ, Geis A, Kok J, Venema G. 1989. Cloning of two bacteriocin genes from a lactococcal bacteriocin plasmid. *Appl. Environ. Microbiol.* 55: 1187–91
3. Benedetti H, Geli V. 1996. Colicin transport, channel formation and inhibition. *Hand. Biol. Phys.* 2:665–91
4. Bhunia AK, Johnson MC, Ray B. 1987. Direct detection of an antimicrobial peptide of *Pediococcus acidilactici* in sodium dodecyl sulfate-polyacrylamide gel-electrophoresis. *J. Ind. Microbiol.* 2:319–22
5. Bouttefroy A, Milliere J. 2000. Nisin-curvaticin 13 combinations for avoiding the regrowth of bacteriocin resistant cells of *Listeria monocytogenes* ATCC 15313. *Int. J. Food Microbiol.* 62:65–75
6. Braun V, Pilsl H, Groß P. 1994. Colicins: structures, modes of actions, transfer through membranes, and evolution. *Arch. Microbiol.* 161:199–206
7. Brotz H, Bierbaum G, Markus A, Molitor E, Sahl HG. 1995. Mode of action of the lantibiotic mersacidin—inhibition of peptidoglycan biosynthesis via a novel mechanism. *Antimicrob. Agents Chemother.* 39: 714–19
8. Bruno MEC, Montville TJ. 1993. Common mechanistic action of bacteriocins from lactic-acid bacteria. *Appl. Environ. Microbiol.* 59:3003–10
9. Buchman G, Banerjee S, Hansen J. 1988. Structure, expression and evolution of gene encoding the precursor of nisin, a small protein antibiotic. *J. Biol. Chem.* 263:16260–66
10. Chao L, Levin BR. 1981. Structured habitats and the evolution of anticompetitor toxins in bacteria. *Proc. Natl. Acad. Sci. USA* 78:6324–28
11. Cheung J, Danna K, O'Connor E, Price L, Shand R. 1997. Isolation, sequence, and expression of the gene encoding halocin H4, a bacteriocin from the halophilic archaeon *Haloferax mediterranei* R4. *J. Bacteriol.* 179:548–51
12. Chung K, Dickson J, Crouse J. 1989. Effects of nisin on growth of bacteria attached to meat. *Appl. Environ. Microbiol.* 55:1329–33
13. Cramer WA, Heymann JB, Schendel SL, Deriy BN, Cohen FS, et al. 1995. Structure-function of the channel-forming colicins. *Annu. Rev. Biophys. Biomol. Struct.* 24:611–41
14. Craven JA, Miniats OP, Barnum DA. 1971. Role of colicins in antagonism between strains of *Escherichia coli* in dual-infected gnotobiotic pigs. *Am. J. Vet. Res.* 32:1775–9
15. Czárán TL, Hoekstra RF, Pagie L. 2002. Chemical warfare between microbes promotes biodiversity. *Proc. Natl. Acad. Sci. USA* 99:786–90
16. de Ruyter PGGA, Kuipers OP, deVos WM. 1996. Controlled gene expression systems for *Lactococcus lactis* with the food-grade inducer nisin. *Appl. Environ. Microbiol.* 62:3662–67
17. Dery KJ, Chavideh R, Waters V, Chamorro R, Tolmasky L, Tolmasky M. 1997. Characterization of the replication and mobilization regions of the multiresistance *Klebsiella pneumoniae* plasmid pJHCMW1. *Plasmid* 38:97–105
18. Dodd HM, Horn N, Gasson MJ. 1990. Analysis of the genetic determinant for production of the peptide antibiotic nisin. *J. Gen. Microbiol.* 136:555–66
19. Dykes GA, Hastings JW. 1998. Fitness costs associated with class IIa bacteriocin

resistance in *Listeria monocytogenes* b73. *Lett. Appl. Microbiol.* 26:5–8
20. Enfedaque J, Ferrar S, Guasch JF, Tomas J, Regue M. 1996. Bacteriocin 28b from *Serratia marcescens* N28b: identification of *Escherichia coli* surface components involved in bacterocin binding and translocation. *Can. J. Microbiol.* 42:19–26
21. Engelke G, Gutowski-Eckel Z, Hammelmann M, Entian KD. 1992. Biosynthesis of the lantibiotic nisin: genomic organization and membrane localization of the nisb protein. *Appl. Environ. Microbiol.* 58: 3730–43
22. Engelke G, Gutowskieckel Z, Kiesau P, Siegers K, Hammelman M, Entian KD. 1994. Regulation of nisin biosynthesis and immunity in lactocoocus-lactis 6F3. *Appl. Environ. Microbiol.* 60:814–25
23. Ennahar S, Sashihara T, Sonomoto K, Ishizaki A. 2000. Class IIa bacteriocins: biosynthesis, structure and activity. *FEMS Microbiol. Rev.* 24:85–106
24. Feldgarden M, Golden S, Wilson H, Riley MA. 1995. Can phage defense maintain colicin plasmids in *Escherichia coli*? *J. Microbiol.* 141:2977–84
25. Feldgarden M, Riley MA. 1998. The phenotypic and fitness effects of colicin resistance in *Escherichia coli* K-12. *Evolution* 53:1019–27
26. Ferrer S, Viejo MB, Guasch JF, Enfedaque J, Regue M. 1996. Genetic evidence for an activator required for induction of colicin-like bacteriocin 28b production in *Serratia marcescens* by DNA-damaging agents. *Am. Soc. Microbiol.* 178:951–60
27. Freter R. 1983. Mechanisms that control the microflora in the large intestine. In *Human Intestinal Microflora in Health and Disease*. New York: Academic
28. Gordon D, Riley MA. 1999. A theoretical and empirical investigation of the invasion dynamics of colicinogeny. *Microbiology* 145:655–61
29. Gordon D, Riley MA, Pinou T. 1998. Temporal changes in the frequency of colicinogeny in *Escherichia coli* from house mice. *Microbiology* 144:2233–40
30. Gouaux E. 1997. The long and short of colicin action: the molecular basis for the biological activity of channel-forming colicins. *Structure* 5:313–77
31. Gross E, Morell JL. 1971. Structure of nisin. *J. Am. Chem. Soc.* 93:4634–35
32. Guasch J, Enfedaque J, Ferrer S, Gargallo D, Regue M. 1995. Bacteriocin 28b, a chromosomally encoded bacteriocin produced by most *Serratia marcesens* biotypes. *Res. Microbiol.* 146:477–83
33. Guder A, Wiedemann I, Sahl HG. 2000. Posttranslationally modified bacteriocins—the lantibiotics. *Biopolymers* 55: 62–73
34. Hardy KG. 1975. Colicinogeny and related phenomena. *Bacteriol. Rev.* 39:464–515
35. Hastings JW, Sailer M, Johnson K, Roy KL, Vederas JC, Stiles ME. 1991. Characterization of leucocin A-UAL 187 and cloning of the bacteriocin gene from *Leuconostoc gelidum*. *J. Bacteriol.* 172: 7491–500
36. Holt JG. 1994. *Bergey's Manual of Determinative Bacteriology*. Baltimore, MD: Williams & Wilkins
37. Hu P, Elliot J, McCready P, Skowronski E, Garnes J, et al. 1998. Structural organization of virulence-associated plasmids of *Yersinia pestis*. *J. Bacteriol.* 180:5192–202
38. Ikari NS, Kenta DM, Young VM. 1969. Interaction in the germ-free mouse intestine of colicinogenic and colicin-sensitive microorganisms. *Proc. Soc. Exp. Med.* 130:1280–84
39. Jack RW, Tagg JR, Ray B. 1995. Bacteriocins of gram-positive bacteria. *Microbiol. Rev.* 59:171–200
40. James R, Kleanthous C, Moore GR. 1996. The biology of E colicins: paradigms and paradoxes. *Microbiology* 142:1569–80
41. James R, Lazdunski C, Pattus F, eds. 1991. *Bacteriocins, Microcins and Lantibiotics*,

Vol. 65. New York: Springer-Verlag. 519 pp.
42. Joerger MC, Klaenhammer T. 1986. Characterization and purification of helveticin-J and evidence for a chromosomally determined bacteriocin produced by *Lactobacillus helveticus*-481. *J. Bacteriol.* 167:439–46
43. Jung G, Sahl HG. 1991. Lantibiotics: a survey. In *Nisin and Novel Lantibiotics*, pp. 1–34. Leiden, The Netherlands: ESCOM
44. Kaletta C, Entian K-D. 1989. Nisin, a peptide antibiotic: cloning and sequencing of the *nis* A gene and posttranslational processing of its peptide product. *J. Bacteriol.* 171:1597–601
45. Kelstrup J, Gibbons RJ. 1969. Inactivation of bacteriocins in the intestinal and oral cavity. *J. Bacteriol.* 99:888–90
46. Kerr B, Riley MA, Feldman MW, Bohannan BJM. 2002. Local dispersal promotes biodiversity in a real-life game of rock-paper-scissors. *Nature* 418:171–74
47. Klaenhammer TR. 1988. Bacteriocins of lactic acid bacteria. *Biochimie* 70:337–49
48. Konisky J. 1982. Colicins and other bacteriocins with established modes of action. *Annu. Rev. Microbiol.* 36:125–44
49. Kuipers OP, Beerthuyzen MM, Deruyter PGGA, Luesink EJ, de Vos WM. 1995. Autoregulation of nisin biosynthesis in *Lactococcus lactis* by signal-transduction. *J. Biol. Chem.* 270:27299–304
50. Kuipers OP, Beerthuyzen MM, Siezen RJ, Devos WM. 1993. Characterization of the nisin gene-cluster *nisABTCIPR* of *Lactococcus lactis*—requirement of expression of the *nisA* and *nisI* genes for development of immunity. *Eur. J. Biochem.* 216:281–91
51. Lau PCK, Parsons M, Uchimura T. 1992. Molecular evolution of E colicin plasmids with emphasis on the endonuclease types. See Ref. 41, 65:353–78
52. Leer RJ, Vandervossen J, Vangiezen M, Vannoort JM, Pouwels PH. 1995. Genetic-analysis of acidocin-B, a novel bacteriocin produced by *Lactobacillus acidophilus*. *Microbiology* 141:1629–35
53. Lenski RR, Riley MA. 2002. Chemical warfare from an ecological perspective. *Proc. Natl. Acad. Sci. USA* 99:556–58
54. Lipsitch M, Bergstrom CT, Levin BR. 2000. The epidemiology of antibiotic resistance in hospitals: paradoxes and prescriptions. *Proc. Natl. Acad. Sci. USA* 97: 1938–43
55. Miller M, Bassler B. 2001. Quorum sensing in bacteria. *Annu. Rev. Microbiol.* 55: 165–99
56. Montville TJ, Winkowski K. 1997. Biologically based preservation systems and probiotic bacteria. In *Food Microbiology: Fundamentals and Frontiers.*Washington, DC: ASM
57. Mota-Meira M, Lapointe G, Lacroix C, Lavoie MC. 2000. MICs of mutacin B-Ny266, nisin A, vancomycin, and oxacillin against bacterial pathogens. *Antimicrob. Agents Chemother.* 44:24–29
58. Muriana PM, Klaenhammer TR. 1991. Cloning, phenotypic expression, and DNA sequence of the gene for lactacin F, an antimicrobial peptide produced by *Lactobacillus* spp. *J. Bacteriol.* 173:1779–88
59. Neu HC. 1992. The crisis in antibiotic resistance. *Science* 257:1064–73
60. Nissenmeyer JH, Havarstein LS, Sletten K, Nes IF. 1992. A novel lactococcal bacteriocin whose activity depends on the complementary action of 2 peptides. *J. Bacteriol.* 174:5686–92
61. Palumbo S. 1986. Is refrigeration enough to restrain foodborne pathogens. *J. Food Prot.* 49:1003–9
62. Pinou T, Riley M. 2001. Nucleotide polymorphism in microcin V plasmids. *Plasmid* 46:1–9
63. Price L, Shand R. 2000. Halocin S8: a 36-amino acid microhalocin from the haloarchaeal strain S8a. *J. Bacteriol.* 182:4951–58
64. Pugsley A. 1984. The ins and outs of colicins. *Microbiol. Sci.* 1:168–75, 203–5
65. Pugsley AP, Oudega B. 1987. Methods for

studying colicins and their plasmids. In *Plasmids, a Practical Approach*, ed. KG Hardy, pp. 105–61. Oxford: IRL
66. Ra R, Beerthuyzen M, de Vos WM, Saris PEJ, Kuipers OP. 1999. Effects of gene disruptions in the nisin gene cluster of *Lactococcus lactis* on nisin production and producer immunity. *Microbiology* 145:1227–33
67. Raloff J. 1998. Staging germ warfare in foods. *Sci. News* 153:89–90
68. Riley M. 1993. Molecular mechanisms of colicin evolution. *Mol. Biol. Evol.* 10: 1380–95
69. Riley MA. 1993. Positive selection for colicin diversity in bacteria. *Mol. Biol. Evol.* 10:1048–59
70. Riley MA. 1998. Molecular mechanisms of bacteriocin evolution. *Annu. Rev. Genet.* 32:255–78
71. Riley M, Cadavid L, Collett M, Neely M, Adams M, et al. 2000. The newly characterized colicin Y provides evidence of positive selection in pore-former colicin diversification. *Microbiology* 146:1671–77
72. Riley MA, Gordon DM. 1992. A survey of col plasmids in natural isolates of *Escherichia coli* and an investigation into the stability of col-plasmid lineages. *J. Microbiol.* 138:1345–52
73. Riley MA, Pinou T, Wertz JE, Tan Y, Valletta CM. 2001. Molecular characterization of the klebicin B plasmid of *Klebsiella pneumoniae*. *Plasmid* 45:209–21
74. Riley MA, Tan Y, Wang J. 1994. Nucleotide polymorphism in colicin E1 and Ia plasmids from natural isolates of *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* 91:11276–80
75. Rodriguez-Valera F, Juez G, Kushner DJ. 1982. Halocins: salt dependent bacteriocins produced by extremely halophilic rods. *Can. J. Microbiol.* 28:151–54
76. Roos U, Harkness RE, Braun V. 1989. Assembly of colicin genes from a few DNA fragments. Nucleotide sequence of colicin D. *Mol. Microbiol.* 3:891–902
77. Ross RP, Galvin M, McAuliffe O, Morgan SM, Ryan MP, et al. 1999. Developing applications for lactococcal bacteriocins. *Antonie Van Leeuwenhoek Int. J. Gen. Mol. Microbiol.* 76:337–46
78. Roy PH. 1997. Dissemination of antibiotic resistance. *Med. Sci.* 13:927–33
79. Saadoun I, al-Momani F, Malkawi HI, Mohammad MJ. 1999. Isolation, identification and analysis of antibacterial activity of soil streptomycetes isolates from north Jordan. *Microbios* 100:41–46
80. Sano Y, Kobayashi M, Kageyama M. 1993. Functional domains of S-type pyocins deduced from chimeric molecules. *J. Bacteriol.* 175:6179–85
81. Sano Y, Matsui H, Kobayashi M, Kageyama M. 1990. Pyocins S1 and S2, bacteriocins of *Pseudomonas aeruginosa*. In *Pseudomonas: Biotransformations, Pathogenesis, and Evolving Biotechnology*, ed. S Silver, pp. 352–58. Washington, DC: ASM
82. Sano Y, Matsui H, Kobayashi M, Kageyama M. 1993. Molecular structures and functions of pyocins S1 and S2 in *Pseudomonas aeruginosa*. *J. Bacteriol.* 175: 2907–16
83. Schillinger U, Lucke F-K. 1989. Antibacterial activity of *Lactobacillus sake* isolated from meat. *Appl. Environ. Microbiol.* 55:1901–6
84. Schuller F, Benz R, Sahl HG. 1989. The peptide antibiotic subtilin acts by formation of voltage-dependent multi-state pores in bacterial and artificial membranes. *Eur. J. Biochem.* 182:181–86
85. Shand R, Price L, O'Connor EM. 1998. Halocins: protein antibiotics from hypersaline environments. In *Microbiology and Biogeochemistry of Hypersaline Environments*, ed. A Oren, Chapter 24, pp. 295–306. Boca Raton, FL: CRC
86. Smajs D, Pilsl H, Braun V. 1997. Colicin U, a novel colicin produced by *Shigella boydii*. *J. Bacteriol.* 179:4919–28
87. Smajs D, Weinstock G. 2001. Genetic organization of plasmid colJs, encoding

colicin Js activity, immunity, and release genes. *J. Bacteriol.* 183:3949–57
88. Song HJ, Richard J. 1997. Antilisterial activity of three bacteriocins used at subminimal inhibitory concentrations and cross-resistance of the survivors. *Int. J. Food Microbiol.* 36:155–61
89. Stewart FM, Antia R, Levin BR, Lipsitch M, Mittler JE. 1998. The population genetics of antibiotic resistance. II: analytic theory for sustained populations of bacteria in a community of hosts. *Theoret. Popul. Biol.* 53:152–65
90. Tagg JR, Dajani AS, Wannamaker LW. 1976. Bacteriocins of gram-positive bacteria. *Bact. Rev.* 40:722–56
91. Tan Y, Riley MA. 1996. Rapid invasion by colicinogenic *Escherichia coli* with novel immunity functions. *Microbiology* 142:2175–80
92. Tan Y, Riley MA. 1997. Nucleotide polymorphism in colicin E2 gene clusters: evidence for nonneutral evolution. *Mol. Biol. Evol.* 14:666–73
93. Tan Y, Riley MA. 1997. Positive selection and recombination: major molecular mechanisms in colicin diversification. *Trends Ecol. Evol.* 12:348–51
94. Torreblanca M, Meseguer I, Rodriguez-Valera F. 1989. Halocin H6, a bacteriocin from *Haloferax gibbonsii*. *J. Gen. Microbiol.* 135:2655–61
95. Torreblanca M, Meseguer I, Ventosa A. 1994. Production of halocin is a practically universal feature of archael halophilic rods. *Lett. Appl. Microbiol.* 19: 201–5
96. Upreti GC, Hinsdill RD. 1975. Production and mode of action of lactocin 27—bacteriocin from a homofermentative *Lactobacillus*. *Antimicrob. Agents Chemother.* 7:139–45
97. Vandermeer JR, Polman J, Beerthuyzen MM, Siezen RJ, Kuipers OP, Devos WM. 1993. Characterization of the *Lactococcus-lactis* nisin-a operon genes *nisP*, encoding a subtilisin-like serine protease involved in precursor processing, and *nisR*, encoding a regulatory protein involved in nisin biosynthesis. *J. Bacteriol.* 175:2578–88
98. Vaughan EE, Daly C, Fitzgerald GF. 1992. Identification and characterization of helveticin V-1829, a bacteriocin produced by *Lactobacillus helveticus* 1829. *J. Appl. Bacteriol.* 73:299–308
99. Vignolo G, Palacios J, Farias ME, Sesma F, Schillinger U, et al. 2000. Combined effect of bacteriocins on the survival of various *Listeria* species in broth and meat system. *Curr. Microbiol.* 41:410–16
100. Walker ES, Levy F. 2001. Genetic trends in a population evolving antibiotic resistance. *Evolution* 55:1110–22
101. Wilson KH. 1997. *Biota of the Human Gastrointestinal Tract*. New York: Chapman & Hall. 680 pp.
102. Wu S, Dornbusch K, Kronvall G, Norgren M. 1999. Characterization and nucleotide sequence of a *Klebsiella oxytoca* cryptic plasmid encoding a CMY-type beta-lactamase: confirmation that the plasmid-mediated cephamycinase originated from the *Citrobacter freundii* Amp C beta-lactamase. *Antimicrob. Agents Chemother.* 43:1350–57