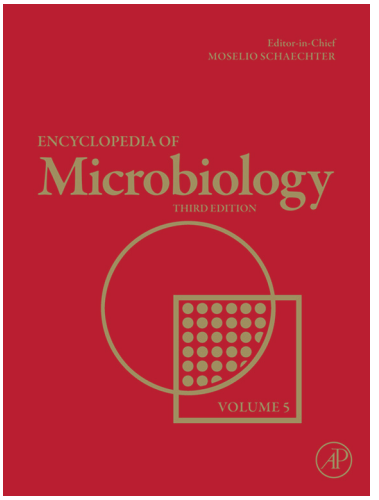

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Bacteriocins, Biology, Ecology, and Evolution

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Glossary

antimicrobial Antimicrobials are substances that kill or inhibit the growth of bacteria, fungi, or viruses.
Archaea Archaea comprise a domain of life, although more closely related to eukaryotes than to bacteria, but are similar to bacteria in that they lack nuclei and are microscopic.
bacteriocin Bacteriocins are proteinaceous toxins produced by bacteria to inhibit the growth of closely related bacterial strains.
colicin Colicins are the bacteriocins produced by *Escherichia coli*.
immunity Bacteriocin immunity is governed by an immunity protein, which binds to the bacteriocin toxin and renders it harmless to self.
probiotics Probiotics are dietary supplements containing potentially beneficial bacteria or yeast.
resistance Bacteriocin resistance is the result of one or more mutations in the cell surface receptors and/or translocation systems that are involved in bacteriocin recognition and uptate by the target cell.
SOS response The SOS response is a DNA repair system that allows DNA replication to bypass errors in the DNA.

Abbreviations

BLIS	bacteriocin-like inhibitory substance
FDA	Food and Drug Administration
GI	Gastrointestinal
GRAS	generally regarded as safe
LAB	lactic acid bacteria
PB	probiotic bacteria
UV	ultraviolet

Defining Statement

The bacteriocin family is the most abundant and diverse group of bacterial defenses. Key information about their evolutionary history and ecological role is emerging from molecular evolutionary studies. The more we learn about this fascinating family of toxins, the greater seems their potential to solve human health and agriculture challenges.

What are Bacteriocins?

Microbes produce an extraordinary array of microbial defense systems. These include broad-spectrum classical antibiotics, metabolic by-products such as lactic acids, lytic agents such as lysozymes, numerous types of protein exotoxins, and bacteriocins, which are loosely defined as biologically active protein moieties with a bacteriocidal mode of action. This biological arsenal is striking not only in its diversity but also in its natural abundance. For instance, lactic acid production is a defining trait of lactic acid bacteria (LAB). 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. Halobacteria universally produce their own version of bacteriocins, the halocins. Streptomyces commonly produce broad-spectrum antibiotics.

The microbial weapons of choice, based on natural abundance and diversity, are the bacteriocins, which comprise a large and functionally diverse family of toxins found in all major lineages of Bacteria. Certain features unite them as a family; they are ribosomally synthesized proteinaceous compounds active against bacteria related to the producing strain. Recent studies reveal that bacteriocins play a critical role in competitive dynamics between bacterial strains and may play a fundamental

role in maintaining microbial diversity. In this article, current knowledge of the biology of this diverse family of toxins is reviewed, as well as studies that seek to explain how such an extraordinary range of bacteriocin diversity arose and is maintained in microbial species, and, finally, the role these toxins play in mediating microbial dynamics. Fascination with bacteriocins is not limited to the evolutionary and ecologically minded; in the latter half of this article, our attention focuses on the growing application of these toxins to address human health concerns and to aid in food preservation.

Bacteriocins were first identified almost 100 years ago as a heat-labile product present in cultures of *Escherichia coli* V and toxic to *E. coli* S, and were given the name colicin to identify the producing species. Fredericq, in 1946, demonstrated that colicins were proteins and that they had a limited range of activity based on the presence of specific receptors on the surface of sensitive cells. Since then, bacteriocins have been found in all major lineages of Bacteria and, more recently, have been described as universally produced by some members of the Archaea. According to Klaenhammer, 99% of all bacteria may make at least one bacteriocin and the only reason we have not isolated more is that few researchers have looked for them.

Two main features distinguish the majority of bacteriocins from antibiotics: (1) bacteriocins are ribosomally synthesized, while antibiotics are not and (2) bacteriocins have a relatively narrow killing spectrum, while most antibiotics have broad killing ranges. The bacteriocin family includes a number of proteins that vary in size, microbial target, mode of action, and release and immunity mechanisms, and can be divided into two main groups, those produced by Gram-negative and Gram-positive bacteria.

Bacteriocins of Gram-Negative Bacteria

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. Colicins, bacteriocins produced by *E. coli*, were found in 30–50% of the strains isolated from human hosts and are often referred to as a virulence factor. Much higher production levels have been found in some enterics, such as *Pseudomonas aeruginosa*, in which 90% or more of both environmental and clinical isolates produce bacteriocins.

Since their discovery, the colicins of *E. coli* have been the most extensively studied Gram-negative bacteriocins and they now serve as a model system for investigating the mechanisms of bacteriocin structure/function, genetic organization, ecology, and evolution. Colicins are high molecular weight proteins that kill target cells through a variety of mechanisms. Nomura showed early on that colicins E1 and K inhibit macromolecular synthesis without arrest of respiration, colicin E2 causes DNA breakdown, and colicin E3 stops protein synthesis. In each case, he showed that the lethal action is reversed by treatment with trypsin. Since his pioneering work, three discrete steps in colicin action have been described. The toxin binds to a specific cell surface receptor on the outer membrane and is translocated through the cell envelope by either the Tol or the TonB machinery to its target, which is the inner membrane for ionophore colicins and the cytoplasm for nuclease colicins. Colicin M is unique in acting on peptidoglycan synthesis through enzymatic degradation of the undecaprenyl phosphate-linked peptidoglycan precursors.

Colicins are usually encoded on one of two types of colicinogenic plasmids. Type 1 plasmids are small (6–10 kb) and are present in numerous copies per cell. They are mobilizable in the presence of a conjugative plasmid and are amplifiable. Type 2 are monocopy plasmids of about 40 kb, which carry numerous genes in addition to those encoding colicin activity, and can conjugate. Plasmid carriage is not a requirement. The nuclease pyocins of *P. aeruginosa*, which show sequence similarity to colicins, are found exclusively on the chromosome. Another close relative of the colicin family, the bacteriocins of *Serratia marcescens*, are found on both plasmids and chromosomes.

The gene organization of almost all known colicin operons was reviewed recently by Riley and is summarized in **Figure 1**. In all colicin operons, the first gene is the gene encoding the toxin, called *cxa* for colicin X activity.

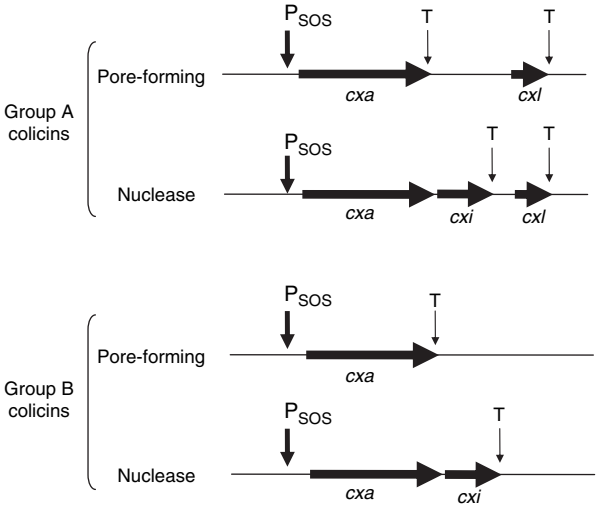

Figure 1 Organization of the colicin operons. The genes are represented by arrowheads. SOS promoters (P_{SOS}) and transcription terminators (T) are indicated by arrows. Names of the colicin gene (*cxa*, in which *x* is specific to the colicin), its immunity gene (*cxi*), and lysis gene (*cxl*) follow the nomenclature.

In operons encoding a nuclease colicin, the gene encoding an immunity protein, designated either *cxi* for colicin X immunity or *immX*, is located downstream of the structural gene. The immunity function is encoded on the opposite DNA strand for ionophoric colicins. In this case, it is transcribed from its own promoter under constitutive regulation. The immunity gene encodes a protein conferring specific immunity to the producer cell that acts by binding to and inactivating the toxin protein. The last gene, which encodes a protein involved in colicin release through lysis of the producer cell, is called the lysis gene and is named *cxl* for colicin X lysis. Exceptions to this organization have been found. Colicin E3 operon encodes two different immunity genes, one to colicin E3 and one to colicin E8, as does that of colicin E6, which contains the immunity genes to E6 and E8. The colicin E9 operon contains the immunity genes to E9 and to colicin E5 and two lysis genes belonging to colicins E9 and E5.

Unlike bacteriocins of Gram-positive bacteria, which are self-regulated with specifically dedicated transport mechanisms facilitating their release, colicins and other bacteriocins of Gram-negative bacteria are dependent on the regulatory pathways of the host cell, mainly the SOS system. Under conditions of stress, a small proportion of the bacteriocin-producing cells are induced. The producing cells commit 'suicide', which results in the release of the toxin molecules that kill the neighboring sensitive cells, and lead to the death of the producing cells. The released proteins bind to specific cell surface receptors on target cells and are translocated into the cell via a set of transport proteins. These specific targeting and transport mechanisms ensure a narrow killing range. The toxin then kills the target cell by one of several mechanisms: (1) channel formation in the cytoplasmic membrane; (2) cellular DNA degradation; (3) protein biosynthesis inhibition by cleaving RNA; or (4) murein and lipopolysaccharide biosynthesis inhibition by interference with lipid carrier regeneration. It is assumed that such targeted killing results in increased resources for the closest relatives of the producing cells, which are immune to the toxin by encoding the specific immunity protein.

Given the intriguing nature of this lethal expression system, it is surprising that relatively few studies have explored the molecular details of bacteriocin induction. Comparison of enteric bacteriocin promoters reveals that the typical bacteriocin promoter comprises a σ^{70} promoter sequence, one or two SOS boxes, a T-rich region, and Shine–Dalgarno sequence. The most prominent feature on induction is the global SOS repressor, the LexA binding site, also known as the SOS box. This appears to be a common mechanism of induction, because other bacteriocins produced by Gram-negative bacteria, such as pyocins (produced by *Pseudomonas* spp.), are also triggered by the SOS response.

The prevalence of SOS boxes within the enteric bacteriocin regulatory region suggests that they play a critical role in regulation. Indeed, for over 50 years, the inducers used to enhance production of these toxins were mutagenic agents such as mitomycin C and UV light. However, it is safe to assume that in their natural habitat, for example, the mammalian intestine, DNA-damaging agents are not abundant. If not mitomycin C or UV, then what are the natural inducers of enteric bacteriocins?

Previous studies investigating colicins E1 and K suggest that their regulation is dependent not only on DNA-damaging agents but also on catabolic repression, stringent response, cell phase, and anaerobiosis. Osmolarity, nutrient depletion, catabolite repression, heavy metals, pH, and temperature triggered low or no response. A very recent study has observed that certain colicin-producing strains trigger the expression of other colicins.

A colicin protein comprises three functionally distinct domains, namely, receptor recognition, protein translocation, and killing. 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 (<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. Most Gram-negative bacteriocins have an identical domain structure. Although the pyocins produced by *P. aeruginosa* share a similar domain structure, the order of the translocation and receptor recognition domains is switched. Further study shows that 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. The Gram-positive bacteriocins resemble many of the antimicrobial peptides produced by eukaryotes; they are generally cationic, amphiphilic, membrane-permeabilizing peptides, approximately 2–6 kDa in size. They differ from bacteriocins of Gram-negative bacteria in two fundamental ways. First, bacteriocin production is not necessarily lethal to the producing cell. This critical difference is due to the transport mechanisms Gram-positive bacteria encode to release bacteriocin toxin. Typically, their biosynthesis is self-regulated with specifically dedicated transport mechanisms facilitating release, although some employ the *sec*-dependent export pathway. Second, the Gram-positive bacteria have evolved bacteriocin-specific regulation, whereas bacteriocins of Gram-negative bacteria rely solely on host regulatory networks.

Bacteriocins produced by LAB, which have a long history of use in fermentation and meat and milk preservation, are the most well-characterized of this group. Four classes of LAB antibiotics are identified: Class I comprises modified bacteriocins, known as lantibiotics; Class II includes heat-stable, minimally modified bacteriocins; Class III includes larger, heat-labile bacteriocins; and Class IV comprises complex bacteriocins carrying lipid or carbohydrate moieties. Classes I and II have been the focus of most probiotic research.

LAB have been used for centuries in the fermentation of food, partly due to the fact that they can prevent the growth of spoilage and pathogenic microorganisms. They produce bacteriocins, the lantibiotics, so named because they are post-translationally modified to contain amino acids such as thioether bridges of lanthionine and 3-methyllanthionine or dehydroalanine. Lantibiotics are ribosomally synthesized bacteriocins that target a broad range of Gram-positive bacteria and are subdivided into three groups: Type A lantibiotics, such as lacticin, are small (2–5 kDa), elongated, screw-shaped proteins that contain positively charged molecules, which kill by membrane polarization. Type B lantibiotics, such as mersacidin, are smaller (about 2 kDa), globular in shape, and kill by interfering with cellular enzymatic reactions, such as cell wall synthesis. Another subgroup is composed of two-component lantibiotics, such as lacticin 3147, consisting of two lantibiotic peptides that synergistically display antimicrobial activity.

Class II LAB bacteriocins are also small, ranging in size from 30 to 60 amino acids, cationic, hydrophobic, heat-stable, non-lanthionine-containing peptides. They are organized into subgroups. Class IIa is the largest group and its members are distinguished by a conserved N-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, sakacin A, and leucocin A. Class IIb bacteriocins such as lacticin F and lactococcin G 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). Class III bacteriocins are large heat-labile proteins such as helveticins J and V and lactacin B. An additional proposed class (VI) requires lipid or carbohydrate moieties for activity. Little is known about the structure and function of this class. Examples include leuconocin S and lactocin 27.

Gram-positive bacteriocins in general and lantibiotics in particular require many more genes for their production than do those of Gram-negative bacteria. 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*). 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.

The conventional wisdom about the killing range of Gram-positive bacteriocins is that they are restricted to killing other Gram-positives. 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*, 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*. 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*.

Production of bacteriocins in Gram-positive bacteria is generally associated with the shift from log phase to stationary phase. For example, nisin production begins during mid-log phase and increases to a maximum as the cells enter stationary phase. 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). 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.

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. The first haolocin discovered, S8, is a short hydrophobic peptide of 36 amino acids, which is processed from a much larger pro-protein of 34 kDa. 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. The mechanism of halocin action has been established only for halocin H6

(a Na+/H+ antiporter inhibitor), and the immunity mechanism is unknown.

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.

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

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. Studies that include DNA and protein sequence comparisons, surveys of DNA sequence polymorphism in natural isolates, experimental evolution, and mathematical modeling have revealed two primary modes of colicin evolution.

The more abundant pore-former colicins are generated by domain shuffling, which is mediated by recombination. All characterized pore-former colicin proteins share one or more regions with high levels of sequence similarity to other pore-former colicins (**Figure 2**). 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 3**), which is a nuclease klebicin that shares sequence similarity with both colicin A-like pore former and pyocin S1-like nuclease sequences. 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. Even altering the domain

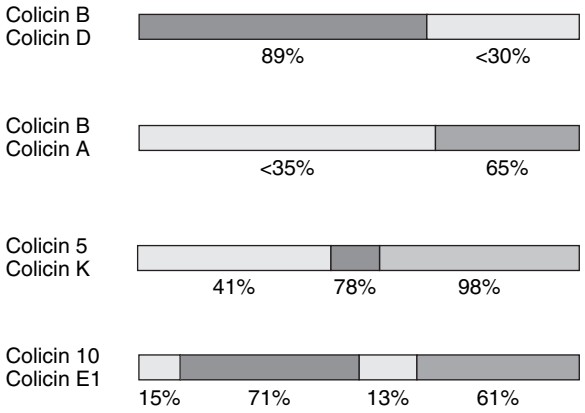

Figure 2 Pairwise comparisons of pore-forming colicin protein sequences. Values below each comparison indicate the percent sequence identity for the region indicated. Colicins are not drawn to scale.

structure of the protein, as seen for pyocins that have switched the receptor recognition and translocation domains relative to the order found in colicins, has not limited the influence of diversifying recombination.

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 4**). To explain this pattern of divergence, a two-step process of mutation and selection was proposed, which posits the action of strong positive selection acting on mutations that generate novel immunity and killing functions (**Figure 5**). 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. A second mutation, this time in the colicin gene, is paired with the immunity mutation. 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.

Colicin Y is a close relative of colicin U, another pore-former colicin isolated from a different continent over 20

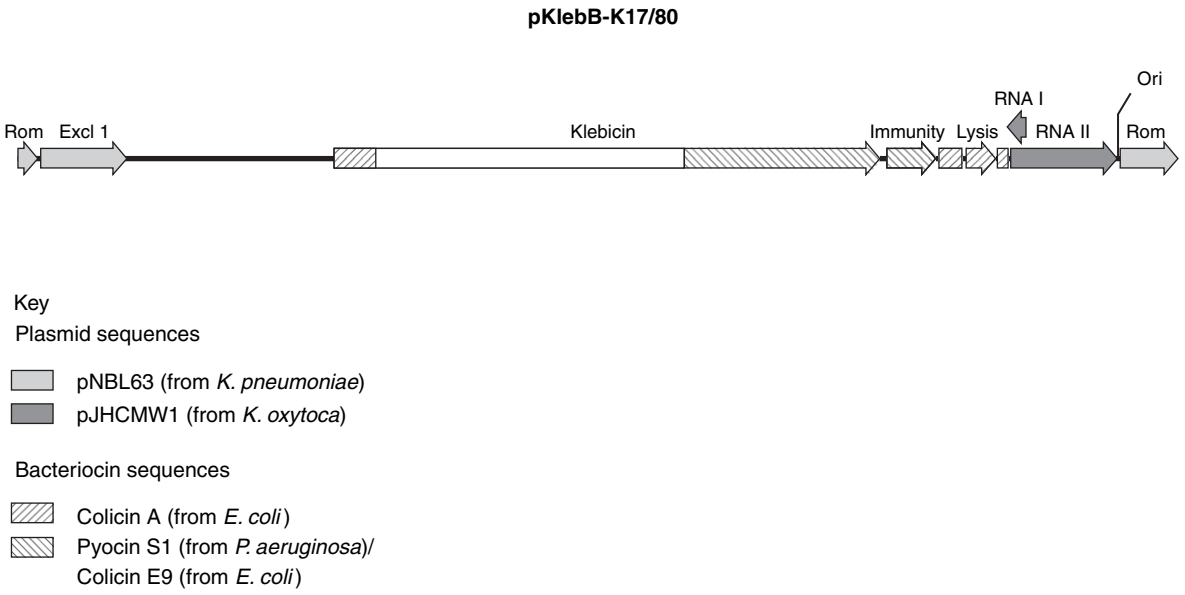

Figure 3 Klebicin recombination. Patterns of sequence similarity in klebicins suggest recombination. The chimeric nature of the pKlebB plasmid sequence is indicated by alternate shadings. The key notes regions of sequence similarity with other bacteriocin gene clusters and plasmids. pKlebB illustrates a pattern typical of other bacteriocin-encoding plasmids where sequences encoding plasmid functions are similar to sequences found on 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.

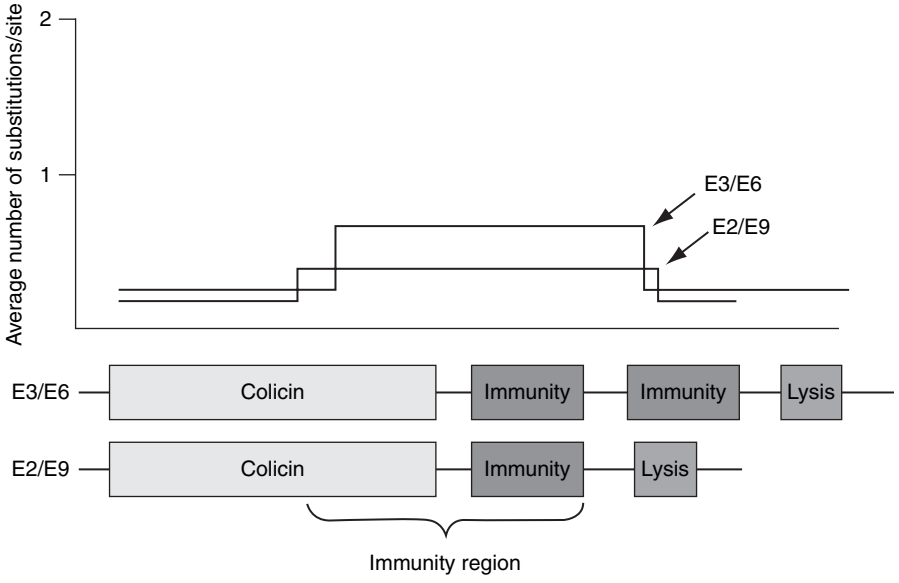

Figure 4 Nuclease diversity. The graph indicates the average number of total nucleotide substitutions between pairs of nuclease-type colicin gene clusters (colicin pairs E2/E9 and E3/E6). Most of the divergence between colicins occurs in the immunity region of the gene cluster (composed of the immunity gene and the immunity-binding region of the colicin gene).

years earlier. 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. Furthermore, several E2 colicins isolated from Australia suggest that diversifying recombination is not restricted to pore-former colicins. 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

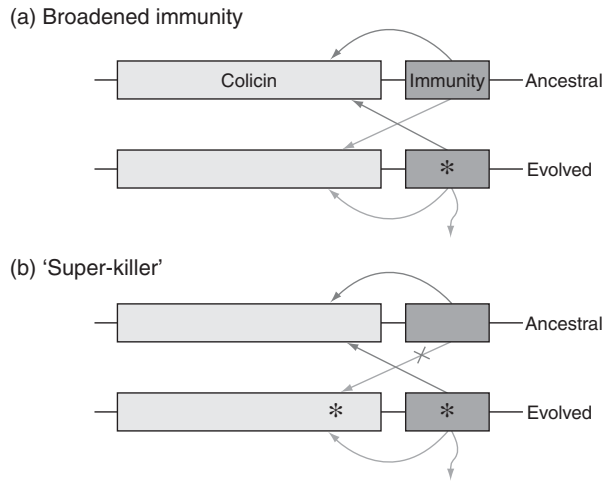

Figure 5 Diversifying selection. The hypothesis of diversifying selection invokes two steps in the generation of a novel immunity function. (a) A point mutation in the immunity gene generates a broadened immunity function (noted with an asterisk). The strain with this colicin gene cluster is immune to 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 'superkiller' (noted with two asterisks). 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 a X).

evolutionary process is more complex than the proposed simple dichotomy suggests.

A two-step process of colicin diversification has been proposed. 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 simply due 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.

Bacteriocin-encoding plasmids, such as pColJs (which encodes colicin Js) and pKlebB (which encodes klebicin B), demonstrate another aspect of bacteriocin evolution. 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 *K. pneumoniae*, the plasmid contains sequences similar to pNBL63 and pJHCMW1, 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 colicins A and E9, originally isolated from *E. coli*. 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*. The colicin Js gene cluster itself has a significantly lower GC content (33.6%) than the rest of the plasmid (52.9%), indicating that it originated from yet a third source, 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

Undoubtedly, 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 anticompetitors, 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. It is likely that whatever roles bacteriocins play, these roles change as components of the environment, both biotic and abiotic, change.

Early experimental studies on the ecological role of bacteriocins were inconclusive and contradictory. More recently, a theoretical and empirical base has been established that has defined the conditions that favor the maintenance of toxin-producing bacteria in both population and community settings. Almost exclusively, these studies have modeled the action of colicins. Chao and 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. 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.

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. 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 that there is a cost to resistance and that this cost is less than the cost of toxin production borne by the killer strain. Owing to this third member, pairwise interactions among the strains have the nontransitive structure of the childhood game of rock-scissors-paper (**Table 1**). The producer strain beats the sensitive strain, owing to the effects of the toxin 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, 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.

Further effects of evolution were incorporated 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. 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 'hyperimmunity' 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 hyperimmunity 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 on initial conditions. If the evolving system begins with the entire population sensitive to all toxins, then the frozen state results. The hyperimmunity state is reached if the system starts with enough diversity that most colonies already have multiple killer and resistance traits.

Table 1 Chemical warfare among microbes as a nontransitive, three-way game similar to the 'rock-scissors-paper' game

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

More recently, experimental tests of several of these theoretical conclusions have been reported. The first used *in vitro* methods (liquid culture, static plate, and mixed plate environments) to assess the impact of local interactions and dispersal on the abundance of three strains of *E. coli* (C – colicin producer; S – colicin sensitive; R – colicin resistant). This study revealed that in environments where interactions and dispersal were not solely local, the resistant strain overtook the community during the course of the experiment. In contrast, in the static plate environment, where interactions and dispersal were solely local, the three phenotypes were maintained at similar densities throughout the experiment. The third environment, mixed plate, revealed that growth on a surface is not the key factor, as resistance overtook the other strains in this plate also. The critical component is whether the interactions are local or not. The second study used a mouse model to investigate precisely the same colicin dynamics in an *in vivo* setting, the mouse gut. The C, S, and R strains in these experiments revealed exactly the same nontransitive interactions described above. When a mouse harbored a sensitive strain, then an introduced C strain was able to invade. When a C strain was resident, then an introduced R strain was able to invade. In both experimental systems, the nontransitive nature of colicin-mediated dynamics was further revealed.

Numerous surveys of colicin production in natural populations suggest that populations of *E. coli* may closely match predictions of these ecological models. 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 cosegregating colicins. Finally, there is a small population of sensitive cells. **Figure 6** provides a summary of phenotype distributions in a population of *E. coli* isolated from wild field mice in Australia. The models predict this distribution of phenotypes results

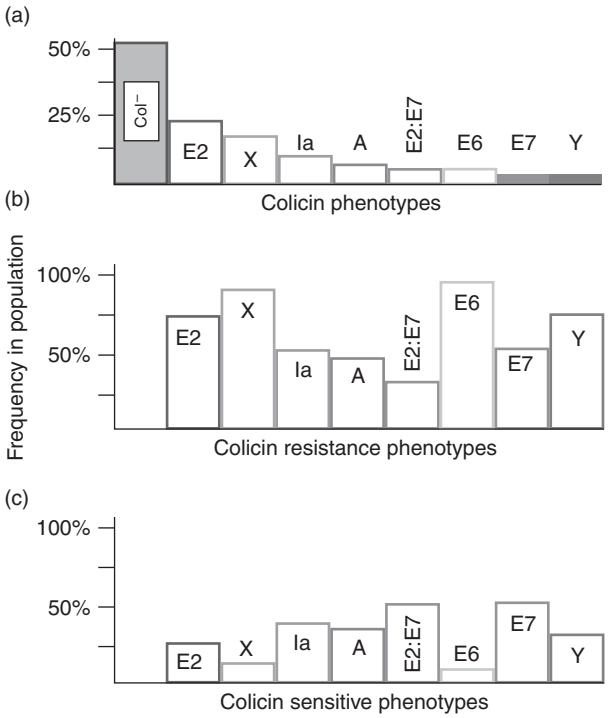

Figure 6 A survey of colicin production and resistance in *Escherichia coli*. Over 400 strains were isolated from two populations of feral mice in Australia over a period of seven months. The isolates were scored for colicin production and resistance. (a) Colicin production is abundant with just under 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-occuring colicins.

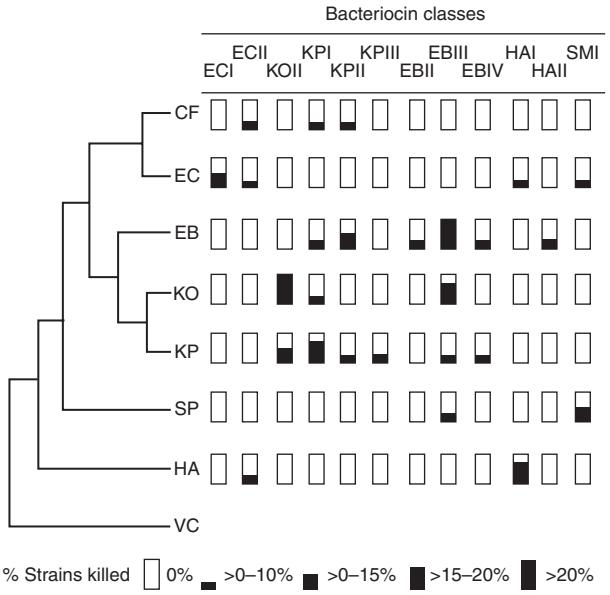

Figure 7 Phylogenetic breadth of bacteriocin killing. The killing spectrum of each class of bacteriocins was cross-referenced with a phylogenetic tree of the enteric species they were screened against. Heights of the black boxes are proportional to the percentage of strains sensitive to each class of 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*, and *pgi*) and 16s ribosomal sequences. The tree is rooted using *Vibrio cholera* as an outgroup. CF, *Citrobacter freundii*; EB, *Enterobacter cloacae*; EC, *Escherichia coli*; HA, *Hafnia alvei*; KO, *Klebsiella oxytoca*; KP, *Klebsiella pneumoniae*; SM, *Serratia marcescens*; VC, *Vibrio cholera*.

from the frequent horizontal transfer of resistance and the significant cost to colicin production. In other words, if a strain can gain resistance and lose production, they will, over a period of time – just as was observed in the *E. coli* isolated from the field mouse population over the course of a summer.

We assume bacteriocins play a role in mediating within-species (or population level) dynamics. This assumption is based on 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. Figure 7 reveals that 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 may play an equally compelling role in mediating both population-level and community-level interactions. A more 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 Used in Human Health

The rapid rise and spread of multiresistant bacterial pathogens have forced the consideration of alternative methods of combating infection. 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. Once resistance appears, it is simply a matter of time and the intensity of

human-mediated selection before human pathogens will acquire resistance.

Current solutions to this dilemma involve developing a more rational 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. 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.

The development and use of such narrow-spectrum antimicrobials not only increase the number of drugs on the pharmaceutical shelf but, more importantly, also extend 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 nonpathogenic species, that is, 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.

The Gastrointestinal (GI) tract is a complex ecosystem in which a delicate balance exists between the intestinal microflora and the host. The microflora serves as a primary stimulus for the development of the mucosal immune system. Two main genera of LAB dominate the intestinal flora, with 56 species of *Lactobacillus* and numerous *Bifidobacterium*, most of which produce bacteriocins *in vitro*. Recent work has shown that at least some of these strains also produce bacteriocins *in vivo*. For example, *Lactobacillus salivarius* strain UCC118 produces a potent broad-spectrum bacteriocin, Abp118, which is active *in vivo* against the food-borne pathogen *Listeria monocytogenes*. When tested in mice infected with the pathogen, the bacteriocin-producing strain provided protection against infection, while a mutant, impaired in its production ability, did not. Furthermore, the bacteriocin-producing strain provided no protection to mice infected with a strain of *L. monocytogenes* expressing the cognate Abp118 immunity protein.

Additional evidence in favor of the proposed beneficial role of bacteriocin-producing probiotic bacteria (PB) is less direct. A strain of *Lactobacillus casei* was shown to significantly inhibit both an enterohaemorragic strain of *E. coli* and one of *L. monocytogenes*, probably due to bacteriocin production. This potential probiont was further demonstrated to survive in a sustained transient state in the GI tract of mice. The release of bacteriocins with anti-*Helicobacter pylori* activity has been chiefly studied in *Lactobacilli* strains. Bacteriocin-like inhibitory substance (BLIS) with anti-*H. pylori* activity were identified in probiotic strains of *Lactobacillus johnsonii* and *Lactobacillus acidophilus*. The latter inhibits both urease activity and pathogen growth. In both cases the inhibitory activity was retained when *H. pylori* was bound to intestinal epithelial cells. Oral treatment of *L. acidophilus* in mice protected the animals from infection with *Hemobartonellosis felis*. This probiont was further shown to inhibit gastric colonization and prevent the development of gastric inflammation. Administration of *L. johnsonii* supernatant to adult patients colonized by *H. pylori* significantly decreased infection, while oral consumption of live bacteria by school children, found to be *H. pylori* positive, proved to moderately, yet significantly, decrease urease production. Mutacin B-Ny266, a lantibiotic produced by *Streptococcus mutans*, was recently shown to inhibit a broad spectrum of multi-resistant pathogens including staphylococci, streptococci, and *Neisseria* strains. It was also shown to be active *in vivo* against the infection of the methicillin-resistant *Staphylococcus aureus* in a mouse model.

Compared to those in class I, most class IIa bacteriocins have relatively narrow killing spectra and only inhibit closely related Gram-positive bacteria. There are exceptions, such as pediocin, which has a fairly broad inhibitory spectrum and can inhibit less closely related Gram-positive bacteria such as *S. aureus* and vegetative cells of *Clostridium* spp. and *Bacillus* spp. and is generally active against *Listeria*. A pediocin-producing strain of *Pediococcus acidilactici* able to survive in the GI tract was recently isolated and is an effective inhibitor of several pathogenic Gram-positive bacteria, such as *Enterococcus* spp., including the vancomycin-resistant strains, and *L. monocytogenes*. Furthermore, it inhibited gastric adhesion of pathogenic Gram-negative strains from the genera *Klebsiella, Pseudomonas*, and *Shigella*.

One weakness of the bacteriocins produced by Gram-positive bacteria, with respect to their use in probiotic applications, is that they do not inhibit the commonly encountered enteropathogenic bacteria such as *Enterobacter, Klebsiella*, or *Salmonella*. However, Gram-negative bacteriocins can accomplish this task. For example, *E. coli* H22 inhibited the growth of seven genera of the family *Enterobacteriaceae* (*Enterobacter, Escherichia, Klebsiella, Morganella, Salmonella, Shigella*, and *Yersinia*). The observed inhibition activity was attributed to the production of microcin C7 and colicins E1 and Ib, as well as aerobin and an unidentified phage. Simultaneous administration of the probiont and the enetric pathogen *Shigella flexneri* to germfree mice resulted

in a strong inhibition of the pathogen, which was attributed to its microcin production. A more widely used enteric probiont is *E. coli* strain Nissle 1917 that was shown to produce microcins H47 and M as well as colicin H. This strain was successfully used in clinical trails treating acute diarrhea in infants caused by enteric pathogens.

Streptococci, in particular *S. mutans* and *Streptococcus salivarius*, are considered the principal etiological agents of dental caries in humans. *S. mutans* has been reported to produce mutacins active against neighboring plaque-forming strains and a positive correlation exists between mutacin 1140 production and the ability of a strain to colonize the oral cavity. A nonpathogenic mutacin-producing strain was constructed for replacement therapy of dental caries, one that lacked one of the primary pathogenic traits, LDH production. Animal model studies showed the resulting strains to be significantly less pathogenic, yet stable, after colonization for 6 months. Initial human trails showed that the strain was apparently retained for 14 years following a single application and appeared to competitively exclude colonization by all other *S. mutans* strains. *S. salivarius* K12 produces two lantibiotics, salivaricin types A and B, that can treat infections of the upper respiratory tract caused by streptococcal organisms, including treatment of dental caries caused at least in part by *Streptococcus sobrinus* and *S. mutans*. Salivaricin B was demonstrated to successfully treat halitosis caused by *Prevotella* spp., *Eubacterium saburreum*, and *Micromonas micros*. A newly developed mouth spray consisting of the salivaricin-producing strain, *S. salivarius* K12, is marketed by BLIS technologies and claims to safely improve halitosis by restoring the 'normal' oral cavity microflora.

Streptococcus pyogenes is one of the most frequent pathogens of humans. It is estimated that between 5 and 15% of normal individuals harbor the bacterium, usually in the respiratory tract, without signs of disease. *S. pyogenes* can become pathogenic when host defenses are compromised or when the organism is able to penetrate the hosts defenses. When *S. pyogenes* is introduced or transmitted to vulnerable tissues, a variety of infections can occur, including pharyngitis (strep throat), scarlet fever, and skin infections. The ability of the normal flora of the upper airways to inhibit growth of potential pathogens *in vitro* has been well documented. In the oral cavity the presence of *S. salivarius* producing the bacteriocin salivaricin has been shown to reduce the frequency of acquisition of *S. pyogenes* in school children. Large populations of *S. salivarius* isolated from the nasopharynx of rarely infected children were found to produce bacteriocins with anti-*S. pyogenes* activity, as well as the ability to kill a range of other pathogens including *Moraxela catarrhalis* and *Haemophillus influenza*. Two potent anti-*S. pyogenes* bacteriocins, salivaricins A and B, were characterized and the producing strain, *S. salivarius* K12, was isolated for use as a dietary supplement. This strain is marketed as a throat guard spray that aims to 'assist in maintaining a healthy throat' and was shown to reduce throat infections in children.

Bacterial vaginosis, candidiasis, and trichomoniasis are common vaginal infections causing considerable discomfort to patients. Conventional antibiotic treatments often fail in the long run, due to the inability of a normal flora to re-establish following therapy. The normal vaginal microbiota is normally dominated by *Lactobacillus* spp., while the infected vagina is dominated by strains such as *Gardnerella vaginalis, Candida albicans, Propionibacterium, Lactobacillus, Streptococcus, Enterococcus*, and *Bacteroides* spp. Production of bacteriocins by probiotic *Lactobacilli* strains inhibits the growth of some of these infectious pathogens. *L. acidophilus* and *Lactobacillus jensenii* 5L08 showed antagonistic activity against *G. vaginalis*. BLIS produced by *Lactobacillus pentosus* and *L. jensenii* 5L08 inhibited the growth of *C. albicans*. *Lactobacillus penteus* strain NCIMB 41114 was patented for used as a probiotic agent that competitively excludes various species of *Candida*. The most promising vaginal probiont seems to be the vaginal isolate of *L. salivarius* CRL 1328 that was found to release a BLIS inhibiting the growth of *Enterococcus* and *Neisseria gonorrhoeae*. This strain was evaluated for pH, temperature, and culture medium impact on bacteriocin production, as well as viability after long-term storage using freeze dry and capsulation, all of which were found to have no apparent affect on bacteriocin synthesis.

Bacteriocins in Livestock Health

To maintain intestinal microflora balance in agricultural animals, it is important to control the overgrowth of potentially pathogenic bacteria. The exclusion of pathogenic bacteria is especially important in newly hatched broiler chickens owing to modern production methods whereby chicks have no contact with the maternal feces and thus receive none of the maternal antigens such that no active immune system can develop. Consequently, probiotic supplements are essential for safe poultry husbandry. *Salmonella* spp. may colonize poultry GI tracts without any deleterious effects on the birds, yet, upon consumption, human may experience severe intestinal diseases. A promising probiont is the enterocin A-producing strain *Enterococcus faecium* EK13 with antagonistic activity against *Salmonella dusseldorf* SA13 in gnotobiotic Japanese quails. Further preventative effects against *Salmonella pullorum* were reported when the bacteriocin producing *E. faecium* J96 was introduced to young broiler chicks. Microcin 24, produced by *E. coli*, holds promise in the prevention of *Salmonella typhimurium* contamination in adult chickens. Wooley and colleagues transformed plasmids containing microcin 24 gene

fragments into a nonpathogenic avian *E. coli* strain. Addition of the recombinant probiont to the drinking water of chickens significantly reduced their intestinal *S. typhimurium* load.

In cattle, the cow's rumen serves as a major reservoir for *E. coli* O157:H7, a pathogen that is difficult to control using antibiotics. In fact, studies have shown that antibiotic therapy increases the amount of shiga toxin released by this pathogen, resulting in higher levels of bacterial virulence. Recently, there have been reports that administration of colicin-producing bacteria into the rumen of cows can reduce the level of enteric pathogens in the animal. For example, *E. coli* O157:H7 cells could not be detected in most calves treated with colicin-producing *E. coli* strains. A second study employed three colicin-producing strains administrated to infected adult cattle and yielded similarly efficacious results. Colicin E7 has been shown to be the one colicin capable of consistently inhibiting the infectious strains. A colicin E7-producing strain was shown to reduce the colonization of *E. coli* pathogenic strains in treated calves upon inoculation. An additional promising strain is the microcin B17-producing strain *E. coli* Nissle 1917 that was able to reduce by half the incidence of calf diarrhea. A mixture of *L. acidophilus* and *Propionibacterium freudenreichii* also reduced *E. coli* O157:H7 colonization in cattle and is currently being marketed as a probiotic under the name of Bovamine.

There is growing interest in producing rabbit meat, as it requires less land, the animals are highly fertile and the meat provides a good protein source low in fat and cholesterol. However, young rabbits are susceptible to infectious agents such as *E. coli* and *Clostridia*. LAB are rarely found in rabbits but *Enterococci* are prevalent in their GI tract. *E. faecium* EK13 is an enterocin A-producing strain with probiotic properties that was found to persistently colonize the rabbit GI tract with an apparent effect on its microflora, reducing colonization of pathogenic staphylococci spp.

Aquatic cultures are continuously exposed to a wide range of microorganisms, some pathogenic. Efforts to prevent and control invasion by disease-causing agents have concentrated on good husbandry and the use of vaccines and antibiotics. These methods can result in an improvement of the organism's immunity by reducing stress but cannot prevent disease outbreak. The use of vaccines is laborious, costly, and highly stressful to the animals. The use of antibiotics will result in the selection for antibiotic-resistant bacteria and the residues of the drugs remain active long after use either as free unused antibiotic or extracted from the water by the cultured animals.

An alternative approach to disease prevention in aquaculture is the use of bacteriocin-producing PB. The definition of PB for aquaculture includes the naturally occurring microbial organisms found in the animal as well as in the aquatic environment. Administration of PB into water was reported to improve water quality by controlling organic matter and algae bloom. Prolonged administration of PB has the potential to serve as an efficacious long-term solution, as the administered bacteria become established in the host and/or the aquatic environment. Early attempts at the use of probiotic species in aquaculture usually employed PB developed for terrestrial animals, which contained the facultative or obligate Gram-positive anaerobes found in the GI tract, specifically of the genera *Bifidobacterium, Lactobacillus*, and *Streptococcus*. Production of PB specifically for the use in aquaculture is now a more popular approach, as these strains are more likely to establish themselves in the aquatic communities.

Often a dominant strain of the Gram-negative facultative anaerobes *Vibrio* and *Pseudomonas* are found in crustaceans, bivalves, and marine fish, while *Aeromonas, Plesiomonas*, and *Enterobacteriaceae* dominate the freshwater environment. Dietary and water enrichment with commercial PB ('Alchem Poseidon': a mixture of *Bacillus subtilis, L. acidophilus, Clostridium butyricum*, and *Saccharomyces cerevisiae*) administered to Japanese flounder (*Phalacrocorax olivaceus*) significantly enhanced lysozyme activity, lowered amounts of protein in mucus, and also improved survival after bacterial immersion challenge with *Vibrio anguillarum*. Previously these bacterial species were shown to produce potent bacteriocins: bacillocin 22 from *B. subtilis*, lacticin B from *L. acidophilus*, and butyricin 7423 from *C. butyricum*. It is likely that these toxins play a role in control of opportunistic pathogenic bacteria.

Two PB strains (*Carnobacterium maltaromaticum* B26 and *Carnobacterium divergens* B33) were isolated from the intestine of rainbow trout. These strains were selected for their activity against the bacterial fish pathogens *Aeromonas salmonicida* and *Yersinia ruckeri* and their safe application to the trout pond. Addition of *A. salmonicida* to a pond resulted in a 60% increase in fish survival. *Aeromonas media* strain A199 producing several BLIS was shown to control infection by *Vibrio tubiashii* in pacific oyster larvae (*Carnobacterium gigas*) and reduce saprolegniosis-related mortality in eels by 17%. Cultures of *Aeromonas hydrophila* and *Vibrio fluvialis* were shown to be effective at controlling infections by *A. salmonicida* in rainbow trout. In addition, Ruiz-Ponte found that BLIS-producing *Roseobacter* sp. BS 107 cell extract can inhibit the pathogenic affect of *Vibrio* spp., resulting in the enhanced survival of scallop larvae.

Bacteriocins and Food Preservation

The only bacteriocins currently employed in food preservation are those produced by LAB used in the

production of fermented foods. Because LAB have been used for centuries to ferment foods, they enjoy GRAS (generally regarded as safe) status by the US Food and Drug Administration (FDA). This permits their use in fermented foods without additional regulatory approval.

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. 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 US foods. Over the past decade the recurrence of listeriosis outbreaks, combined with the natural resistance of the causative agent, *L. 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. This attention has resulted in the isolation of a large number of class IIa bacteriocins, all of which are highly active against *L. monocytogenes*.

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. A gelatin form of pediocin, a class IIa bacteriocin made by lactic acid-producing bacteria, that protects hot dogs from *Listeria* contamination has been produced. A pediocin-producing bacteria was added to sausage and found to reduce *Listeria* numbers to fewer than one in ten thousand of the original number in untreated sausage. Equally compelling, active pediocin was found in the sausage after two months of refrigeration. *Listeria* has been targeted with piscicolin, a bacteriocin from yet another lactic acid-producing bacterium. 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.

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. Treatment with a combination of bacteriocins, for instance nisin and a class IIa bacteriocin, would theoretically reduce the incidence of resistance. There is currently conflicting evidence as to whether resistance to one class of LAB bacteriocins can result in cross-resistance to another class.

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. 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 are limited only by our imagination.

See also: Antibiotic Resistance; Antibiotic Production; Cyanobacterial Toxins; Ecology, Microbial; Exotoxins; Food Webs, Microbial; Bacteriophage Therapy: Past and Present; Plasmids, Bacterial

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