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Bacteriocin diversity: ecological and evolutionary perspectives

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Abstract

The bacteriocin family is the most abundant and diverse group of bacterial defense systems. Bacteriocins range from the well-studied narrow spectrum, high molecular weight colicins produced by *Escherichia coli* and the short polypeptide lantibiotics of lactic acid bacteria to the relatively unknown halocins produced almost universally by the haolobacteria. The abundance and diversity of this potent arsenal of weapons is clear. Less clear is 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 best-characterized family of bacteriocins, the colicins. 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.

Keywords: Bacteriocin; Colicin; Evolution; Ecology; Resistance

1. Introduction

Bacteriocins are loosely defined as biologically active protein moieties with a bacteriocidal mode of action [1,2]. The family includes a diversity of proteins in terms of size, microbial targets, modes of action and immunity mechanisms. They 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 within a species tens or even hundreds of different kinds of bacteriocins are produced [2,3]. According to Klaenhammer, 99% of all bacteria may make at least one bacteriocin and the only reason more have not been isolated is that very few researchers have looked for them [4]. It is clear that microbes invest considerable energy into the production and elaboration of these antimicrobial mechanisms. Less clear is how such diversity arose and what roles these biological weapons serve in microbial communities.

One family of bacteriocins, the colicins, has successfully served as a model for exploring such evolutionary and ecological questions. In this review, current knowledge of how the extraordinary range of colicin diversity arose and is maintained in microbial populations will be assessed and the role these toxins play in mediating microbial dynamics will be discussed.

The most extensively studied bacteriocins, the colicins produced by *Escherichia coli*, share certain key characteristics [5–11]. Colicin gene clusters are encoded on plasmids and are comprised of a colicin gene, which encodes the toxin, an immunity gene, which encodes a protein conferring specific immunity to the producer cell, and a lysis gene, which encodes a protein involved in colicin release from the 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

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449 to 629 amino acids. Nuclease bacteriocins have an even broader size range, from 178 to 777 amino acids.

Although colicins are representatives 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 [12]. The nuclease pyocins of *Pseudomonas aeruginosa*, which share a recent ancestry with colicins, and other, as yet uncharacterized, bacteriocins are found exclusively on the chromosome [13]. Another close relative to the colicin family, the bacteriocins of *Serratia marcesens* are found on both plasmids and chromosomes [14–16].

Many bacteriocins isolated from Gram-negative bacteria appear to have been created by recombination between existing bacteriocins [6,17–19]. 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 (~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 share a similar domain structure, the order of the translocation and receptor recognition domains are switched [20]. As we shall explore further below, the conserved domain configuration of these toxins is responsible for much of the bacteriocin diversity we find in nature.

2. 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 [21]. Studies that include DNA and protein sequence comparisons [6,18], surveys of DNA sequence polymorphism in natural isolates [22–24], experimental evolution [25,26] and mathematical modeling [25] have revealed two primary modes of colicin evolution [27].

2.1. The role of diversifying recombination in colicin evolution

The more abundant pore former colicins are generated by domain shuffling, which is mediated by recombination [6,27]. All characterized pore former colicin proteins share one or more regions with high levels of sequence similarity to other pore former colicins (Fig. 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

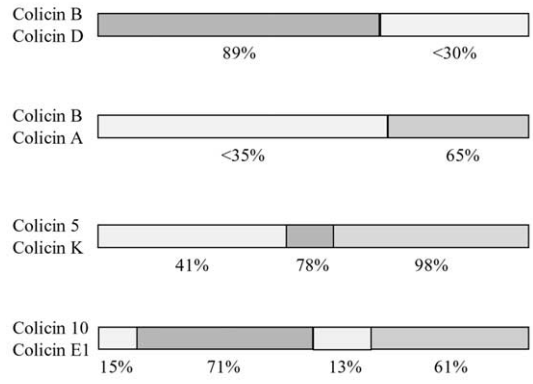

Fig. 1. Pairwise comparisons of pore forming colicin protein sequences. Values below each comparison indicate the percent sequence identity for the region indicated. Colicin proteins are not drawn to scale.

diversifying recombination is seen in the first published klebicin sequence (Fig. 2), which is a nuclease klebicin that shares sequence similarity with both colicin A-like pore former and pyocin S1-like nuclease sequences [28–30]. 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 [13,29]. Even altering the domain structure of the protein, as is seen for pyocins which have switched the receptor recognition and translocation domains relative to the order found in colicins, has not limited the influence of diversifying recombination.

2.2. 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 (Fig. 3). To explain this pattern of divergence, Riley and collaborators have proposed a two-step process of mutation and selection [18,27,31].

The diversifying selection hypothesis posits the action of strong positive selection acting on mutations that generate novel immunity and killing functions (Fig. 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

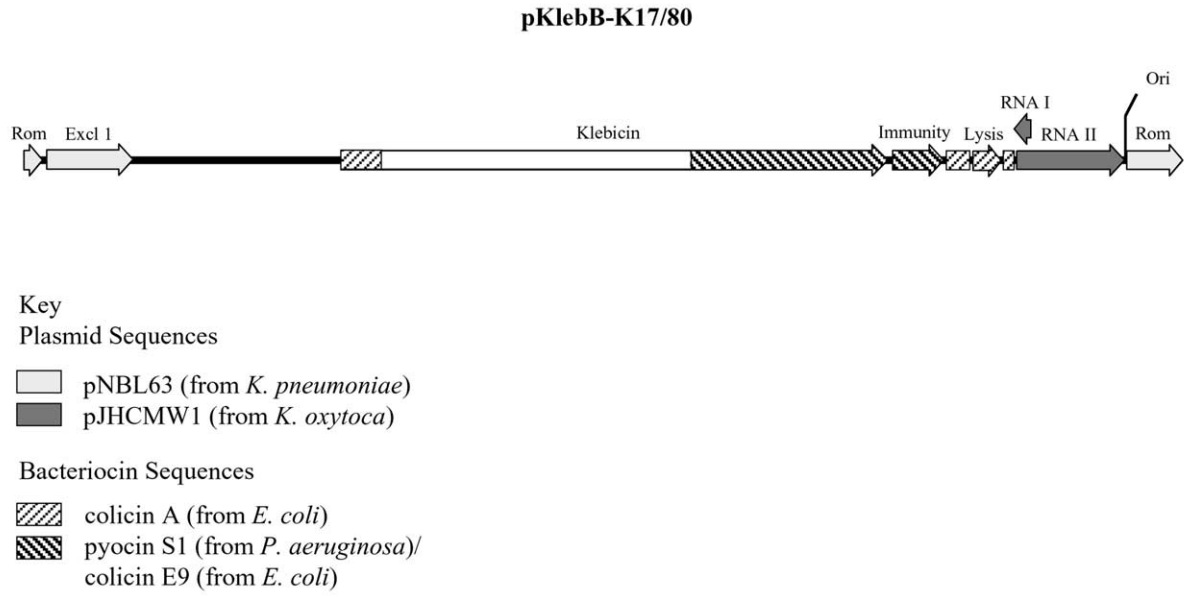

Fig. 2. Patterns of sequence similarity in klebicins suggest recombination. The chimeric nature of the pKlebB plasmid sequence is indicated by alternate shadings. The key notes regions of sequence similarity with other bacteriocin gene clusters and plasmids. pKlebB illustrates a pattern typical of other 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.

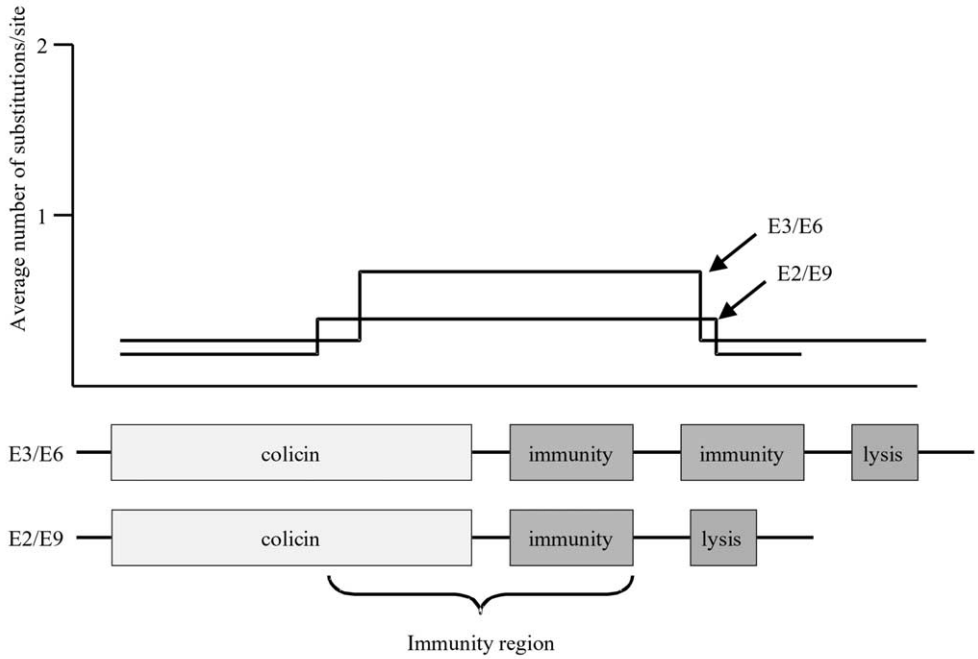

Fig. 3. The graph indicates the average number of total nucleotide substitutions between pairs of nuclease type colicin gene clusters (colicin pairs E2/E9 and E3/E6). Most of the divergence between colicins occurs in the immunity region of the gene cluster (comprised of the immunity gene and the immunity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

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 [3,32]. 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 [31].

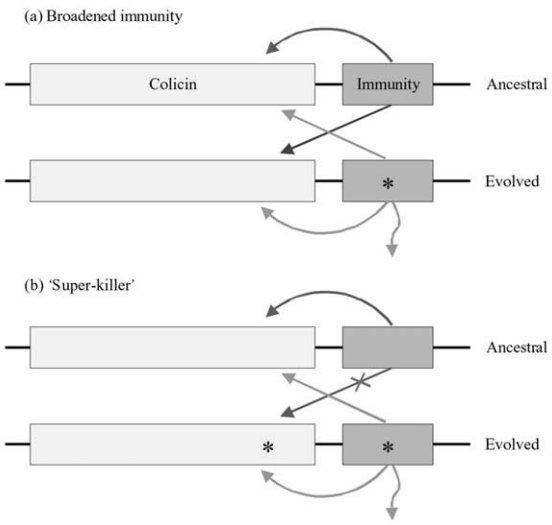

Fig. 4. The hypothesis of diversifying selection invokes two steps in the generation of a novel immunity function. (a) A point mutation in the immunity gene generates a broadened immunity function (noted with an asterisk). The strain with this colicin gene cluster is immune to self, to its ancestor,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

Recently, the DNA sequence of a new pore former colicin, Y, was determined [33]. Colicin Y is a close relative of colicin U, another pore former colicin isolated from a different continent and over 20 years earlier [34]. 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 [24]. 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.

2.3. A two-step process of colicin evolution

Riley has developed a model of colicin diversification that involves two phases [21]. 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 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 currently 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.

We have only just begun to tap 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 [28] (M.A. Riley et al., unpublished information). For still other enteric bacteriocins, the action of diversifying selection has been proposed (M.A. Riley et al., 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 [35]. 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, like pColJs (which encodes colicin Js) and pKlebB (which encodes klebicin B), demonstrate another aspect of bacteriocin evolution [28,35]. 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 [36] and pJHC-MW1 [37], 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* [28,30,38]. 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* [39,40]. 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 [35], perhaps even outside of the Enterobacteriaceae. This type of recombination, while 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.

3. 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, is that role?

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

4. Theoretical and experimental studies of bacteriocin ecology

Early experimental studies on the ecological role of bacteriocins were inconclusive and contradictory [42–48]. 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 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 [49]. 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, goes 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.

4.1. 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 [50,51]. 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 [52]. Owing to this third member, pair-wise interactions among the strains have the non-transitive 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, 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 [50].

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 [50]. 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 very 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

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

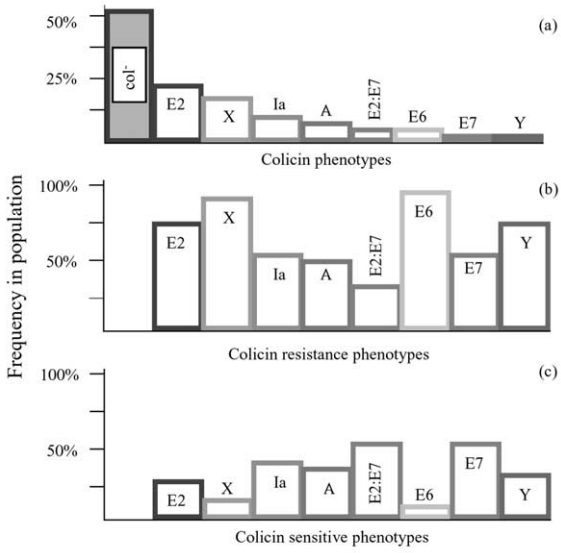

Fig. 5. A survey of colicin production and resistance in *E. coli*. Over 400 strains were isolated from two populations of feral mice in Australia over a period of 7 months. The isolates were scored for colicin production and resistance. (a) Colicin production is abundant with just under 50% of the strains producing eight distinct colicin types. Col⁻ represents non-producer strains. (b) The majority of isolates are resistant to most co-occurring colicins. (c) A small proportion of the population is sensitive to co-occurring colicins.

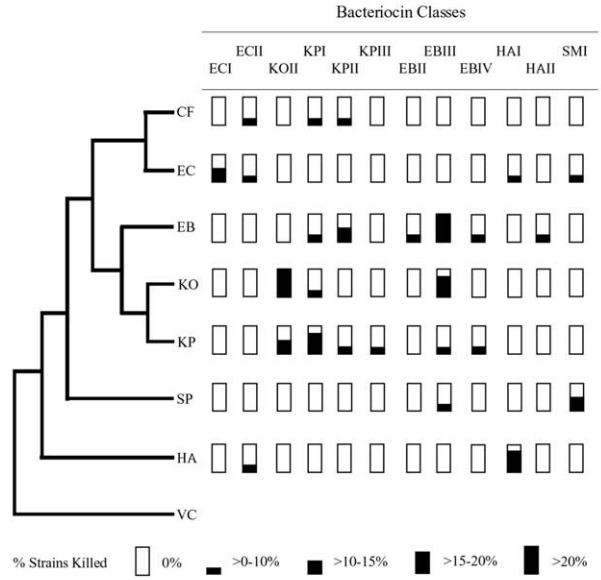

Fig. 6. Phylogenetic breadth of bacteriocin killing. The killing spectrum of each class of bacteriocins was cross-referenced with a phylogenetic tree of the enteric species they were screened against. Heights of the black boxes are proportional to the percentage of strains sensitive to each class of 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 cholera* as an outgroup. Abbreviations are: KO = *Klebsiella oxytoca*, KP = *Klebsiella pneumoniae*, EB = *Enterobacter cloacae*, CF = *Citrobacter freundii*, EC = *Escherichia coli*, SM = *Serratia marcescens*, HA = *Hafnia alvei*, VC = *Vibrio cholera*.

match predictions of the Czárán model [3,32]. 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. Fig. 5 provides a summary of phenotype distributions in a population of *E. coli* isolated from wild field mice in Australia [32]. The Czárán model predicts this distribution of phenotypes results from frequent horizontal transfer of resistance, and the significant cost to colicin production [50]. In other words, if a strain can gain resistance and lose production, they will over time—just as was observed in the *E. coli* isolated from the field mouse population over the course of a summer [32].

4.2. 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 non-producers isolated from the same sources (M.A. Riley et al., unpublished information). Fig. 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. Similar killing patterns have been recently reported for halocins screened against halobacterial strains as well [54]. 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 thorough understanding of how bacteriocins function in the environment 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.

Although colicins have been used as a model system for investigating the ecology of bacteriocins in general, it must be pointed out that they differ from most other bacteriocins in ecologically and evolutionarily significant ways. If we compare them to other bacteriocins, for instance the lantibiotics produced by Gram-positive bacteria, they differ in almost every aspect of their physiology. Gram-positive bacteriocins in general and lantibiotics specifically, have a very broad killing range compared to colicins, even dem-

onstrating efficacy against Gram-negative bacteria [55]. Gram-positive bacteriocins are also secreted from the producing cell rather than released through cell lysis as in colicins, thus they may suffer a lower fitness cost associated with toxin production compared to colicins [56]. Regulation of the expression of Gram-positive bacteriocins is typically under growth phase and/or quorum sensing regulatory control [57,58]. Finally, the genetic organization of Gram-positive bacteriocins is much different from that seen in colicins. There are typically eight to 12 genes in a Gram-positive bacteriocin gene cluster compared to the two or three required for colicins. In addition to the toxin and immunity genes, Gram-positive bacteriocin gene clusters contain genes controlling post translational modification of the toxin, regulatory and export genes [56,59]. Gram-positive bacteriocin toxin genes also lack the clearly defined domain structure on which the current models of colicin evolution are largely based. Clearly, colicins represent a distinct subgroup of bacteriocins. Further study will be required to determine if ecological and evolutionary models based on colicins apply more broadly to other groups of bacteriocins.

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