# 2 The Diversity of Bacteriocins in Gram-Negative Bacteria

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## Summary

The frequency and diversity of bacteriocin production varies greatly among bacterial populations. The dynamic interactions occurring among bacteriocin-producing, sensitive and resistant cells are likely responsible for much of this variation. However, the frequency of bacteriocinogeny and the diversity of bacteriocins produced are also determined by the habitat in which the population lives and by the genomic background of the producing strains. The production by a cell of two or more bacteriocins is a common phenomenon, at least in *Escherichia coli*. Further research is required if we are to understand the nature of fitness advantages accruing from multiple bacteriocin production, and how to best exploit bacteriocins as replacements for traditional antibiotics and in the creation and selection of bacterial strains for use as probiotics.

## 2.1 Introduction

Allelopathy is the production of chemical compounds which are toxic to other organisms but not to the producers of these compounds. For microorganisms, there is a wealth of data demonstrating that allelopathy is an important mediator of intra- and inter-specific interactions and consequently, a significant factor in maintaining microbial biodiversity (Chap. 6, this volume). In bacteria, these allelopathic substances include metabolic by-products such as ammonia or hydrogen peroxide; the 'classical' antibiotics such as bacitracin and polymyxin B, lysozyme-like bacteriolytic enzymes and the bacteriocins.

The bacteriocins produced by Gram-negative bacteria are diverse. Over 30 bacteriocins from *Escherichia coli* have been identified and, undoubtedly, more have yet to be discovered. The diversity present in other Gram-negative species, including other members of the Enterobacteriaceae, is largely unexplored. The molecular mechanisms by which this diversity has arisen, at least

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for that class of bacteriocins known as colicins, is well understood and is discussed elsewhere (Chap. 3, this volume). However, the factors influencing the frequency of bacteriocin production and the diversity of bacteriocins in populations of bacteria are largely unknown. Acquiring this knowledge is essential, not only if we are to understand the role bacteriocins play in shaping bacterial communities in natural environments but also because there is an increasing desire to exploit bacteriocins to solve a range of applied problems.

As a consequence of the rising incidence of resistance to most traditional antibiotics, numerous research programs have been implemented aiming to explore the potential role which naturally produced and genetically modified bacteriocins might have as replacements for traditional antibiotics (Gillor et al. 2004). Other efforts focus on the use of antimicrobial toxins as food preservatives (Gillor et al. 2004). There is also an ever-increasing interest in the use of bacteria as biocontrol agents for the management of fungal and bacterial plant pathogens and, more recently, as the active agent in probiotic formulations. Probiotic therapy is a disease prevention strategy used in humans and domesticated animals, as well as a procedure considered to enhance the growth rate of livestock and poultry. The basis of the method is to ensure the establishment of 'good' bacteria in the gastro-intestinal tract in order to prevent the establishment of bacterial pathogens. One of the most important attributes of a 'good' probiotic strain is thought to be the strain's ability to produce antimicrobial compounds. However, the successful use of bacteria as biocontrol agents will require a sound understanding of microbial ecology and the factors influencing the frequency of bacteriocin production and diversity in populations of bacteria. Thus, the aim of this chapter is not to describe the diversity of bacteriocins which have been characterised from Gram-negative bacteria but, rather, to identify and discuss some of the factors observed to influence bacteriocin diversity. As usual, much of the data relates to bacteriocin production in *E. coli* but reference will be made also to observations concerning other species of the Enterobacteriaceae. The material presented has largely not been published previously, and is based on phenotypic and genotypic surveys of bacteriocin production in three collections of *E. coli*. The first of these collections consists of 496 isolates taken from a variety of mammal species living throughout Australia. The methods used to isolate and characterise the strains in this collection have been described by Gordon and Cowling (2003). The second collection consists of 266 faecal isolates recovered from people living in the Canberra ACT region of Australia, the isolation and characterization of these strains having been described by Gordon et al. (2005). In addition to the human faecal isolates, the collection contains 353 isolates recovered from extra-intestinal body sites of people. The third dataset was collected and characterised using the same methods as those used for the other two, and consists of 208 strains recovered from soil, water and sediment samples from a variety of localities across Australia. All strains in the three collections have been screened using

a PCR-based approach for the presence of 29 virulence-associated traits and to determine their *E. coli* group membership (A, B1, B2, D), as described by Gordon et al. (2005). The majority of the strains have been screened using a combination of phenotypic and genotypic approaches in order to determine the frequency of bacteriocin production and the types of bacteriocins present in these strains, using the methods described by Gordon and O'Brien (2006).

## 2.2 The Frequency of Bacteriocin Production

### 2.2.1 Colicins

Typically, a third of *E. coli* strains produces a mitomycin C-inducible bacteriocin (Riley and Gordon 1996). For example, 24% of the *E. coli* isolates from humans and 33% of the isolates from mammals were colicin producers. Although there have been relatively few representative surveys of other members of the Gram-negative bacteria, similar frequencies of production are observed in these studies (Reeves 1972; Riley et al. 2003). However, the prevalence of colicinogenic strains may vary from 10 to over 70% among different *E. coli* populations (Riley and Gordon 1996; Gordon et al. 1998). Much of the variation in the frequency of colicinogenic strains in populations of *E. coli* is undoubtedly due to the dynamic interactions occurring between colicin-producing, resistant and sensitive cells (Riley and Gordon 1999), which result in temporal fluctuations in the relative frequencies of these three phenotypes. Such temporal fluctuations have been observed in vitro (Kerr et al. 2002) and in a population of *E. coli* isolated from house mice, in which the frequency of colicinogenic isolates declined from 71 to 43% over a 7-month period (Gordon et al. 1998).

Although the frequency of colicinogenic strains in a population is expected to vary naturally, theoretical considerations and empirical observation suggest that the environment in which the cells live also influences the frequency of colicinogenic cells. The predictions of mathematical models indicate that colicin-producing cells will have an advantage in benign habitats (resulting in high population growth rates) whereas harsher habitats will favour non-producers (Frank 1994). There is some empirical evidence to support this prediction. Population growth rates and cell densities are significantly lower in the external environment than in the lower intestine of mammals and, in Australia, 9% of *E. coli* isolated from the environment were producers, a significantly lower proportion than the 30% observed for Australian faecal isolates. The frequency of colicin-producing *E. coli* also depends on the type of host from which the cells are isolated. In Australian mammalian carnivores, the frequency of colicin-producing strains is about 20% whereas colicin production is twice as frequent in strains isolated from herbivorous or omnivorous Australian mammals. For animals of similar body mass, the

turnover rate of the carnivore gastro-intestinal tract is significantly faster than that recorded in herbivorous or omnivorous mammals (Hume 1999). The predictions of mathematical models support the observation that the cost of colicin production can result in colicinogenic strains being disadvantaged when living in hosts with high gut turnover rates (unpublished data). There is additional evidence for the importance of the host environment in determining the likelihood that a strain will be colicinogenic. There are two species in the genus *Hafnia* (Okada and Gordon 2003; Janda et al. 2005). A collection of *Hafnia* species 2 strains, isolated from fish, reptiles and mammals from across Australia, was screened for the presence of a bacteriocinogenic phenotype. The results of this screening showed that 4% of the isolates from fish, 64% of the isolates from reptiles and 29% of the isolates from mammals were bacteriocin producers.

Faecal isolates of *E. coli* can be assigned to one of four main genetic groups (subspecies), designated A, B1, B2 and D (Ochman and Selander 1984; Herzer et al. 1990). Strains of the four groups appear to occupy different ecological niches (Gordon and Cowling 2003; Gordon et al. 2005). For *E. coli*, it is well established that some traits, particularly virulence factors associated with extra-intestinal disease, are largely confined to particular genetic groups (Johnson and Stell 2000). Genetic group membership also appears to predict the frequency of colicin production. Among the Australian mammal isolates, 46% of genetic group B2 strains produce a colicin, compared to only 23–27% of strains in the genetic groups A, B1 or D. The reasons for these differences are unknown.

### 2.2.2 Microcins

There has been far less work on the frequency of microcin production in *E. coli* or other Gram-negative bacteria. All the microcins characterised to date are secreted from the cell, rather than being released as a consequence of cell lysis (Braun et al. 2002). It has also been suggested that as much as 90% of the microcin produced by a cell may be retained within the cell (Braun et al. 2002). Consequently, there is no reliable and simple phenotypic method for assaying microcin production.

The *E. coli* isolated from Australian humans and mammals were screened for seven microcins, using a PCR-based approach. Of the human isolates screened, 32% were microcin producers whereas, among the isolates from mammals, 9% were microcin producers. Why microcin production is less common in *E. coli* isolated from mammals compared to humans is unknown. In the isolates from humans, microcin production is significantly more prevalent among genetic group B2 strains (47%) than among A (16%), B1 (18%) or D strains (9%). The frequency of microcin production in both of these *E. coli* collections has almost certainly been underestimated, as a PCR-screening approach can be used only for those microcins which have been genetically characterised.

## 2.3 Bacteriocin Diversity

### 2.3.1 Colicins

Surveys of colicin diversity in different collections of *E. coli* all give rise to similar results – only a small fraction of the known colicins are present in a given collection and, in general, different colicins are detected in different collections (Riley and Gordon 1996; Table 2.1). There are a few exceptions to this general trend – colicins E1 and Ia are often observed (Riley and Gordon 1996; Table 2.1). Colicin Ia is encoded on a conjugative plasmid and therefore is able to transfer among *E. coli* lineages, so the fact that it is one of the more commonly observed colicins could be expected. However, colicin B is also borne on a conjugative plasmid and is the most common colicin produced by the isolates from mammals, yet it is uncommon in human isolates (Table 2.1).

The available data also suggest that a single colicin type dominates in any particular population of *E. coli*. This observation is expected, based on our understanding of the dynamics of colicin-producing, resistant and sensitive cells. As the frequency of cells resistant to the dominant colicin type in a population increases, that of the dominant producer population will decline, thereby providing the opportunity for the invasion of a different type of producer to which the fraction resistant to the original colicin is susceptible. Thus, theory predicts that there should be a continual flux in the relative frequency of different colicin types in a population of *E. coli*. There is some

**Table 2.1** The frequency of colicin types in three collections of *Escherichia coli* from Australia

| Colicin type | Human isolates % Frequency | Mammal isolates % Frequency | Environmental isolates % Frequency |
|---|---|---|---|
| A | 0 | 0 | 0 |
| B | 1.3 | 10.8 | 3.8 |
| D | 0.2 | 1.2 | 0 |
| E1 | 8.9 | 3.6 | - |
| E2 | 0.6 | 0 | - |
| E6 | 0 | 0 | - |
| E7 | 1.6 | 3.0 | - |
| Ia | 9.9 | 10.7 | 1.0 |
| Ib | 0.5 | 0 | 0 |
| K | 1.6 | 0 | 0 |
| M | 3.9 | 13.3 | 4.8 |
| ?[a] | 9.7 | 25.0 | 7.2 |

[a]Identity of the colicin produced was not determined

evidence to support this prediction. Colicin E2 is rare among strains in the collection of *E. coli* recovered from Australian mammals (Table 2.1). However, in a study of *E. coli* isolated from a single Australian population of house mice, 25% of the colicinogenic strains produced colicin E2. Over a 7-month period, there was a significant decline in the frequency of E2 and a concomitant increase in the frequency of resistance to E2 (Gordon et al. 1998). Additional evidence comes from the distribution of colicin D. Overall, colicin D is rare in *E. coli* isolated in Australia (Table 2.1) but, among mammals, colicin D was detected only from a single population of mountain brush-tailed possum (*Trichosurus canis*).

The ecological niche of a bacterial strain may also play a role in determining bacteriocin diversity. Two new bacteriocins, alvecin A and B (Wertz and Riley 2004), have recently been described from genomic species 2 of the genus *Hafnia*. PCR screening for the presence of alvecins A and B revealed that these bacteriocins were most frequently produced by strains isolated from mammals but were not detected in bacteriocinogenic strains isolated from reptiles (unpublished data). The bacteriocinogenic isolates from reptiles appear to produce several novel, as yet uncharacterised bacteriocins which appear to be absent in isolates from mammals (unpublished data).

The frequency of bacteriocin production in *E. coli* varies depending on the genetic group membership of the producing strains, as does the type of bacteriocin produced by a strain. In the collection of isolates from mammals, colicin Ia is significantly more prevalent among group B2 strains (20%), less common in B1 strains (8%), uncommon in D strains (4%), and absent in group A strains. In the collection of isolates from humans, however, the frequency of colicin Ia producers is independent of a strain's *E. coli* group membership. In both the human and mammal *E. coli* collections, colicin E1 is significantly co-associated with K1, and a strain which is K1 positive is five times more likely to harbour the colicin E1 plasmid than if it is not.

### 2.3.2 Microcins

All of the seven microcins screened for were detected in *E. coli* isolated from humans and all, but one, were detected in the isolates from mammals (Table 2.2). As was the case for colicins, the distribution of a particular microcin varies based upon a strain's group membership. Microcin V is encoded on a conjugative plasmid and, in human isolates, its prevalence does not vary with a strain's *E. coli* group membership. However, V is not randomly distributed among *E. coli* genotypes. Thus, among the group B2 strains isolated from humans, microcin V is never detected in a strain encoding for either of the adhesins *focG* or *iha*, the toxin *hylA*, or the secreted protein *she*. Therefore, microcin V is absent from the 69% of the B2 strains which possess one or more of these traits but present in the 30% of the B2 strains which possess none of these traits.

**Table 2.2** The frequency of microcin types in two collections of *E. coli* from Australia

| Type | Human isolates % Frequency | Mammal isolates % Frequency |
|---|---|---|
| B17 | 1.8 | 3.8 |
| C7 | 0.8 | 2.1 |
| H47 | 21.5 | 3.8 |
| J25 | 1.9 | 0 |
| L | 0.8 | 1.7 |
| M | 18.1 | 2.8 |
| V | 8.7 | 1.4 |

## 2.4 Multiple Bacteriocin Production

We have a good empirical and theoretical understanding of the dynamics of colicin-producing, resistant and sensitive cell populations (Chaps. 6 and 7, this volume). However, most research has focused on populations of producing cells encoding only for a single bacteriocin type, together with cells either resistant or sensitive to the bacteriocin being produced. Theoretical work by Czaran et al. (2002) investigated the dynamics of a community of cells where multiple toxin types were being produced, together with cells which had different sensitivity and resistance profiles to these toxins. Numerical simulations of the model revealed two distinct quasi-equilibriums, which Czaran et al. (2002) termed the "frozen" and "hyper-immunity" states. In the frozen state, all toxins are present in the community but most cells produce only a single toxin to which it is immune. In the hyper-immunity state, most cells produce no toxin, many others produce a single toxin, some produce multiple toxins, and a few produce most of the toxins present in the community. In the latter state, virtually all cells are resistant to most of the bacteriocins present in the population. Which outcome – frozen or hyper-immunity – eventuates depends on initial conditions, recombination rate, and the costs associated with toxin production. The results of the screening of the *E. coli* isolates from Australian human and mammals revealed that multiple bacteriocin production is common. In the human isolates, 35% of the bacteriocinogenic strains produced a single bacteriocin, 46% produced two, 18% produced three, and 1% produced four or more bacteriocins. The production of multiple bacteriocins is also common for strains isolated from mammals, where 52% of the bacteriocinogenic strains produced one type of bacteriocin, 30% produced two, 12% produced three, and 6% produced three or more different bacteriocins. Again, the genetic group membership of a strain influences the likelihood that a strain will produce multiple bacteriocins (Table 2.3). Among human isolates, group B2 strains are more likely to produce multiple

**Table 2.3** The frequency of multiple bacteriocin production by an *E. coli* strain with respect to the strain's genetic group membership in two collections of *E. coli* from Australia

| Collection | # of Bacteriocins | *E. coli* genetic group % of strains | | | |
|---|---|---|---|---|---|
| | | A | B1 | B2 | D |
| Human isolates | 1 | 57 | 60 | 26 | 54 |
| | 2 | 27 | 27 | 52 | 36 |
| | 3 or more | 16 | 13 | 22 | 10 |
| Mammal isolates | 1 | 30 | 77 | 45 | 45 |
| | 2 | 60 | 17 | 29 | 40 |
| | 3 or more | 10 | 6 | 26 | 15 |

bacteriocins compared to group A or B1 strains whereas, in the animal isolates, group A strains are most likely to be multiple producers. Over 40 different combinations of bacteriocins were observed among the *E. coli* strains isolated from Australian humans, and over 30 combinations from mammals (Table 2.4). The different bacteriocins observed in these two collections do not associate at random. A number of the bacteriocins co-occur at a significantly greater frequency than would be expected by chance; these include colicins Ia and E1, colicins B and M, microcins H47 and M, as well as colicin Ia and microcin V. Conversely, microcins H47 and V are significantly less likely to co-occur in a strain than would be expected by chance. One significant three-way association was detected: microcin J25 was most likely to be observed in a strain with colicin Ia and microcin V but not in strains encoding only Ia or V.

Of the combinations found to co-occur more frequently than expected by chance, all have the characteristic that at least one of the co-occurring bacteriocins is secreted by the cell, rather than released via cell lysis (Table 2.4). Few strains appeared to encode for two colicins which are also associated with lysis genes. Those that did – for example, colicins E2 and E7 – may represent chimeras. At least two examples of E2 and E7 chimeras have been reported which resulted from recombination of portions of the E2 and E7 colicin operons in a single plasmid and, in both cases, there is a single copy of the lysis gene (Tan and Riley 1997; Nandiwada et al. 2004). It may be that the co-occurrence of two colicins released via cell lysis imposes too high a cost to the cell due to the expression of two lysis genes.

## 2.5 Overview

For those colicins released via cell lysis, there is a wealth of mathematical theory (Levin 1988; Frank 1994; Durrett and Levin 1997) as well as in vitro (Chao and Levin 1981; Gordon and Riley 1999; Kerr et al. 2002) and

**Table 2.4** Combinations of bacteriocins detected in a single strain of *E. coli* in two Australian collections

| Human isolates Genotype | Frequency (%) | Mammal isolates Genotype | Frequency (%) |
|---|---|---|---|
| ?[a] | 4.6 | ? | 21.1 |
| B/cM[b] | 1.1 | B | 0.6 |
| B17 | 1.4 | B/cM | 19.3 |
| B17/? | 0.4 | B/cM/B17/L | 1.2 |
| D/K | 0.4 | B/cM/C7 | 0.6 |
| E1 | 4.9 | B/cM/E1 | 1.2 |
| E1/B/cM | 0.4 | B/cM/E1/H47/M | 0.6 |
| E1/B17 | 0.4 | B/cM/E1/H47/M/L | 0.6 |
| E1/E2 | 0.4 | B/cM/E7 | 0.6 |
| E1/K | 0.4 | B/cM/H47/M | 0.6 |
| E1/cM | 1.8 | B/cM/H47/M/L | 0.6 |
| E1/cM/B17 | 0.4 | B/cM/Ia | 4.1 |
| E2 | 0.4 | B/cM/Ia/E1/E7 | 0.6 |
| E7 | 2.5 | B/cM/Ia/E7/C7 | 0.6 |
| H47 | 5.3 | B/cM/V | 0.6 |
| H47/E1 | 1.1 | B17/? | 0.6 |
| H47/Ia | 0.4 | D | 2.9 |
| H47/Ia/V | 0.4 | D/C7 | 0.6 |
| H47/J25/E1 | 0.4 | E1 | 4.1 |
| H47/L | 0.4 | E1/B17 | 0.6 |
| H47/L/E1 | 0.4 | E1/B17/L | 0.6 |
| H47/M | 29.0 | E7 | 4.7 |
| H47/M/? | 1.8 | E7/H47/M | 0.6 |
| H47/M/B17 | 1.1 | H47/M/? | 1.8 |
| H47/M/C7 | 1.1 | Ia | 16.4 |
| H47/M/E1 | 3.2 | Ia/B17 | 0.6 |
| H47/M/E1/Ia/B17 | 0.4 | Ia/C7/B17 | 0.6 |
| H47/M/E7 | 0.7 | Ia/E1 | 1.2 |
| H47/M/Ia | 0.4 | Ia/E1/E7/C7/B17 | 0.6 |
| H47/M/K | 0.7 | Ia/E7 | 0.6 |
| H47/V | 0.4 | Ia/V | 1.8 |
| H47/V/E1 | 0.4 | Ia/V/C7 | 0.6 |
| Ia | 4.9 | V | 0.6 |
| Ia/B/cM | 0.7 | V/? | 0.6 |

(*Continued*)

**Table 2.4** Combinations of bacteriocins detected in a single strain of *E. coli* in two Australian collections—Continued

| Human isolates Genotype | Frequency (%) | Mammal isolates Genotype | Frequency (%) |
|---|---|---|---|
| Ia/E1 | 2.1 | cM | 1.8 |
| Ia/E1/cM | 0.4 | cM/? | 1.2 |
| Ia/V | 5.3 | cM/E1 | 0.6 |
| Ia/V/C7 | 0.4 | cM/E7 | 0.6 |
| Ia/V/E1 | 1.8 | cM/Ia | 2.3 |
| Ia/V/E2/E7 | 0.4 | cM/Ia/B17 | 1.2 |
| Ia/V/J25 | 2.5 | | |
| Ia/V/J25/E1 | 0.7 | | |
| Ia/V/K | 0.7 | | |
| Ia/V/L | 0.4 | | |
| Ib | 0.4 | | |
| Ib/V | 0.4 | | |
| Ib/cM | 0.4 | | |
| J25 | 0.4 | | |
| K | 1.1 | | |
| K/E2 | 0.4 | | |
| L | 0.7 | | |
| M | 0.7 | | |
| M/? | 0.4 | | |
| M/C7 | 0.4 | | |
| V | 4.6 | | |
| V/B/cM | 0.4 | | |
| V/E1 | 0.4 | | |
| V/J25 | 0.4 | | |
| cM | 3.2 | | |

[a]The question mark denotes an unidentified colicin producer
[b]Abbreviations: cM denotes colicin M, and M denotes microcin M

in vivo (Kirkup and Riley 2004) experimental evidence which demonstrates the potential importance of colicins in mediating intra-specific interactions, and the highly dynamic nature of these interactions. However, our current understanding of the dynamics of bacteriocin production is restricted to those colicins released via cell lysis. Cell lysis represents a significant cost to the colicin-producing population, in addition to the costs associated with

colicin plasmid carriage and colicin synthesis. The costs associated with colicin production are an important determinant of the fitness hierarchy among colicin-producing, resistant and sensitive cell populations (Riley and Gordon 1999; Kerr et al. 2002). This hierarchy is analogous to the game of Rock, Paper, Scissors, where producers out-compete sensitive cells, resistant cells out-compete producers, and sensitive cells out-compete resistant cells. Microcins are not released as a result of cell lysis, nor are a number of colicins such as B, Ia, Ib and M (Braun et al. 2002). The survey results suggest that the bacteriocins released through cell lysis represent a minority of the bacteriocins produced by *E. coli* (Riley and Gordon 1996). For example, of the bacteriocin-producing *E. coli* strains isolated from Australian humans, 71% do not encode for a bacteriocin released via lysis.

As suggested by Dykes and Hastings (1997), the fitness costs associated with producing a bacteriocin secreted from the cell may be significantly lower than the costs incurred by cells encoding for a bacteriocin released via cell lysis. If true, then one critical component of the Rock Paper Scissors scenario may be invalid for the secreted bacteriocins – that is, strains resistant to a secreted bacteriocin may not experience a universal fitness advantage when in competition with the strain producing a secreted bacteriocin. Although the resistant cells will be unaffected by the bacteriocin, the growth rate disadvantage which resistant cells suffer due to the loss or modification of an important surface receptor may be greater than the cost associated with producing a secreted bacteriocin. To date, no study has quantified the costs associated with producing a secreted bacteriocin. However, there is some evidence suggesting that the costs associated with producing any colicin are substantial, even if the colicin is not released due to cell lysis.

As described above, the frequency of colicin production is significantly less in *E. coli* strains isolated from mammalian carnivores compared to herbivorous or omnivorous mammals. For animals of a given body mass, gut turnover rates are significantly faster in mammalian carnivores, and a mathematical model predicts that, due to the costs associated with bacteriocin production, the fitness advantage accruing from bacteriocin production should decline as the turnover rate of the system increases. Colicins Ia and B represent the majority of the colicins produced by the isolates from Australian mammals. If any strain producing a colicin released via lysis is excluded from the analysis of the effect of diet on the frequency of colicin production, then the same result is obtained.

If, indeed, the lower frequency of colicinogenic strains in carnivorous mammals is due to the rapid turnover rate of the gastro-intestinal tract, then this would suggest that the costs of bacteriocin production without cell lysis are substantial. Evidently, experiments determining the costs associated with the production of secreted bacteriocins are required, as are experiments to determine if cells resistant to bacteriocins such as Ia or B out-compete strains producing these bacteriocins.

The likelihood that an *E. coli* strain will be bacteriocinogenic depends on the environment from which the host was isolated and on the genetic

background of the strain, as does the type of bacteriocin present in a strain. The reasons for these patterns are poorly understood but it is clear that the magnitude of fitness advantage accruing from bacteriocin production is determined by many factors. The development of a successful probiotic strain will depend not only on the host to be targeted but also on the careful choice of the strain and the bacteriocin produced by that strain.

In *E. coli*, most bacteriocin-producing strains encode more than one type of bacteriocin. On the face of it, the advantage of multiple bacteriocin production is obvious. Consider a community initially consisting of a sensitive cell population and two populations of producing cells, each encoding a single bacteriocin type. Each of the producers can kill the other producer as well as sensitive cells. If one of the producing cells acquires, through recombination, the genes for the other colicin type, then this multiple colicin producer can kill sensitive cells and all cells encoding only a single colicin type. There are, however, no data demonstrating that a strain encoding, for example, colicin Ia and microcin V, will out-compete a strain producing only one of these bacteriocins.

There may also be other benefits arising from multiple bacteriocin carriage. In naturally occurring *E. coli* populations, resistance to colicins is a common phenomenon and most cells are resistant to most co-occurring colicins (Riley and Gordon 1992; Gordon et al. 1998; Feldgarden and Riley 1999). All of the E group colicins exploit the BtuB receptor, and a mutation which alters or causes the loss of this receptor will lead to resistance and render all or many E-type colicins ineffective (Feldgarden and Riley 1999). In those strains harbouring multiple bacteriocins, many (albeit not all) of the combinations represent bacteriocins which exploit different surface receptors. For example, the colicins Ia and E1 exploit the receptors Cir and BtuB. A mutation in one receptor is far more likely than the simultaneous occurrence of mutations in two different receptors. Thus, harbouring multiple bacteriocins exploiting different surface receptors may slow the evolution of resistance in populations where the dominant bacteriocinogenic strain produces multiple bacteriocins, compared to populations where the dominant bacteriocinogenic strain produces a single bacteriocin. Nevertheless, an expanded receptor repertoire cannot explain the occurrence of some of the co-associations observed to date. Microcins H47 and M are thought to exploit the same receptors (Cir Fiu IroN and FepA), whilst colicin Ia and microcin V are both thought to exploit the Cir receptor.

Microcin V production is induced when iron is limited. Although it is well established that iron is a limited resource in extra-intestinal body sites (Ratledge and Dover 2000), it is generally not considered to be limiting in the lower intestinal tract of vertebrates. By contrast, colicin Ia is induced under conditions of general nutrient limitation, a state which does occur in the lower intestinal tract. The ability to acquire iron when attempting to establish at extra-intestinal body sites is considered to be an important virulence trait in *E. coli* (Ratledge and Dover 2000). The strains responsible for an

extra-intestinal infection are thought to originate from the *E. coli* community residing in the infected individual's intestinal tract (Mobley and Warren 1996). Therefore, strains causing extra-intestinal infections must have a suite of traits enabling them to invade and establish at extra-intestinal body sites as well as traits facilitating persistence in the intestine. Thus, we conjecture that the joint carriage of an SOS-induced colicin, such as Ia, and an iron-induced microcin (V) each confers a fitness advantage to the strain but in different environments: colicin Ia in the gut and microcin V at extra-intestinal body sites.

The bacteriocins produced by *E. coli* are diverse and this is undoubtedly true of other Gram-negative species, too. Many microcins and some colicins are difficult to detect using conventional phenotypic approaches and, given that overall 45% of the *E. coli* isolated from humans produce one or more detectable bacteriocins, it is conceivable that all strains of *E. coli* produce a bacteriocin. The survey results also illustrate that much of the diversity in bacteriocin production is a consequence of the association of multiple bacteriocins in a single cell. Understanding the adaptive significance of this diversity represents a considerable challenge but one which must be faced, if we are to successfully exploit bacteriocins in order to manage bacteria and the diseases they cause.

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