Selection and fitness in bacteriocin-producing bacteria

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SUMMARY

Bacteriocins are proteinaceous anticompetitor molecules produced by bacteria against closely related species. A number of theoretical models have been used to explain experimental data that indicate high polymorphisms among bacteriocins and a frequency-dependent nature of selection for bacteriocin-producing strains. The majority of these experimental data were, however, obtained from investigations into the colicin group of bacteriocins produced by Gram-negative bacteria. The conclusions drawn from these models have been extrapolated to other bacteriocins and allelopathic compounds in general. Examination of more recent experimental investigations into the bacteriocins of Gram-positive bacteria indicate a lower degree of polymorphism and a less frequency-dependent mode of selection among these strains than among the colicin-producing strains. Here we examine these contradictions in the light of the assumptions and conclusions of the theoretical models and reported data. We show that fitness costs (as indicated by decreased relative maximum growth rate) associated with bacteriocin production may be much lower in many cases than is assumed in the present models. A lower fitness cost associated with bacteriocin production adequately explains the newer data from Gram-positive bacteria cited here, and indicates that extrapolation of existing models to all bacteriocins and other allelopathic compounds is not appropriate.

1. INTRODUCTION

Bacteriocins are allelopathic, proteinaceous compounds produced by bacteria, which act as anticompetitor toxins against the same or closely related species (Reeves 1972; Chao & Levin 1981; Rice 1984; Jack *et al*. 1995; Nes *et al*. 1996). The term bacteriocin encompasses an array of structurally different molecules produced by a number of phylogenetically distinct Gram-positive and Gram-negative bacterial groups (Reeves 1972; De Vuyst & Vandamme 1994; Jack *et al*. 1995). Bacteriocins may act on cells in a variety of different ways (Reeves 1972; Jack *et al*. 1995; James *et al*. 1996). For example, many bacteriocins, such as mesentericin $Y105^{37}$ and the B-colicins are membrane-active peptides which act to form pores in the cell membrane of antagonized cells (Fleury *et al*. 1996; James *et al*. 1996). These compounds cause leakage of ions and other cellular components, and in so doing disrupt the proton motive force, ultimately resulting in cell death (Abee *et al*. 1994; Jack *et al*. 1995). On the other hand, bacteriocins such as colicin E2 and E5 act by crossing the membrane and degrading the nucleic acids present in the cell (James *et al*. 1996).

Historically, much of the work on these substances has focused on the colicins, which are a group of related bacteriocins produced by *Escherichia coli* and some other Gram-negative bacteria (Reeves 1972; Jack *et al*. 1995). More recently, however, there has been a vast increase in research on bacteriocins from other bacterial groups, including the Gram-positive lactic acid bacteria. The increased interest in these compounds has been fuelled by the potential industrial application of bacteriocins for use as safe and novel food preservatives (De Vuyst & Vandamme 1994; Jack *et al*. 1995). This research has generated a wealth of information on the molecular genetics, mode of action, and application of many bacteriocins. Due to the thrust of this research, however, little remains known about the natural ecology of bacteriocins and the role it plays in the evolution of these molecules (Dykes 1995).

A number of theoretical models investigating the population dynamics of bacteriocin-producing bacteria have been described (Chao & Levin 1981; Levin 1988; Frank 1994). These models, which are based on classic Lotka–Volterra reaction–diffusion equations, assume a fitness cost associated with the production of bacteriocin. In so doing these models explain various experimental observations, such as the high degree of polymorphisms among certain bacteriocins and the frequency-dependent nature of interactions between bacteriocin-producing and sensitive strains. While the models adequately explain the data they cite, these data were derived almost solely from work on the colicins and other Gram-negative bacteriocins. With the increase in research on Gram-positive bacteriocins, more data on a wider range of compounds have come to light. We show, using examples from the literature, that some of the assumptions, such as a fitness cost associated with bacteriocin production, used to construct the models, as well as some of the predictions,

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such as the polymorphism maintaining effect of habitat, made from the models may not hold for all bacteriocins.

2. THEORETICAL BACKGROUND

The published theoretical models describing competition between bacteriocin-producing and bacteriocin-sensitive strains are based on a number of assumptions, which in turn are based on empirical evidence. More specifically, a bacteriocin-producing bacterium pays a cost, in the form of a lowered maximum specific rate of growth, for bacteriocin production (Chao & Levin 1981; Levin 1988; Frank 1994). This assumption is justified if experimental investigations into colicin-producing bacteria are examined. Not all the individuals in a population with the ability to produce colicin will do so, but the small percentage that do produce bacteriocin demonstrate lethal synthesis, i.e. individual cells producing the bacteriocin die in the process (Reeves 1972; Jack *et al.* 1995). This feature will clearly affect the fitness of the population to some degree, but due to the small numbers of individuals involved, the relative costs should not be large due to benefits gained from killing a portion of the competing bacteria. In addition, all cells in the population will also pay a cost associated with bacteriocin plasmid carriage and replication (Adams *et al.* 1979; Helling *et al.* 1981; Noack *et al.* 1981; Hartl *et al.* 1983; Seo *et al.* 1985; Nguyen *et al.* 1989). Specifically, the expression of immunity and other plasmid-encoded proteins which assure the survival of these strains in the presence of bacteriocins will impose a cost on the population which will be expressed as a lowered maximum specific growth rate (Adams *et al.* 1979; Frank 1994). A further assumption made in these models is that the bacteriocins of a specific species or group of bacteria are highly polymorphic (Frank 1994). This assumption is understandable because of the large diversity of species against which the bacteriocins are active. Specifically, high levels of bacteriocin variation within single Gram-negative species such as *E. coli* and *Klebsiella pneumoniae* have been demonstrated and widely applied as a typing method in epidemiological studies of bacteria of medical significance (Chhibber *et al.* 1988; Traub 1991; Riley & Gordon 1992).

The models using the above assumptions as a basis for describing the interactions among bacteriocin-producing and bacteriocin-sensitive strains result in three main conclusions. First, that selection in populations of bacteriocin-producing and bacteriocin-sensitive strains is frequency dependent, i.e. selection of bacteriocin-producing strains (or bacteriocin-sensitive strains) is dependent on their relative occurrence within the population (Chao & Levin 1981; Levin 1988). Second, this frequency dependence is disruptive, meaning that rare types, whether they produce bacteriocin or are bacteriocin-sensitive, are unable to invade a population of the more common type, which in turn may result in reduced variation and, ultimately, monomorphic populations (Levin 1988). Third, the maintenance of bacteriocin production and bacteriocin polymorphisms is brought about by spatial variations in habitat which allow selection for rare types. In other words, good or structured habitats favour bacteriocin producers while bad or unstructured habitats favour susceptible strains (Frank 1994). These conclusions explain the results of experimental observations in competition studies between colicin-producing and colicin-sensitive (but otherwise isogenic) strains. Specifically, it has been shown that colicin-producing bacteria have a relatively 'high' unstable equilibrium point at a relative frequency of approximately 10^{-2} in liquid culture, below which they cannot invade populations of sensitive strains, a feature which is consistent with a disruptive frequency dependent hypothesis (Adams *et al.* 1979; Chao & Levin 1981). Additionally, this unstable equilibrium point could be reduced to a relative frequency of as low as 10^{-6} in structured habitats, such as agar, confirming that variations in habitat may allow selection of rare types (Chao & Levin 1981). Structured habitats allow different resource partitioning than liquid culture since bacteria exist as single clone colonies in these environments. By killing sensitive strains in a zone around the colony they increase the concentration of resources available to themselves in a manner not possible in liquid culture (Chao & Levin 1981).

3. EXAMPLES OF CONFLICTING EVIDENCE

A number of examples from studies on bacteriocins and host–plasmid relationships displaying specific features of relevance to the assumptions and conclusions of the above theoretical framework are presented below.

(*a*) *Cost of bacteriocin production*

Although the majority of bacteriocins described to date are plasmid-encoded, the genetic operons for a number of Gram-positive bacteriocins have been shown to be chromosomally encoded (Quadri *et al.* 1994; Hühne *et al.* 1996) or located on transposons (Horn *et al.* 1991). Specific examples of these are sakacin P, a bacteriocin produced by *Lactobacillus sake* Lb674 (Hühne *et al.* 1996), carnobacteriocin B2, produced by *Carnobacterium piscicola* LV 17B (Quadri *et al.* 1994) and nisin, a transposon-encoded bacteriocin produced by *Lactococcus lactis* (Hühne *et al.* 1996). While bacteriocin production in these strains may still impose a cost to the producer, since the bacteriocin is often produced constitutively, this cost will be much reduced because plasmid carriage itself (regardless of what the plasmid encodes) may impose a fitness cost on the host. Since many chromosomally encoded bacteriocins have closely related analogues on plasmids in other bacterial strains (for example, sakacin A, a plasmid-encoded bacteriocin is very similar to the chromosomally encoded sakacin P), this feature raises interesting possibilities regarding the evolution of bacteriocin genes (Hühne *et al.* 1996). In particular, it may be the case that bacteriocin genes that have integrated into the chromosome have been maintained there due to the reduced cost imposed on the host as compared to plasmid encoded analogues.

Although the fitness costs imposed by plasmids on bacterial hosts are a well-described phenomenon, most studies investigating this phenomenon have examined naive plasmid–host relationships, i.e. plasmid–host relationships which are new and in which no history of association between the plasmid and the host exists (Adams *et al*. 1979; Helling *et al*. 1981; Noack *et al*. 1981; Hartl *et al*. 1983; Seo *et al*. 1985; Nguyen *et al*. 1989). It has been demonstrated, however, that a long-term history of association between plasmids and their hosts may result in beneficial coevolutionary changes in both the plasmid and the host (Bouma & Lenski 1988; Lenski *et al*. 1994). In such cases, plasmid carriage no longer imposes a burden on the host, and will in fact result in enhanced fitness of the host relative to the same host after plasmid-curing (Lenski *et al*. 1994). Since bacteriocins are widely distributed among bacterial strains and many bacteriocin-encoding plasmids are non-conjugative, it is likely that many of these plasmid–host relationships are long-term. It may therefore be possible that plasmid-encoded bacteriocin production may not impose a cost on the host relative to plasmid-free segregants, due to plasmid–host co-evolution. In support of this, experiments investigating long-term relationships between colicin plasmids and their hosts have demonstrated a reduced plasmid burden relative to plasmid-free strains after 800 generations of plasmid–host association (Modi & Adams 1991).

(*b*) *Polymorphisms in bacteriocins*

While many bacteriocins, including those from Gram-positive genera such as *Clostridium* (Keis *et al*. 1995) are highly polymorphic, as is indicated by their spectrum of activity, this is not necessarily the case for these substances from all bacterial groups. An example of this can be seen among the class II bacteriocins, which are small, heat-stable, non-lanthionine-containing bacteriocins produced by a number of genera of Gram-positive lactic acid bacteria (Klaenhammer 1993). If the spectrum of activity, as well as the amino acid and nucleotide sequences of bacteriocins from distinct genera and species within this group are examined, little variation is observed as compared to that of the colicins (Klaenhammer 1993; De Vuyst & Vandamme 1994; Jack *et al*. 1995). This variation is even lower if the bacteriocins of the genus *Leuconostoc* are surveyed. The leucocin group of bacteriocins produced by this genus shows very high homology at both the DNA and the amino acid level, and may differ in only one or two amino acids in the bacteriocin molecule between different strains or species (Hastings *et al*. 1991, 1994; Felix *et al*. 1994; Fleury *et al*. 1996). The high degree of similarity between the bacteriocins of the lactic acid bacteria shown across a number of phylogenetically distinct species, and even genera, is further highlighted by other features within this group. In particular, all the leucocins appear to be encoded by plasmids which vary greatly in size and are generally non-conjugative in nature, making it unlikely that recent intraspecific exchange of plasmids has occurred (De Vuyst & Vandamme 1994; Hastings *et al*. 1994). Furthermore, the bacteriocin-producing strains have been isolated from environments which are separated both spatially and temporally. This should result in a high degree of polymorphism among these bacteriocins if theoretical predictions are correct (De Vuyst & Vandamme 1994; Hastings *et al*. 1994). Clearly then, some of the selective forces which act to make the colicins polymorphic are reduced or not acting in the case of these bacteriocins.

(*c*) *Effects of habitat*

Many bacteriocins have potential as novel food preservatives, while others, such as nisin, are already applied in this capacity. For this reason, a number of studies have investigated the survival potential of bacteriocin-producing bacteria in natural food fermentation processes. Some food-related habitats, such as processed meat fermentations, are structured and nutrient rich, and therefore are expected to favour bacteriocin-producing strains since they may be regarded as good habitats. Conversely, others such as olive and pickle fermentations, are unstructured and relatively nutrient poor, and are therefore expected to favour susceptible bacteria, if theoretical predictions are correct. In addition, in both the above cases nutrient availability is expected to be limited since competition for resources with other bacteria will be high. In most food systems, therefore, bacteriocin-producing strains should be expected to be at a relative disadvantage (Chao & Levin 1981; Frank 1994). It has been demonstrated, however, that if a bacteriocin-producing strain of *Lactobacillus plantarum* is added to a natural olive fermentation at levels of around 10^5 bacteria ml^{-1}, this strain will persist in the microbial population associated with the final product (Ruiz-Barba *et al*. 1994). On the other hand, if a bacteriocin plasmid-free (but otherwise isogenic strain) is added to the same system at the same concentration it is undetectable in the final product (Ruiz-Barba *et al*. 1994). The exact frequencies of the bacteriocin-producing and plasmid-free strains were not determined in this study due to the diverse and uncharacterized natural microbial populations associated with the olive fermentation, but they can be estimated to be below 10^{-2}. It is clear from these experiments that bacteriocin-producing strains are able to invade and maintain themselves in a population of sensitive strains even if they are present at relatively low initial frequencies. These results contradict those expected from previous data (Adams *et al*. 1979; Chao & Levin 1981) and may be speculated to result from a lower than expected fitness cost, or higher than expected benefit, associated with bacteriocin production.

(*d*) *Multiple functions of bacteriocins*

Recently, a number of bacteriocins have been shown to act not only as anticompetitor toxins, but also to demonstrate other biological activities. For example, bacteriocin *small*, produced by *Rhizobium leguminosarum*, has been demonstrated to act as an autoinducer molecule, and is involved in the interaction of this bacterium with its plant symbionts (Schripsema *et al*.

1996). In addition, nisin acts not only as an antimicrobial but also as an induction factor for its own expression (Kuipers *et al*. 1995). Cytolysin, on the other hand, is a two-peptide lantibiotic produced by *Enterococcus faecalis* which acts as both an antimicrobial toxin and as a haemolytic agent, which enhances the pathogenic capabilities of this organism (Gilmore *et al*. 1994). Furthermore, many other bacteriocins may have further as yet undiscovered functions which may increase our understanding of the benefits of these compounds to the producer. Bacteriocins which exhibit both antimicrobial and other activities represent an efficient use of resources by the cell and a possible reduced burden of their production to the cell. Clearly then, the presence of bacteriocins with multiple functions could result in an overestimation of the fitness costs associated with their production.

4. DISCUSSION

The above analysis provides an indication of a number of inconsistencies between theoretical and experimental data. These experimental data should be considered before extrapolating the studies based on the population dynamics of colicin-producing bacteria to all bacteriocin-producing bacteria and other allelopathic substances in general. It can be clearly seen that a number of factors may in fact reduce, eliminate, or perhaps reverse the costs associated with bacteriocin production and plasmid carriage. In particular, the likelihood of the coevolution of non-conjugative bacteriocin-encoding plasmids and their hosts to beneficial associations is high (Modi & Adams 1991; Lenski *et al*. 1994). Furthermore, any of the bacteriocin burden-reducing features, or any combination of them, may act synergistically within a single bacteriocin-producing strain. This reduced burden may, consequently, result in the selection of bacteriocin-producing strains at much lower frequencies than expected. Frank (1994), for example, assumes a burden of a 2–5% decrease in maximal growth rate in all numerical studies of his model in explaining the maintenance of bacteriocin (and other allelopath) polymorphisms. In light of the above accumulated evidence, however, this degree of burden may be too large in the case of some bacteriocins. If little or no burden is assumed, the maintenance of polymorphisms by habitat variations may be reduced and the low level of variations observed for particular bacteriocin groups (such as the leucocins) explained (Hastings *et al*. 1994). This possibility is also consistent with the observed selection for certain bacteriocin producers in habitats which should select against them if the burden associated with bacteriocin production was high. These possibilities also have practical implications since the application of bacteriocin-producing bacteria in, for example, food systems may hold greater potential if bacteriocin production is a highly stable trait, which may result if the relative decrease in maximal growth rate due to bacteriocin production is minimal or non-existant. Future experimental and theoretical investigations into the evolutionary ecology of bacteriocins (and other allelopaths) should take these features into account and address individual bacteriocin groups separately based on empirical evidence for that group.

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