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Rapid invasion by colicinogenic *Escherichia coli* with novel immunity functions

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Bacteriocins have been suggested to play an important role in the invasion dynamics of bacteria. Recently, the 'diversifying selection' hypothesis has been proposed, which addresses the origin and diversification of one group of bacteriocins, the colicins of *Escherichia coli*. According to this hypothesis, novel colicin gene clusters arise from mutations generating expanded immunity functions. Positive selection, favouring these novel immunities, then rapidly drives strains carrying the evolved colicin gene clusters to fixation in the local population. To test this fixation step driven by selection, invasion experiments were carried out by introducing novel colicinogenic strains into established colicinogenic populations. In all cases, invasion by strains expressing novel immunity functions occurred rapidly, even when initial frequencies of the invader were quite low. These invasions were attributed primarily to colicin killing effect. Other factors, such as growth rate, level of colicin production and stationary-phase survival rate, were shown to play very minor roles in the invasion process. These results provide direct evidence for the hypothesis of diversifying selection acting on colicin gene clusters and shed light on the ecological role of colicins.

Keywords: *Escherichia coli*, colicin killing, invasion, dual/single immunity

INTRODUCTION

Colicins are toxic proteins produced by, and active against, *Escherichia coli* and related bacteria. The genes encoding colicins and associated proteins exist as tightly linked gene clusters on plasmid replicons. Colicinogenic bacteria are specifically immune to killing by the colicin they carry. Twenty-three colicin types have been identified, each characterized by a corresponding specific immunity (Pugsley & Oudega 1987).

Based upon amino acid and DNA sequence comparisons, the molecular mechanisms involved in the origin and diversification of colicin gene clusters have been inferred (Riley, 1993a, b). It was proposed that diversifying selection, acting on immunity function, might be a primary mechanism generating new colicin gene clusters. Each round of diversification described in this 'diversifying selection' hypothesis encompasses two steps: (i) the co-occurrence of mutations in both the immunity gene and the immunity binding domain of the colicin gene which provides the host cell with an expanded immunity function, and (ii) the rapid fixation of this evolved strain in the ancestral colicinogenic population.

Earlier work by Masaki *et al.* (1991) provides strong support for the first step in the proposed diversification process. They showed that a single point mutation from TGG (Trp-48) to TGT (Cys) in the immunity gene of colicin E6 generated immunity to the closely related colicin, E3, as well as maintaining original immunity to colicin E6.

The second step in the diversification process, which requires the strain carrying the novel colicin gene cluster to rapidly invade the ancestral colicinogenic population, is tested in this study. It is known that several colicin-encoding plasmids naturally possess an additional immunity gene, which expands the hosts' immunity function in a fashion analogous to that determined by point mutations, as assayed by Masaki *et al.* (1991). Specific examples of this expanded immunity function include (i) plasmid pColE3-CA38 (ColE3) which encodes immunity to colicin E3 as well as to colicin E8, (ii) plasmid pColE6-CT14 (ColE6) which encodes immunity to colicin E6 and colicin E8, and (iii) plasmid pColE9-J (ColE9) encoding immunity to colicin E9 and colicin E5 (Fig. 1). Functionally, a strain carrying such a Col plasmid mimics the expanded immunity 'evolved' strain as described in the 'diversifying selection' hypothesis. The difference between the proposed 'evolved' strain and the functional

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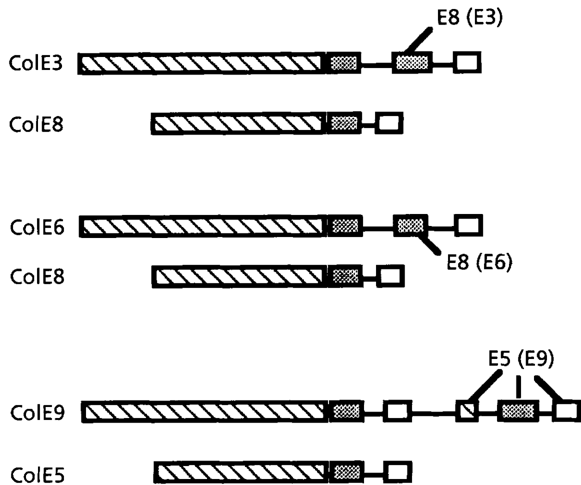

Fig. 1. **Genetic organization of colicin gene clusters for three dual/single-immunity colicinogenic pairs: ColE3 versus ColE8, ColE6 versus ColE8 and ColE9 versus ColE5. ▧, Colicin gene; ▩, immunity gene; □, lysis gene. The extra immunity gene for each colicin gene cluster with dual immunity is shown.**

analogue that we employ here is that, structurally, the 'evolved' strain has a dual immunity due to mutation(s) in a single immunity gene while the Col plasmids employed in this study have a dual immunity due to the presence of two immunity genes. Invasion experiments were carried out employing strains bearing these Col plasmids to test the ability of novel immunity variants to rapidly fix in ancestral, colicinogenic populations.

METHODS

All *E. coli* strains employed were isogenic [BZB1011(*gyrA*)] except for the introduced colicin-encoding plasmid (Pugsley, 1985). Each invasion experiment was initiated by inoculating 10 ml fresh LB (in 50 ml flasks) with invading and ancestral cells obtained from overnight LB monocultures. When the invading strain was introduced at frequencies of 10^{-4}, the total cell density was 4×10^5 ml^{-1}. When the invading strain was introduced at frequencies of 10^{-6}, the total cell density was 4×10^7 ml^{-1}. For invasions by ColE3- and ColE6-carrying strains, ColE8 was designated as the ancestral strain. For invasions by ColE9-carrying strains, ColE5 was designated as the ancestral strain. After these mixed cultures had been incubated for 24 h at 37 °C with agitation, the relative numbers of the two competing strains were measured (detailed below). Successive serial transfer cycles were initiated by adding 0·1 ml 10^{-2} diluted (if the initial frequency of the invading strain was 10^{-4}) or 0·1 ml undiluted (if the initial frequency of the invading strain was 10^{-6}) stationary-phase cultures to 50 ml flasks containing 10 ml fresh LB.

Total numbers of bacteria (including invading and ancestral strains) were estimated by sampling on LB plates. The number of invading bacteria were estimated by sampling on LB plates spread with 0·1 ml crude colicin extract obtained from invading bacteria. From this value, the relative frequency of the invading strain after each transfer cycle was determined. For invasions that occurred within one transfer, frequency estimations were also carried out in the middle of that transfer cycle (i.e. after 12 h growth). To ensure that the invading bacteria sampled on colicin-seeded plates were not resistant ancestors, the colonies appearing on these plates were routinely assayed for colicin production using a patch test (Pugsley & Oudega, 1987).

A selection coefficient of the invading strain was estimated for each invasion experiment, according to the method described by Dykhuizen & Hartl (1980). From the Monod equation, $\ln(p_t/q_t) = \ln(p_0/q_0) + st$, where $p+q = 1$, p_0 and q_0 represent the initial frequencies of two competing strains and p_t and q_t represent their frequencies after t h competition. The slope of the linear regression of $\ln(p_t/q_t)$ against time provides an estimate of the selection coefficient, s. Since sampling was done every 12 or 24 h, fixation of the invader earlier than the final sampling point could not be detected. This could lead to an underestimation of s if the final time point were included. To avoid this problem, the final time point was excluded when s was calculated.

To measure maximal growth rate for each strain, about 4×10^6 cells from an overnight culture were inoculated into 10 ml fresh LB. The culture was then incubated at 37 °C with agitation. Samples were taken every 0·5 h to estimate cell densities.

Stationary phase survival rates for each strain were measured by estimating the maximal cell densities after the exponential phase (8 h growth) and the cell densities after 24 h growth. The density at 24 h was divided by the maximal density to obtain the survival rate.

Both spot and colicin molecule counting assays were used to measure the level of colicin production for each colicinogenic strain. For the spot assay, a crude colicin supernatant was prepared and serially diluted. Dilutions were spotted on a lawn of BZB1011 cells and the inhibition zones were recorded (Riley & Gordon, 1992). The colicin molecule counting assay involved inoculating 100 μl cells from an overnight culture into 10 ml fresh LB for both colicinogenic and BZB1011 strains. After 4 h incubation at 37 °C, cell densities were estimated. Mitomycin C was then added to the colicinogenic culture, followed by 3 h incubation at 37 °C. Crude colicin supernatant was prepared from these mitomycin-C-induced cultures and serially diluted. Dilutions were mixed with 100 μl BZB1011 cells in 1 ml LB. The mixtures were incubated at 37 °C for 0·5 h and plated. By comparing cell densities of BZB1011 on plates with and without colicin, the number of colicin molecules could be estimated, assuming single hit kinetics (Reeves, 1972). The number of colicinogenic cells producing a specified number of colicin molecules could be estimated by comparing cell densities before and after mitomycin C addition.

Six replicates were performed for all assays and invasion experiments.

RESULTS

Invasion by dual-immunity colicinogenic strains of single-immunity colicinogenic populations was studied for three pairs of dual/single-immunity colicinogenic strains: ColE6- versus ColE8-carrying strains, ColE9- versus ColE5-carrying strains, and ColE3- versus ColE8-carrying strains, with initial invasion frequencies of 10^{-4} and 10^{-6} for the dual-immunity colicinogenic strains. In all cases, invasion by the dual-immunity colicinogenic strain occurred rapidly (Fig. 2). With initial frequencies of 10^{-4}, the dual-immunity colicinogenic strains invaded the single-immunity colicinogenic populations after one or two transfers, i.e. after 24 or 48 h (Table 1). With initial frequencies of 10^{-6}, the dual-immunity colicinogenic strains invaded the single-immunity colicinogenic popu-

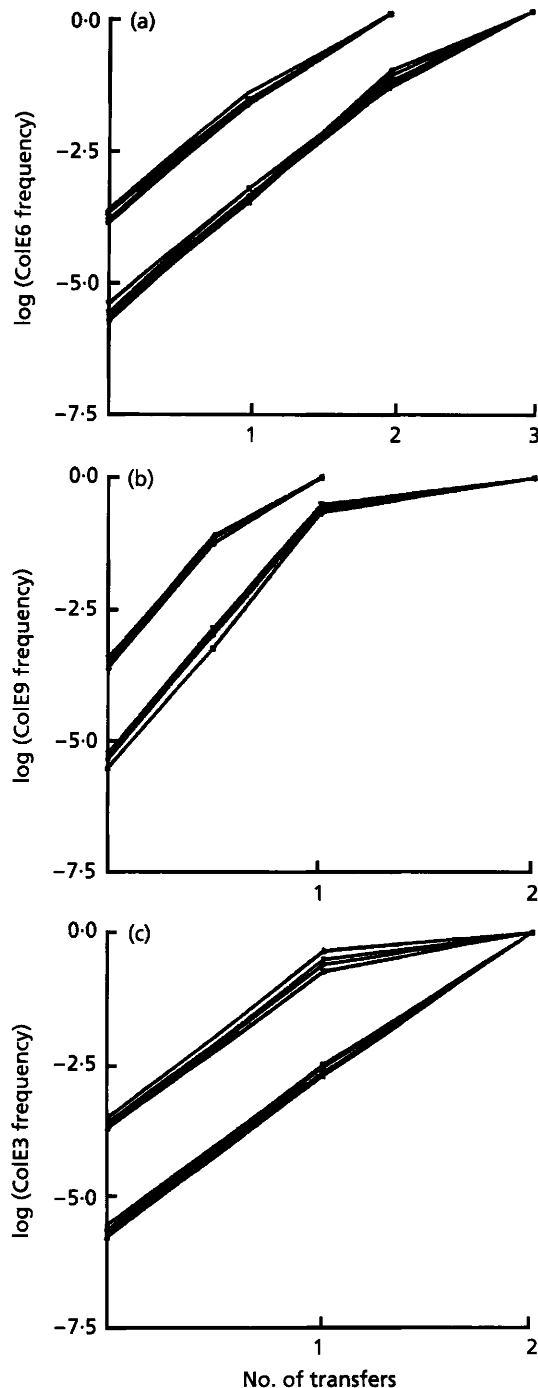

Fig. 2. **Invasion by dual-immunity colicinogenic strains of single-immunity colicinogenic populations. (a) ColE6-carrying strain invading a population of ColE8-carrying cells; (b) ColE9-carrying strain invading a population of ColE5-carrying cells; and (c) ColE3-carrying strain invading a population of ColE8-carrying cells. The initial frequencies of dual-immunity colicinogenic strains were $\sim 10^{-4}$ and $\sim 10^{-6}$ and cells were transferred every 24 h. Each experiment was replicated six times.**

lations after two or three transfers, i.e. after 48 or 72 h (Table 1). The selection coefficient (*s*) for each invading strain is given in Table 1.

As a negative control, the reverse invasions, i.e. by single-immunity colicinogenic strains of dual-immunity colicinogenic populations, were studied for all three single/dual-immunity colicinogenic pairs. As anticipated, at initial frequencies of 10^{-4} or 10^{-6}, all single-immunity colicinogenic strains immediately went extinct after one transfer.

As a positive control, invasions of a population of colicin-sensitive cells (BZB1011) were studied for each of the colicinogenic strains (Table 1). The selection coefficient (*s*) for each colicinogenic strain invading a BZB1011 population is given in Table 1. For all five pairs of colicinogenic versus sensitive strains, invasion by the colicinogenic strain occurred after one or two transfers, i.e. after 24 or 48 h, when initial frequencies were 10^{-4}. With initial frequencies of 10^{-6}, quite different results are obtained. Whilst ColE9-, ColE5- and ColE3-carrying strains invaded a BZB1011 population after 2–4 transfers, ColE6- and ColE8-carrying strains were not able to invade BZB1011 populations, assayed for a total of 14 transfers. They were maintained in BZB1011 populations at frequencies of about 10^{-5} for the duration of the invasion experiments.

To rule out factors other than colicin killing which might affect the invasion dynamics of these strains, a maximal growth rate assay and stationary-phase survival rate assay were carried out for each strain employed in this study (Table 2). In addition, colicin production assays, including a spot assay and colicin molecule counting assay, were also carried out for each colicinogenic strain (Table 2). These assays were compared between the strains within each invasion pair and a *t*-test was carried out for each comparison (Table 1). For each of the dual/single-immunity colicinogenic pairs (except in the case of the ColE3-carrying strain), the maximal growth rate, stationary phase survival rate, selection coefficient when invading a BZB1011 population, and colicin production level of the dual-immunity strain were lower than, or about the same as, those of the single-immunity strain. Similarly, for each of the colicinogenic/sensitive pairs (except in the case of the ColE3-carrying strain), the maximal growth rate and stationary-phase survival rate of the colicinogenic strains are either lower or the same as BZB1011. Thus, except for those cases involving the ColE3-carrying strain, factors other than colicin killing either favour the 'ancestral' strain or do not distinguish between the two strains and therefore could not contribute to the invasion success of the introduced 'evolved' strains.

DISCUSSION

A phylogenetic analysis of colicin proteins suggested that the current diversity of colicins may be, in part, the result of the action of positive selection (Riley, 1993b). The 'diversifying selection' hypothesis was subsequently proposed and elaborated to explain the unusual pattern of DNA sequence evolution observed for colicin gene clusters (Riley, 1993a). A comparison of the pattern of divergence between the colicin gene cluster of ColIa and

Table 1. Dynamics of invasion by colicinogenic *E. coli*

Invasion pair*	Invasion time†		Comparisons within each invasion pair‡			
	Initial frequency of invader 10^{-4}	Initial frequency of invader 10^{-6}	Maximal growth rate	Stationary-phase survival rate	Level of colicin production	s when invading BZB
E6/E8	2 transfers ($s=0{\cdot}205 \pm 0{\cdot}002$)	3 transfers ($s=0{\cdot}212 \pm 0{\cdot}003$)	E6 < E8 ($P<0{\cdot}01$)	E6 = E8 ($P=0{\cdot}77$)	E6 = E8 ($P=0{\cdot}15$)	E6 < E8 ($P<0{\cdot}01$)
E9/E5	1 transfer ($s=0{\cdot}458 \pm 0{\cdot}004$)	2 transfers ($s=0{\cdot}470 \pm 0{\cdot}003$)	E9 = E5 ($P=0{\cdot}37$)	E9 = E5 ($P=0{\cdot}92$)	E9 = E5 ($P=0{\cdot}85$)	E9 = E5 ($P=0{\cdot}39$)
E3/E8	2 transfers ($s=0{\cdot}308 \pm 0{\cdot}006$)	2 transfers ($s=0{\cdot}295 \pm 0{\cdot}003$)	E3 > E8 ($P<0{\cdot}01$)	E3 > E8 ($P<0{\cdot}01$)	E3 < E8 ($P<0{\cdot}01$)	E3 > E8 ($P<0{\cdot}01$)
E6/BZB	2 transfers ($s=0{\cdot}138 \pm 0{\cdot}002$)	NA	E6 < BZB ($P<0{\cdot}01$)	E6 < BZB ($P<0{\cdot}01$)	NA	NA
E8/BZB	2 transfers ($s=0{\cdot}232 \pm 0{\cdot}01$)	NA	E8 < BZB ($P<0{\cdot}01$)	E8 < BZB ($P<0{\cdot}01$)	NA	NA
E9/BZB	1 transfer ($s=0{\cdot}451 \pm 0{\cdot}002$)	2 transfers ($s=0{\cdot}463 \pm 0{\cdot}003$)	E9 < BZB ($P<0{\cdot}01$)	E9 = BZB ($P=0{\cdot}58$)	NA	NA
E5/BZB	1 transfer ($s=0{\cdot}456 \pm 0{\cdot}004$)	2 transfers ($s=0{\cdot}467 \pm 0{\cdot}002$)	E5 = BZB ($P=0{\cdot}22$)	E5 = BZB ($P=0{\cdot}46$)	NA	NA
E3/BZB	2 transfers ($s=0{\cdot}242 \pm 0{\cdot}002$)	4 transfers ($s=0{\cdot}198 \pm 0{\cdot}003$)	E3 > BZB ($P<0{\cdot}01$)	E3 < BZB ($P<0{\cdot}01$)	NA	NA

NA, Not available

* E6, E8, E9, E5 and E3 represent strains carrying ColE6, ColE8, ColE9, ColE5 and ColE3, respectively. BZB represents strain BZB1011. E6/E8 represents the invasion by ColE6-carrying strain of a population of ColE8-carrying strain. Similar representations apply to other invasion pairs.

† Given as the number of transfers for each invasion experiment; the s value for each invading strain is given in parentheses.

‡ For each assay comparisons are given for each invasion pair. The t-test result for each comparison is given in parentheses (d.f. = 10).

Table 2. Assays of maximal growth rate, stationary-phase survival rate and level of colicin production of colicinogenic *E. coli* strains

Strain*	Maximal growth rate (h^{-1}) ± SE	Stationary-phase survival rate ± SE†	10^{-3} × Colicin molecules per cell lysed ± SE	Colicin spot assays‡
E6	1·150 ± 0·010	0·168 ± 0·008	6·952 ± 0·432	1 µl, 10^{-1}
E8	1·328 ± 0·014	0·165 ± 0·008	6·029 ± 0·404	1 µl, 10^{-2}
E9	1·341 ± 0·007	0·533 ± 0·023	3·998 ± 0·109	1 µl, 10^{-1}
E5	1·349 ± 0·008	0·543 ± 0·029	3·970 ± 0·095	1 µl, 10^{-1}
E3	1·434 ± 0·003	0·275 ± 0·021	3·080 ± 0·210	5 µl, 10^{-1}
BZB	1·361 ± 0·004	0·515 ± 0·023	NA	NA

NA, Not available.

* E6, E8, E9, E5 and E3 represent strains carrying ColE6, ColE8, ColE9, ColE5 and ColE3, respectively. BZB represents strain BZB1011.

† Measured by dividing the cell density at 24 h by the maximal cell density.

‡ Results are given as the smallest volume and lowest dilution of a crude colicin supernatant that produced clear inhibition zones on a BZB1011 lawn.

the closely related ColIb and the patterns of DNA sequence polymorphism for seven isolates of ColIa suggests that certain colicin gene clusters may not evolve in a neutral fashion and thus lends support to the hypothesis (Riley *et al.*, 1994).

The present study employed an experimental approach to further evaluate the likelihood of the proposed diversifying selection process of colicin gene cluster evolution in *E. coli*. In particular, experiments on invasion by dual- and single-immunity colicinogenic strains were

carried out to mimic a fundamental step within one round of diversification. This step involves the rapid fixation of a novel immunity variant in an established colicinogenic population. In all cases, it was observed that the dual-immunity colicinogenic strain, even when initially rare, can invade a single immunity colicinogenic population rapidly in LB culture.

The invasion success was primarily attributable to the colicin killing effect of the invaders, i.e. the fact that the dual-immunity colicinogenic cells can kill, but cannot be killed by, the single-immunity cells. For those invasion experiments involving ColE6- versus ColE8-carrying strains, and ColE9- versus ColE5-carrying strains, including the controls of each colicinogenic strain versus the colicin-sensitive isogenic strain, BZB1011, both the growth rates and the stationary-phase survival rates were the same, or even lower for the invading strains relative to the ancestral strains. For invasions by a dual-immunity colicinogenic strain of a single-immunity population, the level of colicin production by the dual-immunity strain is equal to that of the single-immunity strain. Moreover, similar selection coefficients (s) for both dual- and single-immunity colicinogenic strains when invading BZB1011 populations indicate that the single-immunity strain has an equal potential to invade an established population by employing colicin killing function as the dual-immunity strain does. Thus, success in these invasion experiments can be attributed solely to the colicin killing effect of invaders.

In the invasion experiments involving the ColE3-carrying strain, factors other than colicin killing may contribute to the speed of the observed invasions. This is because for the invasion by the ColE3-carrying strain of a population of ColE8-carrying cells, the ColE3-carrying strain has a higher selection coefficient than the ColE8-carrying strain when invading a BZB1011 population, resulting from a combined effect of higher growth and stationary-phase survival rates for the ColE3-carrying strain relative to the ColE8-carrying strain. In the control experiments with invasion by a ColE3-strain of a BZB1011 population, the ColE3-carrying strain has a higher growth rate than BZB1011. However, the growth rate and survival rate effects are clearly minor relative to the effect of colicin killing. If the observed difference in selection coefficients between ColE3-and ColE8-carrying strains when invading a BZB1011 population ($\Delta s = 0{\cdot}01$) is subtracted from the selection coefficient of the ColE3-carrying strain when invading a population of ColE8-carrying cells, the ColE3-carrying strain can still invade a population of ColE8-carrying cells after two transfers (with the initial frequency of ColE3 as 10^{-4}) and the invasion success is solely due to the colicin killing effect. Similarly, if the selective advantage provided by a higher growth rate of the ColE3-carrying strain than BZB1011 ($s = 0{\cdot}01$) is excluded, the invasion speed of a ColE3-carrying strain into a BZB1011 population would not change much (still two transfers). Moreover, if the higher maximal growth rate of the ColE3-carrying strain contributed significantly to its ability to invade a population of ColE8-carrying cells, a higher rate of increase of ColE3-carrying cells during exponential phase than during stationary phase would be expected. This was not observed. In fact, the selection coefficients observed for the ColE3-carrying strain when invading a population of ColE8-carrying cells were not significantly different between the two growth phases (s for exponential phase was $0{\cdot}2957 \pm 0{\cdot}0033$; s for stationary phase was $0{\cdot}2898 \pm 0{\cdot}0025$; d.f. = 10, $t = 1{\cdot}4021$, $P = 0{\cdot}1912$).

All invasion experiments reported here indicate that colicins do confer a significant selective advantage to their host cells when invading an ancestral population. Further, possession of a dual-immunity function allows a novel colicinogenic cell to rapidly sweep to fixation in an ancestral colicinogenic population. These results lend support to the 'diversifying selection' hypothesis (Riley, 1993a, b).

The present study suggests that effects of growth rate, survival rate and level of colicin production play a minor role, relative to colicin killing effect, in invasion by colicinogenic strains. Nevertheless, these factors may help explain the slight differences in invasion behaviour within and between the three sets of experiments. For example, the invasion by a ColE6-carrying strain of a population of ColE8-carrying cells is more rapid than the invasion by a ColE6- or a ColE8-carrying strain of a BZB1011 population when initial invasion frequencies are 10^{-6}. This may be due to the different survival rates among these strains, with a higher rate of survival for BZB1011 relative to ColE6- and ColE8-carrying strains. Similarly, the more rapid invasion by a ColE3-carrying strain of a population of ColE8-carrying cells than its invasion of a BZB1011 population, and the more rapid invasion by a ColE3-carrying strain than either ColE6- or ColE8-carrying strains when invading a BZB1011 population at initial frequencies of 10^{-6} may be accounted for by the lower survival rate of the ColE8-carrying strain than BZB1011, and the lower survival rates of ColE6- and ColE8-carrying strains than the ColE3-carrying strain, respectively. Moreover, similar invasions of a BZB1011 population observed for ColE9- and ColE5-carrying strains can be accounted for by the similar levels of colicin production, growth rates and stationary-phase survival rates for these two strains. Finally, the lower survival rate of ColE6- and ColE3-carrying strains relative to a ColE9-carrying strain may explain why the speed of invasion by the ColE9 strain of a population of ColE5 cells is higher than that observed for either ColE6- or ColE3-carrying strains of a population of ColE8-carrying cells.

The present invasion studies not only provide supporting evidence for the 'diversifying selection' hypothesis (Riley, 1993a, b) but also shed light on the ecological role of colicins in population-level interactions. Colicins have been shown to occur in appreciable frequencies in most *E. coli* populations (Achtman *et al.*, 1983; Riley & Gordon, 1992); however, little is known about their ecological role. Several studies suggest that colicin production may be involved in bacterial competition, the establishment of new strains in bacterial communities and virulence determination (Branche *et al.*, 1963; Ikari *et al.*, 1969;

Kelstrup & Gibbon, 1969; Smith, 1974; Chao & Levin, 1981; Pugsley, 1984; Waters & Crosa, 1991), but the data supporting these claims are limited and often conflicting. Colicin production by *E. coli* seemed clearly associated with its survival in the intestine as a dominant resident (Branche *et al.*, 1963). On the other hand, Ikari *et al.* (1969) concluded that colicin production did not contribute significantly towards providing a competitive advantage in gastrointestinal colonization.

More recently, it has been shown that in the structured habitat (i.e. soft agar surface) of minimal media, the colicinogenic bacteria have a selective advantage over the sensitive bacteria even when initially rare, while in liquid minimal culture the advantage of the colicinogenic bacteria is measurable only when the initial frequencies are higher than 2×10^{-2} (Chao & Levin, 1981). This initial frequency-dependence was attributed to the lower intrinsic growth rate of the colicinogenic strain relative to the sensitive strain and the dilution of colicin in liquid culture.

Similarly, the present studies in LB liquid culture also reveal a generally lower intrinsic growth rate for a colicinogenic strain relative to the isogenic sensitive strain, as also reported by Adams *et al.* (1979). However, in the present studies, all five colicinogenic strains invading sensitive populations show that a disadvantage, resulting from lower growth rate and the lethality of cell lysis for the producing strain can be overcome by the colicin killing effect even when the initial frequencies of the colicinogenic strain are as low as 10^{-4} or even 10^{-6}. The difference between the results of Chao & Levin (1981) and those of the present study in liquid media may be attributable to a higher level of colicin production in LB than in minimal media. It was observed that for the strains in the present study, there was a significant decrease in colicin level in minimal media relative to LB media (Y. Tan & M. A. Riley, unpublished). Mathematical models concerning the population dynamics of colicinogeny suggest that the advantage of colicinogeny is determined by the rate at which sensitive cells are killed by the colicin and that the killing rate is dependent upon the amount of colicin released per lysed cell (M. A. Riley & D. M. Gordon, unpublished). Thus, the increased level of colicin produced in LB media compared to minimal media provides a higher selective advantage to the invading strain.

Rapid invasion by a colicinogenic strain of a sensitive population was observed for all colicinogenic strains in this study. Further, this study suggested for the first time that a novel colicinogenic strain can invade an established ancestral colicinogenic population. These data provide further evidence for the role of colicin production when colicinogenic cells are competing with other populations of the same species. Whether colicins also contribute to interactions between species is an open question. The fact that colicins can sometimes kill species closely related to *E. coli*, such as *Salmonella*, suggests a possibile role in species-level interactions.

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REFERENCES

Achtman, M., Mercer, A., Kusecek, B., Pohl, A., Heuzenroeder, M., Aaronson, W., Sutton, A. & Silver, R. P. (1983). Six widespread bacterial clones among *Escherichia coli* K1 isolates. *Infect Immun* **39**, 315–335.

Adams, J., Kinney, T., Thompson, S., Rubin, L. & Helling, R. B. (1979). Frequency-dependent selection for plasmid-containing cells of *Escherichia coli*. *Genetics* **91**, 627–637.

Branche, W. C., Young, V. M., Robinet, H. G. & Massey, R. D. (1963). Effect of colicin in normal human intestine. *Proc Soc Exp Biol Med* **114**, 198–201.

Chao, L. & Levin, B. (1981). Structured habitats and the evolution of anticompetitors in bacteria. *Proc Natl Acad Sci USA* **78**, 6324–6328.

Dykhuizen, D. & Hartl, D. L. (1980). Selective neutrality of 6PGD allozymes in *E. coli* and the effects of genetic background. *Genetics* **96**, 801–817.

Ikari, N. S., Kenta, D. M. & Young, V. M. (1969). Interaction in the germfree mouse intestine of colicinogenic and colicin-sensitive microorganisms. *Proc Soc Exp Biol Med* **130**, 1280–1284.

Kelstrup, J. & Gibbon, R. J. (1969). Interaction of bacteriocins in the intestinal and oral cavity. *J Bacteriol* **99**, 888–890.

Masaki, H., Akutsu, A., Uozumi, T. & Ohta, T. (1991). Identification of a unique specificity determinant of the colicin E3 immunity protein. *Gene* **107**, 133–138.

Pugsley, A. P. (1984). The ins and outs of colicins. *Microbiol Sci* **1**, 168–175, 203–205.

Pugsley, A. P. (1985). *Escherichia coli* K12 strains for use in the identification and characterization of colicins. *J Gen Microbiol* **131**, 369–376.

Pugsley, A. P. & Oudega, B. (1987). Methods for studying colicins and their plasmids. In *Plasmids, a Practical Approach*, pp. 105–161. Edited by K. G. Hardy. Oxford: IRL Press.

Reeves, P. (1972). *The Bacteriocins*. New York: Springer-Verlag.

Riley, M. A. (1993a). Positive selection for colicin diversity in bacteria. *Mol Biol Evol* **10**, 1048–1059.

Riley, M. A. (1993b). Molecular mechanisms of colicin evolution. *Mol Biol Evol* **10**, 1380–1395.

Riley, M. A. & Gordon, D. M. (1992). A survey of Col plasmids in natural isolates of *Escherichia coli* and an investigation into the stability of Col-plasmid lineages. *J Gen Microbiol* **138**, 1345–1352.

Riley, M. A., Tan, Y. & Wang, J. (1994). Nucleotide polymorphism in colicin E1 and Ia plasmids from natural isolates of *E. coli*. *Proc Natl Acad Sci USA* **91**, 11276–11280.

Smith, H. Williams (1974). A search for transmissible pathogenic characters in invasive strains of *Escherichia coli*: the discovery of a plasmid-controlled toxin and a plasmid-controlled lethal character closely associated, or identical, with colicine V. *J Gen Microbiol* **83**, 95–111.

Waters, V. L. & Crosa, J. H. (1991). Colicin V virulence plasmids. *Microbiol Rev* **55**, 437–450.

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