Host gastro-intestinal dynamics and the frequency of colicin production by *Escherichia coli*

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The production of antimicrobial compounds known as colicins has been shown to be an important mediator of competitive interactions among *Escherichia coli* genotypes. There is some understanding of the forces responsible for determining the frequency of colicin production in *E. coli* populations; however, this understanding cannot explain all of the observed variation. A survey of colicin production in *E. coli* isolated from native Australian mammals revealed that the frequency of colicin production in strains isolated from carnivores was significantly lower than the frequency of production in strains recovered from herbivores or omnivores. The intestine of Australian carnivores is tube-like and gut turnover rates are rapid compared with the turnover rates of the intestinal tracts of herbivores and omnivores, all of which possess a hindgut fermentation chamber. A mathematical model was developed in order to determine if variation in gut turnover rates could determine if a host was more likely to harbour a colicin-producing strain or a non-producer. The model predicted that a colicin producer was more likely to dominate in the gut of a host with lower gut turnover rates, and a non-producer to dominate in hosts with rapid gut turnover rates.

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INTRODUCTION

Colicins are a class of protein antibiotics known as bacteriocins that are produced by, and target strains of, *Escherichia coli* and closely related bacteria. Colicin production is often encoded by three tightly linked plasmid-borne genes: a gene for toxin production, an immunity gene, whose protein protects the producing cell from the action of the colicin, and a lysis gene that causes the cell to rupture, thereby releasing the colicin into the environment. Colicin production is mediated by the SOS system and consequently it typically occurs during times of stress. For those colicins released via cell lysis, there is a wealth of mathematical theory (Levin, 1988; Frank, 1994; Durrett & Levin, 1997), as well as *in vitro* (Chao & Levin, 1981; Gordon & Riley, 1999; Kerr *et al.*, 2002) and *in vivo* (Kirkup & Riley, 2004) experimental evidence, that demonstrates the potential importance of colicins in mediating intra-specific interactions.

There is 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 (Rolfe, 2000). 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 in populations of bacteria.

Typically, 30 % of *E. coli* strains in a population will be colicinogenic (Riley & Gordon, 1996). However, the frequency of colicin production can vary markedly among populations (Riley & Gordon, 1996), although the reasons for this variation are largely unknown. Recent evidence indicates that the frequency of colicinogenic strains in a population of *E. coli* can vary depending on the species of host from which the *E. coli* were isolated. For example, the frequency of colicin production in a population of northern quolls, *Dasyurus hallucatus*, was 11 %; 25 % in a population of mountain possums, *Trichosurus caninus*; 59 % in a feral house mouse, *Mus domesticus*, population; and 81 % in a population of tamar wallaby, *Macropus eugenii* (Gordon *et al.*, 1998, 2006).

The average amount of time it takes for food to enter and leave the gastro-intestinal tract is known as the mean

retention time, which is inversely proportional to the turnover rate of the system (Hume, 1999). A high-quality, easily digestible diet, coupled with a short, simple intestinal tract has the consequence that mean particle retention times in a carnivore are relatively short (Hume, 1999). By contrast, lower quality, more difficult to digest diets, together with a longer, more complex intestinal tract result in herbivores and omnivores having longer mean particle retention times (Hume, 1999). For example, mean fluid digesta retention time in the carnivorous eastern quoll (*Dasyurus viverrinus*) is about 13 h on an insect diet and 17 h on a plant diet (Hume 1999). In the similar-sized omnivorous long-nosed bandicoot (*Perameles nasuta*), the mean fluid digesta retention time on the insect diet was 24 h and on a plant diet 33 h (Hume, 1999).

The aim of this paper is to determine if the turnover rate of the gastro-intestinal tract, a factor that varies with the diet and gastro-intestinal morphology of the host species, can predict the frequency of colicinogenic cells in a population of *E. coli*. Empirical evidence demonstrating that the frequency of colicin production varies depending on the diet of the host from which the strain was isolated is presented first, and then a mathematical model is developed that explores the consequences of variation in gastro-intestinal dynamics on the outcome of competition between a colicin producing strain and a colicin-sensitive strain.

EMPIRICAL DATA

Faecal isolates of *E. coli* from asymptomatic hosts can be assigned to one of four genetic groups, or 'subspecies', arbitrarily designated A, B1, B2 and D (Ochman & Selander, 1984; Herzer *et al.*, 1990; Wirth *et al.*, 2006). Strains of the four groups differ in their phenotypic characteristics, including their ability to use different sugars, their antibiotic-resistance profiles and their growth rate-temperature relationships (Gordon, 2004). Genome size varies among the four *E. coli* groups, with A and B1 strains having smaller genomes than B2 or D strains (Bergthorsson & Ochman, 1998). The distribution (presence/absence) of a range of putative virulence factors thought to be involved in the ability of a strain to cause extra-intestinal disease also varies among the four groups (Johnson *et al.*, 2001). The distribution of strains from the four genetic groups of *E. coli* in native Australian vertebrates varies depending on the host group (Gordon & Cowling, 2003). Group B1 strains dominate in hosts with relatively simple tube-like intestines such as birds and the mammalian carnivores: bats and the marsupial carnivores such as the Tasmanian devil (*Sarcophilus harrisii*). Group B2 strains predominate in herbivorous and omnivorous hosts with hindgut microbial fermentation chambers.

The frequency of mitomycin-inducible colicin production in a previously characterized collection of *E. coli* strains

isolated from more than 60 species of native Australian mammal (Gordon & Cowling, 2003) was determined using the methods described by Gordon *et al.* (1998) and by Gordon & O'Brien (2006).

The frequency with which colicin-producing strains of *E. coli* are detected varies depending on the strains' genetic group membership (A, B1, B2, D) and with the diet of the host (Stepwise Nominal Logistic Regression, minimal model: Genetic Group, $\chi^2_{(3496)}=15.98$, $P<0.002$; Host Diet, $\chi^2_{(2496)}=14.73$, $P<0.001$; Genetic Group*Host Diet $\chi^2_{(6496)}=20.16$, $P<0.003$). Overall, colicinogeny was most common in B2 strains (46 %) and less prevalent in strains from group A (26 %), B1 (27 %) and D (23 %). On average, strains isolated from carnivores were less likely to produce a colicin (21 %) than strains isolated from herbivorous (41 %) or omnivorous (42 %) hosts. However, the frequency of colicinogenic strains varied as both a function of genetic group and host diet (Table 1).

THE MATHEMATICAL MODEL

Although there can be a number of *E. coli* strains (genotypes) present in the gut of a host individual (Gordon, 2004), for simplicity, only the interaction between two strains x and y with densities in the gut of X and Y are considered. While including further strains would alter the dynamics, the mechanisms are the same and the outcome is expected to be qualitatively similar. The gut is assumed to have a fixed volume V , with a constant flow rate F into and out of the gut. *E. coli* cells enter and leave the gut with this flow, but we assume the incoming density of cells varies at random. Thus, the rate at which strain X enters the gut is given by $n_X X_{in}$, where n_X is an uniformly distributed stochastic variable between 0 and 1, and X_{in} is the maximum rate that strain x enters the gut via

Table 1. Proportion of *E. coli* strains producing a colicin with respect to their genetic group membership and the diet of the host from which the strain was isolated

Genetic group	Host diet	Sample size	Colicin producers (%)
A	Carnivorous	46	17.4
A	Herbivorous	11	27.3
A	Omnivorous	17	52.9
B1	Carnivorous	85	14.1
B1	Herbivorous	40	30.0
B1	Omnivorous	38	52.6
B2	Carnivorous	34	35.3
B2	Herbivorous	49	63.3
B2	Omnivorous	95	42.6
D	Carnivorous	34	26.5
D	Herbivorous	15	13.3
D	Omnivorous	33	27.3

the host's diet. Similarly, for strain y , n_Y is uniformly distributed and varies stochastically between 0 and 1, and Y_{in} is the maximum incoming rate of strain y . This treatment of intake of an *E. coli* strain as a random process is intended as a simple approximation of the random nature in which such genotypes enter the gut.

We have assumed a logistical rate of growth for each strain with intrinsic rates of growth given by β_X and β_Y , and with carrying capacities K_X and K_Y , respectively. Competition between strains, due to colicin production, is included as follows. It is assumed that each strain is capable of colicin production and that colicin is produced and cells subsequently lyse at the constant, strain-specific rates α_X and α_Y . Each lysed cell releases about 10^6 colicin molecules (Gordon & Riley, 1999). Each colicin molecule is capable of killing a cell of the opposing strain, destroying itself in the process (Reeves, 1972). Assuming mass-action dynamics, the probability of an encounter between a colicin molecule and a sensitive cell is of the order of 10^{-11} (Reeves, 1972; Gordon & Riley, 1999). Given these assumptions, the model is

$$\frac{dX}{dt} = \beta_X X \left(1 - \frac{X}{K_X}\right) + n_X X_{in} \frac{F}{V} - X \frac{F}{V} - \alpha_X X - \delta_X XY \quad (1)$$

$$\frac{dY}{dt} = \beta_Y Y \left(1 - \frac{Y}{K_Y}\right) + n_Y Y_{in} \frac{F}{V} - Y \frac{F}{V} - \alpha_Y Y - \delta_Y XY$$

where $\delta_X XY = (\text{no. of lysed } Y \text{ cells}) \times (\text{no. of colicin molecules per cell}) \times (\text{probability of contact}) \times X \approx \alpha_Y XY \times 10^{-5}$ and $\delta_Y XY = (\text{no. of lysed } X \text{ cells}) \times (\text{no. of colicin molecules per cell}) \times (\text{probability of contact}) \times Y \approx \alpha_X XY \times 10^{-5}$.

To determine if variation in mean residence time $\tau = V/F$ influences the outcome of competition between a colicin-producing strain and a non-producing strain, all other constraints being equal, we fix parameter values based on typical measurements (Gordon & Riley, 1999; Hume, 1999; Gordon, 2004). Thus we fix $\alpha_X > \alpha_Y$, with $\alpha_X = 0.03$ and $\alpha_Y = 0$. All other parameters are set as constants, equal between the strains, with $\beta_X = \beta_Y = 0.3$, $X_{in} = Y_{in} = 0.01 \times 10^6$, $K_X = K_Y = 1 \times 10^6$ and $V = 20$. The stochastic variables n_X and n_Y are determined randomly. Mean residence times in mammals can vary from 1 h for a small carnivorous marsupial to over 213 h for a large folivorous marsupial, such as the koala (Hume, 1999). Since typical cell densities are of the order 10^6 , X , Y , K_X , K_Y , X_{in} and Y_{in} are rescaled in units of 10^6 cells, which results in $K_X = K_Y = 1$, $X_{in} = Y_{in} = 0.01$, $\alpha_X = 0.03$, $\alpha_Y = 0$, $\delta_X = 10\alpha_Y$ and $\delta_Y = 10\alpha_X$.

PROPERTIES OF THE MODEL

Allowing for the random generation of n_X and n_Y each 30 time steps, Fig. 1 illustrates the output of the model for $F=10$, and thus $\tau=2$. Clearly, the switching behaviour

Fig. 1. Predicted scaled densities ($\times 10^3$) of two *E. coli* strains over time as determined by model (1). Strain x , with density X (black), is a colicin producer and strain y , with density Y (red), is a colicin-sensitive strain. Initial values are set at $X_0 = 0.005 = Y_0$, and $F=10$, $X_{in} = 0.01 = Y_{in}$, $K = K_X = K_Y = 1$, $\beta_X = 0.3 = \beta_Y$, $\alpha_X = 0.03$, $\alpha_Y = 0$, $\delta_X = 10\alpha_Y$, $\delta_Y = 10\alpha_X$, and $V = 20$.

between dominating strains is encapsulated by this formulation.

We now consider relevant steady-state values for the system. Numerically we found that for $0 < F < 100$, there is a single stable steady state (X_e, Y_e) in the positive (X, Y) plane given by

$$X_e = \frac{K_X}{2\beta_X} \left[\left(\beta_X - \frac{F}{V} - \alpha_X \right) + \sqrt{\left(\beta_X - \frac{F}{V} - \alpha_X \right)^2 + 4n_X X_{in} \frac{F\beta_X}{V K_X}} \right]$$

$$Y_e = \frac{K_Y}{2\beta_Y} \left[\left(\beta_Y - \frac{F}{V} - \delta_Y X_e \right) + \sqrt{\left(\beta_Y - \frac{F}{V} - \delta_Y X_e \right)^2 + 4n_Y Y_{in} \frac{F\beta_Y}{V K_Y}} \right]$$

with $X_e < Y_e$ for $F > 5.3$ and $X_e > Y_e$ for $0 < F < 5.3$ when $n_X = 1 = n_Y$ (Fig. 2). Note that, while the figures are plotted for $n_X = 1 = n_Y$, variation in these stochastic variables does not change the dynamics qualitatively, only quantitatively.

Due to the inclusion of stochastic variables, theoretical analysis of the model is not straightforward; however, since n_X and n_Y are assumed equally likely to take on any value on the unit interval, we can consider the relative likelihood of various scenarios. The associated equilibrium point may have $Y_e > X_e$, or vice versa, so that either strain may dominate. After multiple simulations with a variety of initial conditions (X_0 and Y_0 in $[0.02, 0.2]$), for $\tau=2$ ($F=10$) our 2500 simulations covering each combination of n_X and n_Y on the unit interval $[0, 1]$ resulted in $Y_e > X_e$ in 60 % of cases, while for $\tau=3.3$ ($F=6$), $Y_e > X_e$ in 78 % of cases. From numerous simulations we conclude that in general, with n_X and n_Y stochastic, for $F > 5.05$ ($\tau < 3.96$), strain y will dominate; that is, the strain without colicin production will dominate when residence times (τ) are low. As τ increases through 3.96 (F decreases through 5.05), the outcome changes. As before, for each combination of n_X and n_Y on the unit interval $[0, 1]$ we ran simulations for initial values X_0 and Y_0 on $[0.02, 0.2]$. Our results indicate that for the case $F=5$ ($\tau=4$), x dominates 51 % of the time, and for

Fig. 2. Y is plotted against X ; the curve corresponding to $dY/dt=0$ is in red and the curve corresponding to $dX/dt=0$ is in black. The intersection $dY/dt=0=dX/dt$ in the positive plane is the stable steady state. Arrows indicate the direction of trajectories over time. Parameter values are as for Fig. 1, with $F=6$ in (a) and $F=4$ in (b), and $n_X=1=n_Y$.

$F=4$ ($\tau=5$), x dominates 98 % of the time. Thus our conclusions are that for $F < 5.05$ ($\tau > 3.96$), strain x will dominate; that is, the strain with the higher rate of colicin production will, in general, dominate when residence times (τ) are relatively high. We note that, from theoretical considerations, no further structural changes occur. Thus our conclusions hold for smaller and larger values for F . Moreover, we note that the inclusion of a stochastic element alters the theoretical results for the case $n_X=1=n_Y$ quantitatively, but not qualitatively. And the quantitative change is marginal.

While the model presented above takes logistic growth into account and incorporates standard functional responses for the death rates, the phenomenon observed above concerning residence times was observed both in the model with Holling type II functional responses for the death rates [$\delta_X XY/(1+\delta_X XY)$ and $\delta_Y XY/(1+\delta_Y XY)$] and also for the simpler case of exponential growth. For the simpler

systems, the mathematics is more tractable, i.e. the equilibrium point has a straightforward algebraic form, and the point at which the dominating strain changes has a simple expression. We note that the value of F for which the switching occurs in all these models is almost exactly the same.

DISCUSSION

Previous models have not focused on turnover time being a factor in determining the dominant *E. coli* genotype. Other theoretical studies have explored the impact of different factors that might lead to considerable variation in the frequency of colicinogenic cells among different populations of bacteria. Colicin-producing strains are predicted to be favoured in habitats with lower rates of resource competition whilst non-producing strains should be favoured in poor-quality habitats (Frank, 1994). The frequency of colicinogenic cells in a population has also been predicted to depend on the degree to which competing cell lineages are related (Gardner *et al.*, 2004). These phenomena could be incorporated into the framework presented here and would not be expected to change the theoretical results.

As well as varying with gastro-intestinal tract morphology and hence with species, gut turnover rates can also vary substantially in the same species fed diets that differ in the type and quantity of fibre they contain (Hume, 1999), as well as with host age and sex, as occurs in humans (Graff *et al.*, 2001). The theoretical prediction that colicin producers should be favoured in good-quality habitats, while non-producers are favoured in poor-quality habitats (Frank, 1994), suggests that the frequency of colicinogenic cells should change along the length of the intestinal tract because resource concentration also changes along the length of the gut (Ballyk & Smith, 1999). The results of this study indicate that when developing probiotic formulations containing antimicrobial-producing bacteria care will need to be taken to ensure a proper match between the characteristics of the bacterial strain, the pathogens it is targeting, and the gastro-intestinal dynamics of the target host population.

In this paper we have considered a very simple two-dimensional model in order to examine the relationship between the turnover rate of the gastro-intestinal tract and the outcome of the interaction between two strains of *E. coli* that differ in their rates of colicin production. Real systems typically consist of many more interacting strains that may compete for resources, and differ in their reproduction rates, as well as other parameters. However, the model, in spite of its simplicity, appears to encapsulate the observed phenomena. That is, in hosts with fast gut turnover rates, such as carnivores, *E. coli* strains that do not produce colicins are predicted to more frequently dominate in a host. Conversely, in hosts exhibiting slower gut turnover rates, such as omnivores and herbivores,

colicin-producing strains are predicted to be observed more frequently.

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