Effect of diet and gut dynamics on the establishment and persistence of *Escherichia coli*

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Escherichia coli population dynamics and diversity in rats fed diets differing in their crude fibre content were assessed. Female Wistar rats ($n=40$) were fed diets containing 1, 4, 18 or 26 % crude fibre. Animals were housed in pairs, and one animal was inoculated with a phylogroup B1 strain of *E. coli*, the other with a phylogroup B2 strain. Natural strain transmission was allowed to occur between the animals in each cage. As expected, the diets had a significant effect on gut dynamics. Mean gut retention times were shorter in animals fed the 18 and 26 % crude fibre diets compared with animals on the low-fibre diets. The effect of diet on gastrointestinal dynamics in turn affected *E. coli* population dynamics and clonal composition. Animals fed the low-fibre diets had higher cell densities than animals fed the high-fibre diets. *E. coli* populations dominated by phylogroup B2 strains exhibited lower cell densities in animals fed the high-fibre diets compared with cell densities in animals fed the low-fibre diets. Overall, *E. coli* cell densities declined as gut transit times decreased. Results from this experiment support the results garnered from prospective studies examining the distribution of *E. coli* from hosts with differing diets, gut morphology and dynamics.

INTRODUCTION

The lower gastrointestinal tract is home to a diverse microbiota (Palmer *et al.*, 2007). Although the extent of the diversity is increasingly well understood (Eckburg *et al.*, 2005; Durbán *et al.*, 2011), the factors contributing to the observed diversity and community dynamics are only now beginning to be elucidated (Ventura *et al.*, 2007; Khachatryan *et al.*, 2008; Turnbaugh *et al.*, 2009; Jakobsson *et al.*, 2010). Although *Escherichia coli* is a minor representative of the microbiota in numerical terms, it is a very significant species from the perspective of human and animal health. The species is capable of causing both intestinal and extra-intestinal disease, and is believed to cause more than two million deaths per year (Russo & Johnson, 2003; Wennerås & Erling, 2004; Steffen *et al.*, 2005).

E. coli can be isolated from a wide variety of vertebrate hosts, but is most common in birds and mammals (Gordon & Cowling, 2003). However, the probability of detecting *E. coli* in an individual host varies with host species (Gordon & Cowling, 2003). For example, mammals such as bats and small marsupial carnivores are unlikely to harbour *E. coli* (Pupo *et al.*, 2000; Gordon & Cowling, 2003). *E. coli* is a genetically and ecologically diverse species, and strains of the species can be subdivided into at least five distinct phylogroups (Gordon *et al.*, 2008; Tenaillon *et al.*, 2010). Strains of the various phylogroups

differ in their phenotypic and genotypic characteristics, in their ecological niches, as well as in their propensity to cause disease (Tenaillon *et al.*, 2010). Strains of the various phylogroups are non-randomly distributed with respect to host species in non-human mammals (Gordon & Cowling, 2003; Carlos *et al.*, 2010), and within humans are non-randomly distributed with respect to host locality (Tenaillon *et al.*, 2010) and the age and sex of the host (Gordon *et al.*, 2005).

Why might these differences in the prevalence of *E. coli* or the distribution of strains of the different phylogroups exist among or within host species? One way that host species differ is in their diet and body size. Plant material is energy-poor, and through fermentation, microbes provide extra nutrients to herbivores by converting plant cell wall components anaerobically to fatty acids (Hume, 1999). Microbial fermentation often takes place in modified gut structures in either the foregut or the hindgut (Hume, 1999). In contrast, animals provide predators with a protein-rich food source whose digestibility is not dependent on fermentation, and consequently many predators have tube-like intestinal tracts often lacking fermentation chambers (Hume, 1999). Owing to the differences in gut morphology and digestive strategies, retention times also vary between herbivores and carnivores. In general, energy-poor food takes longer to digest and convert to energy, and this increases the mean retention times. Energy-rich food spends less time in the gut because minimal time is spent extracting energy from it, and therefore mean retention times are shorter. Mean

Abbreviations: HSD, honestly significant difference; REP, repetitive extragenic palindromic.

retention time increases with host body mass because it takes longer for food to move through a longer gastrointestinal tract. Among humans, diets vary with country, while gut morphology and dynamics vary between the sexes and with age (Hounnou *et al.*, 2002; Graff *et al.*, 2001; Córdova-Fraga *et al.*, 2008).

The predictions of mathematical models show that differences in gut morphology and dynamics will influence the likelihood that a population of bacteria is able to establish in a host (Ballyk & Smith, 1999; Jones *et al.*, 2002). The models describing batch flow reactors represent a gastrointestinal tract incorporating a fermentation chamber, such as a caecum. The models mimicking a plug flow reactor represent a narrow tube-like gut, void of chambers or space for mixing of digesta. Two steady states have been described for these models. One involves the complete washout of microbes, and the other entails the successful establishment of microbes on the intestinal wall and in the enclosed fluid. Which of the steady states arises depends on numerous factors, including the length of the gastrointestinal tract, the rate of flow through the tract, and the concentration and distribution of nutrients throughout it. Properties of the bacterial strain itself, such as its growth rate, will also influence the success of establishment. The probability of establishment for a strain unable to adhere to the gastrointestinal tract lining increases as a function of gut length, but if a strain has a predisposition to adhere it can establish in a gut of any length (Ballyk & Smith, 1999; Ballyk *et al.*, 2001).

The diversity of gastrointestinal environments observed among different mammal species, or among individuals of a single species differing in sex, age or diet preferences, are a consequence of the interplay of gut morphology, dynamics and host diet. The available empirical evidence and predictions of mathematical theory indicate that the conditions of the gastrointestinal tract will determine whether *E. coli* cells are able to establish and persist, as well as determining the type of *E. coli* that are able to establish. However, there is relatively little direct evidence to indicate that the conditions to be found in the lower gastrointestinal tract influence the dynamics of *E. coli* populations or the types of *E. coli* to be found. Consequently an experiment was undertaken whereby rats were fed on diets differing in their crude fibre content, and the effects of the

diet treatments on *E. coli* population dynamics and diversity were assessed.

METHODS

Experimental design. A randomized complete block design was used, consisting of five blocks of four cages. There were four diet treatments and these were assigned at random to cages within a block. Each block was assigned to its own row in the cage rack and the position of the cages within a row remained the same throughout the experiment. Each cage housed two female outbred (Wistar) rats and all animals within a block were sisters. The ages of the animals at the start of the experiment were as follows: block 1, 77 days; block 2, 48 days; block 3, 42 days; block 4, 27 days; block 5, 57 days. The experiment was approved by the Animal Experimentation Ethics Committee of the Australian National University (protocol number F.BTZ4204).

Diets. The diets used in this study were commercial diets manufactured by the Hill's Pet Nutrition Company. In choosing from the diets available, three criteria were used: each diet supplied the minimal daily nutrient requirements of the laboratory rat (National Research Council, 1972), varied as much as possible in their crude fibre concentration and had similar crude protein concentrations (Table 1). The diets are referred to by their crude fibre content, i.e. 1, 4, 18 and 26 %.

Diet batches were tested twice for bacterial contamination by suspending two 1 g samples of each diet in 10 ml MacConkey agar broth and incubating the culture overnight at 37 °C. A 1 ml sample of the overnight culture was then plated onto a MacConkey agar plate that was then incubated overnight at 37 °C. No bacterial colonies were detected using either test.

Animal husbandry. The rats were housed in standard high-top cages and maintained at 21 °C with a 12:12 day:night cycle. Cages were changed weekly using steam-sterilized bedding (Animal bedding, Fibrecycle) and a small amount of sterile cotton wool was added to provide environmental enhancement. Cages were chemically disinfected and dried at 55 °C overnight prior to their use. Rats were weighed weekly. Latex gloves were worn whenever the animals were handled in order to minimize cage-to-cage and foreign bacterial contamination.

Food consumption. Diets were provided *ad libitum*. A known amount of each diet was weighed out daily, with the amount representing 20–30 % more food than the animals would consume in a day. Fresh food was provided every morning and all food from the previous day was removed using sterile forceps, weighed and discarded. The amount of food consumed by the two rats in a cage

Table 1. Compositions of the diets used in the experiment

Composition (percentage dry matter)	Name of diet			
	Canine i/d	Canine c/d	Light Adult	Canine r/d
Crude fibre	1.5 %	4 %	18 %	26 %
Protein	23 %	19 %	20 %	20 %
Fat	9 %	17 %	6 %	6 %
Carbohydrate	52 %	47 %	47 %	38 %
Energy, kcal kg ⁻¹ (kJ kg ⁻¹)	3849 (16 104)	4195 (17 551)	2997 (12 539)	2699 (11 292)

was calculated as the difference between the amount of food provided and the amount remaining.

Faecal production. Faecal production was determined on three occasions: weeks 3, 7 and 11. Faecal production estimates were obtained by removing all faeces and orts produced by the two animals in each cage over one week, then drying the faeces to a constant weight at 55 °C.

Mean retention times. At the end of the experiment one animal was chosen at random from each of the 20 cages. These animals were housed individually and their daily food consumption and total faecal production over 10 days were determined. These animals were also used for the determination of mean retention times of liquid and particles by the gastrointestinal tract using the methods outlined in Caton (1997). The fluid component of digesta was marked with 0.1 g cobalt-EDTA (Co-EDTA), the particulate component with 0.2 g chromium mordanted onto cell wall constituents (Cr-CWC) for each rat. Both markers were ground with a drop of vegetable oil and the appropriate diet using a mortar and pestle. A pellet was formed in a pellet machine. The animals were deprived of food for 1 h before the pellet was introduced. All animals ate more than 85 % of the pellet containing the markers. After the markers had been ingested, all of the faeces produced by each animal were collected every 3 h for 3 days.

The faeces from each sample were dried to a constant weight at 55 °C. Hot nitric acid digestion was used to break down carbon compounds and this was followed by the addition of hydrogen peroxide (33 %, w/w). The cobalt and chromium concentration of every sample was determined using a Vista AX CCD Simultaneous inductively coupled plasma atomic emission spectrometer (Varian).

Gut morphology. All rats were killed using CO₂ and necropsied once the transit time data were obtained. The gastrointestinal tract was removed from each animal and completely flushed with water to remove the contents and divided into sections: oesophagus and stomach, small intestine, caecum and colon. The tissue of each gut region was dried to a constant weight at 75 °C.

Strain selection and exposure. Survey data indicate that the phylogroup membership (A, B1, B2 or D) of the dominant strain in a host varies with host diet and body mass (Gordon & Cowling, 2003). Phylogroup B1 and B2 strains are among the most common *E. coli* to be isolated from mammals, and in order to determine whether B1 and B2 strains responded differently to diet the rats were exposed to a strain belonging to phylogroup B1 or B2 (Gordon *et al.*, 2008). The strains of *E. coli* used in this study were originally isolated from *Rattus* species and form part of the Australian Enteric Collection (Gordon & Lee, 1999; Gordon & Cowling, 2003). These strains had been assigned to an *E. coli* phylogroup using the method of Clermont *et al.* (2000). Ten B1 and 10 B2 strains, each representing a different REP genotype (see below), were selected at random from those available. Each strain was used in two different pair combinations and the B1 and B2 strains were paired at random. The strains used were: TA063, TA150, TA207, TA217, TA222, TA241, TA367, TA369, TA372, TA374, TA389, M040, M171, M176, M196, M207, M220, M236, M411 and M479.

Starting on day 32 of the experiment all animals were treated with streptomycin for 4 days via their drinking water (250 mg l⁻¹). Fresh faeces were collected from all animals immediately following the cessation of antibiotic treatment. The faeces of each animal were suspended in 10 ml sterile saline (0.85 %), and 1 ml of the faecal suspension was plated onto a MacConkey agar plate that was incubated overnight at 37 °C. No colonies were detected on any of the plates following incubation. The detection limit for this procedure was 100 cells (g faeces)⁻¹. In order for the antibiotic to clear the

gastrointestinal tract, the animals were held for 24 h and fed sterile food and water prior to inoculation. The animals were housed individually and exposed to the appropriate *E. coli* strains via their food and drinking water. About 1.5 × 10¹⁰ cells were added to 10 g of the appropriate diet and 3.0 × 10¹⁰ cells were added to a water bottle containing 100 ml sterile water. After 24 h the animals were paired in their appropriate cages and provided with the appropriate diet and sterile water.

Cell density determinations. *E. coli* cell densities [c.f.u. (g faeces)⁻¹] were determined weekly, with all of the animals in one block being sampled each day. To collect the faecal pellets, each animal was housed in an empty cage until it produced faeces (usually <15 min). In general (more than 95 % of samples), two faecal pellets were collected per animal. The faeces from each animal were transferred aseptically to a pre-weighed sterile 15 ml centrifuge tube containing 0.9 ml sterile 0.85 % saline. The centrifuge tube and faeces were then weighed and a sufficient amount of sterile saline was added to each tube to achieve a total volume of 10 ml. The faeces were broken up with a sterile glass rod and the tubes were then vortexed for 1 min. The faecal suspension was serially diluted and a 100 µl aliquot of each dilution was spread on a MacConkey agar plate. The plates were incubated overnight at 37 °C. Cell counts were determined by counting the number of c.f.u. that were observed on each of the plates.

The moisture content of the faeces varied depending on the diet that the animal was being fed (data not shown). Consequently, from week 3 onwards, estimates were made of the dry weight of the faeces sampled. After the centrifuge tubes containing the 10 ml of faecal suspension had been sampled, the tubes were transferred to a drying oven and dried to a constant weight at 75 °C. The weight of the dry faeces was determined by accounting for the weight of the tube and the salt that had been added. Cell densities were then expressed as cells per gram dry weight of faeces. The moisture content of the faecal pellets was estimated as the difference between the wet and dry faecal weights divided by the wet weight of faeces in the sample.

Genotyping the bacteria. Two *E. coli* isolates were sampled from the faeces of each animal when they arrived from the breeding colony (prior to antibiotic treatment). At the end of the experiment a subsample of cells from each animal were genotyped using PCR-based methods. From the MacConkey plates used to estimate cell densities, 16 colonies were selected at random and transferred to a Luria broth plate for temporary storage. These cells were used to inoculate overnight Luria broth cultures, and the genomic DNA of these cells was extracted using DNAzol (Invitrogen) following the manufacturer's protocol. The two isolates recovered from the rats prior to antibiotic treatment, together with the 16 isolates collected from each animal at the end of the experiment as well as the inoculant strain, were assigned to an ECOR group using the methods of Clermont *et al.* (2000) and genotyped using a PCR-based fingerprinting method known as the repetitive extragenic palindromic (REP) method. Oligonucleotides analogous to highly conserved REP units were used as PCR primers to create DNA fingerprints of each *E. coli* isolate (Versalovic *et al.*, 1991). All samples from an animal were run on the same gel. When the results suggested that the same strain was present in different animals, these isolates were genotyped again and then run side-by-side on a gel.

Statistical analyses. The specific statistical analyses that were used are described in Results. All analyses were carried out using the software package JMP V5 (SAS Institute). Cell densities were transformed (log₁₀) prior to analysis. For all analyses the block effect was maintained in the model, regardless of whether block effects were significant or not.

RESULTS

Host response to diets

Food consumption was expressed as milligrams food consumed per gram total body mass (both animals) per day basis for each cage. A repeated measures analysis of variance (ANOVA) revealed that there was a significant difference in food consumption among animals fed on the four diets (repeated measures ANOVA: diet, $F_{(3)}=248.6$, $P<0.0001$; block, $F_{(4)}=6.55$, $P<0.005$). Predicted consumption was greatest for animals that were fed the 26 % diet [98.7 mg food (g¹ body mass)⁻¹ day⁻¹], intermediate for the animals on the 18 % diet (81.8) and lowest for the animals on the 1 % (60.2) and 4 % (58.5) diets (means adjusted for animal age).

The production of faeces was measured over 1 week on three occasions for all animals, at weeks 6, 10 and 14, and once over 10 days for a single animal from each cage at the end of the experiment. Faecal production was expressed as milligrams faeces per gram rat per day. There was a significant effect of diet on faecal production (ANOVA: diet, $F_{(3,79)}=2016.96$, $P<0.0001$; sample, $F_{(3,79)}=79.04$, $P<0.0001$; sample $\times$ diet, $F_{(9,79)}=9.10$, $P<0.0001$; block, $F_{(4,79)}=9.89$, $P<0.0001$). Predicted average faecal production for animals on the four diets was: 5.9, 7.0, 18.9 and 33.7 mg faeces (g body mass)⁻¹ day⁻¹ for the 1, 4, 18 and 26 % fibre diets, respectively. Overall, predicted average faecal production declined over the course of the experiment from 19.7 to 13.8 mg (g rat)⁻¹ day⁻¹.

ANOVA was used to determine whether diet affected the size of various gastrointestinal tract components, and Tukey's honestly significant difference (HSD) tests were used to investigate a posteriori differences among the means. The size of the gastrointestinal tract may be expected to vary with the body mass of the animal; therefore, body mass was included in the model as a covariate. There was a significant difference in the size of the stomach among animals on the four diets, with animals fed the 4 % diet having the smallest stomachs (Table 2). There was no significant effect of diet on the mass of the small intestine or caecum (Table 2). Colon mass varied depending on diet, and there was an increase in colon mass as the fibre content of the diet increased (Table 2).

Cobalt was used to monitor the rate at which liquids moved through the rat gut, whilst chromium mordanted to plant material was used to monitor the time taken for particles to pass through the gastrointestinal tract. Two measures were used to quantify passage rates: the time at which the marker first appeared in the faeces and mean retention time. Mean retention time was estimated using the formula: $\Sigma M_i T_i / M_i$, where M_i is the amount of marker in the i th sample and T_i is the time (h) at which the sample was taken (Caton, 1997). No relationship between any of the two measures of gut passage rates and the total amount of chromium or cobalt recovered from an animal could be detected (data not presented).

Table 2. Results of ANOVA and Tukey HSD analyses of the effect of diet on the gastrointestinal mass of rats fed diets differing in their crude fibre content

Effect	Stomach	Small intestine	Caecum	Colon
ANOVA $P>F$				
Diet	=0.0428	=0.0860	=0.3208	=0.0054
Body mass	=0.0457	=0.0699	=0.8078	=0.0690
Diet $\times$ body mass	=0.2336	=0.1924	=0.2647	=0.2279
Block	=0.3771	=0.5822	=0.5424	=0.9622
Means (mass, mg)				
1 % fibre	323 ^{AB†}	1311	261	456 ^B
4 % fibre	295 ^B	1445	218	462 ^B
18 % fibre	335 ^{AB}	1530	262	529 ^{AB}
26 % fibre	354 ^A	1433	261	663 ^A

†Predicted means with the same letter are not significantly different ($P<0.05$).

The liquid marker first appeared in the faeces 1 h sooner than the particle marker: 6 versus 7 h (paired comparison t test; $t_{(19)}=2.33$ $P>|t|=0.0308$). However, the first appearance of either marker was independent of diet: liquid marker (ANOVA: diet $F_{(3,12)}=0.056$, $P=0.9815$; block, $F_{(4,12)}=0.51$ $P=0.7309$), particle marker (ANOVA: diet $F_{(3,12)}=0.28$, $P=0.8366$; block, $F_{(4,12)}=0.56$, $P=0.6959$).

The mean retention times of the liquid and particle markers did not differ (paired comparison t test; $t_{(19)}=-1.32$ $P>|t|=0.2031$). Consequently, a repeated measures ANOVA was used to investigate the effect of diet on mean retention time. Mean retention times of the markers for animals fed on the 1 and 4 % diets were significantly longer than the mean retention times for animals fed on the 18 and 26 % diets (Table 3) (repeated measures ANOVA: diet $F_{(3,12)}=5.58$, $P=0.0124$; block, $F_{(4,12)}=2.31$, $P=0.1172$). There was a longer 'tail' to the distribution of marker concentration [mg (g faeces)⁻¹] in the animals fed the low-fibre diets compared with those on the high-fibre diets (Fig. 1).

Given that no difference between the mean retention times of the liquid and particle markers could be detected, all further analyses were based on the means of the retention estimates of the two markers for each animal, and the mean value was used to estimate a gut turnover rate (the inverse of retention time).

Diet effects and temporal variation in *E. coli* cell densities

There was a significant effect of diet on cell numbers per dry weight of faeces (repeated measures ANOVA: diet, $F_{(3, 39)}=17.53$, $P<0.0001$; block, $F_{(4,39)}=6.49$, $P<0.0006$). Cell densities were consistently greater in animals fed the 1 and 4 % diets over the course of sampling (Fig. 2).

Table 3. Passage rate estimates for liquid and particle markers in female Wistar rats fed diets differing in their crude fibre content

Values shown are mean $\pm$ SEM.

Diet	First appearance (h)		Retention time (h)	
	Liquid	Particle	Liquid	Particle
1 % fibre	6.0 $\pm$ 1.01	7.2 $\pm$ 1.37	16.0 $\pm$ 1.12	16.2 $\pm$ 1.17
4 % fibre	6.1 $\pm$ 2.35	7.9 $\pm$ 2.84	17.3 $\pm$ 1.59	16.9 $\pm$ 1.32
18 % fibre	5.7 $\pm$ 0.97	5.7 $\pm$ 0.97	12.9 $\pm$ 1.02	12.6 $\pm$ 0.88
26 % fibre	6.7 $\pm$ 1.85	7.9 $\pm$ 1.63	13.0 $\pm$ 0.79	12.6 $\pm$ 0.79

Cell density and gut turnover

There was a significant negative relationship between the estimated gut turnover rate and cell density estimates at week 11 (ANOVA: turnover rate, $F_{(1,14)}=31.42$, $P<0.0001$; block, $F_{(4,14)}=1.70$, $P=0.1207$). Cell densities varied with diet, but the significant effect of turnover rate remained after the effect of diet was removed (ANOVA, terms added sequentially: diet, $F_{(3,11)}=11.01$, $P=0.0012$, turnover rate, $F_{(1,11)}=25.19$, $P=0.0004$, block, $F_{(4,11)}=2.14$, $P=0.1437$). Overall, turnover rate ranged from 0.046 h^{-1} (retention time 21.8 h) to 0.10 h^{-1} (retention time 10 h), and this twofold range in turnover rates corresponded to a 20-fold decline in cell density (Fig. 3). No significant difference could be detected in the slope of the lines (diet $\times$ turnover rate) describing the relationship between cell density and gut turnover rate, regardless of diet [(ANOVA: diet, $F_{(3,8)}=0.29$, $P=0.8315$; turnover rate, $F_{(1,8)}=12.85$, $P=0.0071$; diet $\times$ turnover rate, $F_{(3,8)}=0.68$, $P=0.591$; block, $F_{(4,8)}=1.37$, $P=0.3259$].

E. coli genotype diversity

At the end of the experiment, 16 *E. coli* clones were isolated from every animal, genotyped using REP PCR and assigned to one of the *E. coli* genetic groups. Among the 640 isolates characterized, 27 genotypes were detected. Of the 27 genotypes, five had REP fingerprints matching the B1 inoculant strains and four had REP fingerprints matching

the B2 inoculant strains. Consequently, of the 27 genotypes detected, 18 did not represent inoculant strains; of these, nine belonged to phylogroup B1, six to phylogroup B2, two to phylogroup D and one to phylogroup A. The REP fingerprinting results suggested that in five animals a strain present prior to antibiotic treatment was also present in the animal at the end of the experiment.

The distribution of each genotype among animals is depicted in Table 4. Genotypes belonging to phylogroup B1 were significantly more likely to be found in multiple animals than were genotypes belonging to phylogroup B2 (likelihood ratio $\chi^2_{(1)}=6.17$, $P=0.013$). There were 14 genotypes belonging to phylogroup B1 and 10 (71%) of these were recovered from more than one animal. By contrast, of the 10 phylogroup B2 genotypes, only four (40%) were detected in more than one rat.

Richness was defined as the number of different genotypes observed in an animal, whilst genotype diversity (H) was estimated as $1 - \sum p_i^2$, where p_i is the frequency of the i th genotype detected in an animal. The median number of genotypes detected in an animal was two; 45% harboured a single genotype, 37% harboured two, 8% harboured three and 10% harboured four genotypes. Genotype diversity varied from 0 to 0.73 and the mean $\pm$ SEM was 0.19 ± 0.035 . The mean number of genotypes (richness) was not found to vary with respect to diet (nominal logistic regression: diet, likelihood ratio $\chi^2_{(9)}=13.22$, $P=0.153$; block, likelihood ratio $\chi^2_{(12)}=13.87$, $P=0.3091$). Diet explained none of the observed variation in genotype diversity (H) (Kruskal–Wallis test; diet, $\chi^2_{(3)}=2.61$, $P=0.4568$).

E. coli phylogroup membership and cell density

ANOVA was used to investigate whether the observed cell density in an animal depended on the phylogroup membership of the dominant strain in an animal and whether diet affected this relationship. To this end, the proportion of isolates present in an animal that belonged to phylogroup B1 was estimated. The majority (92%) of the animals harboured strains belonging to phylogroups B1 and B2 (Table 4). Phylogroup A or D isolates were only

Fig. 1. Change in the average faecal concentration of the particle marker (chromium) over time in female Wistar rats fed on the 1% (dashed line) or 26% (solid line) fibre diets.

Fig. 2. *E. coli* cell densities in female Wistar rats fed different diets over 8 weeks. Day 0 corresponds to the day on which antibiotic treatment of the animals ceased. ◆, 1% fibre; ■, 4% fibre; ●, 18% fibre; ▲, 26% fibre.

found in three animals, and in these animals they represented fewer than 12% of the isolates present. Therefore, if fewer than 100% of the isolates in an animal were phylogroup B1, then the balance of the isolates were, the vast majority of the time, members of phylogroup B2.

The results of the analysis revealed that, as expected, cell densities differed among diets, and there was a significant percentage B1 × diet interaction (ANOVA: diet, $F_{(3,26)}=9.66$, $P=0.0002$; percentage B1, $F_{(1,26)}=0.076$, $P=0.785$; percentage B1 × diet, $F_{(3,26)}=3.82$, $P=0.021$; block, $F_{(4,26)}=6.69$, $P=0.0008$). For animals on the 1 and 4% crude fibre diets, the same cell densities were observed, regardless of whether the *E. coli* cells in an animal were predominantly B1 or not (Fig. 4). In contrast, cell densities observed in animals fed the 18 and 26% diets were significantly lower if the cells in an animal were predominantly B2, but as the proportion of B1 cells in an animal increased so did cell densities (Fig. 4).

DISCUSSION

Of all the components of an animal's diet, fibre has the greatest influence on the dynamics and morphology of the gastrointestinal tract. Traditionally, fibre is considered to

be the food component of plants not broken down by native enzymes or secretions of the gastrointestinal tract, but which may be metabolized by bacteria in the hindgut (FAO/WHO, 1998; AACC, 2001). There are two main types of fibre: soluble and insoluble. Pectins, glucans, gums and some hemicelluloses are soluble and the most easily metabolized by micro-organisms. The insoluble fibre fraction consists of some hemicelluloses, cellulose and lignin – the only non-carbohydrate component of dietary fibre. The hemicelluloses are the most easily metabolized by micro-organisms, followed by cellulose, whilst lignin is highly resistant to fermentation, and high lignin concentrations may inhibit the fermentation process in the hindgut (Cummings, 1997; Van Soest *et al.*, 1991).

Diets containing large amounts of resistant fibre, such as lignin, act as roughage, and significantly increase the amount of faeces produced by an animal as well as the total amount of material present in the gastrointestinal tract (Hume, 1999). In addition, retention times also decrease as the proportion of resistant fibre in the diet increases (Pei *et al.*, 2001a, b; Hume *et al.*, 1993), and there tends to be little difference between particle and liquid retention times (Campbell *et al.*, 2004). By contrast, when animals are fed diets high in fermentable fibre, gut mass or surface area will

Fig. 3. Relationship between *E. coli* cell densities and the turnover rate of the gastrointestinal tract of female Wistar rats fed on diets differing in crude fibre content. Symbols, observed data; solid line, prediction of the regression model. ▲, 1% fibre; ●, 4% fibre; ■, 18% fibre; ◆, 26% fibre.

Table 4. Distribution of *E. coli* genotypes and their phylogroup membership (A, B1, B2, D) among animals and cages at the end of the experiment

The designations B1 and B2 in the Animal column denote the phylogroup membership of the strains to which the animal was exposed.

Block	Diet	Cage	Animal	Genotype*			
1	26 % fibre	1	B1	1:B1	2:B2	3:B1	4:B1*
			B2	5:B2			
	18 % fibre	2	B1	3:B1	6:B1		
			B2	6:B1			
	1 % fibre	3	B1	3:B1	7:B1		
			B2	3:B1	7:B1		
	4 % fibre	4	B1	3:B1	8:B2	9:B2	
			B2	8:B2†	9:B2	10:B1	11:D
2	26 % fibre	1	B1	6:B1			
			B2	6:B1	12:D	13:B2†	
	18 % fibre	2	B1	6:B1	14:B1†		
			B2	6:B1	15:B1	16:B2	17:A
	4 % fibre	3	B1	6:B1			
			B2	6:B1			
	1 % fibre	4	B1	6:B1			
			B2	6:B1			
3	1 % fibre	1	B1	6:B1			
			B2	6:B1			
	26 % fibre	2	B1	6:B1	14:B1†		
			B2	6:B1			
	18 % fibre	3	B1	6:B1	18:B1†		
			B2	18:B1			
	4 % fibre	4	B1	3:B1			
			B2	3:B1	6:B1		
4	1 % fibre	1	B1	19:B2			
			B2	6:B1	19:B2	20:B1	21:B2
	4 % fibre	2	B1	6:B1	22:B1		
			B2	6:B1	22:B1		
	18 % fibre	3	B1	19:B2	23:B1†		
			B2	19:B2	24:B2†		
	26 % fibre	4	B1	25:B1			
			B2	10:B1	25:B1	26:B2†	
5	18 % fibre	1	B1	5:B2	27:B1†		
			B2	5:B2	27:B1		
	1 % fibre	2	B1	4:B1†	5:B2		
			B2	4:B1	5:B2		
	4 % fibre	3	B1	19:B2			
			B2	19:B2			
	26 % fibre	4	B1	19:B2			
			B2	19:B2			

*The number corresponds to a unique REP fingerprint, A, B1, B2 and D to the phylogroup membership of the strain.

†Denotes the establishment of the inoculant strain in the animal exposed to that strain.

increase, and these changes are often most noticeable in the caecum (Pei *et al.*, 2001a, b; Lee & Houston, 1993; Karasov & Diamond, 1988). Also, retention times increase overall (Felicetti *et al.*, 2000; Chilcott & Hume, 1985), and the

mean retention time of the liquid marker is increased relative to the mean retention time of the particle marker (Björnhag, 1994; Felicetti *et al.*, 2000; Sakaguchi, 2003).

Peanut hulls were used to achieve the fibre concentration of the 18 and 26 % fibre diets employed in the present study, and peanut hulls contain a high concentration of lignin (Cummings, 1997; AACC, 2001; FAO/WHO, 1998). Thus, as expected, the diets used in this experiment largely affected gastrointestinal transit times, and did not significantly alter gut morphology or cause the selective retention of the liquid component of the digesta. The only systematic changes in gut morphology with diet were seen in the colon, and these changes were almost certainly a consequence of the need to cope with the significantly larger faecal pellets produced by the animals on the 18 and 26 % crude fibre diets.

Cell density declined as gut turnover rates increased, and this accords with the predictions of chemostat theory (Smith & Waltman, 1995). Although extrapolating beyond the data is not recommended practice, the observed relationship between cell density and gut turnover rates would predict that *E. coli* cell densities would decline to undetectable levels if turnover rates were 0.25–0.33 h⁻¹ (mean residence times 3–4 h). Many species of bats, small insectivores and passerine birds have mean gut retention times of less than 3 h and for some species mean retention times may be as little as 1 h (Herrera & Martínez Del Río, 1998; Hume, 1999; Mancina *et al.*, 2005). *E. coli* is seldom detected in such species (Klite, 1965; Pupo *et al.*, 2000; Gordon & Cowling, 2003), and the results of this study would suggest that this is because their rapid gut transit times make it unlikely that *E. coli* cells would be able to establish and maintain a population.

As well as the turnover rate, chemostat theory predicts that steady-state cell densities will depend on the incoming resource concentration, the efficiency with which resource is converted to cell biomass, and the nature of the relationship between the population rate of cell division, $r_{(t)}$, and resource concentration, $R_{(t)}$. If a Monod function is assumed, then the rate of population change, $r_{(t)} = r_{\max} R_{(t)} / (Q + R_{(t)})$, where $r_{\max}$ is the maximum growth rate, R is the limiting resource concentration at time t and Q is the substrate concentration at $r_{\max}/2$. There is no significant difference in the slope of the lines describing the relationship between cell density and gut turnover rate among the four diets. This outcome suggests that resource concentration, conversion efficiency, $r_{\max}$ and Q are little influenced by the exact constituents of the diets used in these experiments, despite the fact that carbohydrate concentrations and energy content vary substantially among the diets.

Cell densities in this experiment varied, depending on diet, from 5.2×10^5 to 6.3×10^6 cells per gram wet faeces. In humans, from 10^6 to 10^7 cells per gram wet faeces are typical, but this can vary over time by over 1000-fold (McOrist *et al.*, 2005). To confirm that the cell densities in this experiment were lower than expected, 25 female Wistar

Fig. 4. Relationship between the proportion of *E. coli* B1 cells in female Wistar rats and the cell densities achieved for animals on diets differing in crude fibre content. Symbols, observed data; lines, predictions of the statistical model. $\blacktriangle$, 1% observed; $\bullet$, 4% observed; $\blacksquare$, 18% observed; $\blacklozenge$, 26% observed. Long- and short-dashed line, 1% predicted; short-dashed line, 4% predicted; solid line, 18% predicted; long-dashed line, 26% predicted.

rats housed in the breeding colony from which the experimental animals were sourced were sampled. The cell densities observed in these breeding females ranged from 10^7 to 10^8 cells per gram faeces.

The reasons for these differences are unknown, but may reflect differences in the diets of the animals. The diets used in the present experiment either had a low fibre content or a large fraction of the fibre component was composed of lignin. Furthermore, most of the protein in these diets is animal-derived. The ingredients of the commercial rat chow fed to the breeding females are to a much greater extent derived from plant material and include lucerne, soybean meal, sorghum, bran and wheat. Alternatively, there are data that show that *E. coli* cell densities may increase with the age of the human host by almost 100-fold (McOrist *et al.*, 2005). Breeding females would be significantly older than the females used in these experiments.

The *E. coli* genotypes recovered from the animals at the end of study were clearly derived from different sources. In addition to the inoculant strains, some of the genotypes were detected in the animals prior to and after antibiotic exposure. As no *E. coli* were detected in the faeces of any animal immediately following antibiotic treatment, it is possible that the densities of these strains declined to below detectable frequencies during treatment and increased in density once antibiotic treatment ceased. Alternatively, these strains may have been present on the skin or hair of the animals, particularly in the perianal region. The grooming behaviour of the animals in the 24 h between the end of antibiotic treatment and inoculation may have resulted in the animals re-infecting themselves during this window of opportunity. An additional source of strains was the external environment. Although attempts were made to minimize contamination, the animals were not housed in sealed cages supplied with filtered air. Open cages also meant that faecal material and bedding could move between cages as a result of an animal's activity, and this may account for the sharing of genotypes among cages. However, regardless of

the source of the strains inhabiting these rats, the results clearly show that the *E. coli* cells are responding to the environment of the lower intestine.

It is well established that *E. coli* differ in their phenotypic and genotypic characteristics as well as in their life history characteristics (Tenaillon *et al.*, 2010). The relative abundances of the main phylogroups of *E. coli* have been shown to vary with body mass and diet in mammals (Gordon & Cowling, 2003; Escobar-Páramo *et al.*, 2006), with host age and sex in humans (Gordon *et al.*, 2005), and with geographical locality in humans (Tenaillon *et al.*, 2010).

It was not the purpose of this study to compare the establishment success of the phylogroup B1 and B2 inoculant strains, but to determine whether the dynamics of established strains belonging to different phylogroups differed in their response to host diet. The present study shows that strains belonging to the different *E. coli* phylogroups respond directly to changes in diet, and that B2 strains appear to be unable to maintain cell densities similar to those of B1 strains in animals fed on high-fibre diets.

E. coli strains belonging to phylogroup B2 cause a significant fraction of community-acquired urinary tract infections in humans, and the strains responsible for infection are thought to originate from the intestinal *E. coli* community of the infected host (Mobley & Warren, 1996). The results of the present study show that it is possible to modify the abundance of phylogroup B2 strains in the lower gastrointestinal tract. Whether it is possible to reduce the abundance of B2 strains in a human population through diet manipulation to an extent sufficient to diminish the incidence of uncomplicated urinary tract infection is unknown.

In conclusion, the results of the present experimental study support the conclusions of the retrospective studies, in that strains of *E. coli* differ in their response to the gastrointestinal environment of the host in which they find themselves.

REFERENCES

- AACC (2001). The definition of dietary fibre. Report of the dietary fibre definition committee to the board of directors of the American Association of Cereal Chemists. *46*, 112–126.
- Ballyk, M. M. & Smith, H. L. (1999). A model of microbial growth in a plug flow reactor with wall attachment. *Math Biosci* **158**, 95–126.
- Ballyk, M. M., Jones, D. A. & Smith, H. L. (2001). Microbial competition in reactors with wall attachment: a mathematical comparison of chemostat and plug flow models. *Microb Ecol* **41**, 210–221.
- Björnhag, G. (1994). Adaptations in the large intestine allowing small animals to eat fibrous foods. In *The Digestive System in Mammals: Food, Form and Function*. Edited by D. J. Chivers & P. Langer. Cambridge, UK: Cambridge University Press.
- Campbell, J. L., Williams, C. V. & Eismann, J. H. (2004). Use of total dietary fiber across four lemur species (*Propithecus verreauxi coquereli*, *Haplemur griseus griseus*, *Varecia variegata*, and *Eulemur fulvus*): does fiber type affect digestive efficiency? *Am J Primatol* **64**, 323–335.
- Carlos, C., Pires, M. M., Stoppe, N. C., Hachich, E. M., Sato, M. I., Gomes, T. A., Amaral, L. A. & Ottoboni, L. M. (2010). *Escherichia coli* phylogenetic group determination and its application in the identification of the major animal source of fecal contamination. *BMC Microbiol* **10**, 161.
- Caton, J. M. (1997). *Digestive strategies of non-human primates*. PhD thesis, Australian National University, Australia.
- Chilcott, M. J. & Hume, I. D. (1985). Coprophagy and selective retention of fluid digesta: their role in the nutrition of the common ringtail possum, *Pseudocheirus peregrinus*. *Aust J Zool* **33**, 1–15.
- Clermont, O., Bonacorsi, S. & Bingen, E. (2000). Rapid and simple determination of the *Escherichia coli* phylogenetic group. *Appl Environ Microbiol* **66**, 4555–4558.
- Córdova-Fraga, T., De la Roca-Chiapas, J. M., Solís, S., Sosa, M., Bernal-Alvarado, J., Hernández, E. & Hernández-González, M. (2008). Gender difference in the gastric emptying measured by magnetogastrography using a semi-solid test meal. *Acta Gastroenterol Latinoam* **38**, 240–245.
- Cummings, J. H. (1997). *The Large Intestine in Nutrition and Disease*. Danone Chair Monograph. Brussels, Belgium: Institut Danone.
- Durbán, A., Abellán, J. J., Jiménez-Hernández, N., Ponce, M., Ponce, J., Sala, T., D'Auria, G., Latorre, A. & Moya, A. (2011). Assessing gut microbial diversity from feces and rectal mucosa. *Microb Ecol* **61**, 123–133.
- Eckburg, P. B., Bik, E. M., Bernstein, C. N., Purdom, E., Dethlefsen, L., Sargent, M., Gill, S. R., Nelson, K. E. & Relman, D. A. (2005). Diversity of the human intestinal microbial flora. *Science* **308**, 1635–1638.
- Escobar-Páramo, P., Le Menac'h, A., Le Gall, T., Amorin, C., Gouriou, S., Picard, B., Skurnik, D. & Denamur, E. (2006). Identification of forces shaping the commensal *Escherichia coli* genetic structure by comparing animal and human isolates. *Environ Microbiol* **8**, 1975–1984.
- FAO/WHO (1998). Carbohydrates in human nutrition: report of a joint FAO/WHO expert consultation. *FAO Food Nutr Pap* **66**, 1–140.
- Felicetti, L. A., Shipley, L. A., Witmer, G. W. & Robbins, C. T. (2000). Digestibility, nitrogen excretion, and mean retention time by North American porcupines (*Erethizon dorsatum*) consuming natural forages. *Physiol Biochem Zool* **73**, 772–780.
- Gordon, D. M. & Cowling, A. (2003). The distribution and genetic structure of *Escherichia coli* in Australian vertebrates: host and geographic effects. *Microbiology* **149**, 3575–3586.
- Gordon, D. M. & Lee, J. (1999). The genetic structure of enteric bacteria from Australian mammals. *Microbiology* **145**, 2673–2682.
- Gordon, D. M., Stern, S. E. & Collignon, P. J. (2005). Influence of the age and sex of human hosts on the distribution of *Escherichia coli* ECOR groups and virulence traits. *Microbiology* **151**, 15–23.
- Gordon, D. M., Clermont, O., Tolley, H. & Denamur, E. (2008). Assigning *Escherichia coli* strains to phylogenetic groups: multi-locus sequence typing versus the PCR triplex method. *Environ Microbiol* **10**, 2484–2496.
- Graff, J., Brinch, K. & Madsen, J. L. (2001). Gastrointestinal mean transit times in young and middle-aged healthy subjects. *Clin Physiol* **21**, 253–259.
- Herrera, M. L. & Martínez Del Río, C. (1998). Pollen digestion by New World bats: effects of processing time and feeding habits. *Ecology* **79**, 2828–2838.
- Hounnou, G., Destrieux, C., Desmé, J., Bertrand, P. & Velut, S. (2002). Anatomical study of the length of the human intestine. *Surg Radiol Anat* **24**, 290–294.
- Hume, I. D. (1999). *Marsupial Nutrition*. Cambridge: Cambridge University Press.
- Hume, I. D., Morgan, K. R. & Kenagy, G. J. (1993). Digesta retention and digestive performance in sciurid and microtine rodents: effects of hindgut morphology and body size. *Physiol Zool* **66**, 396–411.
- Jakobsson, H. E., Jernberg, C., Andersson, A. F., Sjölund-Karlsson, M., Jansson, J. K. & Engstrand, L. (2010). Short-term antibiotic treatment has differing long-term impacts on the human throat and gut microbiome. *PLoS ONE* **5**, e9836.
- Jones, D., Le, D., Smith, H. & Kojouharov, H. V. (2002). Bacterial wall attachment in a flow reactor. *SIAM J Appl Math* **62**, 1728–1771.
- Karasov, W. H. & Diamond, J. M. (1988). Interplay between physiology and ecology in digestion: intestinal nutrient transporters vary within and between species. *Bioscience* **38**, 602–611.
- Khachatryan, Z. A., Ktsoyan, Z. A., Manukyan, G. P., Kelly, D., Ghazaryan, K. A. & Aminov, R. I. (2008). Predominant role of host genetics in controlling the composition of gut microbiota. *PLoS ONE* **3**, e3064.
- Klite, P. D. (1965). Intestinal flora and transit time of three neotropical bat species. *J Bacteriol* **90**, 375–379.
- Lee, W. B. & Houston, D. C. (1993). The effect of diet quality on gut anatomy in British voles (Microtinae). *J Comp Physiol B* **163**, 337–339.
- Mancina, C. A. & Balseiro, F. Herrera, M. (2005). Pollen digestion by nectarivorous and frugivorous Antillean bats. *Mamm Biol* **70**, 282–290.
- McOrist, A., Veulett, G., Vuaran, M., Bird, A., Noakes, M. & Topping, D. (2005). Population and virulence factor dynamics in fecal *Escherichia coli* from healthy adults consuming weight control diets. *Can J Microbiol* **51**, 467–475.
- Mobley, H. L. T. & Warren, J. W. (1996). The vagina flora and urinary tract infections. In *Urinary Tract Infections: Molecular Pathogenesis and Clinical Management*, vol. 3, pp. 67–94. Edited by T. M. Hooton & W. E. Stamm. Washington, DC: American Society for Microbiology.
- National Research Council (1972). Nutrient requirements of the laboratory rat. In *Editorial Committee on Animal Nutrition. Nutritional Requirements of Laboratory Animals*, pp. 56–93. Washington, DC: National Academy of Science.
- Palmer, C., Bik, E. M., DiGiulio, D. B., Relman, D. A. & Brown, P. O. (2007). Development of the human infant intestinal microbiota. *PLoS Biol* **5**, e177.
- Pei, Y. X., Wang, D. H. & Hume, I. D. (2001a). Selective digesta retention and coprophagy in Brandt's vole (*Microtus brandti*). *J Comp Physiol B* **171**, 457–464.

- Pei, Y. X., Wang, D.-H. & Hume, I. D. (2001b).** Effects of dietary fibre on digesta passage, nutrient digestibility, and gastrointestinal tract morphology in the granivorous Mongolian gerbil (*Meriones unguiculatus*). *Physiol Biochem Zool* **74**, 742–749.
- Pupo, G. M., Lan, R., Reeves, P. R. & Baverstock, P. R. (2000).** Population genetics of *Escherichia coli* in a natural population of native Australian rats. *Environ Microbiol* **2**, 594–610.
- Russo, T. A. & Johnson, J. R. (2003).** Medical and economic impact of extraintestinal infections due to *Escherichia coli*: focus on an increasingly important endemic problem. *Microbes Infect* **5**, 449–456.
- Sakaguchi, E. (2003).** Digestive strategies of small hindgut fermenters. *Anim Sci J* **74**, 327–337.
- Smith, H. L. & Waltman, P. (1995).** *The Theory of the Chemostat*. Cambridge: Cambridge University Press.
- Steffen, R., Castelli, F., Dieter Nothdurft, H., Rombo, L. & Zuckerman, J. N. (2005).** Vaccination against enterotoxigenic *Escherichia coli*, a cause of travelers' diarrhea. *J Travel Med* **12**, 102–107.
- Tenaillon, O., Skurnik, D., Picard, B. & Denamur, E. (2010).** The population genetics of commensal *Escherichia coli*. *Nat Rev Microbiol* **8**, 207–217.
- Turnbaugh, P. J., Ridaura, V. K., Faith, J. J., Rey, F. E., Knight, R. & Gordon, J. I. (2009).** The effect of diet on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice. *Sci Transl Med* **1**, 6–14.
- Van Soest, P. J., Robertson, J. B. & Lewis, B. A. (1991).** Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J Dairy Sci* **74**, 3583–3597.
- Ventura, M., Canchaya, C., Tauch, A., Chandra, G., Fitzgerald, G. F., Chater, K. F. & van Sinderen, D. (2007).** Genomics of Actinobacteria: tracing the evolutionary history of an ancient phylum. *Microbiol Mol Biol Rev* **71**, 495–548.
- Versalovic, J., Koeuth, T. & Lupski, J. R. (1991).** Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. *Nucleic Acids Res* **19**, 6823–6831.
- Wennerås, C. & Erling, V. (2004).** Prevalence of enterotoxigenic *Escherichia coli*-associated diarrhoea and carrier state in the developing world. *J Health Popul Nutr* **22**, 370–382.

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