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Molecular Characterization of Commensal *Escherichia coli* Adapted to Different Compartments of the Porcine Gastrointestinal Tract

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The role of *Escherichia coli* as a pathogen has been the focus of considerable study, while much less is known about it as a commensal and how it adapts to and colonizes different environmental niches within the mammalian gut. In this study, we characterize *Escherichia coli* organisms ($n = 146$) isolated from different regions of the intestinal tracts of eight pigs (dueodenum, ileum, colon, and feces). The isolates were typed using the method of random amplified polymorphic DNA (RAPD) and screened for the presence of bacteriocin genes and plasmid replicon types. Molecular analysis of variance using the RAPD data showed that *E. coli* isolates are nonrandomly distributed among different gut regions, and that gut region accounted for 25% ($P < 0.001$) of the observed variation among strains. Bacteriocin screening revealed that a bacteriocin gene was detected in 45% of the isolates, with 43% carrying colicin genes and 3% carrying microcin genes. Of the bacteriocins observed (H47, E3, E1, E2, E7, Ia/Ib, and B/M), the frequency with which they were detected varied with respect to gut region for the colicins E2, E7, Ia/Ib, and B/M. The plasmid replicon typing gave rise to 25 profiles from the 13 Inc types detected. Inc F types were detected most frequently, followed by Inc HI1 and N types. Of the Inc types detected, 7 were nonrandomly distributed among isolates from the different regions of the gut. The results of this study indicate that not only may the different regions of the gastrointestinal tract harbor different strains of *E. coli* but also that strains from different regions have different characteristics.

Escherichia coli is one of the commonly occurring enteric bacteria in the mammalian gastrointestinal tract (GIT), exhibiting a lifestyle that ranges from that of an obligate pathogen to a commensal (20). *E. coli*'s role as a pathogen has been the focus of considerable study; however, much less is known about its function as a commensal (30). Unraveling the population structure of commensal *E. coli* may reveal how the acquisition or loss of particular genes can enhance its capacity to adapt and colonize different environmental niches within the mammalian gut (17, 30).

There is a growing body of evidence demonstrating that a significant fraction of the genetic variation observed in *E. coli* recovered from the feces of an animal is explained, in part, by the morphology and dynamics of the host's gastrointestinal tract (11–13). Although the evidence is much less extensive, it has also been shown that there is a nonrandom distribution of *E. coli* genotypes among the different regions of the gastrointestinal tract (8, 27). This diversity is not unexpected, as epithelial cell types differ among the regions of the GIT (26). In addition, the quality and quantity of nutrients varies along the length of the gut, as do transit times of material moving through the gut (16, 28).

Plasmids harbor a variety of genes that contribute to a strain's establishment and persistence in particular environments. Such traits include toxin production, iron uptake systems, antibiotic resistance, and bacteriocin production (4, 18–20). Bacteriocins are antimicrobial peptides produced following exposure to iron-limiting conditions or stressful situations that elicit SOS responses (25). Empirical and theoretical studies have shown that bacteriocins mediate competitive interactions among cells, and bacteriocin production has been shown to play a significant role in the colonization of the gastrointestinal tract by *E. coli* (10, 14). Mathematical models have also shown that the production of colicins is not universally advantageous (9). Bacteriocin producers tend to be favored in situations where resource competition is less intense, while nonproducers are favored when the rate of resource competition is high (9). The predictions of mathematical models have also shown that colicin production will be favored when gut turnover rates are low, while nonproducers dominate when gut turnover rates are high (3). Consequently, given that nutrient concentrations and turnover rates vary along the length of the gastrointestinal tract, the frequency of bacteriocin-producing strains also should vary along the gastrointestinal tract.

To test this hypothesis, *E. coli* strains isolated from different regions of the porcine gut were screened for the presence of genes associated with bacteriocin production (8). In addition, given the importance of plasmids in the adaptation of the *E. coli* host to its environment, the strains were screened to determine the number and diversity of plasmid replicon types among the isolates.

MATERIALS AND METHODS

Bacterial strains. Eight 13-week-old male pigs (hybrids of Large White and Landrace) from eight different litters, weighing 35 to 45 kg at the start of the experiment, were used in this study. The pigs were housed in separate pens and provided with water and commercial pelleted diet (Vella Stock Feeds, New South Wales, Australia) *ad libitum*. After 3 weeks, the animals were killed and the GITs removed. A section of gut was removed

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TABLE 1 Frequency of bacteriocins and plasmid replicons in a collection of commensal *E. coli* isolates from different intestinal sections of grower pigs

Bacteriocin or plasmid replicon type	Prevalence (%) in:					Contingency analysis[a] ($P > \chi^2$)
	Overall ($n = 146$)	Duodenum ($n = 30$)	Ileum ($n = 36$)	Colon ($n = 36$)	Feces ($n = 44$)	
Bacteriocin						
Microcin H47	3.4	0.0	5.6	2.8	4.5	0.439
Colicin E3	2.1	0.0	8.3	0.0	0.0	ND
Colicin E1	8.9	3.3	8.3	13.9	9.1	0.482
Colicin E2	15.1	0.0	8.3	11.1	34.1	<0.0001
Colicin E7	20.5	0.0	16.7	19.4	38.6	<0.0001
Colicin Ia/Ib	13.7	6.7	33.3	11.1	4.5	0.002
Colicin B	8.2	6.7	22.2	5.6	0.0	0.002
Colicin M	8.2	6.7	22.2	5.6	0.0	0.002
Plasmid replicon						
B/O	2.1	0.0	0.0	2.8	4.5	ND
FIC	8.9	10.0	13.9	0.0	11.4	0.045
P	4.8	0.0	0.0	13.9	4.5	0.012
K/B	2.7	6.7	0.0	5.6	0.0	ND
FIA	17.8	0.0	13.9	5.6	43.2	<0.0001
FIB	47.9	53.3	47.2	30.6	59.1	0.071
Y	7.5	20.0	11.1	0.0	2.3	0.004
I1	22.6	16.7	36.1	22.2	15.9	0.156
Frep	54.8	60.0	72.2	50.0	40.9	0.034
X	2.1	0.0	8.3	0.0	0.0	ND
HI1	28.8	16.7	47.2	13.9	34.1	0.005
N	30.8	16.7	55.6	13.9	34.1	0.0004
H12	0.7	3.3	0.0	0.0	0.0	ND

[a] ND, not determined.

from each of three regions: duodenum, the proximal 4-cm region immediately after the stomach; ileum, the distal 4-cm region immediately before the ileocecal junction; and colon, a 4-cm region immediately past the cecum. A fecal sample was also removed from the rectum. The methods that were used to isolate and identify *E. coli* are described by Dixit et al. (8). A total of 146 *E. coli* isolates from duodenum ($n = 30$), ileum ($n = 36$), colon ($n = 36$), and feces ($n = 44$) were characterized in this study (8).

Characterization of the isolates. These isolates had been previously characterized using the technique of multilocus enzyme electrophoresis, screened for a range of putative virulence factors (*fimH*, *iha*, *kpsMTII*, *hlyA*, *fyuA*, *ompT*, *traT*, K5, stx_1, and *eae*), and assigned to phylogroups according to the method of Clermont et al. (7, 8). However, as the isolates had only been characterized at 10 enzyme loci, they were further characterized using the random amplified polymorphic DNA (RAPD) profiling method.

DNA extraction was performed using 6% percent Chelex 100 (Bio-Rad, Australia) with 100 μl of overnight culture from a single colony as previously described (2).

Plasmid replicon typing was carried out to determine the presence of 18 replicons using three multiplex and one uniplex PCR. These assays were performed using primers (5), a multiplex pool, and PCR conditions as previously described (1).

The presence of the following 16 bacteriocins was assessed for each isolate using multiplex and uniplex reactions: colicins A, B, D, E1, E2, E3, E6, E7, Ia/Ib, K, and M and microcins B17, H47, J25, M, and V (1).

RAPD analysis was carried out to assess the clonality of the commensal *E. coli* strains using RAPD primers 1247, 1254, and 1290 (21, 22).The PCR was performed using methods previously described (1). Gel-to-gel variations of the RAPD patterns were minimized by running the samples on a 1.5% agarose gel with the same dimensions at 120 V for 2 h. BioNumerics 6.1 was used to normalize the gels using the same DNA ladder.

Statistical analysis. BioNumerics 6.1 (Applied Maths, Belgium) was used to convert the RAPD profiles into band presence/absence data. Comparisons of the frequency of traits among isolates taken from different gut regions were based on contingency table analysis and likelihood ratio tests as implemented in JMP (V9.0). Molecular analysis of variance and Mantel correlation tests were executed using GenAlEx (6.4) (23). Multiple correspondence analysis was carried out using the software Tanagra (24).

RESULTS

RAPD profiling. The presence/absence of RAPD bands was used to construct a Euclidean distance matrix, and the amount of variation explained by gut region within each pig was determined using a nested molecular analysis of variance. These results revealed that, on average, gut region accounted for 25.3% ($P < 0.001$) of the observed variation, while differences among pigs accounted for 1.4% ($P = 0.02$) of the variation.

Bacteriocin profiling. The bacteriocin gene assay targeting 16 colicin and microcin genes revealed that a bacteriocin gene was detected in 45% of the 146 isolates, with 43% of the isolates carrying colicin genes and 3% carrying microcin genes. Of the 66 strains that carried bacteriocin genes, 27% carried one type of bacteriocin, 59% carried two, 11% carried three, and two strains were found to carry four different bacteriocin types. Overall, 18 different bacteriocin profiles were observed.

The proportion of isolates harboring one or more colicin genes varied significantly among gut regions (according to contingency analysis of the likelihood ratio, $\chi^2 = 19.887$ and $P = 0.0002$), with 13.3% of the isolates from the duodenum, 63.9% from the ileum, 38.9% from the colon, and 50.0% from feces being positive for one or more colicins. Among the isolates that possessed one or more bacteriocin genes, the average number of bacteriocins detected per

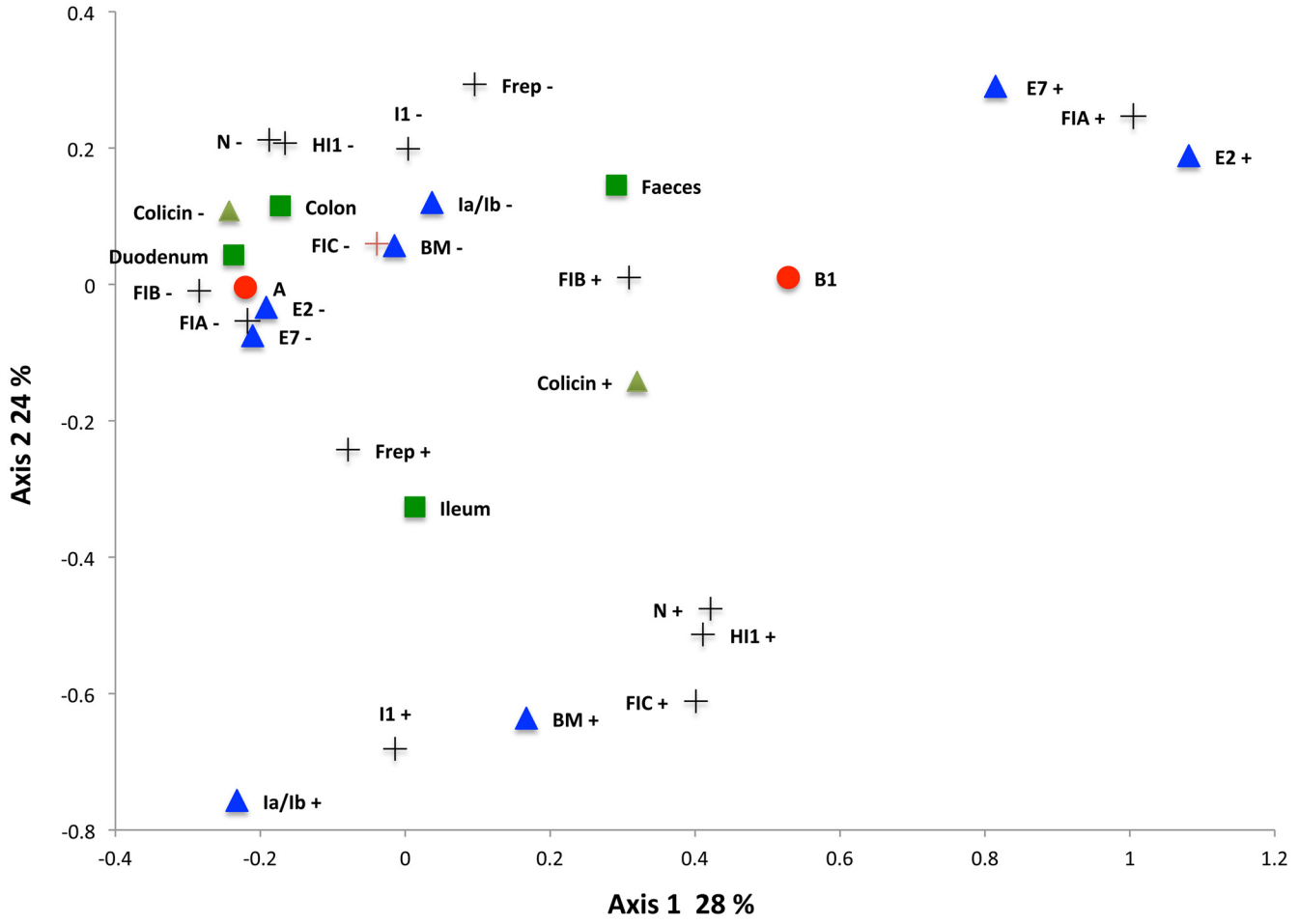

FIG 1 Multiple correspondence analysis of bacteriocin genes, plasmid replicon types, and Clermont phylogenetic groups of *E. coli* isolated from duodenum, ileum, colon, and feces of grower pigs. Triangles, crosses, circles, and squares represent bacteriocin genes, plasmid types, Clermont grouping, and intestinal sections, respectively.

isolate did not vary with respect to the region of the gut from which they were isolated ($\chi^2 = 14.516$; $P = 0.1051$).

Neither the microcins M, B17, J25, and V nor the colicins A, D, E6, and K were detected. Of the bacteriocins observed (H47, E3, E1, E2, E7, Ia/Ib, and B/M), the frequency with which they were detected varied with respect to gut region for the colicins E2, E7, Ia/Ib, and B/M (Table 1). Colicins E2 and E7 were detected more frequently in fecal isolates, while the colicins Ia/Ib, B, and M were more frequent among isolates taken from the ileum (Table 1).

Plasmid profiling. The prevalence of plasmid replicons among the *E. coli* isolates was analyzed using single and multiplex PCR assays targeting 18 plasmid replicons (Table 1). Replicons possessing Inc A/C, FIIA, L/M, T, and W were not detected. The 13 detected Inc types gave rise to 25 profiles. Inc F types were detected most frequently, followed by Inc HI1 and N types (Table 1). Of the Inc types detected, 7 were nonrandomly distributed among isolates from the different regions of the gut (Table 1). For example, Inc FIA was detected most frequently in isolates taken from feces, while Inc Y was detected most often in isolates from the small intestine (Table 1).

DISCUSSION

Our previous investigation using multilocus enzyme electrophoresis (MLEE) indicates that among the same 146 isolates, there were 95 distinct MLEE profiles (8). Combining the MLEE results with the bacteriocin and Inc group profiles suggests that the 146 isolates represent at least 130 distinct genotypes. Thus, the nonrandom distribution of traits among isolates from different gut regions is unlikely to reflect the overrepresentation of a few genotypes in one or more gut regions. However, the traits that were screened for do not occur independently of one another within a strain. Most of the bacteriocins investigated are plasmid encoded. Further, most of the plasmids encoding the bacteriocins Ia/Ib, B, and M are F-type plasmids (6, 18). Further, it is well known that colicins B and M almost always co-occur (6). Colicins E2 and E7 are typically encoded on small nonconjugative plasmids. In this study, colicin E2 or E7 was detected in 33 isolates; however, in 19 of the isolates both E2 and E7 were detected. The cooccurrence of colicins E2 and E7 on separate plasmids in a single strain has not been previously reported. Strains harboring a single 6-kb plasmid able to produce a toxin to which K-12 strains harboring either colicin E2

or E7 plasmid are immune have been reported from Australia (15), as have colicin E2/colicin E7 chimeras (29).

Due to the coassociation of many of the traits examined in this study, the data were analyzed using the technique of multiple correspondence analysis (Fig. 1). The results of this analysis support the basic conclusion that the traits considered in the study are nonrandomly distributed among *E. coli* isolated from different gut regions. *E. coli* isolates from the ileum are more likely to encode colicin Ia/Ib and colicins B and M and to be Inc I1, FIC, HI1, or Inc N positive. Fecal isolates are more likely to be positive for colicins E2 and E7 and to be Inc FIA positive.

The extent to which the nonrandom distribution of Inc types reflects the nonrandom distribution of plasmid-encoded traits other than bacteriocins is not clear. That is, does the variety of Inc profiles observed among this collection of isolates simply yield a fingerprint of the isolate, much as RAPD profiling does, or do the different Inc profiles represent markers for unknown traits that are nonrandomly distributed among gut regions? Mantel correlations between the Euclidian distance matrices based on the RAPD data, bacteriocin profiles, and Inc profiles reveal that the correlation between the distance matrices based on the RAPD and Inc group data was 0.219 ($P < 0.001$), while the correlation between the bacteriocin profiles and RAPD distance matrices was 0.082 ($P < 0.032$). This result suggests that the nonrandom distribution of Inc groups is more of a reflection of the nonrandom distribution of *E. coli* isolates as reflected by the RAPD data rather than the nonrandom distribution of other, as-yet undetermined, plasmid-associated traits.

Colicin-producing strains were least prevalent among isolates from the duodenum. A previous study has shown that colicin-producing strains are less frequent among *E. coli* isolated from mammalian hosts with rapid gut transit times (carnivores) compared to those with slower gut transit times (herbivores or omnivores) (3). Mathematical models also predict that colicin-producing strains are less likely to have a competitive advantage compared to nonproducers when gut transit times are short (3). In the present study, the fact that colicin-producing strains were least prevalent among those isolated from the duodenum is in accord with these previous observations. Transit times in the duodenum are shorter than those in the large intestine (16, 28).

Strains producing the bacteriocins Ia/Ib, B, and M were more frequent among isolates taken from the ileum, while strains producing colicin E2 or E7 were more common among fecal isolates. Colicins E2 and E7 target the vitamin B_{12} receptor and are released as a consequence of cell lysis. Colicins Ia/Ib, B, and M are excreted from the cell and target siderophore receptors. The significance of the differences in colicin types being produced by strains from different gut regions is unknown. Do these differences reflect differences in the nature of the competitive interactions occurring in different gut regions, where competition for iron is occurring in the ileum, while more generalized nutrient competition is occurring among fecal isolates? Or do the differences reflect differences in the cost of colicin production relative to gut transit times in the different gut regions? Colicins released as a consequence of cell lysis may impose a higher cost on the producing population than those that are excreted from the cells.

The results of this study show that not only may the different regions of the gastrointestinal tract harbor different strains of *E. coli* but also that strains from different regions have different characteristics. The observation that the frequency of colicin production and type of colicins produced varies with gut region has implications for the design or selection of probiotic strains.

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