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Molecular analysis of bacterial populations in the ileum of broiler chickens and comparison with bacteria in the cecum

Jianhua Gong [a,*], Robert J. Forster [b], Hai Yu [a], James R. Chambers [a], Roger Wheatcroft [a], Parviz M. Sabour [a], Shu Chen [c]

[a] *Food Research Program, Agriculture and Agri-Food Canada, Guelph, ON, Canada N1G 5C9*
[b] *Lethbridge Research Center, Agriculture and Agri-Food Canada, Lethbridge, AB, Canada T1J 4B1*
[c] *Laboratory Services Division, University of Guelph, Guelph, ON, Canada N1H 8J7*

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Abstract

Bacterial populations in the ileum of broiler chickens were analyzed by molecular analysis of 16S rRNA genes and compared to those in the cecum. Bacteria found in the ileal mucosa were mainly Gram-positive with low G+C content. There were 15 molecular species among 51 cloned sequences. More than 70% of the cloned sequences were related to lactobacilli and *Enterococcus cecorum*. Two sequences had 95% or less homology to existing database sequences. Terminal restriction fragment length polymorphism (T-RFLP) analysis revealed differences among bacterial populations present in the mucosa and lumen of the ileum. Comparative studies by T-RFLP and sequence analyses of 16S rRNA genes indicated a less diverse bacterial population in the ileum (mucosa and lumen) than in the cecum. Lactobacilli, *E. cecorum*, and butyrate-producing bacteria related (including both identified and unidentified species) sequences were the three major groups detected in ilea and ceca. Although butyrate-producing bacteria may have good potential in the development of novel probiotics for poultry, verifying the presence of the bacteria in the chicken gut is required to warrant further investigation.

Keywords: Chicken ileum; Cecum; 16S rRNA; Phylogeny; Diversity

1. Introduction

The normal gut microbiota in farm animals is important because of its effect on the production of livestock and the quality and safety of livestock products. In poultry, while the cecal microbiota can protect chickens against bacterial infection, a healthy microbiota present in the small intestine contributes significantly to small intestinal function, including digestion and nutrient absorption, which is the limiting factor determining growth rate. Modern commercial poultry production practices, including artificial egg incubation, hatching and rearing, routine medication and facility hygiene, however, impede natural transmission of microbiota constituents between generations of birds. Chickens are consequently more susceptible to colonization by bacterial pathogens. To prevent chickens from acquiring infection and to promote growth, poultry producers in North America currently rely on sub-therapeutic use of antibiotics in diets. Increased public concern over the development and spread of antibiotic resistance in bacteria and the possible presence of antibiotic residuals in poultry products has led to a search for alternatives to the use of antibiotics in chicken diets. Popular alternatives to the use of antibiotics have been probiotics, which have been used in poultry for 'competitive exclusion' of bacterial pathogens [1]. However, a better understanding of the microbial ecology of chicken gut microbiota is required for the development of novel probiotics and to achieve the most effective use of commercial probiotics.

The chicken gut microbiota has previously been studied by culture-based methods (reviewed in [1]). Because these methods are inapplicable to non-cultivable bacteria and are selective for readily cultivated bacteria [2,3], our understanding of the gut microbiota in the past may be inaccurate and certainly incomplete. To overcome these limitations, molecular approaches are being increasingly used to characterize the gut microbiota, including cultiva-

* Corresponding author. Tel.: +1 (519) 829-2400 ext. 3107; Fax: +1 (519) 829-2600.
E-mail address: gongj@em.agr.ca (J. Gong).

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ble and non-cultivable bacteria, although some drawbacks have been recognized [4–6]. Recently, we reported a study on the diversity and phylogenetic relationship of bacteria in the cecum of broiler chickens using molecular analysis of 16S rRNA genes [7]. Cecal bacteria were found to be more diverse and to have a more complicated community structure than previously reported using the culture-based approach. A similar observation was also reported very recently by Zhu et al. [8]. In our study, 25% of the sequences exhibited less than 95% homology to 16S rRNA sequences available in databases, and these may represent new species [9] from the chicken gut. We also reported that a significant proportion of cloned sequences were related to uncultured bacteria from human feces [10] or from bovine rumen [11]. To broaden our understanding of chicken gut microbial ecology and to provide a scientific base for the effective development and use of probiotics, we investigated the diversity and community structures of bacterial populations in the ileum (the lower part of the small intestine) of broiler chickens. To our knowledge, this is the first report that describes the diversity and phylogenetic relationship of bacteria present in the ileal mucosa by molecular analysis of 16S rRNA genes. Terminal restriction fragment length polymorphism (T-RFLP) analyses are presented of mucosal and lumen bacteria. Populations of both mucosa-associated and unassociated bacteria are compared in the two gut regions.

2. Materials and methods

2.1. Chicken maintenance and sample collection

Chickens used in this investigation were those of the previous study on the cecal microbiota [7]. In brief, broiler chickens (Ross/Ross) were reared under controlled management conditions similar to those used in commercial practice. Management and experimental procedures were carried out in accordance with the welfare guidelines of the Animal Care Committee, University of Guelph (AUP 98R161). The birds were fed non-medicated corn-soy broiler diets [12] containing 18–22% crude protein and 3073–3195 kcal metabolizable energy per kg. Gut samples were collected from the ilea and ceca of 10 six-week-old broiler chickens. All gut samples were kept on ice and processed immediately after dissection. All collected ileal or cecal samples from 10 chickens were combined for preparation of bacterial samples that were subsequently used for DNA extraction.

Bacterial samples from digesta were prepared essentially by the method of Apajalahti et al. [13]. Ileal and cecal wall-associated bacterial samples, which we referred to as mucosal bacteria, were prepared as described recently [7]. Briefly, ilea and ceca were opened longitudinally and washed three times in saline to remove unattached or loosely attached bacteria from the wall. Bacterial cells were then released from the wall by two washes in saline containing 0.1% Tween 80 with vigorous hand shaking for 30 s per wash followed by centrifugation (27 000 × *g*, 20 min) at 4°C to pellet the cells. This procedure was shown to release about 95% of the wall-associated bacterial cells. Samples for DNA extraction were frozen in liquid nitrogen and stored at −70°C.

2.2. Cell lysis and DNA extraction

Bacterial samples prepared from 10 chickens were subjected to five freeze–thaw cycles, alternating between liquid nitrogen temperature and 65°C for 5 min in the presence of β-mercaptoethanol (5 μl ml^{-1}), followed by bead-beating as described by Stahl et al. [14] to lyse cells. DNA was extracted from cell lysates using the method of phenol/chloroform extraction and ethanol precipitation as described previously [11].

2.3. Random cloning of 16S rRNA genes

16S rRNA genes were amplified by PCR from genomic DNA of mucosal bacteria using eubacterial primers F8 (5′-AGAGTTTGATCCTGGCTCAG-3′) and R1492 (5′-GGTTACCTTGTTACGACTT-3′) [15]. PCR reaction mixtures were the same as described previously [11]. The thermocycle program was 30 s at 94°C, 30 s at 50°C and 2 min at 72°C for 25 cycles followed by 10 min at 72°C. PCR products were cloned into vector, pCR®4-TOPO®, using the TOPO TA cloning kit (Invitrogen) according to the manufacturer's instructions, and partially sequenced with an ABI PRISM™ 377 Automated DNA Sequencer.

2.4. Sequence and phylogenetic analysis

Partial 16S rDNA sequences corresponding to *Escherichia coli* 16S rRNA bases 400–1050 were compared directly with the GenBank, EMBL and DBJI non-redundant nucleotide databases using BLAST. Sequence alignment and phylogenetic analysis were conducted as described previously [11]. Briefly, cloned 16S rDNA sequences and closely related reference sequences were aligned using the program tkDCSE [16]. Phylogenetic trees were generated using a neighbor-joining method [17], in the PHYLO_WIN package [18], with pairwise gap removal and Jukes–Cantor correction [19]. In order to validate the tree, statistical bootstrapping [20] was carried out with data resampled 1000 times. Sequences were also compared by generating similarity matrices. Putative chimeric sequences were identified using the program Check_Chimera [21].

2.5. T-RFLP analysis

Eubacterial primers, F8 and R1492, were used for T-RFLP. F8 was labeled with 6-FAM (6-carboxyfluorescein,

Applied Biosystems), while R1492 was labeled with NED (Applied Biosystems). PCR conditions were the same as those used for random cloning. Aliquots of amplified rDNA products were separately digested with *Alu*I, *Hha*I and *Msp*I (New England Biolabs) according to the manufacturer's instructions. The lengths of terminal restriction fragments (70–600 bp) were determined by comparison with Rox-labeled internal standards using an ABI PRISM™ 377 Automated DNA Sequencer and GeneScan® Analysis Software (Applied Biosystems).

2.6. Sequence accession numbers

The sequences of two cloned 16S rRNA genes (CIAJG45 and CIAJG49) showing 95% or less homology to the existing database sequences have the GenBank accession numbers AF461498 and AF461499.

3. Results

3.1. Diversity and phylogenetic analysis of bacteria in the ileal mucosa

Combined gut samples (ileal digesta and mucosa) from 10 chickens were examined. The average microscope count of bacterial cells was 10^8–10^9 cells per gram of ileal digesta and about 10^{11} cells for the total bacteria recovered from the ileal mucosa of one bird. More than 95% of bacterial cells stained Gram-positive.

Partial sequences of 51 random 16S rDNA clones generated from bacteria present in the ileal mucosa of 10 birds were analyzed. The presumptive relationships of these sequences were obtained from database comparison. The cloned sequences represented at least 15 molecular species including *E. coli* (Table 1). Twelve of these species were identified in the chicken cecum in our previous study [7]. There were two cloned sequences (CIAJG45 and CIAJG49) having 95% or less homology to the existing database sequences, which may represent new species in the chicken gut [9]. Phylogenetic analysis showed that the cloned sequences were mainly Gram-positive bacteria with low G+C content (Fig. 1). Lactobacilli and *Enterococcus cecorum*-related sequences were the predominant groups of molecular species. Twenty-three of the 51 cloned sequences were related to lactobacilli and 19 were closely related to *Lactobacillus aviaries*.

3.2. T-RFLP analysis of bacterial populations present in the mucosa and lumen of ilea

T-RFLP analysis with restriction enzymes, *Alu*I, *Hha*I and *Msp*I, was used to compare bacterial populations in the mucosa and lumen of ilea from 10 birds. Both *Alu*I and *Hha*I generated similar T-RFLP profiles from the two bacterial populations (e.g. Fig. 2, panels 1–4). However, a significant level of polymorphism was observed between the mucosa and lumen bacteria upon digestion with *Msp*I (Fig. 2, panels 5–8). Three most significant bands (90, 405 and 415 bp) were typically found in samples from the mucosa or lumen. The 90-bp band was present only in the mucosal sample (panel 7), while the 405- and 415-bp bands were largely in the sample from the lumen (panels 6 and 8).

Table 1
Molecular species found in the mucosa of chicken ilea[a]

Closest species	Closest database sequence	Similarity (%)	Number of clones[b]
E. cecorum	AF061009	99	15 (29)
E. coli	AE005555	99	1 (2)
F. prausnitzii	X85022	96	1 (2)
	AB001836	99	19 (37)
Lactobacilli	AF243165	99	1 (2)
	AF335475	99	3 (6)
Streptococcus alactolyticus	AF201899	99	1 (2)
Uncultured bacteria from chicken ceca[c]	AF376209	99	1 (2)
	AF376213	99	2[d] (4)
	AF376217	98	2 (4)
	AF376227	97	1 (2)
Uncultured bacterium from mouse gut[e]	AJ400260	94	1 (2)
Unidentified butyrate-producing bacteria	AJ270484	94–95	2 (4)
Unidentified filamentous bacterium	X80834	99	1 (2)
Total			51 (100)

[a]Based on a BLAST analysis (conducted in December of 2001) of the random clone pool of 16S rRNA genes from bacteria present in the mucosa of ilea prepared from 10 chickens.
[b]Numbers in parentheses represent percentages of total clones.
[c]Does not include our previously reported sequences (AF429354–AF429382) of 16S rRNA genes cloned from chicken ceca [7].
[d]These two clones showed 96% similarity to the sequence of AJ270469 from a butyrate-producing bacterium that had been identified as *F. prausnitzii* [10], while they had a higher similarity (99%) to that identified in the chicken cecum [8].
[e]The clone (CIAJG17) showing 94% similarity to AJ400260 had a counterpart (CCAJG151, 98% similarity) in the mucosa of chicken cecum [7].

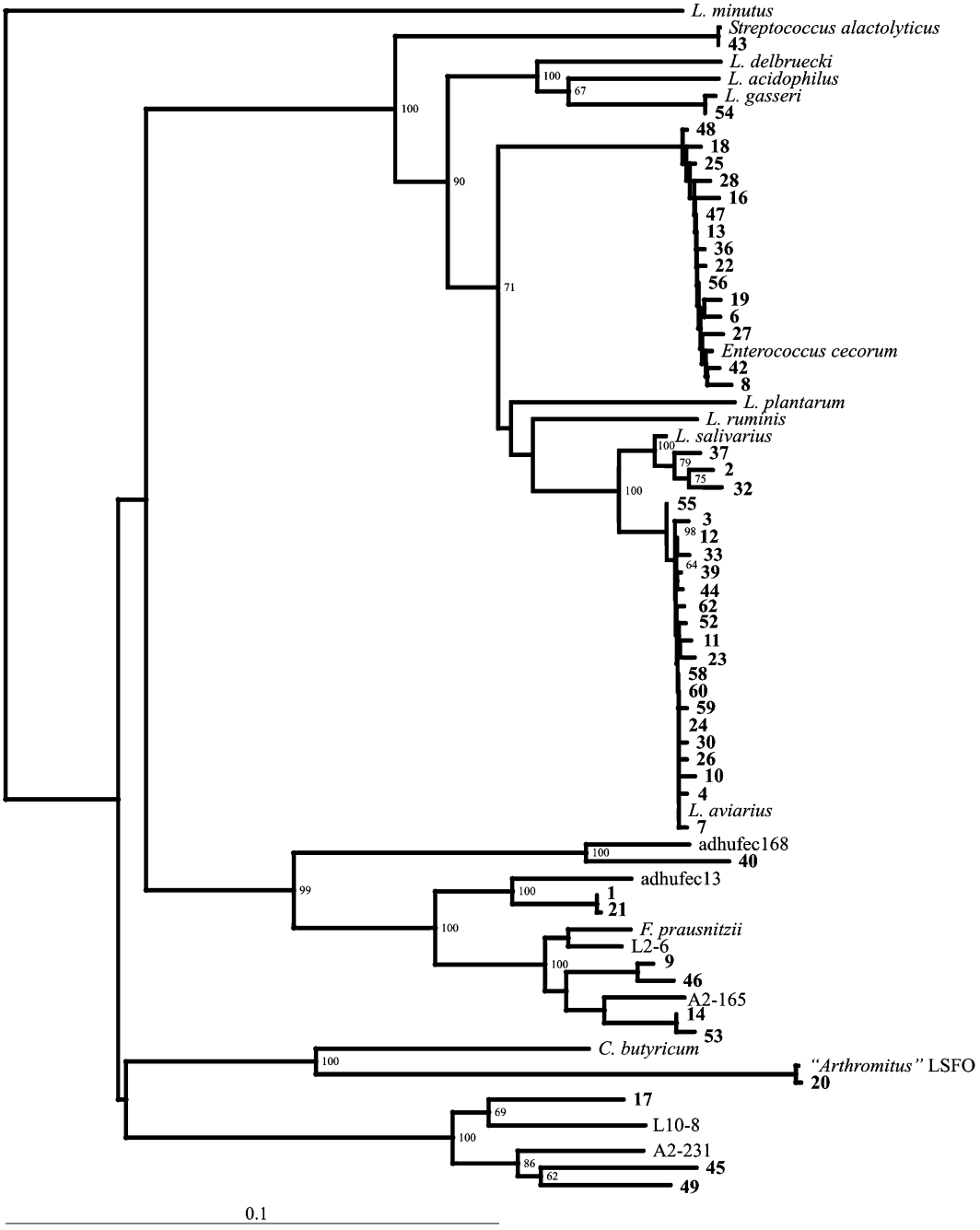

Fig. 1. Unrooted phylogenetic tree of mucosal bacteria in the chicken ilea (from 10 chickens) constructed using a neighbor-joining method. Our cloned sequences (named ciajg) are shown in bold numbers. Bootstrap values for 1000 trees are shown at branch points. Only values of 60% or above are shown. The bar represents a sequence divergence of 0.1%.

3.3. Comparison of bacterial populations in the mucosa of ilea and ceca

Bacterial populations in the mucosa of ilea and ceca were compared by analysis of two sets of random 16S rDNA clones prepared from the mucosa of the two gut regions from 10 birds. As shown in Table 2, the diversity and community structure of the bacterial population in the ileal mucosa were significantly different from those in the cecal mucosa, although bacteria of both populations were largely Gram-positive with low G+C content. The population in the ileal mucosa was much less diverse than that in the cecal mucosa. For example, sequences homologous to those of uncultured bacteria from human feces and from bovine rumen were found in the cecum, but were not detected in the ileum. Fifteen bacterial species were found in the ileal mucosa as opposed to 49 estimated in the cecal mucosa. Four percent of the cloned sequences from the ileal mucosa versus 25% from the cecal mucosa exhibited less than 95% homology to 16S rRNA sequences

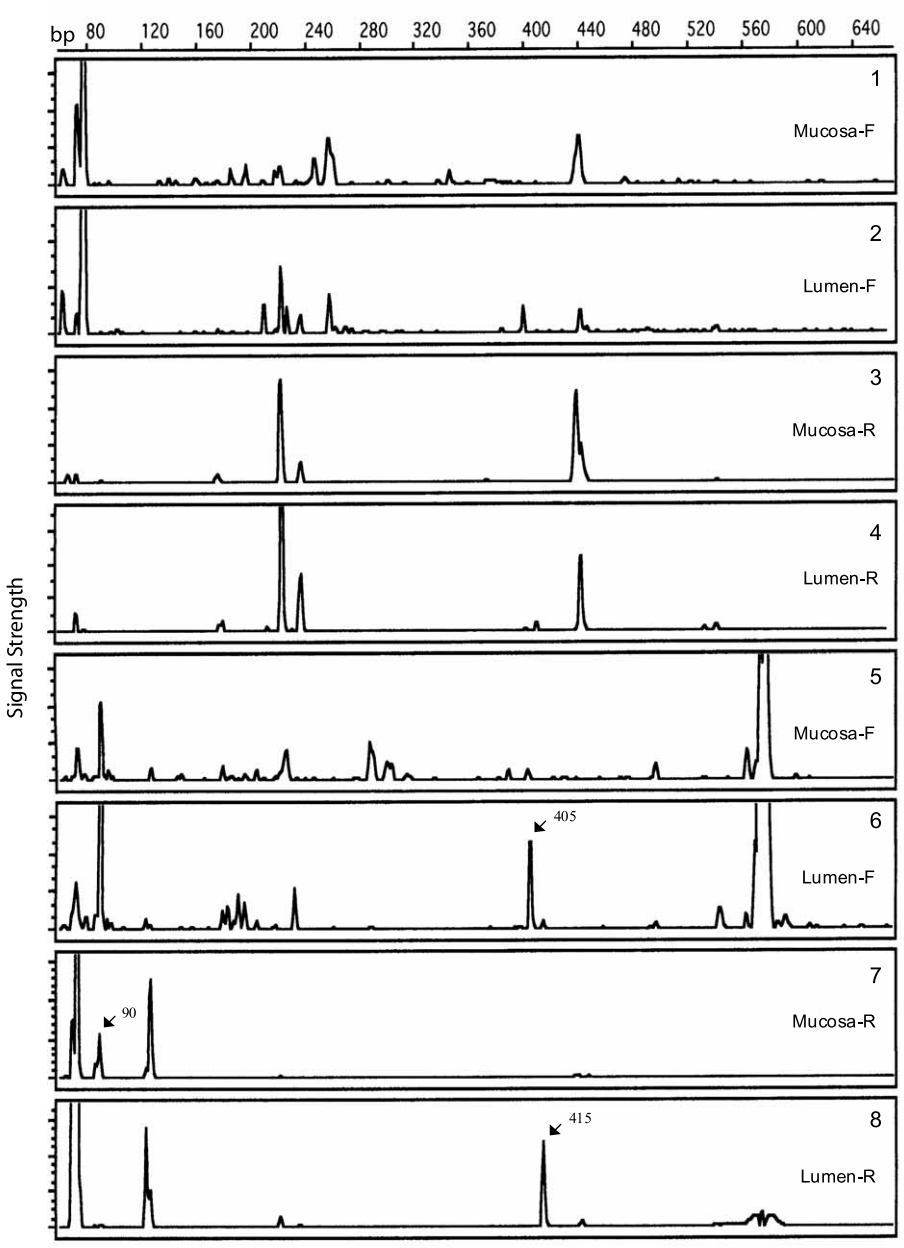

Fig. 2. T-RFLP analysis of 16S rDNA amplified by primers F8 and R1492 from bacteria present in the mucosa and lumen of chicken ilea prepared from 10 chickens. PCR products were digested with *Alu*I (panels 1–4) or *Msp*I (panels 5–8). F: forward primer, F8. R: reverse primer, R1492. Major T-RFLP bands with a preferable location are indicated by arrows. The numbers represent the size of bands in bp.

in the databases. Of the cloned sequences from the sample of ileal mucosa, lactobacilli and *E. cecorum* represented more than 70%, while butyrate-producing bacteria (including those related to *Fusobacterium prausnitzii*), ruminococci, clostridia and *E. cecorum* were predominant groups of defined bacteria in the cecal mucosa.

3.4. T-RFLP analysis of bacterial populations from the lumen of ilea and ceca

Fig. 3 shows the T-RFLP profiles of bacterial populations in the lumen of ilea and ceca. Bacterial DNA was amplified by PCR and then digested with *Alu*I (panels 1–4) or *Msp*I (panels 5–8). Both restriction enzymes produced more bands from DNA isolated from ceca compared to DNA isolated from ilea. Multiple major bands of 74, 187, 247, 258, 346, 360, 423, 178, 407, 424 and 439 bp (panels 1 and 3) were identified upon *Alu*I digestion of bacterial DNA isolated from the lumen of ceca. The lumen of ceca also contained a polymorphic band less than 70 bp (panel 3). Digestion with *Alu*I generated two major bands (78 and 237 bp) that largely appeared in the lumen of ilea (panels 2 and 4). Polymorphisms were also observed in *Msp*I-generated T-RFLP profiles. Six major bands (87,

Table 2
Comparison of molecular species present in the mucosa of ilea and ceca[a]

Closest relative	Cecum		Ileum	
	Number of clones	Similarity (%)	Number of clones	Similarity (%)[b]
Bacillus	3 (3)	93–96	0 (0)	0
Eubacteria	5[c] (4)	93–97	0 (0)	0
Clostridia	7 (6)	91–99	0 (0)	0
E. cecorum	7 (6)	95–99	15 (29)	99
E. coli	1 (1)	99	1 (2)	99
F. prausnitzii	21[d] (18)	94–96	1 (2)	96
Lactobacilli	4 (3)	99	23 (45)	99
Ruminococci	13 (11)	93–97	0 (0)	0
S. alactolyticus	2 (2)	96–99	1 (2)	99
Uncultured bacteria from chicken cecum[e]	17[f] (15)	93–99	6[g] (12)	97–99
Uncultured bacteria from human feces	17 (15)	91–97	0 (0)	0
Uncultured bacteria from bovine rumen	7 (6)	88–97	0 (0)	0
Uncultured bacteria from mouse gut	1 (1)	94	1 (2)	94
Unidentified butyrate-producing bacteria	8 (7)	94–95	2 (4)	94–95
Other species	3 (3)	94–96	1 (2)	99
Total	116 (101)		51 (100)	

[a]Based on a BLAST analysis (conducted in December of 2001) of two random clone sets of 16S rRNA genes from bacteria present in the mucosa of cecum and ileum prepared from 10 chickens. Sequences were assigned to the most closely related bacterial group.
[b]Represents sequence homology to those of closest relatives in the databases.
[c]One of the clones showed 97% similarity to the sequence (AJ270471) from a butyrate-producing bacterium that had been identified as *Eubacterium* spp. [10].
[d]Sixteen of the clones were related to the sequence of AJ270469 (95–96% similarity) from a butyrate-producing bacterium that had been identified as *F. prausnitzii* [10].
[e]Does not include our previously reported sequences (AF429354–AF429382) of 16S rRNA genes cloned from chicken cecum [7].
[f]Four of the clones showed a similarity of 94–96% to the sequence of AJ270469 from a butyrate-producing bacterium that had been identified as *F. prausnitzii* [10], while they had a higher similarity (97–99%) to those identified in the chicken cecum [8].
[g]Two of the clones showed 96% similarity to the sequence of AJ270469 from a butyrate-producing bacterium that had been identified as *F. prausnitzii* [10], while they had a higher similarity (99%) to that identified in the chicken cecum [8].

227, 290, 302, 315 and 390 bp, panels 5 and 7) were identified in ceca while four major polymorphic bands (74, 91, 415 and 573 bp, panels 6 and 8) were identified in the lumen of ilea. These data suggest that bacterial populations in the lumen of the two gut regions, ilea and ceca, are significantly different.

4. Discussion

In the present study, ileal and cecal samples were collected from 10 chickens that were subsequently combined for DNA preparation. Since the samples were from the same chickens, the bacterial populations represented the gut microbiota in the ileum and cecum of these birds as a whole, regardless of differences in microbiota of individual chickens. It is known that the gut microbiota can significantly be influenced by diets and other factors, such as the hosts and environment. The data presented in this study should be considered to be case-specific.

We analyzed 51 partial sequences of 16S rRNA genes cloned from ileal bacteria, which represented 15 molecular species. More than 70% of these sequences were related to lactobacilli and *E. cecorum*, suggesting that the sample size of sequences might be sufficient to determine predominant species of the bacterial community in the ileum. Nevertheless, more molecular species could be identified if different pairs of universal PCR primers were used to amplify 16S rRNA genes, as reported recently by Zhu et al. [8].

The present study has revealed the heterogeneity of bacterial populations present in the ileum and cecum. The differences of bacterial distribution in these two regions likely resulted from the interactions of different animal host tissues/cells and gut microbiota. The function of the ileum (the lower end of the small intestine) is mainly nutrient absorption, while the cecum is the site where extensive bacterial fermentation occurs, resulting in further nutrient absorption and detoxification of substances that are harmful to the host [22,23]. Since these regions function differently and provide different environments, it is expected that different types of bacteria would colonize them and distinct microbiota would develop. In this study, we identified some differences of the bacterial populations in the two gut regions, particularly of those attached to the mucosa.

Previous studies on chicken gut microbiota using culture-based methods suggested that lactobacilli were dominant in the small intestine of adult birds (reviewed in [1]). Molecular analysis of ileal bacteria presented here supports this observation. It is noteworthy that lactobacilli,

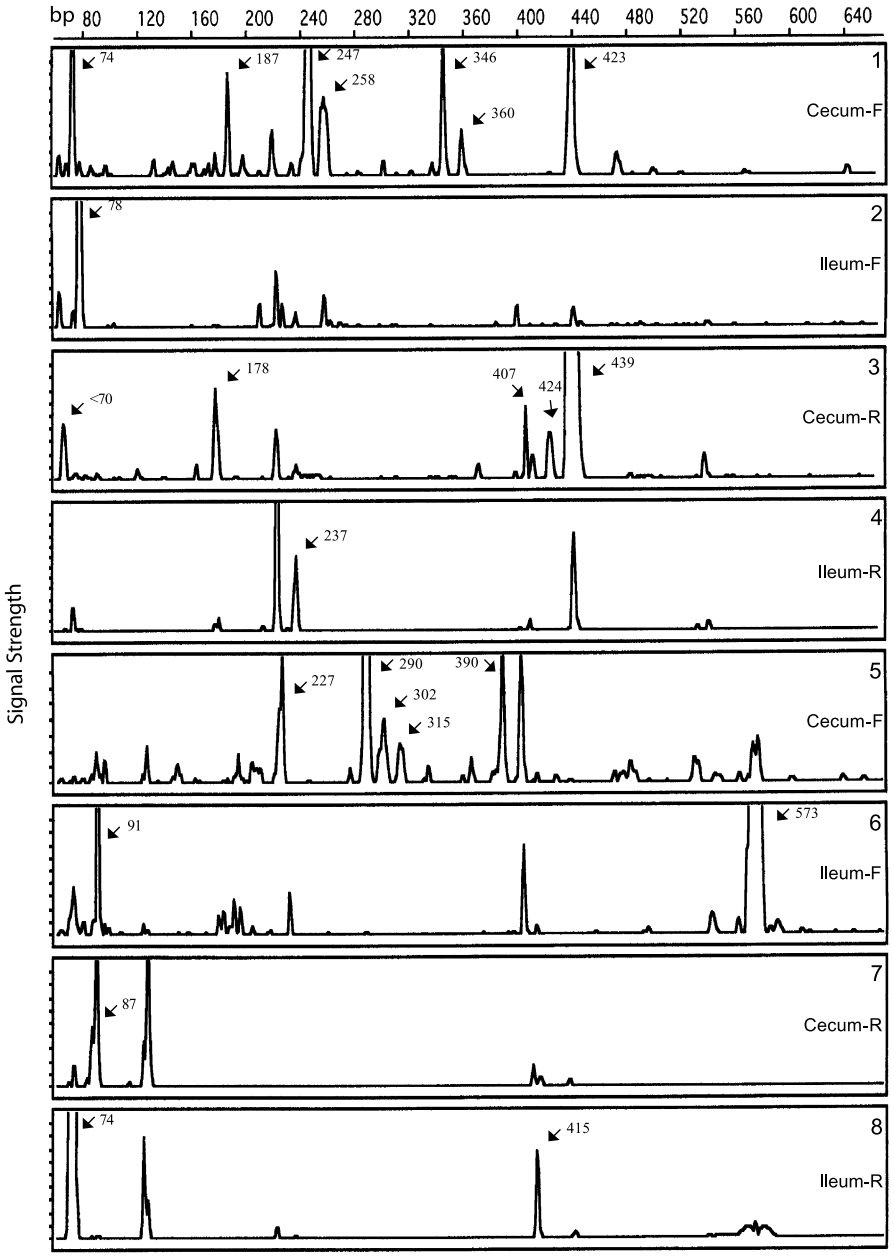

Fig. 3. T-RFLP analysis of 16S rDNA amplified by primers F8 and R1492 from bacteria present in the lumen of chicken ilea and ceca prepared from 10 chickens. PCR products were digested with *Alu*I (panels 1–4) or *Msp*I (panels 5–8). F: forward primer, F8. R: reverse primer, R1492. Major T-RFLP bands with a preferable region location are indicated by arrows. The numbers represent the size of bands in bp.

E. cecorum and butyrate-producing bacteria (identified and unidentified species) are the three major groups of bacteria found in ilea and ceca in our studies. Butyrate is preferentially transported by gut epithelial cells [24] and known to confer beneficial effects on animals [23,25–27]. Butyrate-producing bacteria in the human gut, therefore, have recently attracted research attention [10]. The potential of butyrate-producing bacteria as probiotics for poultry may warrant further investigation after verification of the presence of the bacteria in the chicken gut.

In this study, 12% of the sequences cloned from the ileal mucosa were found to have counterparts of uncultured bacteria in the cecum ($\geq$98% homology). Moreover, most of these sequences were also closely related to unidentified butyrate-producing bacteria and uncultured bacteria reported in human feces ($\geq$95% homology). Ninety-five percent 16S rRNA homology is generally used as a cutoff for the definition of operational taxonomic units in cloned sequences [9]. Thus these sequences may belong to the same bacterial species as those reported in the human gut.

T-RFLP analysis is becoming a useful tool in microbial ecology. Such restriction profiles can serve as 'community fingerprints' to characterize a particular microbial community [28]. In this study, we compared bacteria present in the lumen of different gut regions (ilea and ceca), and also bacteria in the mucosa and lumen of ilea by T-RFLP analysis of combined gut samples from 10 chickens. Polymorphisms shown in T-RFLP profiles indicated that the bacterial populations were different. It is unclear, however, which bacterial groups contribute to the polymorphisms. Sequence analysis of the polymorphic bands may lead to a clarification of their species composition.

When we analyzed the two sets of random 16S rRNA clones generated by PCR and gene cloning from the mucosal bacterial samples, we presented groups of sequences as percentages of the total number of cloned sequences. This was based on an assumption that the proportions of different groups of bacteria were conserved in the PCR step. Alternative molecular methods, such as real time PCR, dot blot or in situ hybridization, will be needed to confirm the assumption and provide sequence quantification.

The gut-surface-associated microbiota has long been studied because of its importance in pathogen control, immune modulation, and its effects upon nutrient absorption by their hosts. In this study, the diversity and community structure of bacterial populations in the mucosa of chicken ileum were determined and were also compared to those in the cecal mucosa. The predominant bacterial groups with potential to be used as probiotics were also identified. Our data may have significant implications for the health and nutrition of chickens and are particularly relevant for the development of probiotics and their most effective use for poultry.

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References

[1] Barrow, P. (1992) Probiotics for chickens. In: Probiotics, the Scientific Basis (Fuller, R., Ed.), pp. 225–257. Chapman and Hall, London.
[2] Ricke, S.C. and Pillai, S.D. (1999) Conventional and molecular methods for understanding probiotic bacteria functionality in gastrointestinal tracts. Crit. Rev. Microbiol. 25, 19–38.
[3] Theron, J. and Cloete, T.E. (2000) Molecular techniques for determining microbial diversity and community structure in natural environments. Crit. Rev. Microbiol. 26, 37–57.
[4] Farrelly, V., Rainey, F.A. and Stackebrandt, E. (1995) Effect of genome size and *rrn* gene copy number on PCR amplification of 16S rRNA genes from a mixture of bacterial species. Appl. Environ. Microbiol. 61, 2798–2801.
[5] Suzuki, M.T. and Giovannoni, S.J. (1996) Bias caused by template annealing in the amplification of mixtures of 16S rRNA genes by PCR. Appl. Environ. Microbiol. 62, 625–630.
[6] Qiu, X., Wu, L., Huang, H., McDonel, P.E., Palumbo, A.V., Tiedje, J.M. and Zhou, J. (2001) Evaluation of PCR-generated chimeras, mutations, and heteroduplexes with 16S rRNA gene-based cloning. Appl. Environ. Microbiol. 67, 880–887.
[7] Gong, J., Forster, R.J., Yu, H., Chambers, J.R., Sabour, P.M., Wheatcroft, R. and Chen, S. (2002) Diversity and phylogenetic analysis of bacteria in the mucosa of chicken ceca and comparison with bacteria in the cecal lumen. FEMS Microbiol., 208, 1–7.
[8] Zhu, X.Y., Zhong, T., Pandya, Y. and Joerger, R.D. (2002) 16S rRNA-based analysis of microbiota from the cecum of broiler chickens. Appl. Environ. Microbiol. 68, 124–137.
[9] Amann, R.I., Ludwig, W. and Schleifer, K.H. (1995) Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol. Rev. 59, 143–169.
[10] Barcenilla, A., Pryde, S.E., Martin, J.C., Duncan, S.H., Stewart, C.S., Henderson, C. and Flint, H.J. (2000) Phylogenetic relationships of butyrate-producing bacteria from the human gut. Appl. Environ. Microbiol. 66, 1654–1661.
[11] Whitford, M.F., Forster, R.J., Beard, C.E., Gong, J. and Teather, R.M. (1998) Phylogenetic analysis of rumen bacteria by comparative sequence analysis of cloned 16S rRNA genes. Anaerobe 4, 153–163.
[12] Leeson, S. and Summers, J.D. (1997) Commercial Poultry Nutrition. University Books, Guelph, ON.
[13] Apajalahti, J.H., Sarkilahti, L.K., Maki, B.R., Heikkinen, J.P., Nurminen, P.H. and Holben, W.E. (1998) Effective recovery of bacterial DNA and percent-guanine-plus-cytosine-based analysis of community structure in the gastrointestinal tract of broiler chickens. Appl. Environ. Microbiol. 64, 4084.
[14] Stahl, D.A., Flesher, B., Mansfield, H.R. and Montgomery, L. (1988) Use of phylogenetically based hybridization probes for studies of ruminal microbial ecology. Appl. Environ. Microbiol. 54, 1079–1084.
[15] Eden, P.A., Schmidt, T.M., Blakemore, R.P. and Pace, N.R. (1991) Phylogenetic analysis of *Aquaspirillum magnetotacticum* using polymerase chain reaction-amplified 16S rRNA-specific DNA. Int. J. Syst. Bacteriol. 41, 324–325.
[16] De Rijk, P. and De Wachter, R. (1993) DCSE, an interactive tool for sequence alignment and secondary structure research. Comput. Appl. Biosci. 9, 735–740.
[17] Saitou, N. and Nei, M. (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4, 406–425.
[18] Galtier, N., Gouy, M. and Gautier, C. (1996) SEAVIEW and PHYLO_WIN: two graphic tools for sequence alignment and molecular phylogeny. Comput. Appl. Biosci. 12, 543–548.
[19] Jukes, T.H. and Cantor, C.R. (1969) Evolution of protein molecules. In: Mammalian Protein Metabolism (Munro, N., Ed.), pp. 21–132. Academic Press, New York.
[20] Felsenstein, J. (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39, 783–791.
[21] Maidak, B.L., Cole, J.R., Parker Jr., C.T., Garrity, G.M., Larsen, N., Li, B., Lilburn, T.G., McCaughey, M.J., Olsen, G.J., Overbeek, R., Pramanik, S., Schmidt, T.M., Tiedje, J.M. and Woese, C.R. (1999) A new version of the RDP (Ribosomal Database Project). Nucleic Acids Res. 27, 171–173.
[22] Moran Jr., E.T. (1982) Comparative Nutrition of Fowl and Swine, the Gastrointestinal Systems. University of Guelph, Guelph, ON.
[23] Csordas, A. (1995) Toxicology of butyrate and short-chain fatty acids. In: Role of Gut Bacteria in Human Toxicology and Pharmacology (Hill, M.J., Ed.), pp. 105–125. Taylor and Francis, London.
[24] Ritzhaupt, A., Ellis, A., Hosie, K.B. and Shirazi-Beechey, S.P. (1998) The characterization of butyrate transport across pig and human colonic luminal membrane. J. Physiol. 507, 819–830.

[25] Scheppach, W., Bartram, H.P. and Richter, F. (1995) Role of short-chain fatty acids in the prevention of colorectal cancer. Eur. J. Cancer 31A, 1077–1080.
[26] Hague, A., Singh, B. and Paraskeva, C. (1997) Butyrate acts as a survival factor for colonic epithelial cells: further fuel for the in vivo versus in vitro debate. Gastroenterology 112, 1036–1040.
[27] Tran, C.P., Familari, M., Parker, L.M., Whitehead, R.H. and Giraud, A.S. (1998) Short-chain fatty acids inhibit intestinal trefoil factor gene expression in colon cancer cells. Am. J. Physiol. 275, G85–G94.
[28] Liu, W.T., Marsh, T.L., Cheng, H. and Forney, L.J. (1997) Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA. Appl. Environ. Microbiol. 63, 4516–4522.