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Postprint: PCR-DGGE Analysis of Small Intestinal Bacterial Microbiota in Caged and Free-Range Laying Hens

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Abstract

This study analyzed the bacterial species in the small intestines of caged and free-range layer chicks and adult laying hens. The entire small intestinal contents were collected from 8-week-old chicks and 30-week-old laying hens, and polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) analysis combined with fingerprint cloning was performed to investigate the DNA fingerprinting profiles of the bacterial flora in the chicken small intestine. The results showed that a total of 39 bacterial strains were isolated from the intestinal contents of laying hens, belonging to 4 phyla: Proteobacteria (2 strains, 5.1%), Bacteroidetes (4 strains, 10.2%), Actinobacteria (5 strains, 12.8%), and Firmicutes (21 strains, 53.8%), along with 7 environmental samples (unculturable bacteria). Different bacterial species were present in different intestinal segments of laying hens at different developmental stages, with the intestinal bacterial species being more diverse in adult hens and free-range chickens compared to chicks and caged chickens. Among all isolated bacteria, 10 lactic acid bacteria strains were obtained. Except for Coprococcus comes, the remaining 9 Lactobacillus strains were preserved as candidate strains for probiotic preparations. The results suggest that rearing mode and feeding stage have a significant influence on the distribution of bacterial community species in the intestines of laying hens, with free-range mode harboring richer bacterial communities and adult chickens possessing more intestinal bacteria than chicks.

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Full Text

Analysis of Intestinal Bacterial Flora in Cage-Reared and Free-Range Laying Hens by PCR-DGGE

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Abstract: This study analyzed bacterial species in the small intestines of both young and adult laying hens under cage-reared and free-range conditions. Intestinal contents were collected from 8-week-old chicks and 30-week-old laying hens, and polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) analysis was performed, combined with fingerprint cloning, to investigate the DNA fingerprint profiles of chicken intestinal bacterial flora. The results revealed that a total of 39 bacterial strains were isolated from the intestinal contents, representing four bacterial phyla: Proteobacteria (2 strains, 5.1%), Bacteroidetes (4 strains, 10.2%), Actinobacteria (5 strains, 12.8%), and Firmicutes (21 strains, 53.8%), along with seven environmental samples (unculturable bacteria). Different bacterial species were present in different intestinal segments of hens at various developmental stages, with adult hens and free-range chickens harboring richer bacterial diversity than young chicks and cage-reared birds. Among all isolated bacteria, ten lactic acid bacterial strains were obtained, and nine of these Lactobacillus strains were preserved as candidate probiotics, excluding *Coprococcus comes*. These findings indicate that both feeding mode and developmental stage significantly influence the distribution of bacterial community types in the intestines of laying hens, with free-range systems supporting more diverse bacterial communities and adult birds possessing greater intestinal bacterial richness than young chicks.

Keywords: laying hens; cage rearing; free range; PCR-DGGE; flora identification

Introduction

The avian intestinal tract represents a complex and diverse ecological environment harboring over 400 microbial species that play crucial roles in host development and nutrient digestion. For many years, the simplicity of microbial morphology and lack of distinct external features limited our understanding of bacterial community structures in various environments. However, the development of 16S rDNA-based molecular technologies has enabled more comprehensive and in-depth investigation of microbial community structures. Among these approaches, denaturing gradient gel electrophoresis/temperature gradient gel electrophoresis (DGGE/TGGE) has been employed to examine the struc-

ture and diversity of dominant bacteria in the gastrointestinal tracts of humans, pigs, and chickens. Intestinal dysbiosis can impair nutrient absorption efficiency, reduce immune function, and compromise intestinal barrier integrity, ultimately affecting livestock growth and health. Consequently, research investigating whether intestinal microorganisms cause functional disturbances in animal guts has become increasingly urgent. Currently, limited understanding of host-microbe interactions in the intestine directly constrains research on the relationship between intestinal microbiota and gut function. Therefore, this study utilized PCR-DGGE with species-specific bacterial primers to compare and analyze 16S rDNA V3 region fingerprints of bacterial communities from the entire intestinal contents of cage-reared and free-range chicks and laying hens. The objective was to investigate developmental changes in bacterial population structure and diversity in the duodenum, jejunum, ileum, and cecum of laying hens under different feeding regimes, providing a theoretical foundation for understanding poultry intestinal microbiota structure and isolating specific beneficial bacteria.

Materials and Methods

1.1 Experimental Animals and Sample Collection

Experimental animals were selected from HaiLan Gray laying hens raised at Daqing Xinghe Poultry Farm (cage-reared) and Daqing Lindian free-range facility. Twenty-five birds each of 8-week-old and 30-week-old cage-reared and free-range laying hens with similar body weights were randomly selected and euthanized. The entire small intestine was collected, and intestinal contents from the duodenum, jejunum, ileum, and cecum were separately pooled from every five chickens of the same age and feeding system. The pooled samples were aliquoted at 1 g per tube into 5 mL centrifuge tubes and stored at -20°C.

1.2 DNA Extraction

Genomic DNA was extracted from samples using the sodium dodecyl sulfate (SDS) high-salt extraction method. The extracted DNA was further purified and dissolved using a Bacterial Genomic DNA Extraction Kit (Shanghai Haibo Biological Co., Ltd.). The final total DNA was dissolved in 30 L sterile water and stored at -20°C.

1.3 PCR Amplification of Bacterial 16S rDNA Fragments

Using the extracted bacterial genomic DNA as template, bacterial universal primers GC-338F and 518R were employed to amplify hypervariable regions of the 16S rDNA sequence (Table 1). The 50 L PCR reaction mixture contained: 5 L 10× PCR buffer, 3.2 L dNTPs (2.5 mmol/L), 0.4 L rTaq polymerase (5 U/ L), 1 L GC-338F primer (20 mmol/L), 1 L 518R primer (20 mmol/L), 50 ng template DNA, and ddH_2O to a final volume of 50 L. The PCR amplification

program consisted of: initial denaturation at 94°C for 5 min; 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 45 s, and extension at 72°C for 1 min; followed by a final extension at 72°C for 10 min. PCR products were purified and recovered using the OMEGA DNA Gel Extraction Kit. The PCR instrument was a T-Gradient from Biometra, and gel imaging was performed using a Gel-Doc2000 system from Bio-Rad.

Table 1 Primers and sequences

Primer	Sequence
GC-338F	CCT ACG GGA GGC AGC AG
518R	ATT ACC GCG GCT GCT GG
GC-clamp	CGCCCGGGGCGCGCCCCGGGCGGGGCGGGGGCGCGGGGGG CCT ACG GGA GGC AGC AG

1.4 DGGE Analysis of PCR Products

Ten microliters of PCR product were analyzed by DGGE using 8% polyacrylamide gels with a denaturing gradient of 35%–55%. The chemical denaturant consisted of 100% urea at 7 mol/L and 40% (v/v) acrylamide. Electrophoresis was performed at 150 V and 60°C for 5 h in 1× TAE buffer.

1.5 Recovery and Sequencing of Dominant DGGE Bands

Target DGGE bands were excised with a sterile scalpel, and DNA was recovered using the OMEGA Poly-Gel DNA Extraction Kit. Two microliters of the recovered product were used as template for PCR amplification with primers 338F/518R. The re-amplified DNA fragments were gel-purified, ligated into the pMD18-T vector, and transformed into DH5α competent cells. Positive clones were selected and sent to Beijing Huada Gene Research Center for sequencing of the inserted bacterial 16S rDNA fragments.

1.6 Data Analysis

Quantity One software was used for band counting and simulation of PCR-DGGE fingerprints from bacteria isolated from various chicken intestinal segments. Homology comparisons were performed using the Blast program in GenBank to obtain 16S rDNA sequences of the most similar reference strains.

Results

2.1 DGGE Analysis of PCR Products

Total DNA was extracted from duodenal, jejunal, ileal, and cecal contents of cage-reared and free-range laying hens at 8 and 30 weeks of age. Electrophoretic analysis showed that total DNA from all intestinal contents had similar molecular weights above 2,000 bp, indicating consistent DNA extraction. Using the

extracted bacterial total DNA from each intestinal segment as template and GC-338F/518R as primers, approximately 200 bp DNA fragments were amplified for DGGE analysis.

The DGGE analysis results of 16S rDNA PCR products are shown in Figure 1 [Figure 1: see original paper]. Analysis of specific flora in small intestinal contents revealed that bacterial universal primers successfully amplified 16S rDNA V3 region fragments from all 16 samples. These fragments were used for PCR-DGGE fingerprinting and cluster analysis. Bacterial species in the small intestines of chickens were abundant and varied across different feeding modes and ages. A total of 35 bands were isolated. The total number of bacterial bands in the duodenum, jejunum, ileum, and cecum were 8, 12, 8, and 9, respectively, for 8-week-old cage-reared chicks; 8, 13, 14, and 9 for free-range chicks; 13, 12, 9, and 9 for adult cage-reared hens; and 16, 15, 13, and 10 for adult free-range hens. Although some common bands existed across chicken groups, 30-week-old adult hens harbored more intestinal bacterial species than 8-week-old chicks, and free-range chickens showed greater total bacterial numbers than cage-reared birds, with the most pronounced difference observed in the duodenum.

Figure 1 Analysis results of DGGE bands

Note: 8 and 30 represent weeks of age; letter F indicates free-range, C indicates cage-reared; bands 1-4, 5-8, 9-12, and 13-16 represent bacteria from duodenum, jejunum, ileum, and cecum of chickens, respectively.

2.2 Bacterial Community Structure Similarity Among Samples

A total of 39 bacterial strains were isolated from chicken small intestines. Genetic similarity coefficients were calculated based on the proportion of shared bacterial taxa among total bacteria in each intestinal segment, and the unweighted pair-group method with arithmetic means (UPGMA) dendrogram is shown in Figure 2 [Figure 2: see original paper].

Bacterial community composition differed substantially among intestinal segments of chicks and adult hens under different feeding modes. Genetic similarity coefficients were low between cage-reared and free-range chickens of the same age and intestinal tissue, ranging from 18.9% to 33.5% for 30-week-old hens and 17.0% to 33.3% for 8-week-old chicks. Similarity coefficients were relatively high between different ages within the same feeding mode: 19.4%–35.2% for free-range hens and 33.1%–49.1% for cage-reared hens. The latter values were significantly higher than those for free-range hens, indicating that cage-reared environments are relatively stable, whereas free-range environments are more complex and expose chickens to more variable bacterial categories. Similarity indices measure the degree of similarity between communities or samples, and their values reflect bacterial community resemblance and indirectly indicate non-shared flora composition. These results demonstrate substantial differences in intestinal microbiota composition across different feeding environments and ages, with feeding mode, age, and intestinal location all influencing bacterial

diversity in chickens.

Figure 2 Dendrogram based on unweighted pair-group method analysis (UPGMA)

2.3 Sequence Determination of Major Electrophoresis Bands

After recovering DGGE gel bands, PCR amplification with primers 338F/518R yielded approximately 200 bp DNA fragments. Following purification, PCR products were ligated into pMD18-T vectors and transformed into DH5α competent cells. Positive clones were selected for sequencing, with results presented in Table 2 . Sequences were compared against GenBank database entries, revealing bacterial types represented by each band and enabling construction of a phylogenetic tree (Figure 3 [Figure 3: see original paper]). Among the 39 sequencing results, homology with microorganisms in the GenBank database exceeded 92% in most cases, with some showing 100% identity.

The identified bacteria from laying hen small intestines belonged to four phyla: Proteobacteria, Bacteroidetes, Actinobacteria, Firmicutes, and environmental samples (uncultured bacteria). The two Proteobacteria strains (5.1% of total) were *Acinetobacter lwoffii* (band 8) and *Pseudoxanthomonas mexicana* (band 21), detected only in free-range chickens in the jejunum of 8-week-old chicks and ileum of 30-week-old hens, respectively. Four Bacteroidetes strains (10.2% of total) included *Bacteroides plebeius* (band 2), *Butyricimonas virosa* (band 13_2), *Paraprevotella clara* (band 14), and *Alistipes putredinis* (band 33). Band 2 represented a *Bacteroides plebeius* subspecies with 89% similarity, while band 13_2 corresponded to *Butyricimonas virosa* present across all chicken small intestines. Band 14 was a *Paraprevotella clara* variant with 72% similarity, detected in free-range chickens and the jejunum of 8-week-old cage-reared chicks. Band 33 corresponded to *Alistipes putredinis*, found only in adult hens.

Five Actinobacteria strains (12.8% of total) were identified: two *Gardnerella vaginalis* strains (bands 28 and 29) and three *Streptomyces spiralis* strains (bands 30, 31, and 32). Bands 28 and 29 were 92% similar variants of *G. vaginalis*, while bands 30, 31, and 32 were 100% homologous *S. spiralis* strains. *G. vaginalis* (band 28) and *S. spiralis* (bands 30, 31, 32) were present in all chicken small intestines, whereas *G. vaginalis* (band 29) was detected only in 30-week-old free-range hens.

Among the 39 bacterial strains analyzed, 21 were Firmicutes (53.8% of total), representing the dominant flora in laying hen small intestines. The *Lactobacillus* genus comprised 10 strains (25.6% of total bacteria and 47.6% of Firmicutes), including three *Lactobacillus aviarius* strains, three *Lactococcus raffinolactis* strains, one *Lactobacillus agilis* strain, one *Coprococcus comes* strain, one *Lactobacillus equigenerosi* strain, and one *Lactobacillus acidophilus* strain. Seven *Lactobacillus* strains were detected in 30-week-old free-range and cage-reared hens, while five and six strains were found in 8-week-old free-range and cage-reared chicks, respectively. Three common *Lactobacillus* species were present

across all chicken intestines: *L. aviarius* (bands 4, 5, and 23), *L. raffinolactis* (bands 6 and 25), and *L. equigenerosi* (band 15_1). Bands 4 and 23 showed only 31% similarity to each other and 27% similarity to *L. aviarius*, but 98% similarity to band 5, suggesting they represent novel *L. aviarius* species. Band 15 showed only 36% similarity to *L. equigenerosi* but was detected in all chicken intestines, indicating a common chicken gut species. *L. raffinolactis* (bands 6_1 and 6_2) was present throughout chicken small intestines but absent from the duodenum, representing a common species in the lower small intestine. *L. agilis* (band 11), *C. comes* (band 12), and *L. acidophilus* (band 34) were specific to certain age groups. Band 11 (87% similar to *L. agilis*) was a subspecies isolated only from 8-week-old cage-reared and free-range chicks, not from adult hens. Band 12 (70% similar to *C. comes*) was a variant isolated only from 30-week-old cage-reared and free-range adult hens, absent in chicks. Band 34 (84% similar to *L. acidophilus*) was a subspecies detected only in the duodenum of 30-week-old free-range hens, representing a species unique to adult free-range chickens.

Six *Clostridium* strains were identified within Firmicutes (15.4% of total bacteria): *Fusobacterium plautii* (band 1), *Clostridium tertium* (band 7), *Clostridium lentocellum* (band 17), *Clostridium difficile* (band 24), *Clostridium sartagoforme* (band 26), and *Clostridium irregulare* (band 27). *F. plautii*, *C. tertium*, and *C. sartagoforme* were detected across all chicken small intestines, representing common clostridial species. Band 1 (92% similar to *F. plautii*), band 17 (90% similar to *C. lentocellum*), and band 26 (90% similar to *C. sartagoforme*) were subspecies. Band 7 showed only 32% similarity to *C. tertium* and was detected only in adult hens, being absent or undetectable in chicks. Band 27 (89% similar to *C. irregulare*) was a variant found only in free-range chicks, not detected in other chickens. Band 24 (85% similar to *C. difficile*) was a variant common to chick small intestines but absent from adult free-range hens.

Four additional Firmicutes strains were identified: *Rummeliibacillus stabekisii*, *Faecalibacterium prausnitzii*, and *Streptococcus gallolyticus*. These three strains were detected in adult free-range hens, with band 9 (homologous to *F. prausnitzii*) being unique to adult free-range hens, while band 3 (homologous to *R. stabekisii*) and band 10 (homologous to *S. gallolyticus*) were also detected in free-range chicks.

Seven environmental samples (17.9% of total bacteria) representing uncultured bacteria (bands 13_1, 16, 18, 19, 20, 22, and 35) were detected across all chicken small intestines, with similarity values above 97%, indicating their ubiquitous presence in the chicken gut.

These results demonstrate that feeding mode and intestinal location significantly influence chicken gut bacterial structure.

Table 2 Analytic results of bacterial species

Band No.	Most similar strain	Accession No.	Similarity/%	Bacterial phylum
1	*Fusobacterium plautii*	NR_{029356}	92	Firmicutes
2	*Bacteroides plebeius*	NR_{041277}	89	Bacteroidetes
3	*Rummeliibacillus stabekisii*	NR_{043992}.1	90	Firmicutes
4	*Lactobacillus aviarius*	NR_{044703}.1	27	Firmicutes
5	*Lactobacillus aviarius*	NR_{044703}.1	98	Firmicutes
6	*Lactococcus raffinolactis*	NR_{044359}.1	95	Firmicutes
7	*Clostridium tertium*	NR_{037086}.1	32	Firmicutes
8	*Acinetobacter lwoffii*	NR_{026209}.1	94	Proteobacteria
9	*Faecalibacterium prausnitzii*	NR_{028961}.1	96	Firmicutes
10	*Streptococcus gallolyticus*	NR_{074849}.1	93	Firmicutes
11	*Lactobacillus agilis*	NR_{044700}.1	87	Firmicutes
12	*Coprococcus comes*	NR_{044048}.1	70	Firmicutes
13_1	Uncultured bacterium	HM192239.1	98	Environmental samples
13_2	*Butyricimonas virosa*	NR_{041691}.1	91	Bacteroidetes
14	*Paraprevotella clara*	NR_{041626}.1	72	Bacteroidetes
15_1	*Lactobacillus equigenerosi*	NR_{041566}.1	36	Firmicutes
15_2	*Pediococcus claussenii*	NR_{075029}.1	89	Firmicutes
16	Uncultured bacterium	JQ013040.1	97	Environmental samples
17	*Clostridium lentocellum*	NR_{026101}.1	90	Firmicutes
18	Uncultured bacterium	JN021901.1	98	Environmental samples
19	Uncultured bacterium	AB666120.1	99	Environmental samples

Band No.	Most similar strain	Accession No.	Similarity/%	Bacterial phylum
20	Uncultured bacterium	EU473569.1	97	Environmental samples
21	*Pseudoxanthomonas mexicana*	NR_{025105}.193		Proteobacteria
22	Uncultured bacterium	AB506418.1	98	Environmental samples
23	*Lactobacillus aviarius*	NR_{044703}.131		Firmicutes
24	*Clostridium difficile*	NR_{074454}.185		Firmicutes
25	*Lactococcus raffinolactis*	NR_{044359}.196		Firmicutes
26	*Clostridium sartagoforme*	NR_{026490}.190		Firmicutes
27	*Clostridium irregulare*	NR_{029249}.189		Firmicutes
28	*Gardnerella vaginalis*	NR_{044694}.192		Actinobacteria
29	*Gardnerella vaginalis*	NR_{044694}.192		Actinobacteria
30	*Streptomyces spiralis*	NR_{044142}.1100		Actinobacteria
31	*Streptomyces spiralis*	NR_{044142}.1100		Actinobacteria
32	*Streptomyces spiralis*	NR_{044142}.2100		Actinobacteria
33	*Alistipes putredinis*	NR_{025909}.194		Bacteroidetes
34	*Lactobacillus acidophilus*	NR_{075049}.184		Firmicutes
35	Uncultured bacterium	JX183818.1	98	Environmental samples

Figure 3 Phylogenetic tree of chicken intestinal flora

Discussion

Intestinal microorganisms influence host nutrition, health, and growth performance through nutrient utilization and gastrointestinal system development. The composition of gut microbiota affects host health and growth, as confirmed by studies in humans, pigs, and chickens showing that different individuals exhibit distinct intestinal flora fingerprint profiles. Even chickens of the same age,

housed in identical environments and fed the same diet, display different banding patterns, indicating strong host-specific influences on gut microbiota composition. Since animal species, feeding environment, and temperature all affect intestinal flora structure, and traditional culture-based methods cannot comprehensively reflect or compare gut microbiota characteristics, this study employed advanced and effective DGGE technology to investigate bacterial composition in duodenal, jejunal, ileal, and cecal contents of 8- and 30-week-old HaiLan Brown laying hens under cage-reared and free-range conditions. This approach enabled analysis of developmental changes in microbial groups in laying hen intestines across different feeding systems.

A total of 35 bacterial genera were identified from the entire small intestines of all chickens. Free-range hens exhibited relatively greater bacterial taxonomic diversity than cage-reared birds, with free-range chicks harboring seven more bacterial strains than cage-reared chicks, and free-range adult hens showing eleven more strains than their cage-reared counterparts. Within the same feeding system, adult hens possessed richer gut microbiota than chicks, primarily reflected in greater bacterial numbers in the duodenum, while total bacterial counts in post-jejunal segments were similar. For instance, under free-range conditions, chicks had eight duodenal bacterial strains versus sixteen in adult hens, while cage-reared chicks had eight strains compared to thirteen in adult hens, confirming that living environment significantly affects bacterial survival in the avian gut.

To develop probiotic preparations for animal use, *Lactobacillus* strains surviving across all intestinal segments were targeted as candidate probiotics. This study isolated ten *Lactobacillus* strains from various chicken intestinal segments. Currently, only three reports exist on *Lactobacillus equigenerosi*, all isolated from horse intestines or used as equine probiotics. Biological characterization revealed that *L. equigenerosi* exhibits high acid and bile salt tolerance, surviving at pH 3.0, with intestinal epithelial cell adhesion rates exceeding 60%. The strain is non-pathogenic and can reduce blood cholesterol and urea levels in horses, making it a promising probiotic candidate. This study marks the first isolation of *L. equigenerosi* from cage-reared chick intestines, and based on these findings, it warrants further investigation as a candidate probiotic for laying hens.

Lactobacillus aviarius has only been mentioned in a review by Waters et al. as a potential probiotic, with no other relevant studies reported. Research on *Lactobacillus agilis* includes three key studies: Palop et al. first reported that *L. agilis* strain R16 could grow in mustard seed extract-containing environments and degrade glucosinolates, demonstrating stress resistance and biological activity. Baele et al. confirmed *L. agilis* as an important component of pigeon gut flora, while Stephenson et al. most recently identified *L. agilis* as a resident *Lactobacillus* species in broiler intestines with high adhesion and colonization capacity and efficient antimicrobial protein expression. The identification of *L. agilis* in this study, combined with these previous findings, suggests this species possesses

excellent colonization ability and adaptability in avian intestines, making it a promising probiotic candidate for further research.

Studies on *Lactococcus raffinolactis* have emerged only in the past two years, focusing exclusively on milk fermentation. No previous reports have isolated this species from poultry intestines, though Meslier et al. noted its presence as a common environmental lactic acid bacterium. Its isolation from chicken intestines in this study, combined with its documented fermentation-promoting properties, suggests *L. raffinolactis* possesses characteristics suitable for probiotic development, though specific application effects require further investigation.

Most research on *Coprococcus comes* dates from before the 1990s, with only one recent study by Graessler et al. associating increased abundance of this species with Crohn' s disease. Therefore, *C. comes* is not suitable as a probiotic candidate.

Additionally, three non-*Lactobacillus* common strains were identified: one *Rummeliibacillus* and two *Gardnerella vaginalis* strains. Multiple studies have identified *G. vaginalis* as a pathogenic bacterium causing reproductive tract inflammation, making it unsuitable for probiotic development. Only two reports directly investigate *Rummeliibacillus*, both isolating strains from soil. Vaishampayan et al. isolated a strictly aerobic, salt-tolerant *Rummeliibacillus* strain with optimal growth at 28–32°C, while Her et al. identified a strain that was neither salt-tolerant (NaCl < 1.5%) nor acid-tolerant (pH 5–10), lacking desirable probiotic characteristics. Therefore, whether the *Rummeliibacillus* strain isolated in this study is suitable for probiotic preparation requires further investigation.

In conclusion, feeding mode and developmental stage significantly influence bacterial community distribution in chicken intestines, with free-range systems supporting richer bacterial communities and adult hens harboring greater bacterial diversity than chicks. Intestinal location determines bacterial diversity and specificity, particularly in the duodenum, where the most pronounced differences among chicken groups were observed. This study isolated and identified nine *Lactobacillus* strains and one *Bacillus* strain. Except for *L. equigenerosi*, which has been applied as an equine probiotic, the suitability of *L. aviarius*, *L. agilis*, *L. raffinolactis*, and *Rummeliibacillus* as probiotic candidates requires further investigation, though they have been preserved as potential strains for future research.

References

[1] ZWIELEHNER J, LASSL C, HIPPE B, et al. Changes in human fecal microbiota due to chemotherapy analyzed by TaqMan-PCR, 454 sequencing and PCR-DGGE fingerprinting[J]. PLoS One, 2011, 6(12): e28654, doi:10.1371/journal.pone.0028654.

[2] KONSTANTINOV S R, ZHU W Y, WILLIAMS B A, et al. Effect of fermentable carbohydrates on piglet faecal bacterial communities as revealed by

denaturing gradient gel electrophoresis analysis of 16S ribosomal DNA[J]. FEMS Microbiology Ecology, 2003, 43(2): 225–235.

[3] WIELEN P W J J, KEUZENKAMP D A, LIPMAN L J A, et al. Spatial and temporal variation of the intestinal bacterial community in commercially raised broiler chickens during growth[J]. Microbial Ecology, 2002, 44(3): 286–293.

[4] FLÓREZ A B, MAYO B. PCR-DGGE as a tool for characterizing dominant microbial populations in the Spanish blue-veined Cabrales cheese[J]. International Dairy Journal, 2006, 16(10): 1205–1210.

[5] ZOETENDAL E G, COLLIER C T, KOIKE S, et al. Molecular ecological analysis of the gastrointestinal microbiota: a review[J]. The Journal of Nutrition, 2004, 134(2): 464–472.

[6] TAPIA-PANIAGUA S T, CHABRILLÓN M, DÍAZ-ROSALES P, et al. Intestinal microbiota diversity of the fish *Solea senegalensis* (Kaup, 1858) following probiotic administration[J]. Microbial Ecology, 2010, 60(2): 310–319.

[7] FLIEGEROVÁ K, MRÁZEK J, KAJAN M, et al. The effect of maize silage as co-substrate for swine manure on the bacterial community structure in biogas plants[J]. Folia Microbiologica, 2012, 57(4): 281–284.

[8] NADAL I, DONANT E, RIBES-KONINCKX C, et al. Imbalance in the composition of the duodenal microbiota of children with coeliac disease[J]. Journal of Medical Microbiology, 2007, 56(12): 1669–1674.

[9] WANG S P, BO M J, KONG X F, et al. 16S rRNA gene-based analysis of ileal bacterial community and phylogeny in nursing and weaned piglets[J]. Animal Husbandry and Feed Science, 2009, 1(4/5): 12–17.

[10] LI X M, YU Y H, FENG W S, et al. Host species as a strong determinant of the intestinal microbiota of fish larvae[J]. The Journal of Microbiology, 2012, 50(1): 29–37.

[11] LÜ Y C, LI N, GONG D L, et al. The effect of temperature on the structure and function of a cellulose-degrading microbial community[J]. Applied Biochemistry and Biotechnology, 2012, 168(2): 219–233.

[12] ENDO A, ROOS S, SATOH E, et al. *Lactobacillus equigenerosi* sp. nov., a coccoid species isolated from faeces of thoroughbred racehorses[J]. International Journal of Systematic and Evolutionary Microbiology, 2008, 58(Pt 4): 914–918.

[13] MORITA H, NAKANO A, SHIMAZU M, et al. *Lactobacillus hayakitensis*, *L. equigenerosi* and *L. equi*, predominant lactobacilli in the intestinal flora of healthy thoroughbreds[J]. Animal Science Journal, 2009, 80(3): 339–346.

[14] BOTHA M, BOTES M, LOOS B, et al. *Lactobacillus equigenerosi* strain Le1 invades equine epithelial cells[J]. Applied and Environmental Microbiology, 2012, 78(12): 4248–4254.

[15] WATERS S M, MURPHY R A, POWER R F G. Characterisation of prototype Nurmi cultures using culture-based microbiological techniques and PCR-DGGE[J]. International Journal of Food Microbiology, 2006, 110(3): 268–277.

[16] PALOP M L, SMITS J P, BRINK B T. Degradation of sinigrin by *Lactobacillus agilis* strain R16[J]. International Journal of Food Microbiology, 1995, 26(2): 219–229.

[17] BAELE M, DEVRIESE L A, HAESEBROUCK F. *Lactobacillus agilis* is an important component of pigeon flora[J]. Journal of Applied Microbiology, 2001, 91(3): 488–491.

[18] STEPHENSON D P, MOORE R J, ALLISON G E. Transformation of, and heterologous protein expression in, *Lactobacillus agilis* and *Lactobacillus vaginalis* isolates from the chicken gastrointestinal tract[J]. Applied and Environmental Microbiology, 2011, 77(1): 220–228.

[19] MESLIER V, LOUX V, RENAULT P. Genome sequence of *Lactococcus raffinolactis* strain 4877, isolated from natural dairy starter culture[J]. Journal of Bacteriology, 2012, 194(22): 6364.

[20] KIMOTO-NIRA H, AOKI R, MIZUMACHI K, et al. Interaction between *Lactococcus lactis* and *Lactococcus raffinolactis* during growth in milk: development of a new starter culture[J]. Journal of Dairy Science, 2012, 95(4): 2176–2185.

[21] BULL K, MATTHEWS N, RHODES J. Antibody response to anaerobic coccoid rods in Crohn' s disease[J]. Journal of Clinical Pathology, 1986, 39(10): 1130–1134.

[22] GRAESSLER J, QIN Y, ZHONG H, et al. Metagenomic sequencing of the human gut microbiome before and after bariatric surgery in obese patients with type 2 diabetes: correlation with inflammatory and metabolic parameters[J]. The Pharmacogenomics Journal, 2013, 13(6): 514–522.

[23] MACHADO A, JEFFERSON K K, CERCA N. Interactions between *Lactobacillus crispatus* and bacterial vaginosis (BV)-associated bacterial species in initial attachment and biofilm formation[J]. International Journal of Molecular Sciences, 2013, 14(6): 12004–12012.

[24] TOMUSIAK A, HECZKO P B, JANECZKO J, et al. Bacterial infections of the lower genital tract in fertile and infertile women from southeastern Poland[J]. Ginekologia Polska, 2013, 84(5): 352–358.

[25] VAISHAMPAYAN P, MIYASHITA M, OHNISHI A, et al. Description of *Rummeliibacillus stabekisii* gen. nov., sp. nov. and reclassification of *Bacillus pycnus* Nakamura et al. 2002 as *Rummeliibacillus pycnus* comb. nov[J]. International Journal of Systematic and Evolutionary Microbiology, 2009, 59(Pt 5): 1094–1099.

[26] HER J, KIM J. *Rummeliibacillus suwonensis* sp. nov., isolated from soil

collected in a mountain area of South Korea[J]. Journal of Microbiology, 2013, 51(2): 268–272.

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