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APPLIED MICROBIAL AND CELL PHYSIOLOGY

Changes in the diversity and composition of gut microbiota of weaned piglets after oral administration of *Lactobacillus* or an antibiotic

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Abstract The gut microbiota plays important roles in the health and well-being of animals, and high-throughput sequencing facilitates exploration of microbial populations in the animal gut. However, previous studies have focused on fecal samples instead of the gastrointestinal tract. In this study, we compared the microbiota diversity and composition of intestinal contents of weaned piglets treated with *Lactobacillus reuteri* or chlortetracycline (aureomycin) using high-throughput sequencing. Nine weaned piglets were randomly divided into three groups and supplemented with *L. reuteri*, chlortetracycline, or saline for 10 days, and then the contents of three intestinal segments (jejunum, colon, and cecum) were obtained and used for sequencing of the V3–V4 hypervariable region of the 16S rRNA gene. The microbiota diversity and composition in the jejunum were different from those in the colon and cecum among the three treatments. In the jejunum, treatment with *L. reuteri* increased the species richness of the microbiota, as indicated by the ACE and Chao1 indexes, compared with the chlortetracycline group, in which several taxa were eliminated. In the colon and cecum, relative abundances of the phylum *Firmicutes* and the genus *Prevotella* were higher in the chlortetracycline group than in the other groups. Distances between clustered samples revealed that the *L. reuteri* group was closer to the chlortetracycline group than the control group for jejunum samples, while colon and cecum samples of the *L. reuteri* group were clustered with those of the control group. This study provides fundamental knowledge for future studies such as the development of alternatives to antibiotics.

Keywords Gut microbiota · Diversity and composition · *Lactobacillus reuteri* · Antibiotics · Weaned piglets · High-throughput sequencing

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Introduction

Immediately after birth, the mammalian gastrointestinal (GI) tract is colonized by a complex and diverse microbial ecosystem. The GI microbiota produces essential products, forms a barrier against pathogens, and plays multiple roles in intestinal morphology, immunity, digestion, and modulation of host gene expression (Turnbaugh et al. 2006; Guo et al. 2008). Furthermore, the GI microbiota is dynamic, and its composition is changing continually in response to new microbes in the individual's environment. The number of studies of the composition of the intestinal microbiome is increasing rapidly, reflecting a growing interest in understanding the function of the intestinal microbiota in animal health.

During the process of weaning, piglets are abruptly forced to adapt to nutritional, immunological, and psychological disruptions (Hu et al. 2014), and the microbiota of the GI tract can change sharply, resulting in poor appetite, lower feed intake, and growth retardation (Isaacson and Kim 2012). Fecal microbiota analyses have demonstrated that the use of antibiotics can destroy pathogens as well as other members of the intestinal microbiota (Cecilia et al. 2007), and the microbiota of the GI tract does not immediately return to normal after cessation of antibiotic treatment (Yin et al. 2015). *Lactobacillus* species, which are members of normal intestinal flora, have become a topic of great interest because they can alter the host intestinal microbiota, thereby affecting the

physiological functions of animals and the general health of weaned pigs (Liu et al. 2015; Jiao et al. 2014). However, microbiota sampled from feces do not fully represent the microbial profile of the GI tract (Zhao et al. 2015), and the effect of *Lactobacillus* and antibiotics on the diversity and composition of gut microbiota during the weaning process remains unclear.

It has been estimated that at least 50 % of the microbiota of the GI tract cannot be grown outside the gut, and indeed, the vast majority of gut bacteria have never been cultivated outside the gut (Shanahan 2002; Sears 2005). Therefore, it is difficult to extensively explore the microbial diversity of the healthy gut using culture-dependent methods. High-throughput DNA sequencing methods, such as Illumina sequencing, provide a way to directly detect microbial taxa, especially those with low-abundance species changes (Oberauner et al. 2013; Uroz et al. 2013). Furthermore, Illumina sequencing is cost-effective and can generate tenfold or more sequences per sample than pyrosequencing 454, thereby allowing detailed analysis of taxonomic profiles from samples (Kozich et al. 2013).

Lactobacillus reuteri ZLR003, isolated from the cecum mucosa of a healthy weaned pig, exhibited tolerance to acid and swine bile as well as antimicrobial activity against *Escherichia coli* in vitro. In addition, dietary supplementation with *L. reuteri* ZLR003 increased feed intake and weight gains and improved fecal microbial composition and immune regulation in weaned pigs (unpublished data). To gain insight into the mechanism behind the probiotic properties of *L. reuteri* ZLR003, we performed high-throughput sequencing to compare the diversity and composition of the microbiota of three intestinal segments of weaned piglets treated with *L. reuteri*, chlortetracycline (aureomycin), or sterile saline (control). This study provides us with key information that will be used to elucidate the relationship between the three treatments and the intestinal microbiota. It also provides fundamental knowledge that will facilitate studies such as the development of alternatives to antibiotics.

Materials and methods

Lactobacillus and antibiotic preparation

The strain *L. reuteri* ZLR003 was isolated from the cecum mucosa of a healthy weaned piglet in our laboratory. The strain was identified through standard morphological, biochemical, and physiological tests as well as 16S rDNA sequence analysis by the China Center of Industrial Culture Collection (Beijing, China). The strain is preserved at the China General Microbiological Culture Collection Center (CGMCC), and the CGMCC number is 11,530.

The strain was inoculated 1:100 into MRS broth (Merck, Darmstadt, Germany) and incubated for 18 h at 37 °C. Bacterial cells was harvested by centrifugation (6738×*g* for 10 min) at 4 °C under aseptic conditions and then resuspended in 0.85 % sterile saline solution. The viable cell count of the bacterial suspension was 2.0×10^9 cfu/mL. The concentration of chlortetracycline (Mellon, Biological Technology Co., Ltd., Dalian, China; >95 %) was adjusted to 100 mg/kg using 0.85 % sterile saline solution. The 0.85 % sterile saline solution without *L. reuteri* ZLR003 or chlortetracycline was used as the control treatment. The three preparations were prepared once every 2 days at the same time and stored at 4 °C.

Animals

Crossbred (Landrace × Large White) sibling piglets were used in this study. Nine weaned piglets (four males and five females) with an average initial body weight of 8.57 ± 1.28 kg were housed in three separate cages of identical construction under the same environmental conditions. Littermates were used to minimize differences arising from maternal microbiota. Piglets were weaned at 30 days of age. After weaning, piglets were randomly divided into three groups and subsequently treated with sterile saline (control), chlortetracycline, or *L. reuteri*. All piglets were given a basic diet specifically designed for the stage (Feeding Standard of Swine 2004) (Table 1). Piglets in the *L. reuteri* group received 5 mL of *L. reuteri* ZLR003 (2.0×10^9 cfu/mL) orally every morning. Piglets in the chlortetracycline group received 5 mL of chlortetracycline (100 mg/kg), and those in the control group received 5 mL of 0.85 % sterile saline solution at the same time. Piglets were housed in a controlled environment with stainless steel beds and were allowed ad libitum access to feed and water through a feeder and nipple drinker throughout the experimental period. The room temperature was maintained between 25 and 28 °C with 60 % relative humidity. The experimental period was 10 days.

Sample collection

At the end of the experiment, all piglets were euthanized under anesthesia, by exsanguination. Then, the contents of three intestinal segments (jejunum, colon, and cecum) were collected simultaneously. The 27 samples were snap-frozen in sterile containers on liquid nitrogen and stored at −80 °C.

DNA extraction, PCR, and sequencing

Total bacterial DNA was extracted from the collected intestinal contents using the E.Z.N.A. ® Stool DNA Kit (Omega

Table 1 Ingredients and composition of the basal diet

Ingredients (g/kg)	content
Corn	600
Soybean meal	230
Wheat bran	50
Fish meal	20
Whey	50
Soybean oil	10
Premix	40
Chemical composition	
Digestible energy[a], MJ/kg	13.75
Crude protein[b], g/kg	190
Lysine, g/kg	11.7
Methionine, g/kg	3.5
Salt, g/kg	4.4
Calcium[b], g/kg	8.0
Total phosphorus[b], g/kg	6.5

Each kg of complete feed contains: vitamin A, 11,000 IU; vitamin D_3, 2800 IU; vitamin E, 36 mg; menadione, 2.5 mg; vitamin B_1, 2.5 mg; vitamin B_2, 6.6 mg; vitamin B_6, 3.0 mg; vitamin B_{12}, 0.025 mg; niacin, 25 mg; pantothenic acid, 13 mg; biotin, 0.2 mg; Mn, 55 mg; Fe, 120 mg; Zn, 100 mg; Cu, 12 mg; I, 0.50 mg; Se, 0.30 mg

[a] Calculated nutrient levels [b] Measured nutrient levels

Bio-tek, Norcross, GA, USA) according to the manufacturer's protocols. The DNA quality was determined using agarose gel electrophoresis and a NanoDrop 8000 spectrophotometer (Thermo Fisher Scientific, Scoresby, Australia). Amplification of the V3–V4 region of the bacterial 16S ribosomal RNA gene was performed with the following the PCR cycling conditions: an initial denaturation at 95 °C for 3 min, 27 cycles of 95 °C for 30 s; 55 °C for 30 s, and 72 °C for 45 s; and a final extension at 72 °C for 10 min. The primers 338F (5′-ACTCCTACGGGAGGCAGCA-3′ and 806 R 5′-GGAC TACHVGGGTWTCTAAT-3′ were designed with an attached eight-base barcode sequence that was unique to each sample. The PCR was carried out in triplicate using 4 μL of 5× FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase, and 10 ng of template DNA in a final volume of 20 μL.

The PCR products were extracted from 2 % agarose gels, purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to the manufacturer's instructions, and quantified using QuantiFluor™-ST (Promega, Madison, WI, USA). Purified amplicons were pooled in equimolar amounts and paired-end sequenced (2 × 250) on an Illumina MiSeq platform according to standard protocols. The raw reads were deposited into the NCBI Sequence Read Archive database (accession number: SRP071319).

Processing of sequence data

Raw fastq files were demultiplexed and quality filtered using QIIME (version 1.17) according to the following criteria: (i) the 300-bp reads were truncated at any site receiving an average quality score < 20 over a 50-bp sliding window, discarding the truncated reads that were shorter than 50-bp; (ii) exact barcode matches, two- nucleotide primer mismatchea, and reads containing ambiguous characters were removed; and (iii) only sequences that overlapped more than 10 bp were assembled. Reads that could not be assembled were discarded. Operational units (OTUs) were clustered using UPARSE (version 7.1, http://drive5.com/uparse/) at a 97 % similarity level and chimeric sequences were identified and removed using UCHIME. Taxonomic classification of phylotypes was determined using the Ribosomal Database Project Classifier (http://rdp.cme.msu.edu/) against the Silva (SSU115)16S rRNA database at a 70 % confidence threshold (Amato et al. 2013).

Results

DNA sequence data

A total of 1,523,335 paired-end reads comprising 917,047,670 bp were generated from the raw data, and 1,354,684 valid sequences remained after chimeras were filtered out and low-quality sequences were removed. Among the high-quality sequences, about 99.98 % were longer than 400 bp, and most were between 400 and 460 bp, with an average of 441.22 bp. There was an average of 39, 478 reads for each sample. A total of 9021 OTUs were identified from all samples, with an average of 334 per sample. Rarefaction analysis was used to standardize and compare observed taxon richness among samples and to determine whether the contents of intestinal segments were unequally sampled. The results shown in Fig. 1 suggest that this sequencing depth was sufficient to cover the microbial diversity of each sample.

Alpha diversity of the GI microbiota

Sequence information and calculated microbial diversity indexes of the samples are shown in Table 2. The ACE, Chao1, Shannon, and Simpson indexes indicate that species richness and species diversity were significantly lower in the jejunum than in the hindgut (colon and cecum) in all three treatment groups. In addition, the ACE and Chao1 indexes indicate that species richness in the jejunum was higher in the *L. reuteri* group than in the chlortetracycline group. However, there were no significant differences in the alpha diversity of microbiota of the colon or cecum between either the

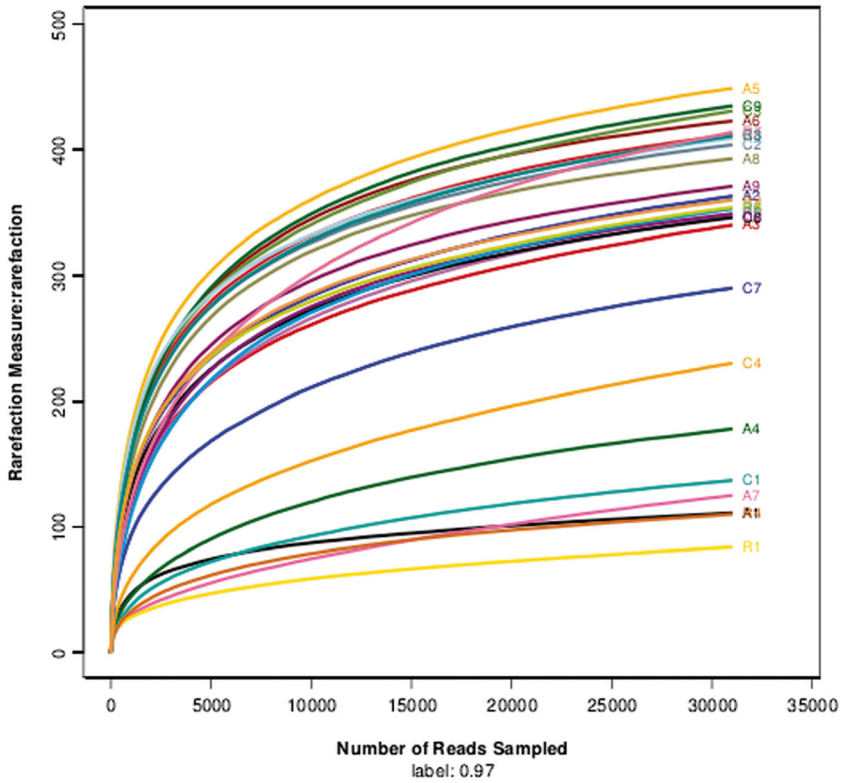


Fig. 1 Rarefaction curves of the OTUs number at 97 % similarity box plot for every sample. C1–C9, A1–A9 and R1–R9 indicate the 9 samples of control, chlortetracycline, and *L. reuteri* groups, respectively

chlortetracycline group or the *L. reuteri* group and the control group based on the ACE, Chao1, Shannon, and Simpson indexes.

Venn diagrams were used to evaluate the distribution of OTUs among the different treatment groups. As shown in Fig. 2a–c, the total number of OTUs was higher in the colon and cecum than in the jejunum of piglets. In the jejunum, a total of 135 OTUs were shared by the three treatment groups. Most of them belonged to the phyla *Proteobacteria*, *Firmicutes*, and *Fusobacteria*. Furthermore, the number of unique OTUs in the *L. reuteri* group was significantly higher than that in the other two groups, especially the chlortetracycline group, which had the lowest number of unique OTUs among the three groups. In the colon and cecum, 437 and 431 OTUs were shared by the three treatments, respectively. Most of them belonged to *Bacteroidetes*, *Proteobacteria*, *Firmicutes*, *Spirochaetae*, and *Fusobacteria*.

Table 2 Different microbial diversity indices in different treatment groups

Sample	ACE	Chao1	Shannon	Simpson	Coverage
Jejunum					
Control	344.7	303.0	2.45	0.201	0.9978
Chlortetracycline	244.3	218.0	1.64	0.411	0.9985
L. reuteri	269.0	301.3	1.87	0.421	0.9980
Colon					
Control	435.0	427.0	3.96	0.058	0.9980
Chlortetracycline	451.0	450.3	4.02	0.067	0.9979
L. reuteri	425.3	438.3	3.84	0.060	0.9978
Cecum					
Control	456.3	472.7	3.94	0.059	0.9977
Chlortetracycline	427.7	447.3	3.75	0.081	0.9979
L.reuteri	426.7	430.3	3.69	0.079	0.9978

The ACE, Chao1, Shannon and Simpson indexes are presented for a similarity of 0.97 between reads

Microbiota compositions in the three treatment groups

Proteobacteria, *Firmicutes*, *Bacteroidetes*, *Fusobacteria*, and *Verrucomicrobia* were the dominant phyla, representing more than 96 % of taxa in the jejunum, colon, and cecum in all three treatment groups. However, there were significant differences among the microbiota profiles of the three intestinal segments in the three treatment groups. In the jejunum, *Proteobacteria* was the dominant phylum in the *L. reuteri* and chlortetracycline groups (73.1 and 86.1 %, respectively), while it represented only 27.7 % of taxa in the control group. *Firmicutes* was the dominant phylum in the control group (53.8 %). Conversely, *Bacteroidetes* was dominant in the colon in all three treatment groups (43.5, 47.7, and 35.0 % in the control, chlortetracycline, and *L. reuteri* groups, respectively). In the cecum,

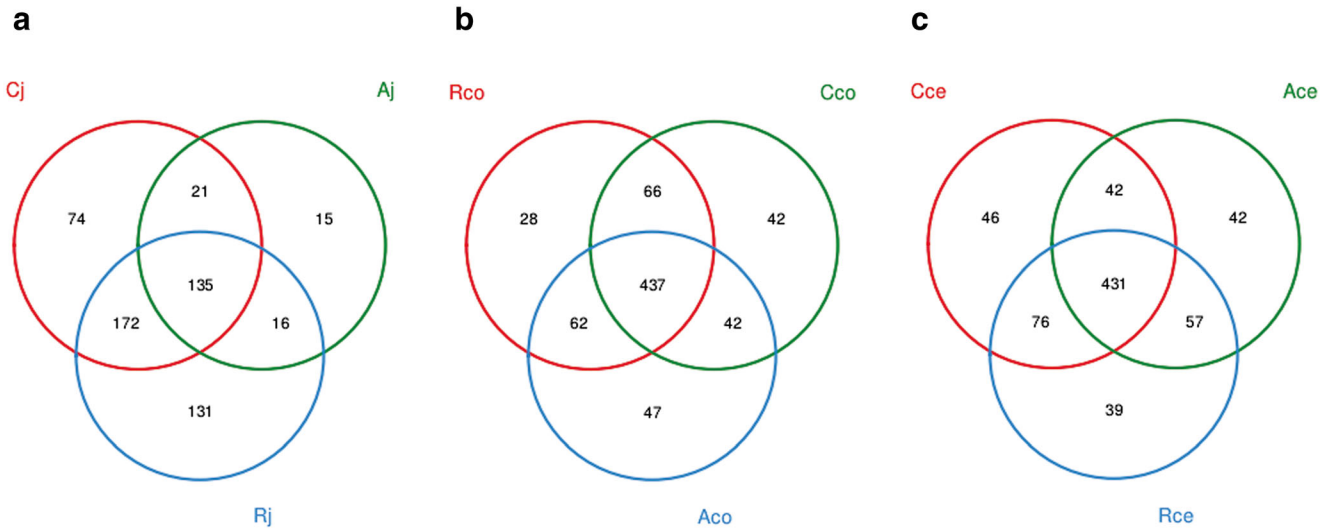


Fig. 2 The Venn diagram of common and unique OTUs among the three groups. The numbers of observed OTUs sharing ≥97 % nucleotide sequence identity. **a** The jejunum of piglets. **b** The colon of piglets. **c** The cecum of piglets. *Aj*, the jejunum samples of chlortetracycline group. *Cj*, the jejunum samples of control group. *Rj*, the jejunum samples of *L. reuteri* group. *Aco*, the colon samples of chlortetracycline group. *Cco*, the colon samples of control group. *Rco*, the colon samples of *L. reuteri* group. *Ace*, the cecum samples of chlortetracycline group. *Cce*, the cecum samples of control group. *Rce*, the cecum samples of *L. reuteri* group

Bacteroidetes was the dominant phylum in the control and *L. reuteri* groups (45.4 and 35.2 %, respectively), while *Firmicutes* was the most abundant phylum in the chlortetracycline group (42.8 %). In addition, *Verrucomicrobia* represented a high percentage of taxa in the jejunum of the control group (13.3 %) and was also observed in the colon and cecum of the control and *L. reuteri* groups. The relative abundance of *Verrucomicrobia* was lowest in the chlortetracycline group in all three intestinal segments (Fig. 3a–c).

A hierarchically clustered heatmap of the microbiota composition of the jejunum at the genus level is shown in Fig. 4a. The relative abundance of the genus *Actinobacillus* was highest in the chlortetracycline group (73.54 %), followed by the *L. reuteri* group (21.13 %). *Escherichia-Shigella*, *Lactobacillus*, and *Streptococcus* were less abundant in the chlortetracycline group than in the *L. reuteri* group, and other genera, such as *Comamonas* and *Enterococcus*, were not detected in the chlortetracycline group. Conversely, the relative abundances of *Parabacteroides* and *Lachnospiraceae incertae sedis* were higher in the *L. reuteri* group than in the chlortetracycline and control groups. Furthermore, while the relative abundance of *Akkermansia* was 13.28 % in the control group, this genus was not detected in the chlortetracycline and *L. reuteri* groups.

In the colon and cecum, the genus *Prevotella* was more abundant than it was in the jejunum in all three treatment groups. The OTUs uncultured *Prevotellaceae* and uncultured *Ruminococcaceae* were also abundant in the chlortetracycline group. The relative abundances of *Treponema*, *Parabacteroides*, and *Desulfovibrio* were higher in the *L. reuteri* group than in the other two groups. Furthermore, *Escherichia-Shigella* was more abundant in the control and *L. reuteri* groups than in the chlortetracycline group (Fig. 4b, c).

Relationships among microbiota of the three treatment groups

A multiple sample similarities tree was constructed to identify the similarities and differences among the three treatments (Fig. 5). Comparison of the microbiota of the different intestinal segments showed that the taxonomic composition of the jejunum was separate from the compositions of the colon and cecum in all three treatment groups. The taxonomic composition of the jejunum in the *L. reuteri* group was closest to that in the chlortetracycline group, indicating that these two treatments resulted in more similar community structures. The taxonomic compositions of the colon and cecum were clustered according to treatment (control, *L. reuteri*, or chlortetracycline), but the compositions in the *L. reuteri* group were closer to those in the control group than to those in the chlortetracycline group.

The results of nonmetric multidimensional scaling (NMDS) analysis (Fig. 6) were consistent with the multiple samples similarity tree. The samples were clustered into the following five groups: jejunum samples of the control group, jejunum samples of the chlortetracycline group, jejunum samples of the *L. reuteri* group, colon and cecum samples of the chlortetracycline group, and colon and cecum samples of the control and *L. reuteri* groups.

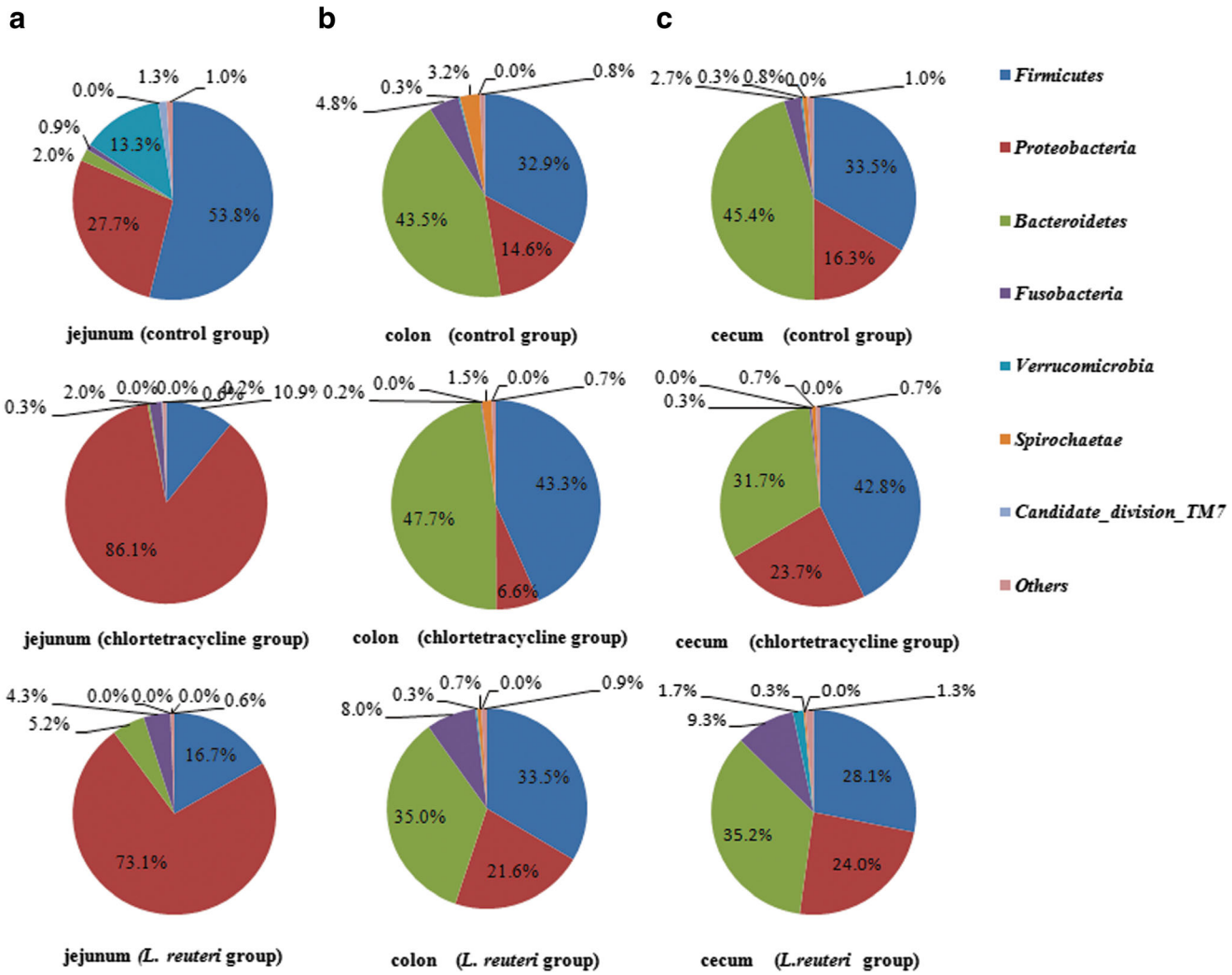


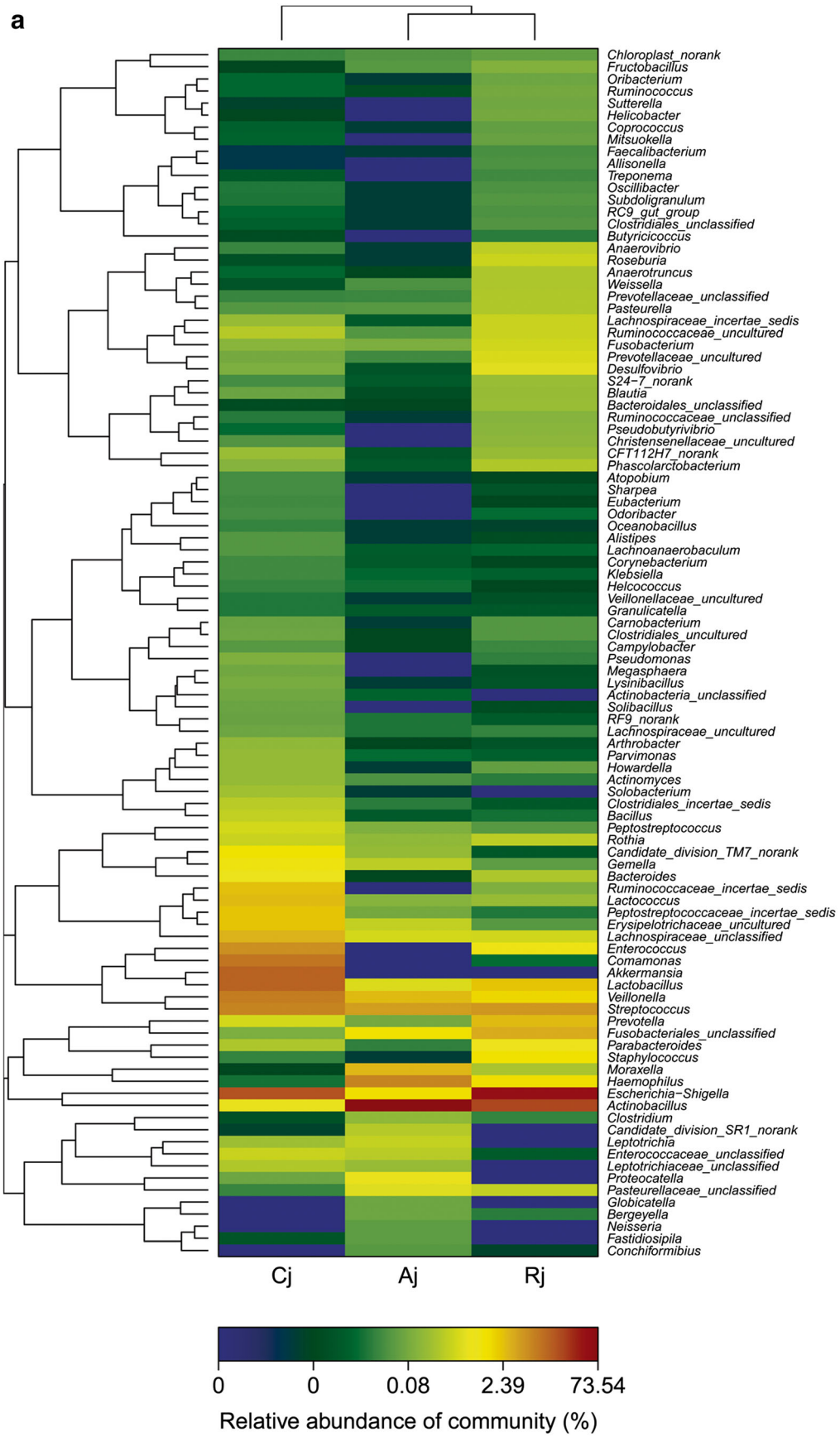
Fig. 3 Profiles of gut microbiota in GI tract segments at the rank of phylum. **a** The jejunum of piglets. **b** The colon of piglets. **c** The cecum of piglets

Discussion

Previous studies have identified changes in microbiota during weaning using culture-dependent methods, denaturing gradient gel electrophoresis (DGGE), and reverse transcription PCR (Eckburg et al. 2005; Liu et al. 2014; Sattler et al. 2015). High-throughput (next-generation) sequencing methods provide a more direct way to analyze microbiota taxa, especially the changes of the low-abundant species. Recent studies of fecal microbial shifts in pigs, using Roche 454 GS FLX Titanium protocols or the Illumina MiSeq platform, have contributed to our understanding of changes in microbiota that occur during weaning, a physiologically stressful time for pigs (Lu et al. 2014; Pajarillo et al. 2014; Zhao et al. 2015; Niu et al. 2015). These previous studies have suggested that improvement of the overall health of pigs requires a better understanding of the intestinal environment, particularly the interactions among microorganisms within the gut microbiota and between microorganisms and the host animal. Therefore, in the present study, we evaluated the microbiota of three intestinal segments in weaned piglets treated with *L. reuteri*, chlortetracycline, or sterile saline (control) using the Illumina MiSeq platform.

Alpha diversity is the diversity of a community within one site (or one sample), that is, the number of species and their relative abundances within one sampling site. High bacterial diversity is favorable for the overall health and productivity of animals (Hildebrand et al. 2013). We used ACE, Chao1, Shannon, and Simpson indexes as well as Venn diagrams to compare the alpha diversity among different treatment groups.

Fig. 4 Relative abundance of community. **a** The jejunum of piglets. **b** ▶ The colon of piglets. **c** The cecum of piglets. *Aj*, the jejunum samples of chlortetracycline group. *Cj*, the jejunum samples of control group. *Rj*, the jejunum samples of *L. reuteri* group. *Aco*, the colon samples of chlortetracycline group. *Cco*, the colon samples of control group. *Rco*, the colon samples of *L. reuteri* group. *Ace*, the cecum samples of chlortetracycline group. *Cce*, the cecum samples of control group. *Rce*, the cecum samples of *L. reuteri* group


a
Chloroplast_norank
Fructobacillus
Oribacterium
Ruminococcus
Sutterella
Helicobacter
Coprococcus
Mitsuokella
Faecalibacterium
Allisonella
Treponema
Oscillibacter
Subdoligranulum
RC9_gut_group
Clostridiales_unclassified
Butyricicoccus
Anaerovibrio
Roseburia
Anaerotruncus
Weissella
Prevotellaceae_unclassified
Pasteurella
Lachnospiraceae_incertae_sedis
Ruminococcaceae_uncultured
Fusobacterium
Prevotellaceae_uncultured
Desulfovibrio
S24−7_norank
Blautia
Bacteroidales_unclassified
Ruminococcaceae_unclassified
Pseudobutyrivibrio
Christensenellaceae_uncultured
CF112H7_norank
Phascolarctobacterium
Atopobium
Sharpea
Eubacterium
Odoribacter
Oceanobacillus
Alistipes
Lachnoanaerobaculum
Corynebacterium
Klebsiella
Helcococcus
Veillonellaceae_uncultured
Granulicatella
Carnobacterium
Clostridiales_uncultured
Campylobacter
Pseudomonas
Megasphaera
Lysinibacillus
Actinobacteria_unclassified
Solibacillus
RF9_norank
Lachnospiraceae_uncultured
Arthrobacter
Parvimonas
Howardella
Actinomyces
Solobacterium
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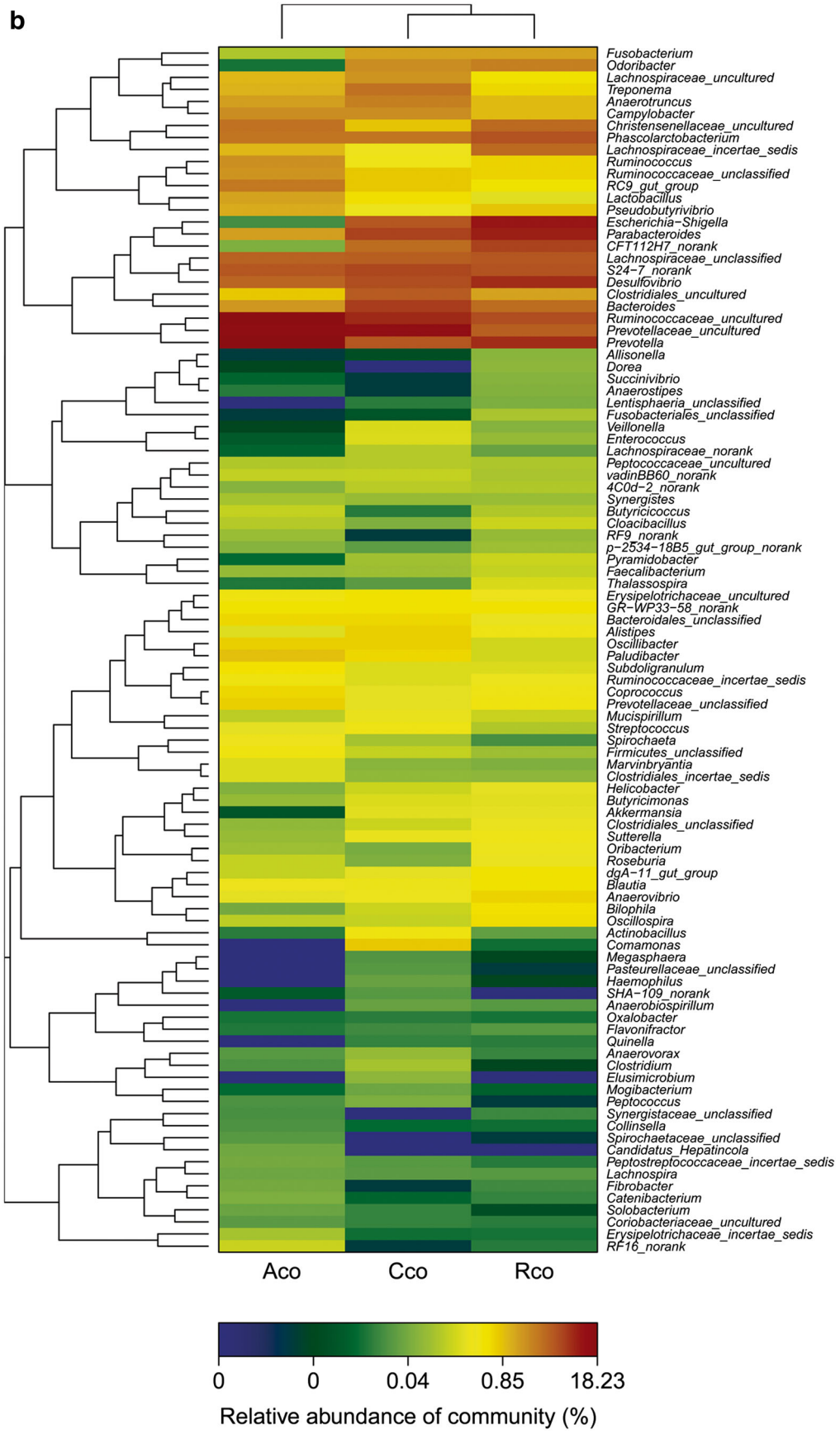
Bacillus
Peptostreptococcus
Rothia
Candidate_division_TM7_norank
Gemella
Bacteroides
Ruminococcaceae_incertae_sedis
Lactococcus
Peptostreptococcaceae_incertae_sedis
Erysipelotrichaceae_uncultured
Lachnospiraceae_unclassified
Enterococcus
Comamonas
Akkermansia
Lactobacillus
Veillonella
Streptococcus
Prevotella
Fusobacteriales_unclassified
Parabacteroides
Staphylococcus
Moraxella
Haemophilus
Escherichia−Shigella
Actinobacillus
Clostridium
Candidate_division_SR1_norank
Leptotrichia
Enterococcaceae_unclassified
Leptotrichiaceae_unclassified
Proteocatella
Pasteurellaceae_unclassified
Globicatella
Bergeyella
Neisseria
Fastidiosipila
Conchiformibius
Cj
Aj
Rj
0
0
0.08
2.39
73.54
Relative abundance of community (%)



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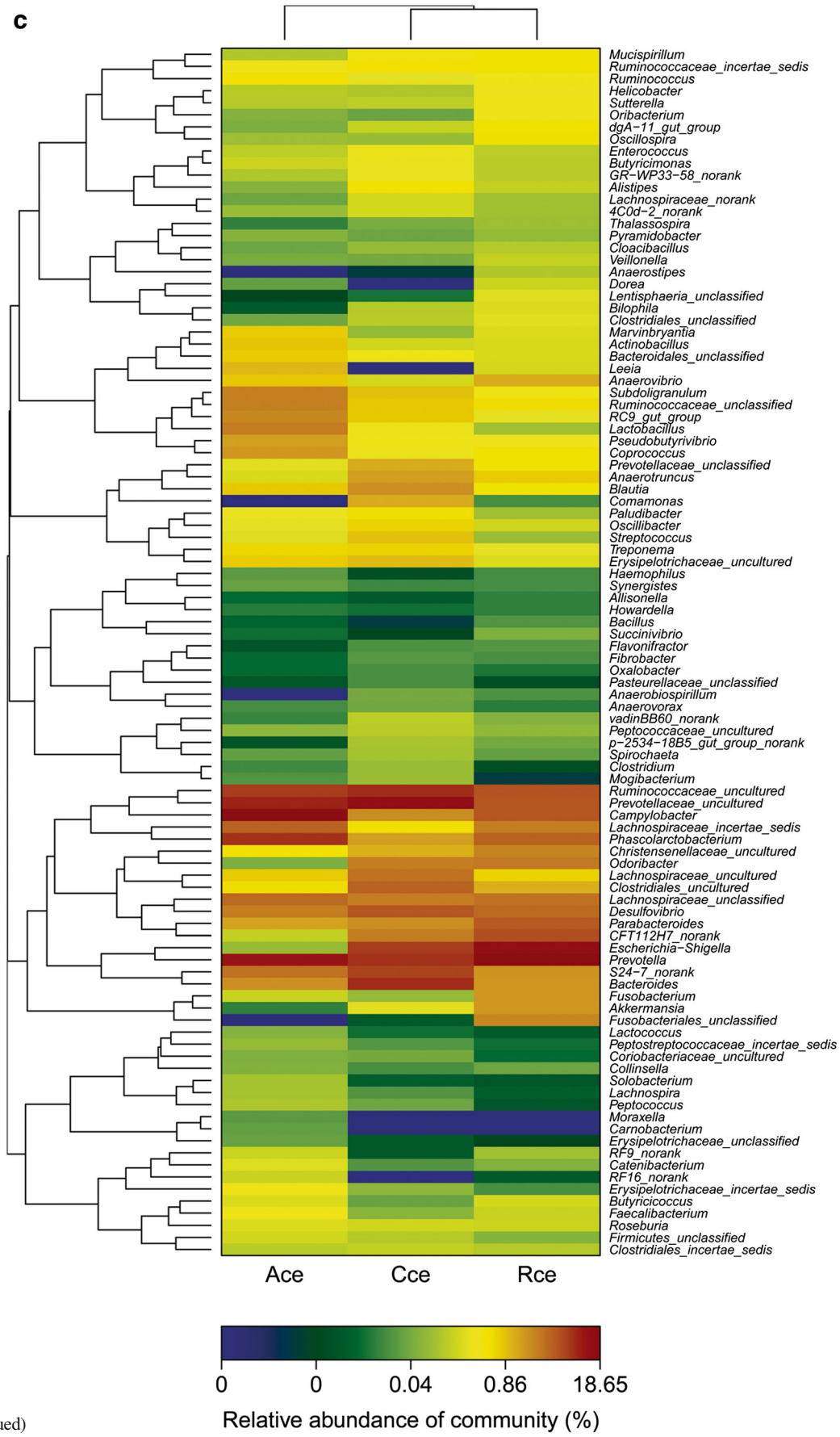

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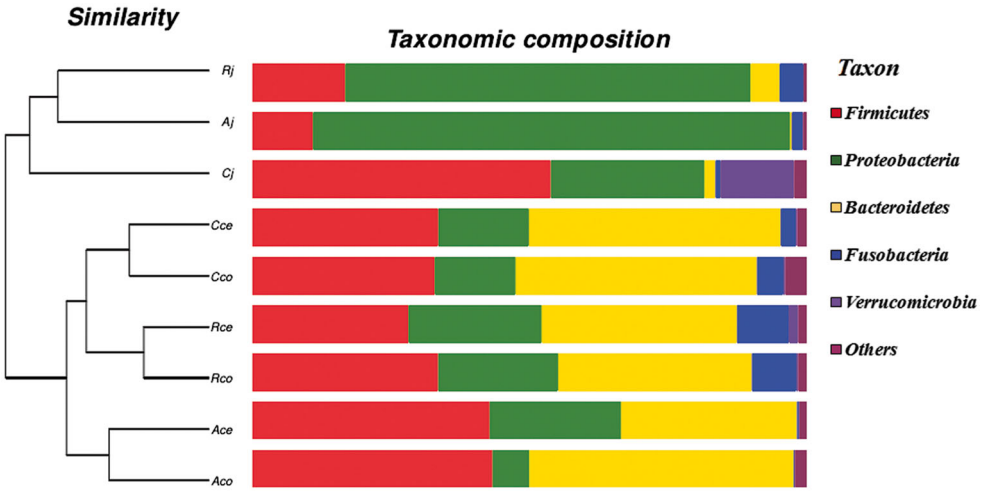

Fig. 5 Multiple samples similarity tree analysis. *Aj*, the jejunum samples of chlortetracycline group. *Cj*, the jejunum samples of control group. *Rj*, the jejunum samples of *L. reuteri* group. *Aco*, the colon samples of chlortetracycline group. *Cco*, the colon samples of control group. *Rco*, the colon samples of *L. reuteri* group. *Ace*, the cecum samples of chlortetracycline group. *Cce*, the cecum samples of control group. *Rce*, the cecum samples of *L. reuteri* group

The results showed that species richness and species diversity of GI microbiota were lower in the jejunum than in the hindgut (colon and cecum). These results are consistent with data reported by Zhao et al. (2015), who analyzed microbiota in the jejunum, ileum, and colon and cecum of pigs over a 6-month period using the Illumina MiSeq platform. Hou et al. (2015) also found that the Shannon diversity index was higher in colonic digesta than in ileal digesta of piglets after oral administration of *L. reuteri* I5007 at days 7, 14, and 21. It has been reported that the use of antibiotics can reduce alpha diversity (Knecht et al. 2014). Similarly, Manichanh et al. (2010) found that intake of an antibiotic cocktail for a short period of time had profound long-term effects on the rat intestinal microbiome. Our study indicated that changes in alpha diversity resulting from the different treatments were most pronounced in the jejunum. The ACE and Chao1 indexes indicated that species richness of the microbiolta in the jejunum was higher in the *L. reuteri* group than in the chlortetracycline group, and the Venn diagrams showed that the *L. reuteri* group had more unique OTUs than the chlortetracycline group. In the colon and cecum, there was no significant difference in alpha diversity among the *L. reuteri*, control, and chlortetracycline groups. Previous studies using PCR-DGGE (Simpson et al. 1999; Su et al. 2008) showed that *L. reuteri* MM53 and *Lactobacillus sobrius* S1 had no significant effects on microbial diversity in the hindgut of piglets or weaned piglets at days 7, 14, 21, and 24. However, reports of the effect of *Lactobacillus* species on the alpha diversity of the small intestine are limited.

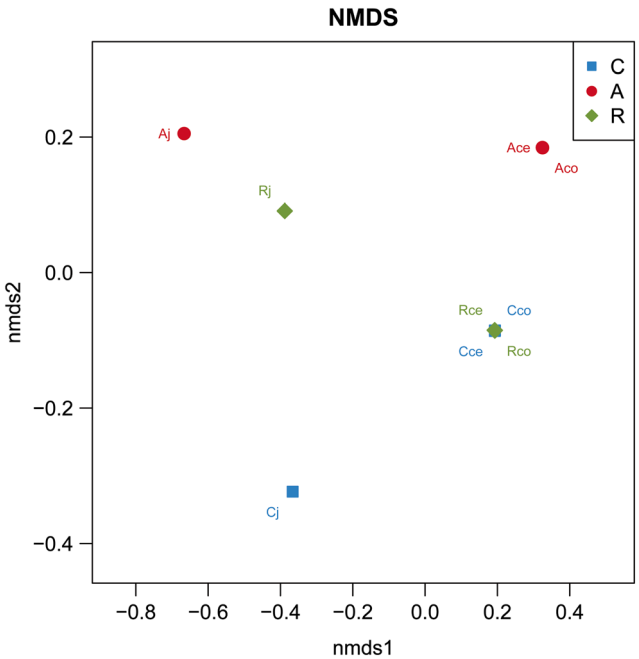

Fig. 6 Multiple samples NMDS analysis. *Aj*, the jejunum samples of chlortetracycline group. *Cj*, the jejunum samples of control group. *Rj*, the jejunum samples of *L. reuteri* group. *Aco*, the colon samples of chlortetracycline group. *Cco*, the colon samples of control group. *Rco*, the colon samples of *L. reuteri* group. *Ace*, the cecum samples of chlortetracycline group. *Cce*, the cecum samples of control group. *Rce*, the cecum samples of *L. reuteri* group

In mammals, the dominant phyla in the GI tract are *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, and *Fusobacteria*. These four taxa were also the dominant phyla in the GI tract of weaned piglets after 10 days of treatment in our study. However, the relative abundances of the taxa in our study were not consistent with the results of previous studies in which microbiota were sampled from pigs at the ages of 6 months (Zhao et al. 2015) and 11–12 weeks (Isaacson and Kim 2012). The differences are due to variability in the distribution of intestinal microbes, which is influenced by pig species, feed, husbandry, and age (Zhao et al. 2015). In our study, there were marked differences in microbiota profiles between the jejunum and the hindgut (colon and cecum), while there was only slight variation between the colon and the cecum. These results are consistent with those of Zhao et al. (2015). The small intestine is mainly responsible for digestion and

absorption, while the large intestine is the site of microbial fermentation and has high numbers of microorganisms, especially in the cecum. The dominant genera in the small intestine belonged to aerobe or facultative anaerobe categories, whereas the main genera in the large intestine were all anaerobes. Therefore, the specificity of the microbial community is related to the function of each intestinal tract segment.

It has been proposed that alteration of gut microbiota is one mechanism by which antibiotics enhance the growth of livestock (Schwarz and Chaslus-Dancla 2001; Dibner and Richards 2005). The microbiota profile of the jejunum was affected by the three treatments in our study. *Proteobacteria* were significantly more abundant in the chlortetracycline and *L. reuteri* groups than in the control group. The multiple samples similarity tree and the NMDS analysis showed that the taxonomic composition in the jejunum was more similar between the chlortetracycline and *L. reuteri* groups than between either of these groups and the control group. However, while the increase in the relative abundance of *Proteobacteria* was mostly correlated with an increase in the relative abundance of *Actinobacillus* in the chlortetracycline group, it was mostly correlated with increases in the relative abundances of *Actinobacillus* and *Escherichia-Shigella* in the *L. reuteri* group. Zhao et al. (2015) reported that *Escherichia-Shigella* is a dominant group in the small intestine, where it mostly takes part in digestion. The difference in the relative abundance of *Escherichia-Shigella* between the *L. reuteri* and chlortetracycline groups in our study suggests that *Lactobacillus* and the antibiotic may act differently to improve the growth performance of animals, but this requires further study.

The relative abundance of *Firmicutes* in the porcine large intestine is higher than that in the small intestine, suggesting that the large intestine might undertake some tasks of fat deposition. It has been shown that fat pigs have more *Firmicutes* but fewer *Bacteroidetes* (Ley et al. 2006), the latter of which are important for the degradation of carbohydrates (Arumugam et al. 2011). In our study, the relative abundance of *Firmicutes* in both the colon and the cecum was higher in the chlortetracycline group than in the control and *L. reuteri* groups. The multiple sample similarity tree and the NMDS analysis illustrated that colon and cecum samples from the *L. reuteri* group were clustered with samples from the control group rather than samples from the chlortetracycline group. At the genus level, the most obvious difference among the three treatments in our study was in the abundance of *Prevotella*. The increase in the relative abundance of *Prevotella* in the colon and cecum compared with the jejunum was greater in the chlortetracycline group than in the other groups. Pajarillo et al. (2014) showed that *Prevotella* became one of the most abundant genera in pigs after weaning. *Prevotella* represented up to 30 % of all classifiable bacteria at 10 weeks of age, but by the time the pigs were 22 weeks old, *Prevotella* accounted for only 3.5–4.0 % of the bacteria (Isaacson and Kim 2012). It has been suggested that the increase in the relative abundance of *Prevotella* during the post-weaning period is due to the ability of these bacteria to degrade hemicelluloses such as xylans in plant-based feed (Hayashi et al. 2007; Lamendella et al. 2011). The changes in the relative abundances of *Firmicutes* and *Prevotella* in the chlortetracycline group in our study may indicate that the antibiotic sped up the development and maturation of the microbiota to the adult "climax" community, as reported by Kim et al. (2012).

We also found that some taxa were detected in the control and *L. reuteri* groups, but not in the chlortetracycline group, especially in the jejunum. Examples include uncultured *Christensenellaceae*, *Ruminococcaceae incertae sedis*, *Comamonas*, and *Enterococcus*. These results may indicate that several genera were eliminated by the antibiotic. We also found that bacteria in the phylum *Verrucomicrobia* were less abundant in the chlortetracycline group than in the other groups among all three intestinal tract segments. Yin et al. (2015) found that the GI microbiota did not return to normal immediately after cessation of antibiotic treatment. For example, following treatment with ceftriaxone sodium, cefoperazone/sulbactam, meropenem, or vancomycin, the Shannon index and the number of OTUs returned to normal on the 14th and 90th days after drug withdrawal, respectively, and dysbiosis and recovery were determined by the antibiotics' mode of action on the bacterial cell wall. Furthermore, in our study, the relative abundances of *Lactobacillus*, *Streptococcus*, unclassified *Fusobacteriales*, *Prevotella*, and *Staphylococcus* in the jejunum were higher in the *L. reuteri* group than in the chlortetracycline group. In the colon and cecum, *Treponema* was significantly less abundant in the *L. reuteri* group than in the chlortetracycline and control groups. *Treponema* species have been shown to induce colitis in the infected host (Molbak et al. 2006). A recent metagenomic study showed that there are unusually high levels of certain *Treponema* species (*Spirochaetes*) in the porcine gut, relative to levels in other mammals (Lamendella et al. 2011). Our results are in line with the findings of Riboulet-Bisson et al. (2012), who showed that a *Lactobacillus salivarious* strain had the ability to modulate the level of *Treponema*.

In conclusion, changes in the diversity and composition of gut microbiota of weaned piglets were distinct under the three treatments in this study (control, chlortetracycline, and *L. reuteri* ZLR003). To our knowledge, this is the first study to compare gut microbiota between pigs treated with *Lactobacillus* and pigs treated with chlortetracycline. There is a lot more to explore in this field, and our findings will facilitate the application of *Lactobacillus* strains in animal production.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures involving animals were approved by the Ethics Committee of the Institute of Animal Husbandry and Veterinary Medicine, Beijing Academy of Agriculture and Forestry Sciences.

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