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The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Jabłonna

Comparative analysis of the rectal and caecal microbial community composition and function in adult Erhualian and Sushan pigs

B. Li[1], S. Ren[1], W. Zhao, X. Wang and F. Tu

Jiangsu Academy of Agricultural Sciences, Institute of Animal Science, Zhongling Street 50, Xuanwu, Nanjing, Jiangsu, China

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[1] Corresponding author:
e-mail: 1026563909@qq.com; shouwenren@163.com

ABSTRACT. Intestinal microbiota plays an important role in nutrition, metabolism and immunity in all mammals. It is comprised of diverse populations of bacteria and other microorganisms whose abundances are impacted by both environmental and host genetic factors. However, the understandings of the intestinal microbiota in different pig breeds remain largely undefined. To examine the differences in intestinal microflora between two pig breeds with different genetic backgrounds under the same environment, 16S rRNA gene amplification and sequencing were performed to investigate the structural composition and potential functions of microbial communities in rectum and caecum of Erhualian and Sushan pigs. The results revealed that the diversity of intestinal microflora in two pig breeds was similar, but the abundance of specific intestinal microflora was different. At the phylum level, the dominant bacteria in caecum and rectum of Erhualian and Sushan pigs were Firmicutes, Acidobacteria and Bacteroides, but their expression abundance was different. Firmicutes and Bacteroidetes in Erhualian pigs were higher than those in Sushan pigs. At the genus level, *Lactobacillus* was the most abundant in caecum of Sushan pigs (6.83%) and rectum of Erhualian pigs (9.61%), while *Ruminococcaceae* UCG-005 were dominant in caecum of Erhualian pigs (10.89%) and *Streptococcus* in rectum of Sushan pigs (24.89%). This study further confirmed the existence of specific microbial community diversity and abundance in different pig breeds. The microbial community diversity and abundance in Erhualian and Sushan pigs were closely related to pig fat deposition and nutrient absorption.

Introduction

The stable microbial diversity of the pig intestine can promote the absorption of nutrients, prevent the occurrence of diseases, and promote the growth and development of pigs (Bergen, 2015). There is a close correlation between the composition of intestinal microflora and intestinal development, immune characteristics, glucose and lipid metabolism and meat quality (Choy et al., 2014). The intestinal microbiota plays an important role in nutrient digestion. For example, the abundances of *Anaerofustis* and *Robinsoniella* in sow faecal samples were positively correlated with the apparent crude fibre digestibility (Niu et al., 2019). *Clostridium* is associated with dietary fibre metabolism, and *Turicibacter* is correlated with butyric acid (Woting et al., 2014). Pig breed is also an important factor affecting intestinal microbial diversity. Yang et al. (2014) showed that there were differences in intestinal microflora among different breeds of pigs. There is a high similarity among the intestinal microbes

of Landrace, Yorkshire and Duroc pigs. However, Bama mini, Erhualian and Xiaomeishan pigs from Chinese local breeds have high similarity when it comes to microorganisms presence and type. Diao et al. (2016) showed that intestinal microbial abundances in Rongchang, Tibetan and Landrace pigs were different. Tibetan and Rongchang pigs had a higher proportion of Firmicutes and Spirochaetes and a lower proportion of Bacteroidetes than Landrace pigs, and the proportion of Spirochaetes in Tibetan pigs was significantly higher than that in Rongchang pigs. Compared with Tibetan pigs, Landrace and Rongchang pigs contained a higher proportion of Tenericutes and a lower proportion of Fibrobacteres and Elusimicrobia. The above results indicated that the composition of intestinal microorganisms in pigs was probably related to the host's metabolic type, feeding characteristics and immune function.

Erhualian pig is a well-known local pig breed in China, with strong lactation, good motherhood, a high feeding rate, early sexual maturity, especially high resistance to rough feeding and a docile temperament. Sushan pig, as a new hybrid breed of Erhualian and Yorkshire pigs, has the advantages of delicious meat and a certain level of resistance to rough feeding. In pig production, the tolerance to rough feeding and crude fibre of Sushan pigs was found to be lower than that of Erhualian pigs, and the demand of Sushan pigs for nutrients in feed is higher than that of Erhualian pigs. In order to study the difference of tolerance to rough feeding and crude fibre between Erhualian and Sushan pigs, characterization and comparative analysis were performed to investigate the structural composition and potential functions of microbial communities in these two breeds. This study provides a powerful theoretical basis on the potential roles of intestinal microbial communities in resistance to rough feeding, nutrition metabolism and crude fibre digestion for safe and healthy pork production.

Material and methods

This experiment was reviewed and approved by the Institutional Animal Ethics Committee from the Research Integrity and Ethics Administration of Jiangsu Academy of Agricultural Sciences, China.

Animals and sampling

Erhualian and Sushan pigs (five barrows each) were fed at the Sushan Pig Breeding Farm (Nanjing, China) under the same conditions and provided with standard diets in accordance with the feeding standard of swine (NY/T 65-2004) issued by the Ministry of Agriculture of the People's Republic of China (Table 1). At the rapid growth stage (175th day of age), the adult Erhualian (75 kg) and Sushan (90 kg) pigs were slaughtered according to standard procedures. The pigs were dissected for collecting the intestinal contents of the rectum and caecum. The samples were kept at −80 °C for 16S rRNA gene analysis.

Table 1. Composition and nutrient levels of basal diets (air-dry basis), %

Indices	Content
Ingredients	
maize	61.00
soyabean meal	9.00
wheat bran	27.50
$CaHPO_4$	0.50
limestone	0.50
NaCl	0.50
premix[1]	1.00
Nutrient levels[2]	
DM	85.43
ash	6.24
CP	15.41
EE	4.31
CF	3.02
DE, MJ /kg	18.32
Ca	0.54
P	0.47

DM – dry matter, CP – crude protein, EE – ether exract, CF – crude fibre, DE – digestible energy; [1] the premix provided the following per kg of diets: mg: Fe 100, Zn 100, Mn 30, Cu 10, Se 0.3, I 0.5, vit. K 3.0, vit. B_1 2.0, vit. B_2 6.0, vit. B_6 3.0, nicotinic acid 30, pantothenic acid 30, folic acid 1.0, biotin 0.2, choline 300; IU: vit. A 8 000, vit. D 31 000, vit. E 20; µg: vit. B_{12} 30; [2] DE was calculated, while the other values were calculated

DNA extraction and PCR amplification

Microbial DNA was extracted from rectal and caecal samples using an E.Z.N.A.® soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) according to the manufacturer's protocols. The final DNA concentration and purification were determined by a NanoDrop 2000 UV-Vis spectrophotometer (Thermo Scientific, Waltham, MA, USA), and the DNA quality was checked by 1% agarose gel electrophoresis. The V3/V4 hypervariable regions of the bacterial 16S rRNA gene were amplified using primers 341F (5'-CCTAYGGGRBGCASCAG-3') and 806R (5'-GGACTACNNGGGTATCTAAT-3') based on a thermocycler PCR system (GeneAmp PCR System 9700, Applied Biosystem, Foster City, CA, USA). The PCRs were conducted with the following program: initial denaturation at 94 °C for 4 min; 94 °C denaturation for 30 s, 50 °C annealing for 45 s and 72 °C extension for 30 s, repeated for 25 cycles;

and final extension at 72 °C for 5 min. PCRs were performed in triplicate with 20 µl of a mixture containing 4 µl of 5× FastPfu Buffer (Transgen Biotech, Beijing, China), 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. The final PCR products were extracted from a 2% agarose gel, further purified with an AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA), and quantified using a QuantiFluor ™-ST (Promega, Madison, WI, USA) according to the manufacturer's protocol.

Illumina MiSeq sequencing

The purified amplicons were pooled in equimolar amounts and paired-end sequenced (2 ×300 bp) on an Illumina MiSeq platform (Illumina, San Diego, CA, USA) according to the standard protocols of the Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database (Accession Number: SRP279885).

Data processing

Raw FastQ files were demultiplexed, quality-filtered using a fastp (version 0.20.0, https://github.com/OpenGene/fastp; Chen et al., 2018), and merged using a FLASH (version 1.2.7, http://ccb.jhu.edu/software/FLASH; Magoč and Salzberg, 2011) with the following criteria: (i) the reads were truncated at any site receiving an average quality score <20 over a 50 bp sliding window; (ii) primers were accurately matched, allowing 2 nucleotides to be mismatched, and reads containing ambiguous bases were removed; and (iii) sequences whose overlap was longer than 10 bp were merged according to their overlap sequence.

Operational taxonomic units (OTUs) were clustered with a 97% similarity cut-off using a UPARSE (version 7.1, http://drive5.com/uparse; Edgar, 2013), and chimeric sequences were identified and removed using a UCHIME (version 4.2.40, http://www.drive5.com/uchime). The taxonomy of each 16S rRNA gene sequence was analysed with an RDP Classifier algorithm (http://rdp.cme.msu.edu) against the SILVA (SSU123) 16S rRNA database at a confidence threshold of 70%.

Data analysis

Community diversity at the inter- and intragroup levels was assessed using a combination of bias Sobs, Shannon diversity indices, Simpson's diversity index, the abundance-based coverage estimator (ACE), the Chao1 richness estimator and the coverage percentage. Based on the OTU expression profile, the alpha diversity of different samples at the OTU level was calculated. All of the aforementioned analyses were conducted using a MOTHUR (Kemp and Aller, 2004; Schloss et al., 2009). The principal component analysis (PCA) was performed based on the expression profile of OTUs at the taxonomic level using the R package (R Core Team, 2020). To identify differentially abundant taxa in multiple segments within different pig breeds, the linear discriminant analysis (LDA) effect size (LEFse) method was applied (Segata et al., 2011). To identify differentially abundant microbial taxa in the same segment among samples of different pig breeds a Metastats was used (White et al., 2009). Phylogenetic investigation of communities by reconstruction of unobserved states (PICRUS) (Langille et al., 2013) was applied to predict the functional enrichment of the microbial communities against the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Du et al., 2014). Correlation coefficients of the pathway enrichment for the samples of different pig breeds were calculated by the Spearman method. The R package 3.3.1, edgeR (Robinson et al., 2010), was used to determine differentiating molecular functions and pathways with a threshold of Log2 fold change > 2 and FDR < 0.01. Volcano plots and heatmaps were generated for the differentiating pathways.

Results

Species annotation and assessment

OTU analysis. In total 1 346 719 tags were obtained from all samples, covering 560 597 784 base pairs (Table 2). The average tag count per sample was 67 336, and 1397 OTUs at 97% identity were obtained, with the number of OTUs ranging from 523 to 1395 per sample. Coverage was determined to be greater than 95% in each sample. The sparse curve showed an obvious asymptote, which indicated that the sampling of the microbial community was close to complete and the sequencing depth was sufficient for diversity evaluation.

Alpha diversity analysis. The results of Sobs and Chao1 indices showed that the relative abundances of bacteria in rectum were higher than those in caecum, and the relative abundances of bacteria in rectum and caecum of Sushan pigs were higher than those of Erhualian pigs. The results of Shannon and Simpson indices showed that the bacterial community diversity of rectum was higher than that of caecum in Erhualian pigs, while an opposite result was found in Sushan pigs (Table 3).

Table 2. Tag number and length of the segmented samples in rectum and caecum of Erhualian and Sushan pigs

Sample ID	Total tag number	Total tag length, bp	Mean length, bp	Min length, bp	Max length, bp
EC-1d	61639	25431211	413	261	514
EC-2d	58044	24031647	414	328	514
EC-3d	56668	23591214	416	319	483
EC-4d	57618	23729682	412	254	463
EC-6d	72425	29913072	413	232	501
ER-1f	58732	24502833	417	317	483
ER-2f	57826	24081958	416	297	492
ER-3f	49790	20837327	419	327	465
ER-5f	69731	29145798	418	335	522
ER-6f	70596	29493081	418	321	445
SC-7	73715	30499096	414	219	509
SC-9	72662	30311299	417	245	458
SC-10	73502	30548412	416	216	436
SC-11	74671	31081964	416	234	444
SC-12	73643	30693287	417	269	434
SR-1	74642	31147919	417	258	520
SR-3	71455	29729693	416	220	463
SR-4	73513	30881879	420	214	473
SR-5	71684	29931980	418	270	432
SR-6	74163	31014432	418	231	478

EC – Erhualian pig caecum, ER – Erhualian pig rectum, SC – Sushan pig caecum, SR – Sushan pig rectum

Table 3. Richness and diversity estimates of 16S rRNA genes from the sequencing analysis in rectum and caecum of Erhualian and Sushan pigs

Sample	Species richness indices		Species diversity indices	
	Sobs	Chao1	Shannon	Simpson
EC	649.4 ± 107.39	783.50 ± 85.99	4.39 ± 0.47	0.038 ± 0.032
ER	699.8 ± 32.39	819.68 ± 65.45	4.62 ± 0.15	0.023 ± 0.004
SC	755.4 ± 62.28	863.67 ± 76.95	4.94 ± 0.11	0.020 ± 0.005
SR	840.4 ± 52.33	963.03 ± 58.46	4.51 ± 0.17	0.068 ± 0.017

EC – Erhualian pig caecum, ER – Erhualian pig rectum, SC – Sushan pig caecum, SR – Sushan pig rectum

Species composition analysis. Figure 1 reveals that 1397 different OTUs were distributed in rectum and caecum of Erhualian and Sushan pigs. In total 842 OTUs were shared by all of the samples, and the number of unique OTUs in rectum of Sushan pigs was the highest. The microbial populations of *Lactobacillus* (9.61%) were the largest in rectum

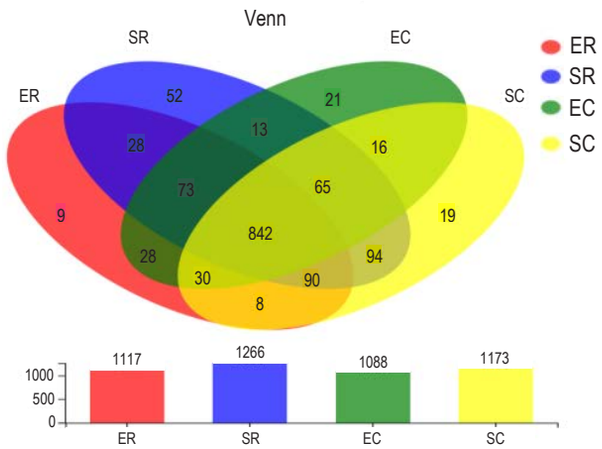

Figure 1. Venn diagrams of the operational taxonomic units (OUT)
EC – Erhualian pig caecum, ER – Erhualian pig rectum, SC – Sushan pig caecum, SR – Sushan pig rectum

of Erhualian pigs (Figure 2A), *Ruminococcaceae* UCG-005 (10.89%) in caecum of Erhualian pigs (Figure 2B), *Streptococcus* (24.89%) in rectum of Sushan pigs (Figure 2C) and *Lactobacillus* (6.83%) in caecum of Sushan pigs (Figure 2D), respectively. Among the dominant bacteria, *Lachnospiraceae* XPB1014 was the unique species identified in rectum of Erhualian pigs; *Coprococcus*, *Phascolarctobacterium* and *Lachnospiraceae* NK4A136 in caecum of Erhualian pigs; *Fusobacterium* in caecum of Sushan pigs; and *Prevotella* and *Ruminococcaceae* NK4A214 in rectum of Sushan pigs, respectively. *Alloprevotella*, *Romboutsia*, *Christensenellaceae* R-7 and *Rikenellaceae* RC9 were among the dominant bacteria in caecum of Erhualian and Sushan pigs. *Prevotellaceae* UCG-003 was not found in rectum of Erhualian pigs, while *Prevotellaceae* NK3B31 was not found in caecum of this breed. *Clostridium* was not in the dominant bacteria found in caecum of Sushan pigs.

Species difference analysis. The significance of microbial compositional differences in caecum and rectum of Erhualian and Sushan pigs was tested (Figure 3). The results showed that four genera were significantly different in caecum of Erhualian and Sushan pigs, and *Ruminococcaceae* UCG-005 and *Christensenellaceae* R-7 exhibited highly significant differences (Figure 3A); three genera were significantly different in rectum of Erhualian and Sushan pigs, and *Streptococcus* exhibited highly significant differences (Figure 3B); seven genera were significantly different in caecum and rectum of Erhualian pigs, and *Christensenellaceae* R-7 exhibited highly significant differences (Figure 3C); four genera were significantly different in caecum and rectum of Sushan pigs, and *Streptococcus* and

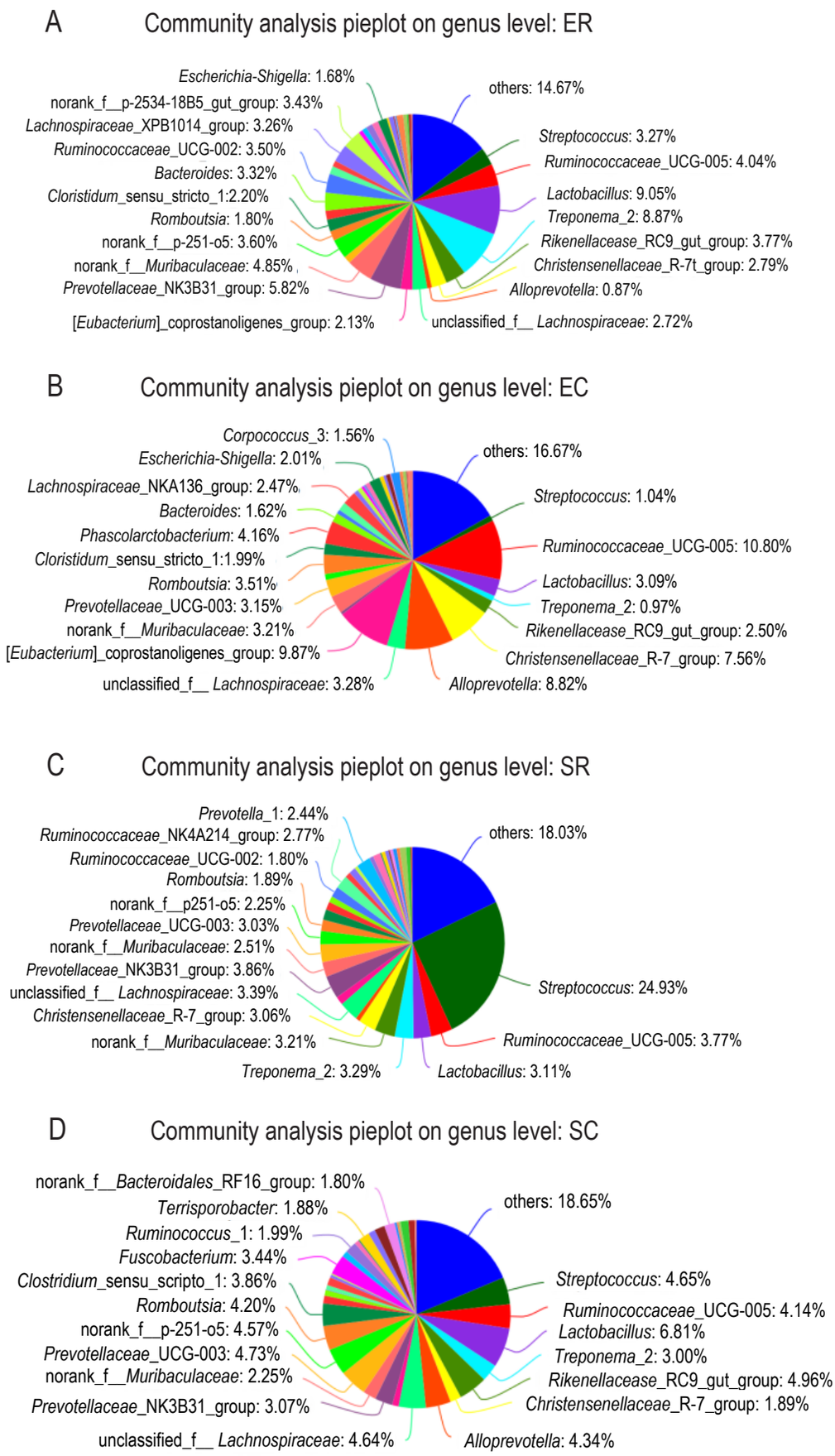

Figure 2. The community pieplot on genus level in rectum and caecum of Erhualian and Sushan pigs
EC – Erhualian pig caecum, ER – Erhualian pig rectum, SC – Sushan pig caecum, SR – Sushan pig rectum

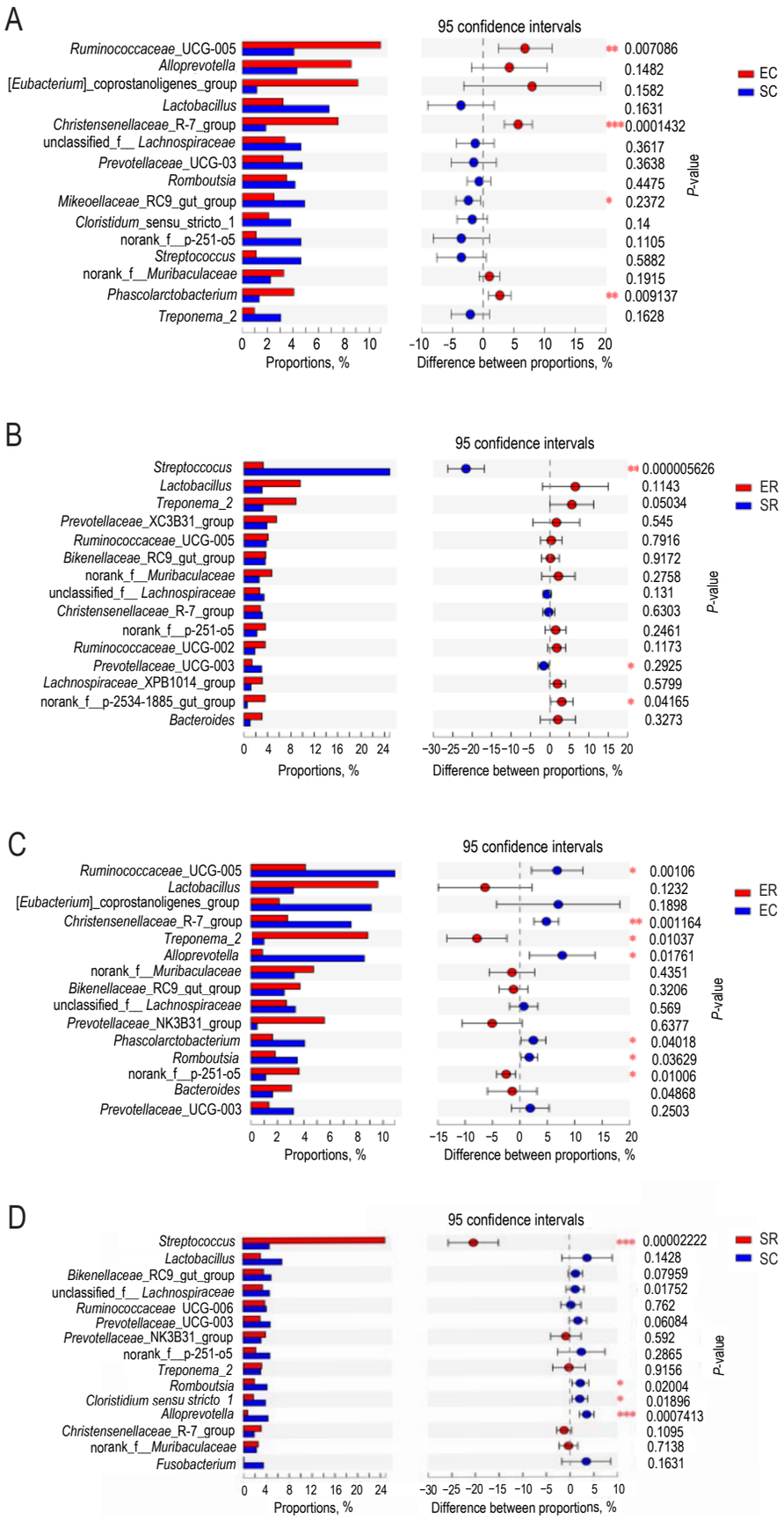

Figure 3. Phylotypes significantly different between EC and SC (A), ER and SR (B), ER and EC (C), SR and SC (D) groups at the genus level
EC – Erhualian pig caecum, ER – Erhualian pig rectum, SC – Sushan pig caecum, SR – Sushan pig rectum; statistical analysis was performed by the Student's t-test; n = 5, in each group; * $P \leq 0.05$, ** $P \leq 0.01$ and *** $P \leq 0.001$

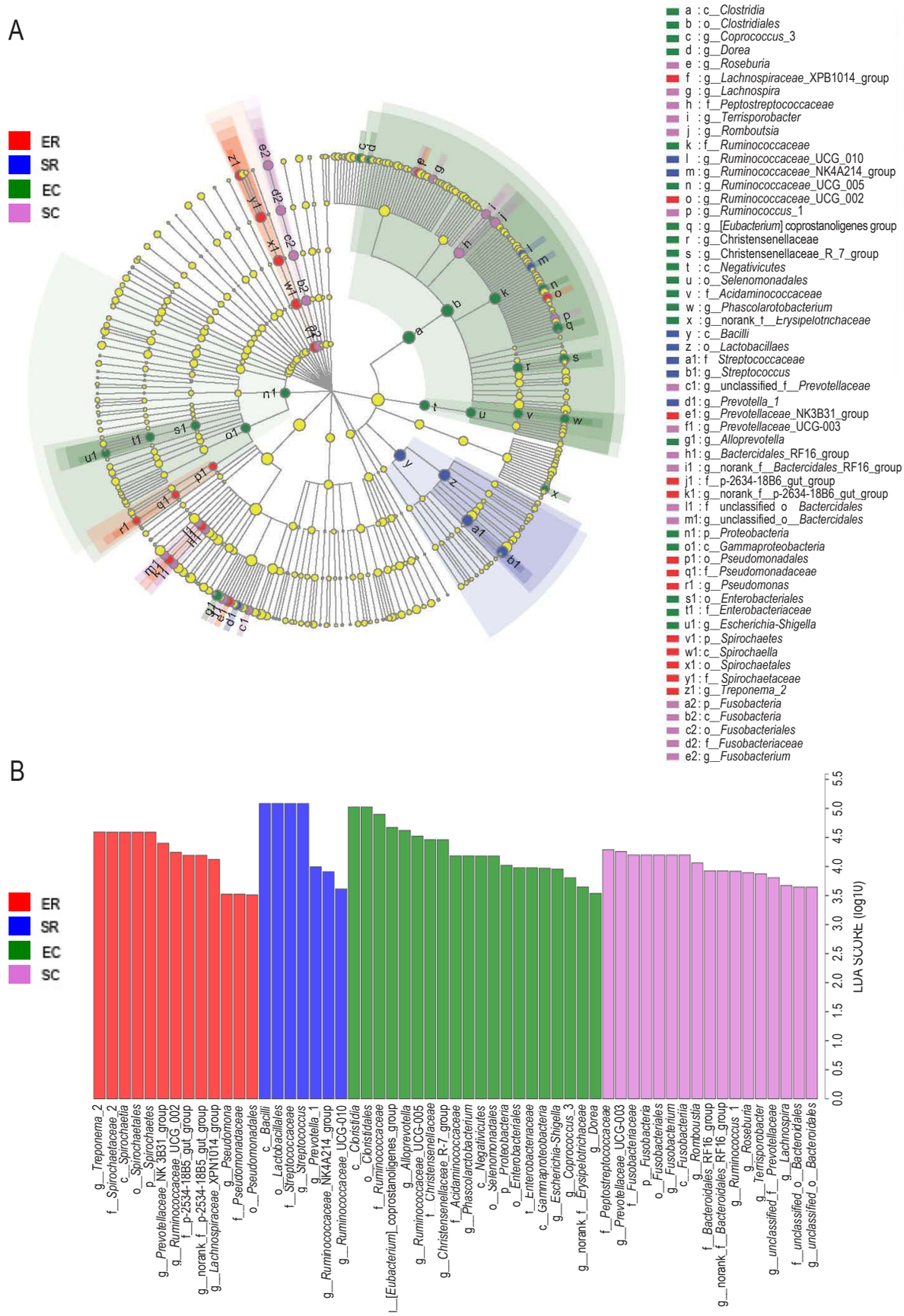

Figure 4. Cladogram (A) and LDA (B) analyses of bacterial communities associated with different portions of intestines in Sushan and Erhualian pigs. Different-coloured regions represent different intestinal parts of two breeds (red, ER; blue, SR; green, EC; pink, SC). Circles indicate phylogenetic levels from phylum to genus. The diameter of each circle is proportional to the abundance of the group.
EC – Erhualian pig caecum, ER – Erhualian pig rectum, SC – Sushan pig caecum, SR – Sushan pig rectum

Alloprevotella exhibited highly significant differences (Figure 3D). Cladogram exhibited a distinct phylogenetic distribution of the bacterial lineages in different intestinal segments of Erhualian and Sushan pigs (Figure 4A). Indicator bacteria with LDA scores of 3.5 in bacterial communities were associated with different intestinal parts of two breeds (Figure 4B). In rectum of Erhualian pigs, enriched bacterial groups included *Treponema* (genus), Spirochaetes (from phylum to family), *Prevotellaceae* (genus), *Ruminococcaceae* (genus), Lachnospiraceae and Pseudomonadales (from order to genus). In rectum of Sushan pigs, enriched bacterial groups included Bacilli (class), Lactobacillales (order) and Streptococcaceae (from family to genus). In caecum of Erhualian pigs, Clostridia (from class to order), Ruminococcaceae (from family to genus), *Alloprevotella* (genus), Christensenellaceae (from family to genus) and Enterobacteriales (from order to genus) were significantly enriched. In caecum of Sushan pigs, enriched bacterial groups included Fusobacteria (from phylum to genus), Bacteroidales (from family to genus), Peptostreptococcaceae (family) and *Prevotellaceae* UCG-003.

Sample comparison analysis. The bacterial community structures of different intestinal segments of the two breeds of pigs were clearly separated, and two coordinates (PC1 and PC2) explained 41.37% of the total variation of bacteria (Figure 5A). In addition, the Jensen-Shannon distance was calculated according to the abundance of the microflora at the genus level and clustered by PAM (partitioning around medoids) to obtain the optimal clustering K value of 3. Then, the PCA results were visually displayed. The analysis of microflora typing showed three types of intestinal microflora clustering (Figure 5B). Intestinal type 1 was the *Streptococcus* intestinal type, which was mainly present in rectum of Sushan pigs; intestinal type 2 was the *Lactobacillus* intestinal type, which was mainly present in rectum of Erhualian pigs and caecum of Sushan pigs; and intestinal type 3 was the *Ruminococcaceae* UCG-005 intestinal type, which was mainly present in caecum of Erhualian pigs.

Community function prediction and pathway enrichment analyses. Using the present OTU data, PICRUS was applied to determine the potential pathway enrichment of intestinal samples *via* annotation against the KEGG database. In all samples, the majority of OTUs were assigned to 24 gene families, which were mainly involved in carbohydrate transport and metabolism, general function prediction, amino acid transport and metabolism, transcription, replication, recombination and repair,

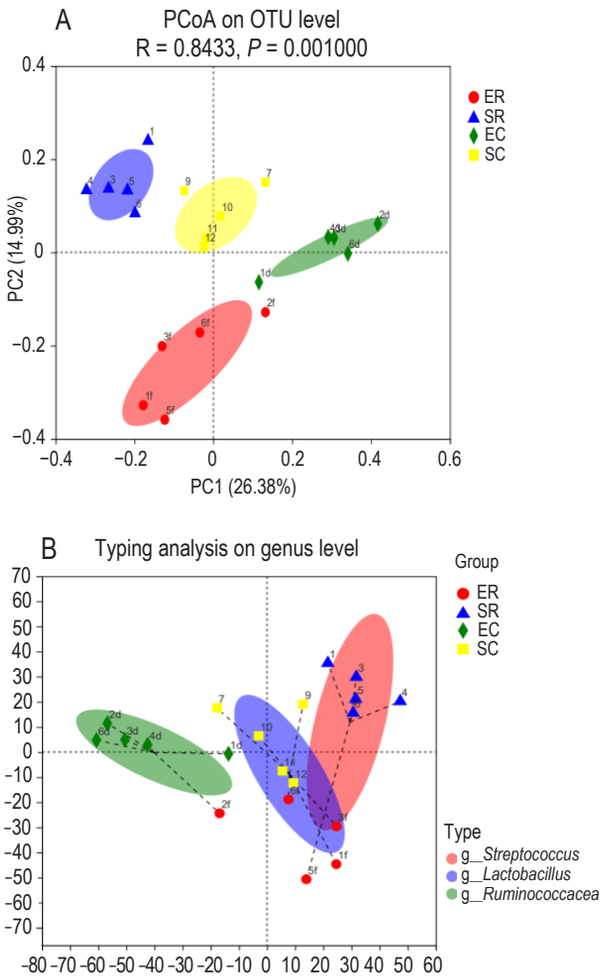

Figure 5. Principal coordinate analysis (PCA) plots (A) and microflora typing (B) of bacterial communities in rectum and caecum of Erhualian and Sushan pigs

EC – Erhualian pig caecum, ER – Erhualian pig rectum, SC – Sushan pig caecum, SR – Sushan pig rectum

translation, ribosomal structure and biogenesis. Compared with breed composition, the COG functional composition of all the samples was relatively similar. No significant difference was found in different samples (Figure 6). The prevalence of pathways at the KEGG 1 class level was similar among different samples, and their abundance values of metabolism were the highest (Figure 7A). The results of pathway level 2 revealed that relatively few microbial communities in rectum of Erhualian and Sushan pigs were involved in amino acid metabolism, biosynthesis of other secondary metabolites, cell motility, cellular processes and signalling, energy metabolism, environmental adaptation, enzyme families, folding, sorting and degradation, genetic information processing, immune system, lipid metabolism, metabolism of cofactors and vitamins, metabolism of other amino acids, nervous system and nucleotide metabolism. However, in rectum of Erhualian and Sushan pigs, the abundance values

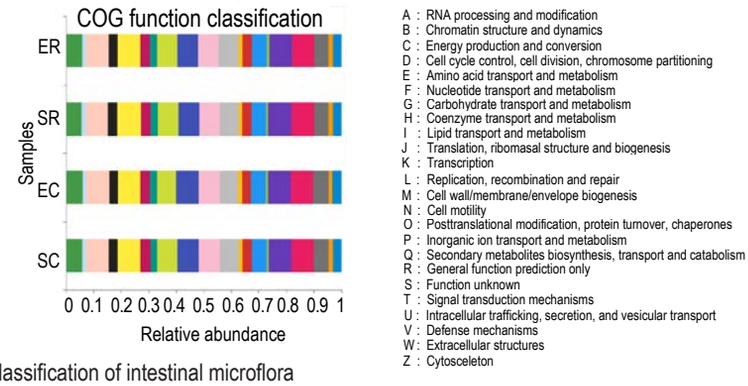

Figure 6. COG functional classification of intestinal microflora
EC – Erhualian pig caecum, ER – Erhualian pig rectum, SC – Sushan pig caecum, SR – Sushan pig rectum

A. Pathway Level 1	SR	ER	EC	SC
Cellular Processes	617977	724002	842920	723858
Environmental Information Processing	3112432	2840912	3339407	2942520
Genetic Information Processing	4348365	4342549	4683729	4447764
Human Diseases	141720	156029	160694	153693
Metabolism	9883882	9731451	10427453	10008479
None	32639	38751	47084	41760
Organismal Systems	136408	152716	158261	152356
Unclassified	2813418	2863250	3175188	2902365
B. Pathway Level 2	**SR**	**ER**	**EC**	**SC**
Amino Acid Metabolism	1963875	1985592	2175436	2057995
Biosynthesis of Other Secondary Metabolites	177009	179655	192018	189747
Cancers	17934	22866	22474	21937
Carbohydrate Metabolism	2308818	2132593	2275523	2192341
Cardiovascular Diseases	38	4	3	24
Cell Growth and Death	116425	117600	121245	115652
Cell Motility	451164	545009	671375	553286
Cellular Processes and Signaling	751544	795898	912650	814521
Circulatory System	203	217	177	266
Digestive System	9210	9162	7115	7489
Endocrine System	55167	63860	62709	60819
Energy Metabolism	1165120	1193364	1283636	1226272
Environmental Adaptation	32627	34591	40187	37658
Enzyme Families	450738	449165	496025	466523
Excretory System	3761	5517	4074	5072
Folding, Sorting and Degradation	502762	517206	543762	524182
Genetic Information Processing	565289	561103	627559	568535
Glycan Biosynthesis and Metabolism	444381	463056	427921	453128
Immune System	15785	17725	20848	18844
Immune System Diseases	10984	9795	8299	10599
Infectious Diseases	71195	79471	83927	78229
Lipid Metabolism	588861	576657	625829	589847
Membrane Transport	2725893	2460862	2910865	2556710
Metabolic Diseases	22013	23128	23432	22893
Metabolism	504694	502443	548473	508443
Metabolism of Cofactors and Vitamins	828549	865515	958616	904101
Metabolism of Other Amino Acids	305602	306390	317523	311206
Metabolism of Terpenoids and Polyketides	365909	346408	366867	356661
Nervous System	19655	21644	23151	22208
Neurodegenerative Diseases	19556	20765	22559	20011
Nucleotide Metabolism	895015	884927	933098	906858
Poorly Characterized	991891	1003806	1086506	1010866
Replication and Repair	1955028	1964034	2102088	2011869
Signal Transduction	349635	345258	395374	348842
Signaling Molecules and Interaction	36904	34792	33168	36968
Transcription	603448	577145	690833	606730
Translation	1287127	1284164	1347046	1304983
Transport and Catabolism	50388	61393	50300	54920
Xenobiotics Biodegradation and Metabolism	390005	348129	374961	353800

Figure 7. Pathway count heatmap of bacterial community at the KEGG-1 class level (A) and KEGG-2 class level (B) in rectum and caecum of Erhualian and Sushan pigs
EC – Erhualian pig caecum, ER – Erhualian pig rectum, SC – Sushan pig caecum, SR – Sushan pig rectum

of the digestive system were significantly higher than those in caecum of Erhualian and Sushan pigs. On the other hand, in rectum and caecum of Erhualian pigs relatively more microbial communities were involved in cell growth, cell death and the endocrine system than those in Sushan pigs (Figure 7B).

Discussion

The main function of intestinal microorganisms is to help animals digest and utilize nutrients in the diet, assist host metabolism, make nutrients better used by animals, provide nutrition for intestinal epithelial cells, strengthen their immune and disease resistance functions, and help the host resist the invasion of harmful pathogens (Kim et al., 2011; Thaiss et al., 2016). The main factors affecting the changes of intestinal microflora are the host itself, dietary factors and the interaction of microflora. Among them, the host itself is the most direct and important factor.

There are great differences in the structure of intestinal microflora and the abundance of microflora among different breeds of pigs. Some studies have shown that the intestinal microflora of pigs is affected by the genetic background, and there are differences in the intestinal microflora of different breeds of pigs. Yang et al. (2014) found that the number of total bacteria, Firmicutes and Bacteroidetes in the faeces of Chinese local pig breeds (Bama mini, Meishan and Erhualian pigs), was significantly higher than that of Duroc pigs. Pig breeds had a significant effect on the structure of intestinal microflora of adult sows. Bama mini pigs had the most abundant intestinal microflora. There was a significant difference between Chinese local pig breeds and Duroc pig breeds. Xiao et al. (2018) found that the microbial diversity in caecum and colon was higher than that in duodenum, jejunum and ileum in Jinhua and Landrace pigs. In this study, the diversity of intestinal microflora in two pig breeds was similar, but the abundance of specific intestinal microflora was different. The relative abundance of rectal microflora was higher than that of caecum, and there were differences between the two breeds. The abundance of rectal and caecum microflora in Sushan pigs was higher than that of Erhualian pigs, but the rectal microflora polymorphism in Sushan pigs was lower than that in Erhualian pigs. The caecum microflora diversity in Sushan pigs was higher than that in Erhualian pigs. The above results indicated that the abundance and diversity of microorganisms in the pig intestine were highly correlated with breeds and specific intestinal segments.

At the phylum level, it was found that the dominant bacteria in caecum and rectum of Erhualian and Sushan pigs were Firmicutes, Acidobacteria and Bacteroides, but the expression abundance of different bacteria was different. Firmicutes and Bacteroidetes in Erhualian pigs were higher than those in Sushan pigs. Some studies have found that the abundance of Firmicutes in Jinhua pigs was higher than that in Duroc, Yorkshire and Landrace pigs, but the abundance of Bacteroidetes was lower (Pajarilla et al., 2014; Yang et al., 2018). Bacteroides, Firmicutes, Spirochaetae and Proteus were the dominant flora in the faeces of Tibetan, Rongchang and Yorkshire pigs. The abundance of Firmicutes in Tibetan and Rongchang pigs was higher than that in Yorkshire pigs, but the abundance of Bacteroides was lower (Diao et al., 2016). Firmicutes and Bacteroidetes are associated with crude fibre digestion and carbohydrate degradation. The ratio of Firmicutes/Bacteroidetes can reflect the host's lipid metabolism, and a higher ratio will cause obesity and other complications (Kim and Isaacson, 2015; Mathur and Barlow, 2015). This may be because Erhualian, Tibetan and Rongchang pigs are obese ones that have a strong ability to deposit fat and digest crude fibre.

At the genus level, it was found that the abundance of *Christensenellaceae*, *Ruminococcaceae*, *Alloprevotella*, *Phascolarctobacterium*, *Treponema*, *Bacteroides* in Erhualian pigs was significantly higher than that in Sushan pigs, and the abundance of *Rikenellaceae*, *Streptococcus*, *Prevotellaceaein* in Sushan pigs was significantly higher than that in Erhualian pigs. The results of this study are different from previous ones. Guo et al. (2008) found that the abundance of *Bacteroides* in the obese pig is less than that in lean pig, and the increase of *Bacteroides* has a negative impact on body weight (Simpson et al., 1999; Dowarah et al., 2017). This may be due to the fact that Sushan pig is a new hybrid breed of Erhualian and Yorkshire pigs, and belongs to the obese pig. The abundance of *Streptococcus* in rectum of Sushan pig was significantly higher than that in Erhualian pig. In this context, our results are not different to previous ones. Xiao et al. (2018) also found that the abundance of *Streptococcus* in jejunum, ileum and colon of Jinhua pigs was higher than that in Landrace pigs, indicating that *Streptococcus* has various specificity. *Streptococcus* is related to inflammation and diseases. Whether disease resistance of different pig breeds is related to the abundance of *Streptococcus* in the intestine remains to be studied.

Christensenellaceae, *Ruminocaceae*, *Alloprevotella* and *Phascolarctobacterium* are all closely related to fat deposition (Bian et al., 2016; Yang et al., 2018). Erhualian pig is a typical Chinese local obese pig with high-fat deposition ability. Whether these bacteria affect fat deposition in Erhualian pigs needs further study. The results of this study further confirmed the existence of specific microbial community diversity and abundance in different breeds of pigs. The microbial community diversity and abundance in the pig intestine were closely related to fat deposition and nutrient absorption of pigs.

Conclusions

In summary, comparisons between different intestinal segments of the two pig breeds showed distinct structural compositions and predicted functions of microbial communities. *Christensenellaceae* R-7, *Ruminococcaceae* UCG-005, *Alloprevotella* and *Phascolarctobacterium* were dominant in caecum of Erhualian pigs, which were associated with fat deposition and crude fibre digestion in pigs. These results may indicate that Erhualian pigs might have stronger fat deposition and crude fibre tolerance than Sushan pigs. *Streptococcus* number in the intestine of Sushan pigs was significantly higher than that in Erhualian pigs. Whether the stronger disease resistance of Sushan pig is related to the abundance of *Streptococcus* in the intestine needs further study.

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Conflict of interest

The authors declare that there is no conflict of interest.

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