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ORIGINAL ARTICLE

The significance of the diversity and composition of the cecal microbiota of the Tibetan swine

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Abstract

The Tibetan swine (TIS) is a non-ruminant herbivore with high disease resistance. Also, it has the ability to digest plants with high fiber content. However, it is not known whether any relationship exist between these characteristics of the TIS and its cecal microbiota. Thus, this study aims to investigate the cecal microbiota of the adult TIS using high-throughput sequencing techniques in order to explore possible relationships between these unique characteristics of the TIS (high disease resistance and ability to digest high fiber plants) and its cecal microbiota. PIC pigs (lean type) were chosen as controls. The results show that 75,069 valid sequences of the 16S rRNA gene at V4-V5 region were obtained in the cecal content of TIS. They were composed of 15 phyla, 70 genera and divided into 660 Operational Taxonomic Units (OTUs). *Bacteroidetes* and *Firmicutes* were the predominant phyla in both breeds, but TIS had more *Bacteroidetes* than *Firmicutes*. Also, 42.4% of the cecal bacteria were found to be unclassified and uncultured. Many cellulolytic bacteria were also found in the two breeds. TIS (88.10%) had much higher abundance in the core bacterial communities than PIC pigs (81.29%), and the proportion of *Bacteroides* and *Spirochaetes* that can effectively degrade cellulose were 6.01 and 6.40% higher than PIC pigs, respectively, while *Proteobacteria* that are closely related to gastrointestinal diseases were 1.61% lower than PIC pigs. Thus, the disease resistance of the TIS and its ability to digest plants with high fiber content may be related to high abundance of core bacterial communities as well as the large number of unknown and unclassified bacteria.

Keywords Cecal microbiota · 16S rRNA gene · High-throughput sequencing · Tibetan swine · Disease resistance · Herbivorous characteristics

Highlights
- Comparing the cecal microbiota of a Tibetan and PIC (lean-type) pig.
- The core bacterial communities were different in these two breeds.
- Tibetan swine had a higher proportion of digestion related bacteria phyla.

Weiping Yang and Haiyun Xin are joint first authors.

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Introduction

The microbial communities in the gastroenteric tracts of humans and animals greatly influence their digestion, metabolism, and disease resistance (Turnbaugh et al. 2009). *Firmicutes* and *Bacteroidetes* are the two dominant phyla in the gut bacterial communities of many ruminant and non-ruminant animals. Changes in their composition and ratio greatly affect digestion, metabolism, and substance absorption of animals (Turnbaugh et al. 2009; Li et al. 2012; Looft et al. 2012). Moreover, changes in the proportion of *Lactobacilli* and *Enterobacteria* could be used as a gut health indicator for animals (Castillo et al. 2007). The diversity and composition of the gut microbial communities of animals are related to several factors, including animal species (Pajarillo et al. 2014), age (Castillo et al. 2007), environment (Wu et al. 2012), diet type (Yan et al. 2013; Chen et al. 2014), dietary fiber content (Castillo et al. 2007; Liu et al. 2012; Zhang et al. 2016), as well as antibiotics content in the diet (Looft et al. 2012, 2014).

The Tibetan swine (TIS) is a Qinghai (China) native, plateau-dwelling herbivore. Ninety percent of its nutrients can be obtained from forage grasses. The TIS has a large intestine like other non-ruminant monogastric animals, and its structure and function are at an intermediate stage between those of carnivorous and herbivorous animals. Most undigested feed components and endogenous secretions are fermented by microorganisms in the large intestine to provide the necessary nutrients for the animal (Wenk 2001).

About 10^{11}–10^{12} microbial cells live in each gram of cecal content of pigs, comprising of 400 to 500 different types (Castillo et al. 2007). However, more than 80% of the bacterial species are not yet identified (Leser et al. 2002). At present, studies on TIS microbiota mainly focus on the isolation and identification of bacteria that can degrade cellulose (Meng et al. 2014; Yang et al. 2014; Ma et al. 2015) or secrete antibacterial peptide (Xin et al. 2017). Xiao et al. (2017) found that the immunologic characteristics can be transferred by gut microbiota, suggesting the vital role of microbiota in immune phenotype programming, and making us suppose the relationship of host microbiota with disease resistance and other characteristics. Therefore, we investigated the diversity and composition of the bacterial community in the cecum of the TIS using Miseq high-throughput sequencing analysis in order to explore possible relationships between the bacterial community of the TIS and its unique characteristics.

Materials and methods

Collection of cecal content samples

Five healthy male adult TIS (8 months) and five healthy male adult PIC (5 months) pigs were obtained from two different farms. All the five pigs of each breed were reared under the same standard feeding and management conditions. The diet of the TIS consisted of 90% of green fodder and 10% of soybeans and wheat bran, while the diet of PIC pigs consisted of compound feed. The pigs had no history of gut infectious disease, and no antimicrobial administration occurred during the feeding process. They were fed an antibiotic-free diet. The cecal content samples from the TIS and PIC pigs were collected from the Tibetan swine Slaughterhouse of Shaanxi Huayi Industrial Co., Ltd. and the Shaanxi Benxiang Pig Slaughterhouse, respectively. After the pigs were killed with sodium pentobarbital (50 mg/1 kg BW), the cecum was ligated at both ends (~ 100 g/sample) and was immediately removed from the peritoneal cavity, placed into aseptic ziplock baggies, and stored in foamed plastic containers filled with dry ice. All samples were transported to the laboratory within 30 min for microbial genomic DNA extraction. The DNA was kept frozen at − 80 °C until it was needed.

The experimental design and procedures were approved by the Animal Care and Use Committee of Northwest A&F University. The cecal samples were collected with the permission of Hongzhou Wang and Junfang Yan, the director of Tibetan Pig Slaughterhouse of Shaanxi Huayi Industrial Co., Ltd. and Shaanxi Benxiang Pig Slaughterhouse, respectively. The study did not involve endangered or protected species.

DNA extraction and PCR amplification

After mixing the cecal contents of each pig, the genomic DNA was extracted from 200 mg of samples using the E.Z.N.A.® Stool DNA Kit (OMEGA, USA) according to the manufacturer's protocols and the concentration was measured using a NanoDrop Spectrophotometer ND1000 (Thermo Scientific, USA). The V4-V5 region of the bacterial 16S rRNA gene was amplified by PCR using primers 515F 5′-barcode-GTGCCAGCMGCCGCGG)-3′ and 907R 5′-CCGT CAATTCMTTTRAGTTT-3′ (Sun et al. 2013; Pitta et al. 2014). The PCR procedure was as follows: initial denaturation at 95 °C for 2 min; 30 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, and a final extension at 72 °C for 5 min where the barcode is an eight-base sequence unique to each sample. PCR reactions were performed in triplicate involving 20 μL mixtures containing 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.

Illumina MiSeq sequencing

Amplicons were extracted from 2% agarose gels and purified using the AxyPrep DNA Gel Extraction Kit (TaKaRa) according to the manufacturer's instructions. They were quantified using QuantiFluor™ -ST (Promega, USA). The purified amplicons were pooled in equimolar concentrations and

paired-end sequenced (2 × 250) on an Illumina MiSeq platform according to the standard protocol.

Processing and analysis of sequencing data

Raw fastq files were demultiplexed and quality-filtered using QIIME (Caporaso et al. 2010) (version 1.17) in accordance with the following criteria: (i) 250 bp reads were truncated at any site receiving an average quality score of < 20 over a 10-bp sliding window, and the truncated reads below 50 bp were discarded. (ii) The exact barcode matching, two nucleotide mismatch in primer matching, and reads containing ambiguous characters were removed. (iii) Only sequences with an overlap of more than 10 bp were assembled according to their overlap sequence. Reads that could not be assembled were discarded.

OTUs were clustered at 97% similarity cutoff using UPARSE (Edgar 2013) (version 7.1, http://drive5.com/uparse/), and chimeric sequences were identified and removed using UCHIME (Edgar et al. 2011) (version 4.2.40, http://drive5.com/usearch/manual/uchime_algo.html). The phylogenetic affiliation of each 16S rRNA gene sequence was analyzed by RDP Classifier (http://rdp.cme.msu.edu/) and compared with the Silva (Release115 http://www.arb-silva.de) 16S rRNA database using a confidence threshold of 70% (Wang et al. 2007) to determine the bacterial community composition of each sample at the different levels. OTUs at a 97% similarity level were used for alpha diversity (Shannon, Simpson), richness (ACE and Chao1), Venn diagram, rarefaction curve, and Shannon curve analyses using the Mothur program (http://www.mothur.org).

Statistical analysis

According to the relative abundance values of the bacterial community, metastats (http://metastats.cbcb. umd.edu/) was used to assess the differences between the two groups at the different taxonomic levels. Alpha levels below 0.05 were considered significant. All data were shown as mean ± SD.

Results

Community richness and diversity

The raw reads of Miseq high-throughput sequencing were deposited into the NCBI Sequence Read Archive (SRA) database (accession numbers: SRP058661).

Up to 169,766 valid sequences at V4-V5 region of 16S rRNA gene were obtained from the 10 samples using Illumina Miseq high-throughput sequencing analysis. The average length of these valid sequences was 393.8 bp. OTUs were analyzed at a 97% similarity level using UPARSE; chimeric sequences were removed using UCHIME; 75,069 and 74,759 sequences were left for further analysis of the TIS and PIC pigs, respectively. Further analysis identified 660 and 668 OTUs from the TIS and PIC pigs, respectively. The total number of optimized reads, OTUs, statistical species richness, and diversity estimations for each sample are presented in Table 1.

OTUs and microbial diversity indices were used to construct rarefaction and Shannon curves to compare the microbial community richness and diversity of the different samples at different sequencing depths by means of the Mothur software. The rarefaction curves (Fig. 1) and the community richness indices (chao1 and ace) (Table 1) indicate that the community richness of the TIS was higher than that of PIC pigs, slightly. However, the Shannon curves (Fig. 2) and indices of community diversity (Shannon and Simpson) (Table 1) indicate that the two breeds of pig were similar with respect to their microbial community diversities. All the Shannon curves tended to reach a plateau shape when the sequencing depth was 10,000 reads for each sample (Fig. 2). Therefore, the sequencing results could completely reflect the microbial community richness and diversity of the samples and can be used for the following analysis.

Taxonomic composition of bacterial communities

All the sequences were classified by the RDP classifier. The cecal bacteria were divided into 15 different phyla in both breeds. In the TIS, almost 98.42% of the bacteria accounted for more than 1% of the total cecal bacterial sequences (*Bacteroidetes*, *Firmicutes*, *Spirochaetae*, and *Tenericutes*), while the corresponding proportion was 98.98% in the PIC pigs (*Bacteroidetes*, *Firmicutes*, *Proteobacteria*, *Spirochaetae*, *Fusobacteria*, *Planctomycetes*, and unclassified). *Bacteroidetes* and *Firmicutes* were the most abundant bacteria in both breeds, but *Bacteroidetes* was more abundant than *Firmicutes*. However, the TIS had a higher *Bacteroidetes* abundance (59.79%) and a lower *Firmicutes* abundance (28.90%) compared with the PIC pigs (Fig. 3, S1 Table).

Statistical significance test showed that *Verrucomicrobia* and *Fusobacteria* were the unique bacterial taxa for the TIS and PIC pigs, respectively; the TIS had a significantly higher abundance of *Spirochaetae* ($p < 0.01$) and lower *Fibrobacteres* as well as *Proteobacteria* ($p < 0.01$) than the PIC pigs. The other bacteria phyla did not show any significant difference between the two breeds (S1 Table).

All the cecal bacteria could be divided into 70 different genera in the TIS, among which 14 genera accounted for more than 1% of the total cecal bacterial sequences and a total proportion of 92.32% of all the bacteria, which mainly included the uncultured bacteria (27.06%), unclassified bacteria (15.30%), *S24-7_norank* (11.89%), *RF16_norank* (7.28%), *Prevotella* (6.48%), *Spirochaeta* (5.31%), *RC9_gut_group*

Table 1 Numbers, abundance, and diversities of OTUs in the cecal microbiota of Tibetan pigs and PIC pigs. The identity value used for all the analyses in this study were 97%

Sample name	Optimize reads	OTUs	Chao1	ace	Shannon index	Simpson index
T1	10,425	418	515.68	485.34	4.66	0.02
T2	19,043	543	600.75	590.53	4.89	0.02
T3	12,026	440	542.10	503.08	4.44	0.03
T4	9342	462	528.94	535.11	4.68	0.02
T5	24,233	549	614.07	595.5	4.85	0.02
P1	22,923	423	498.00	473.35	4.19	0.04
P2	11,727	525	586.12	585.46	5.11	0.01
P3	11,048	412	473.39	464.8	4.80	0.02
P4	11,048	395	480.00	460.81	4.59	0.02
P5	18,013	583	686.06	659.85	5.16	0.01

Note: T1–T5 are the cecal samples from five Tibetan pigs; P1–P5 are the cecal samples from five PIC pigs, respectively

(3.68%), *Bacteroides* (3.57%), *Clostridiales* of *the incertae sedis* (2.26%), *Parabacteroides* (2.26%), *Treponema* (2.25%), *Phascolarctobacterium* (1.86%), *Anaerovibrio* (1.83%), and *RF9_norank* (1.29%). Meanwhile, in the PIC pigs, 74 genera were identified, among which up to 88.88% of all the bacteria made up more than 1% of total cecal bacterial sequences, including the uncultured bacteria (28.67%), *Prevotella* (20.78%), *S24-7_norank* (9.60%), unclassified bacteria (8.24%), *Parabacteroides* (3.00%), *Phascolarctobacterium* (2.95%), *RC9 gut group* (2.73%), *Treponema* (1.80%), *Clostridiales* of *the incertae sedis* (1.77%), *Oscillospira* (1.58%), *Clostridium* (1.48%), *p-1088-a5_gut_group* (1.48%), *Anaerovibrio* (1.38%), *Roseburia* (1.29%), *Fusobacterium* (1.12%), and *Bacteroides* (1.07%) (Fig. 4, S2 Table).

Among all the identified genera in the two breeds, 24 genera showed significant or extremely significant differences between the two breeds ($p < 0.05$ or $p < 0.01$), while all the other genera displayed similar percentages. A number of cellulolytic bacteria were found in the cecum of both breeds, such as *Ruminococcus*, *Bacteroides*, *Prevotella*, *Clostridium*, *Butyricicoccus*, *Fibrobacter*, *Lachnospira*, *Anaerovibrio*, *Parabacteroides*, and *Pseudobutyrivibrio* (S2 Table).

Clustered heatmap analysis depending on the bacterial community profiles at the genus level revealed that the samples obtained from the TIS (T1–T5) were highly similar in bacterial community composition and were classified as a single group, while the samples from the PIC pigs were classified into two groups (Fig. 5). The heatmap rows not only reflect the relative abundance and

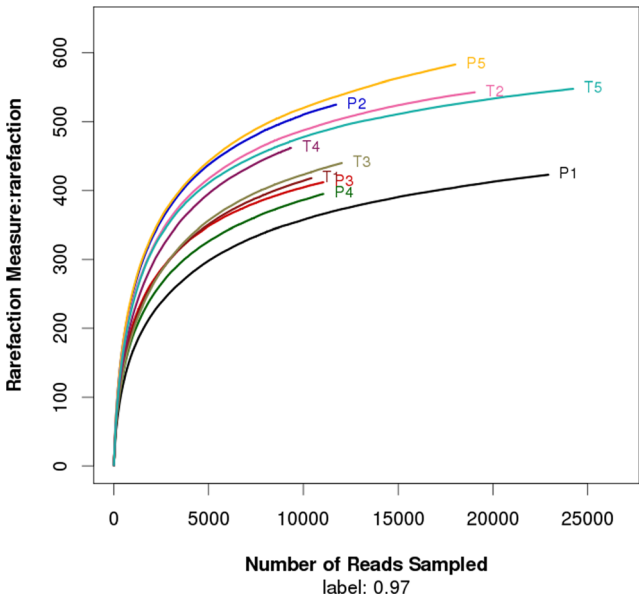

Fig. 1 Rarefaction curves of different samples. The abscissa represents the different sequencing depths and the ordinate represents the numbers of the OTUs

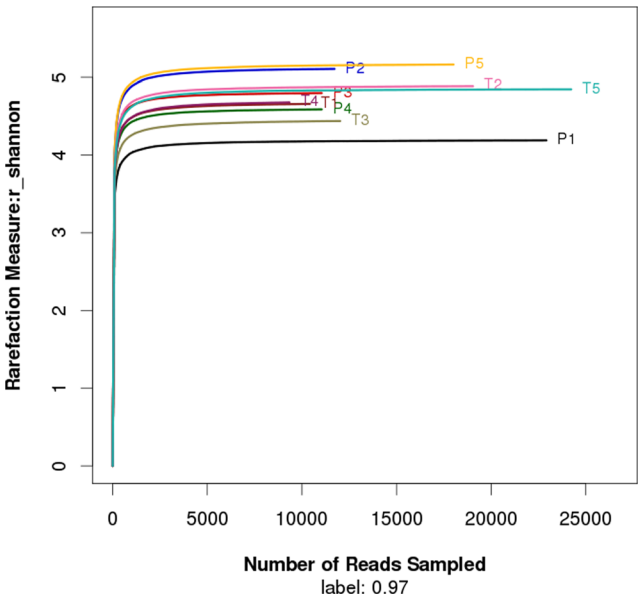

Fig. 2 Shannon-Wiener curves of the different samples. The abscissa represents the different sequencing depths and the ordinate represents the Shannon indices

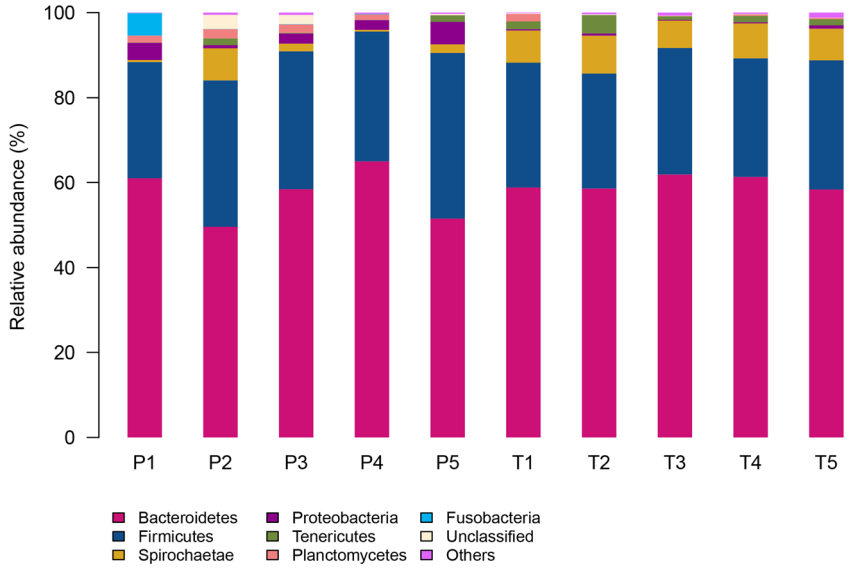

Fig. 3 Compositions of the bacterial communities at the phylum level. Relative abundance of bacterial groups (phylum level) in the cecum of the five Tibetan pigs and the five PIC pigs

clustering of OTUs in the different samples but also show the similarities and differences of the bacterial community compositions in the different samples. The red regions in the heatmap indicate bacterial communities with high relative abundance. Bacterial communities with high relative abundances in the different TIS included the unclassified bacteria, *S24-7_norank*, *RF16_norank*, *Spirochaeta*, *RC9_gut_group*, *Bacteroides*, *Incertae_Sedis*, *Treponema*, *Anaerovibrio*, and *RF9_norank*, while those in the different PIC pigs included the uncultured bacteria, *Prevotella*, *Parabacteroides*, *Phascolarctobacterium*, and *Ruminococcu* (Fig. 5).

Core bacterial communities

The presence of core bacterial communities was assayed further in both breeds. The result shows that 256 OTUs were shared among the different TIS (Fig. 6), and their sequences accounted for 88.1% of all the bacterial sequences. The shared reads made up 86.23, 86.12, 90.00, 89.87, and 88.29% in T1–T5 samples of the TIS, respectively (Fig. 6, Table 2). Moreover, the shared 256 OTUs were identified in seven phyla, and *Bacteroidetes*, *Firmicutes*, and *Spirochaetes* were the dominant phyla (Table 2). For all PIC pigs, 242 OTUs were shared, and

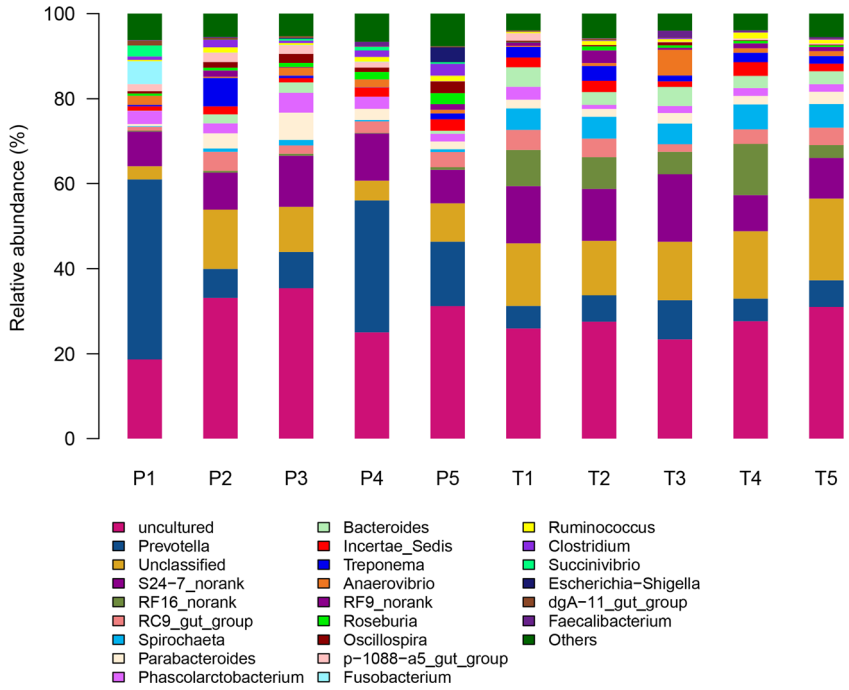

Fig. 4 Compositions of the bacterial communities at the genus level. Relative abundance of bacterial groups (by genus) in the cecum of the five Tibetan pigs and the five PIC pigs

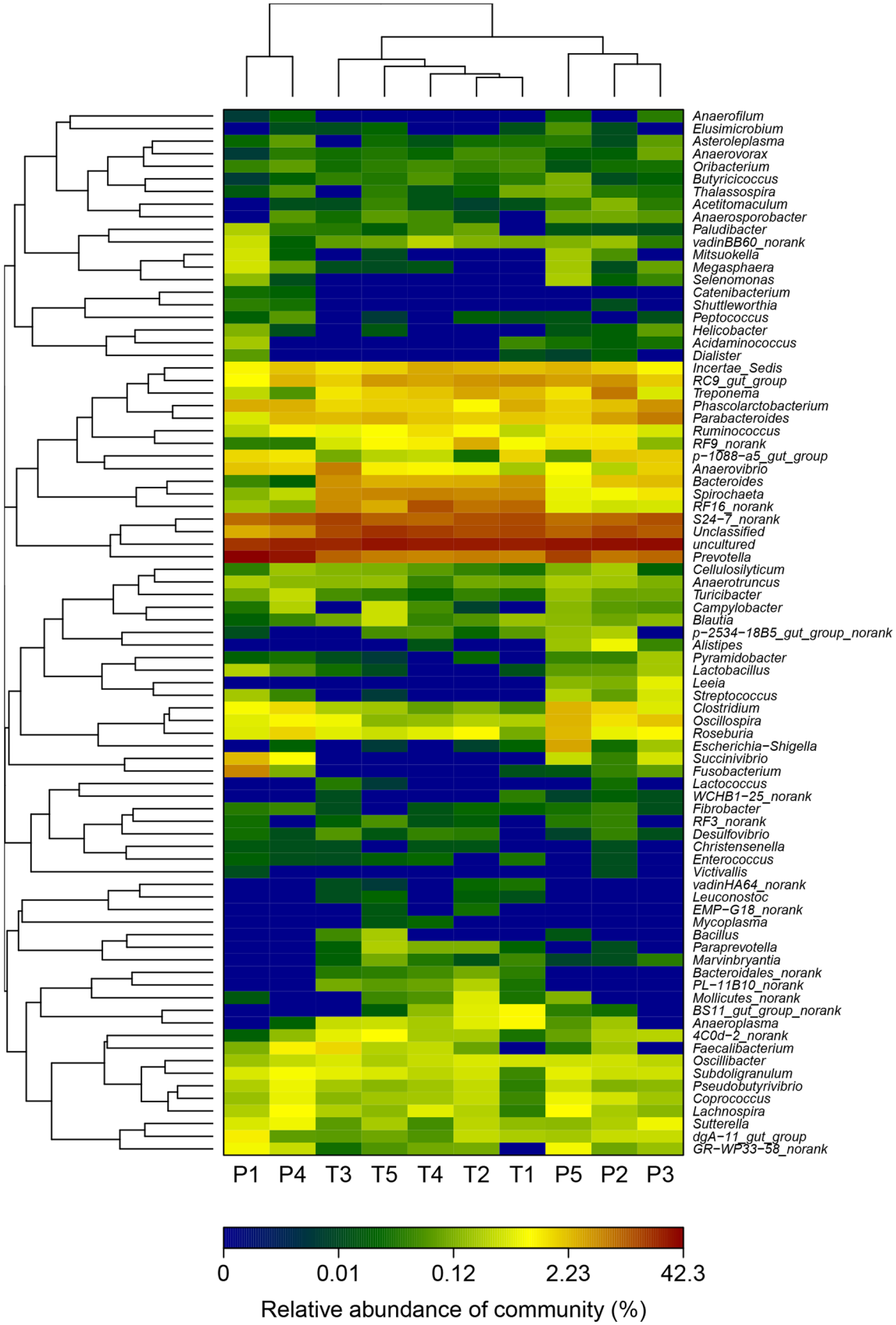

Fig. 5 Bacterial distributions of the gut bacteria communities by heatmap analysis. Bacterial distributions in the ten samples. Columns represent the different samples, while rows represent the OTUs

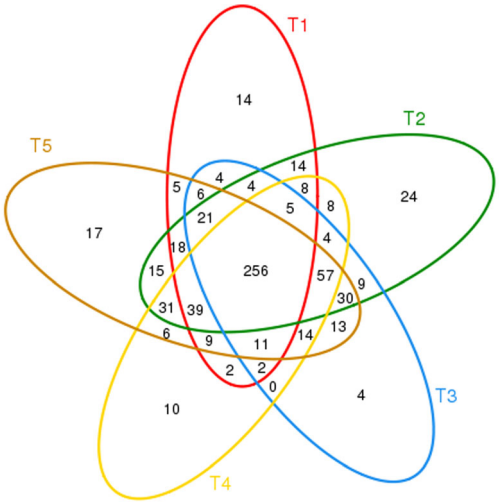

Fig. 6 Shared OTUs in the five TIS. Venn diagram shows the unique and shared OTUs in the different Tibetan pigs

their sequences accounted for 81.29% of all the bacterial sequences. The shared reads were different among the samples obtained from the PIC pigs (P1–P5) (Fig. 7, Table 3). The shared 242 OTUs involved six phyla, with *Bacteroidetes*, *Firmicutes*, *Planctomycetes*, and *Proteobacteria* as the dominant phyla (Table 3).

Firmicutes and *Bacteroidetes* were the core bacterial communities in both breeds, and *Bacteroidetes* made up a higher proportion than *Firmicutes* (Tables 2 and 3). *Firmicutes* contained some dominant families, including *Ruminococcaceae*, *Lachnospiraceae*, and *Erysipelotrichaceae*, while *Bacteroidetes* were mainly dominated by S24-7_norank, *Rikenellaceae*, and *Prevotellaceae* families. In other core bacterial communities, *Spirochaetae* was dominated by the *Treponema* and *Spirochaeta* genera in the TIS, while *Proteobacteria* in the PIC pig samples was dominated by *Campylobacter*, *Helicobacter*, *Succinivibrio*, GR-WP33-58, and *Sutterella* genera. The *Planctomycetaceae* family belongs to the *Planctomycetes* phylum.

Discussion

Diversity of bacterial communities in the cecum of the two breeds

Bacterial 16S rRNA genes contain nine "hypervariable regions" (V1–V9) that demonstrate considerable sequence diversities among different bacteria. Species-specific sequences within a given hypervariable region are useful targets of diagnostic assays and other scientific investigations, but a single region cannot tell all the bacteria (Chakravorty et al. 2007). A large number of scientific reports demonstrate that the combination of V4–V5 is the optimal region combination for diversity and evenness identifications of microbes (Sun et al. 2013; Pitta et al. 2014). Using the Miseq high-throughput sequencing, 660 and 668 OTUs were founded in the cecal bacteria of the TIS and PIC pigs, respectively. These values are lower than the number of OTUs reported in rhinoceros' rumen (Jami and Mizrahi 2012) and the gut of pigs (Kim et al. 2012) but higher than the number in the gut of pandas (Zhu et al. 2011). In addition, the percentage of OTUs (59.23%) presented in some of the TIS (less than 5) were lower compared with those of the PIC pigs (62.87%). The higher OTUs number in PIC pigs mainly because most OTUs were found in individuals, not in all the samples, which was also reported in a study performed on cattle (Jami and Mizrahi 2012). Thus, the TIS had more stable cecal bacterial communities.

Core bacterial communities in the cecum of the two pig breeds

In animals, the core cecal microbiota have a great effect on the normal gut functions (Turnbaugh et al. 2009). Our study found

Table 2 Core bacteria in the cecum of five Tibetan swines

Phylum	Shared OTUs	Reads of shares OTU					Reads of shared OTU/total reads (%)				
		T1	T2	T3	T4	T5	T1	T2	T3	T4	T5
Tenericutes	10	67	234	65	39	160	0.64	1.23	0.54	0.42	0.66
Spirochaetae	14	733	1436	749	737	1746	7.03	7.54	6.23	7.89	7.21
Proteobacteria	2	18	63	9	5	60	0.17	0.33	0.07	0.05	0.25
Planctomycetes	1	131	5	7	30	70	1.26	0.03	0.06	0.32	0.29
Firmicutes	138	2697	4047	3002	2244	6070	25.87	21.25	24.96	24.02	25.05
Cyanobacteria	1	1	3	7	2	13	0.01	0.02	0.06	0.02	0.05
Bacteroidetes	90	5342	10,612	6985	5339	13,276	51.24	55.73	58.08	57.15	54.78
Total shared sequences	256	8989	16,400	10,824	8396	21,395	86.23	86.12	90.00	89.87	88.29

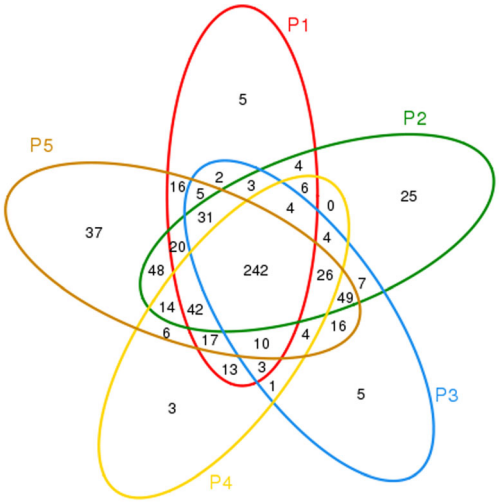

Fig. 7 Shared OTUs in the five PIC pigs. Venn diagram shows the unique and shared OTUs in the different individual of the PIC pigs

that the relative abundance of core bacterial communities was higher in the cecum of the TIS (88.10%) than in the PIC pigs (81.29%). Both *Bacteroidetes* and *Firmicutes* were the most abundant bacteria in both breeds, but the former was more abundant than the latter. Moreover, the distribution of these two dominant bacteria is contrary to other reports involving rhino (Bian et al. 2013), pig (Kim et al. 2012), and herbivorous rodents (Kohl et al. 2014) but consistent with the studies on the rumen of dairy animal (Jami and Mizrahi 2012; Li et al. 2012) and pigs (Looft et al. 2012). In contrast to the situation in TIS and PIC pigs, *Firmicutes* was more abundant than *Bacteroidetes* in the gut of obese mice and obese people. *Firmicutes* showed greater ability to obtain energy from the diet and get volatile fatty acids (SCFAs) during fermentation, thereby promoting the deposition of fat, while increased *Bacteroidetes* was significantly associated with weight-loss in humans (Turnbaugh et al. 2009). Looft et al. also reported that changes in *Firmicutes*/*Bacteroidetes* proportion, which may be affected by antibiotics additives (Looft et al. 2012), in the intestine is related to the improvement of diet utilization efficiency.

Proteobacteria are the most diverse in bacterial phyla. They are well-known for their clinical importance in human gastrointestinal disease diagnosis; they play a role in luminal dysbiosis and in the imbalance between pathogenic bacteria and functionally defensive commensal bacteria (Walujkar et al. 2014). In the PIC pig, *Proteobacteria* were dominant in the cecum and reached 2.96% of all the bacteria, significantly higher than the value in the TIS ($p < 0.01$). Also, lots of *Burkholderiales*, *Campylobacterales*, *Desulfuromonadales*, and *Aeromonadales* belonging to *Proteobacteria* were found to be the core microbiota in the PIC pig. The proportion of *Escherichia* bacteria was also very high, close to 0.75% in the PIC pig. Previous studies indicate that adding antibiotics (Looft et al. 2012; Looft et al. 2014) and soybean fiber (Chen et al. 2014) to pig diet induced an increase in *Proteobacteria* in the intestine, especially an increase in *Escherichia coli*.

Spirochaete, a phylum of bacteria capable of degrading polymers (xylan, pectin, arabinogalactan) and hemicellulose effectively, was found to be dominant among the core microbiota in the cecum of the TIS. *Treponema*, a genus of *Spirochaete* phylum, not only participated in cellulose degradation (Shinkai et al. 2010) but also degraded pectin in the plant cell wall to produce acetic acid, propionic acid, or other short-chain fatty acids to provide energy for the animals (Liu et al. 2014; Niu et al. 2015). Additionally, *Treponema* is a vital and beneficial genus in cattle rumen because of its capability to inhibit *Salmonella* and *Escherichia coli* (Edrington et al. 2012). This may be the reason why the TIS has a high disease resistance.

Bacterial community compositions in the cecum and grazing characteristics of the TIS

The bacterial community compositions in the cecum of the TIS and PIC pigs were highly similar, but their distribution and quantities differed significantly. Cellulolytic bacteria were

Table 3 Core bacteria in the cecum of five PIC pigs

Phylum	Shared OTUs	Reads of shares OTUs					Reads of shared OTUs/total reads (%)				
		P1	P2	P3	P4	P5	P1	P2	P3	P4	P5
Spirochaetae	5	64	105	157	42	168	0.28	0.90	1.42	0.38	0.93
Proteobacteria	7	909	59	151	231	262	3.97	0.50	1.37	2.09	1.45
Planctomycetes	1	378	255	219	141	12	1.65	2.17	1.98	1.28	0.07
Firmicutes	147	5201	3363	3203	2904	5818	22.69	28.68	28.99	26.29	32.30
Cyanobacteria	1	1	5	5	6	2	0.00	0.04	0.05	0.05	0.01
Bacteroidetes	81	13,357	4478	5091	6762	7779	58.27	38.19	46.08	61.21	43.19
Total shared sequences	242	19,910	8265	8826	10,086	14,041	86.86	70.48	79.89	91.29	77.95

detected in the cecum of the two breeds, including *Ruminococcus*, *Bacteroides*, *Prevotella*, *Clostridium*, *Butyricicoccus*, *Fibrobacter*, *Lachnospira*, *Anaerovibrio*, *Parabacteroides*, and *Pseudobutyrivibrio* (Zhu et al. 2011; Jami and Mizrahi 2012; Wu et al. 2012; Bian et al. 2013). The number of *Bacteroidales* was significantly higher in the TIS than in the PIC pigs ($p < 0.01$), but *Prevotella*, *Clostridium*, and *Fibrobacter* were significantly lower in the TIS than in the PIC pigs ($p < 0.05$); the other bacterial genera capable of degrading cellulose displayed no significant difference between the two breeds. At the genus level, the uncultured and unclassified bacteria accounted for 42.4% of all the bacteria in the TIS and 36.9% of the bacteria in the PIC pigs. Studies have shown that high fiber diets can promote gut development in pigs, including increasing the integrities of the small intestinal mucosa, the height of the intestinal villi, the number of beneficial gut microorganisms, and, finally, changing the intestinal mucosal digestive physiology of the pig (Wenk 2001; Chen et al. 2014). Thus, the advantageous characteristics of TIS are related not only to the breed properties but also to the unique intestinal microflora formation resulting from long-term dietary fiber intake (Edrington et al. 2012).

Conclusions

Our study analyzed the diversity and composition of cecal microbiota in the TIS and presents initial observations for further understanding of the microbial ecology of this intestinal habitat. Our results indicate that the high disease resistance of the TIS and its ability to digest high fiber plants may be related to its gut microbiota. Future studies should focus on the characterization of the community composition variations with the changes in temporal and spatial factors as well as dietary changes.

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Compliance with ethical standards

Conflict of interest None declare

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