

Bacterial community and composition in Jiang-shui and Suan-cai revealed by high-throughput sequencing of 16S rRNA

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ABSTRACTS

Fermented vegetables have a long history in many cultures. Jiang-shui and Suan-cai are two of the most well-known instances in North China. They are made by a process of natural lactic acid fermentation. However, they have the different characteristics, i.e. acidity, taste and flavor, which are influenced by the specific bacterial community. Therefore, we used high-throughput sequencing methods to identify the bacterial community structure of Jiang-shui and Suan-cai in this study. Subsequently, we figured out the bacterial differences of these two products using the statistical analysis. *Firmicutes* and *Proteobacteria* were the dominant phyla in both Jiang-shui and Suan-cai. However, *Lactobacillus* was the main genus in Jiang-shui samples, whereas both *Lactobacillus* and *Pediococcus* were the major genera in the Suan-cai samples. At the species level, *Lactobacillus amylolyticus*, *Lactobacillus fermentum* and *Lactobacillus pontis* were the major species in Jiang-shui samples, while *Pediococcus parvulus*, *Lactobacillus coryniformis*, *Lactobacillus pentosus* and *Lactobacillus parabrevis* were the dominant species in the Suan-cai samples. These results suggested that Jiang-shui and Suan-cai had their own unique bacterial community, leading to the specific characteristics. Furthermore, the bacterial communities of both fermented vegetables varied at different locations. This study revealed the flora present in the Jiang-shui and the Suan-cai, providing a deep insight of the microbial species of Chinese fermented vegetables and guidance for the production of the Jiang-shui and the Suan-cai.

1. Introduction

China has a long tradition of fermented vegetable-making. Jiang-shui (also called “serofluid dish”) and Suan-cai are two popular fermented vegetables in China. They distinguish from each other with the production processes and the consequent characteristics e.g. acidity, taste and flavor (Q.Q. Li et al., 2017; Wu et al., 2015). Jiang-shui is a saltless fermented vegetable, which has a history of over two thousand years in northwest China (Zhang et al., 2009). It can be made from various vegetables, e.g. celery, mustard and potherb (Q.Q. Li et al., 2017). In last decades, it has been proven that serofluid dish has many advantages to human health such as promoting digestion, decreasing the cholesterol level, and improving the intestinal function (Chen et al., 2016a, 2016b). On the other hand, Suan-cai is another well-known fermented vegetable in the northeast China and has been widely consumed for centuries (Zhou et al., 2018). Different from Jiang-shui, Suan-cai commonly uses Chinese cabbage as the major raw material. The anaerobic fermentation prevents the vegetables from rotting in

saline with low salt concentration (5–35%) (Yang et al., 2019). In the meantime, the fermentation process improves the taste and nutritional values of the product in winter (Zhou et al., 2018). Therefore, Suan-cai is quite popular in northeast China due to its unique flavor and texture (Wu et al., 2015).

The change in taste, texture and flavor of fermented vegetables highly relies on the bacteria growing during the fermentation process, because they contribute to the flavor formation, acidification of vegetables, and nitrite degradation (Q.Q. Li et al., 2017). Therefore, various measures have been employed to figure out the structure and composition of the microbial communities in these products. Previous studies used culture-dependent methods to identify the microorganisms in the fermented vegetables, indicating that *L. brevis*, *L. plantarum* and *L. parabuchneri* were the main lactic acid bacteria (LAB) (Jun et al., 2018). The results of denaturing gradient gel electrophoresis (DGGE) showed that the genera *Lactobacillus* and *Weissella* are the major bacterial communities in Jiang-shui fermentation (Jun et al., 2018). Additionally, high-throughput sequencing (HTS) technique revealed that

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the major bacterial communities were *Lactobacillus* and *Pediococcus* (Chen et al., 2016a) in serofluid dish. On the other hand, 14 bacterial species have been identified, which are related to the Suan-cai fermentation, including *L. acidophilus*, *L. fermentum*, *L. sp.*, *Leuconostoc sp.*, *L. minor*, *L. taiwanensis*, *L. gasseri*, *L. plantarum*, *Lysinibacillus sp.*, *L. jensenii*, *L. curvatus*, *Pseudomonas sp.* and *L. coryniformis* (Wu et al., 2015). These investigations provided many valuable information of the bacterial community of the Jiang-shui and Suan-cai. However, it still lacks a large picture that compares the overall differences of bacterial community between these two products. Additionally, the characteristics of these fermented vegetables e.g. taste and flavor, often vary remarkably from region to region. Although several studies have been reported in terms of the bacterial diversity of Jiang-shui and Suan-cai in China (Jun et al., 2018; Zhou et al., 2018), the systematic investigation in a large geographic scale has rarely been reported in China. A crucial reason is the lack of an appropriate measure for the reliable and efficient determination of the microbes in the fermented vegetables.

Over the past decades, culture-independent methods have been successfully employed to describe the microbial community in Chinese fermented vegetables. For example, the technique of denaturing gradient gel electrophoresis (DGGE) was widely used to study the microbial ecology of Suancai and Serofluid dish in China (Jun et al., 2018; Wu et al., 2015; Zhou et al., 2018). Real-time quantitative PCR (qPCR) is also used to detect both bacteria and yeast in Chinese Paocai (Xiong et al., 2018). Recently, HTS technique has been successfully applied as main technique to investigate the microbial diversity of fermented food in particularly, the microbial community of Chinese fermented vegetables (Cao et al., 2017; Q.Q. Li et al., 2017; Liu et al., 2018; Motato et al., 2017), because it can provide more detailed information and deeper insight of the microbial communities compared to other molecular methods (Cao et al., 2017; H.F. Li et al., 2017; Motato et al., 2017).

In this study, HTS technique was used to identify both the bacterial communities of 10 different Jiang-shui samples and 10 different Suan-cai samples originated from different areas in China. We systematically compared the differences of the bacterial communities between these two fermented vegetables products. The microbial compositions were determined by the high-throughput DNA sequencing of 16S rDNA gene (V3-V4). This work aimed to deepen the understanding of the microbial communities in Jiang-shui and Suan-cai, which is helpful to stabilize and enhance the characteristics of Chinese Jiang-shui and Suan-cai.

2. Materials and methods

2.1. Sample collection

Ten Jiang-shui samples were collected from different areas in Gansu and Shaanxi Provinces in northwest China, and Ten Suan-cai samples were collected from different areas in Liaoning Provinces, northeast China.

Traditionally, the manufacturing process of Jiang-shui is as follows: first, pakchoi, celery, flour, potherb mustard, radish sprouts and mountain rape were washed, quickly boiled and then placed into fermentation jars (Lü et al., 2014; Q.Q. Li et al., 2017); subsequently, the starter culture called Yinzi together with cold noodle soup were added into the jars for fermentation (Jun et al., 2018). After a spontaneous fermentation about 4–10 days (Q.Q. Li et al., 2017), the Jiang-shui could be cooked through various ways and is widely consumed in northwest China. Jiang-shui is usually fermented in summer in northwest China. On the other hand, the Suan-cai manufacturing process is as follows: Chinese cabbages were washed, cut in halves and put into the jar; afterwards, cool boiled water with 0.5–3.5% salt was added to the jar and submerged the vegetable materials; finally, the jar was pressed with stones and sealed with plastic to exclude air (Yang et al., 2019). Suan-cai is usually fermented in winter in Northeast China, and the fermentation time of Suan-cai was > 30 days (Wu et al., 2015).

Generally, Jiang-shui is a saltless product (Jun et al., 2018), while Suan-cai is a low salt food with low salt concentration (0.5%–3.5%) (Yang et al., 2019; Zhou et al., 2018). In addition, the fermentation time of Suan-cai is usually longer than that of Jiang-shui.

2.2. Physicochemical properties

The pH value and the titratable acidity (TA) of samples were measured using a pH meter as described in our previous study (Xiong et al., 2012). TA was calculated by titrating the brine with 0.1 NaOH to pH 8.2 ± 0.2 and the results were expressed as the concentration of lactic acid, g/L (Chuah et al., 2016). Salt concentration was measured with a salinity meter (SA287, Qingdao Tlead International Co., Ltd., China). The concentrations of sucrose, glucose, fructose, lactic acid and acetic acid were determined with HPLC as described in our previous study (Xiong et al., 2014).

2.3. DNA extraction, PCR amplification and pyrosequencing

Genomic DNA was extracted using the Rapid Bacterial Genomic DNA Isolation Kit (Sangon Biotech, Shanghai, China). The V3-V4 hypervariable region of the 16S rRNA gene was amplified with the following primers: 341 F: 5'-CCTAYGGGRBGCASCAG-3', 806R: 5'-GGACTACHVGGGTWTCTAAT-3 (H.F. Li et al., 2017). For the bacterial DNA amplification, the PCR was carried out with 30 µL reactions solution containing 10 ng template DNA, 6 µM primers, 15 µL 2 × Phusion Master Mix (New England BioLabs Inc., Ipswich, MA) and 2 µL H₂O. The PCR conditions included: i. an initial denaturation at 98 °C for 1 min, ii. 30 cycles of denaturation at 98 °C for 10 s, annealing at 50 °C for 30 s and extension at 72 °C for 30 s, iii. a final extension at 72 °C for 5 min. Subsequently, the PCR products were examined using agarose gel electrophoresis with 2% denaturing agarose gels. After agarose gel electrophoresis, the amplicons were extracted from 2% agarose gels and purified with GeneJET PCR Purification Kit (Thermo Scientific, U.S.) according to the manufacturer's instructions. The amplicon library was prepared with TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, USA), and quantified with QuantiFluor™-ST (Promega, U.S.). Finally, the library was sequenced by Novogene Co., Ltd. (Beijing, China) using the Illumina HiSeq 2500.

2.4. Data analysis

Raw 16S rRNA gene sequence reads were filtered by using the QIIME software to remove primers and barcodes, and then were merged using the FLASH software (Magoc and Salzberg, 2011). The Operational Taxonomic Units (OTUs) were based on a 97% sequence similarity (Q.Q. Li et al., 2017). Alpha-diversity (the rarefaction, Chao1 richness, Ace richness estimators, Simpson and Shannon diversity indices) was calculated with QIIME (Version 1.7.0) (Cao et al., 2017; Caporaso et al., 2010). Beta diversity analysis based on Unifrac was used to compare the difference of all samples (Lozupone et al., 2011). Based on the UniFrac distances, principal coordinates analysis (PCoA) was used to compare the differences between the sample types (Liu and Tong, 2017). Linear discriminant analysis (LDA) effect size (LEfSe) (Kozik et al., 2017) was performed to determine the differences of the microbiomes between the Jiang-shui and Suan-cai samples.

2.5. Statistical analysis

The Differences of physicochemical properties and alpha-diversity between groups were performed using *t*-test (implemented in Sps 22.0), while the *P* value < 0.05 was considered to be significant. All data are presented in mean ± S.D. The graphic illustrations were generated with the GraphPad Prism 7 (GraphPad Software).

Table 1
The physicochemical properties of Jiang-shui and Suan-cai.

Sample name	Sampling location	pH	TA (g/L)	Salinity (g/L)	Saccharides (mg/g)			Organic acids (mg/g)		
					Sucrose	Glucose	Fructose	Lactic	Acetic	Oxalic
Jiang-shui										
J1	Longnan, Gansu	3.60	5.60	2.60	0.19	0.03	0.02	6.91	5.06	0.33
J2	Anqing, Gansu	3.24	3.10	0.70	ND	ND	ND	3.00	0.60	0.03
J3	Ankang, Shaanxi	3.32	2.10	0.90	ND	ND	ND	2.49	1.08	0.08
J4	Gangu, Gangu	3.98	6.60	2.50	0.36	ND	0.40	7.34	1.83	3.19
J5	Lanzhou, Gansu	3.43	1.90	3.40	ND	ND	0.11	3.38	1.15	0.05
J6	Hanzhou, Gansu	3.99	6.40	1.60	0.11	0.01	2.24	8.39	2.72	4.61
J7	Qinzhou, Gansu	3.27	4.10	0.80	ND	0.06	0.38	4.29	2.58	0.30
J8	Hanzhong, Shaanxi	4.23	6.20	3.30	0.14	ND	0.01	7.38	4.11	0.62
J9	Xian, Shaanxi	4.00	8.70	2.50	0.57	1.04	1.45	9.83	3.70	0.85
J10	Pingliang, Gansu	3.69	4.80	2.90	0.18	0.04	0.02	2.65	3.99	0.05
Suan-cai										
S1	Huludao, Liaoning	3.86	8.50	15.60	ND	0.10	0.00	8.03	4.21	6.84
S2	Jinzhou, Liaoning	3.80	7.79	8.40	ND	ND	ND	8.00	3.45	5.26
S3	Panjin, Liaoning	5.08	3.12	5.90	ND	ND	ND	2.95	4.90	0.43
S4	Fuxin, Liaoning	3.49	13.58	22.70	ND	ND	ND	4.60	3.32	0.14
S5	Pengwu, Liaoning	3.74	5.28	14.50	ND	ND	ND	7.51	4.13	21.75
S6	Shenyang, Liaoning	6.50	1.04	20.40	ND	0.09	2.53	13.31	3.65	4.83
S7	Benxi, Liaoning	3.80	9.30	22.20	ND	0.07	1.77	12.21	3.47	3.48
S8	Liaoyang, Liaoning	4.35	3.99	10.90	ND	ND	ND	3.42	3.12	0.17
S9	Fencheng, Liaoning	4.64	2.11	4.10	ND	ND	0.07	3.43	7.93	0.49
S10	Dandong, Liaoning	5.09	3.89	13.30	ND	ND	0.10	3.70	2.10	0.82

Values are mean of duplicate measurements. ND: not detected.

3. Results

3.1. Physicochemical properties

As shown in Table 1, the pH values of the Jiang-shui samples and the Suan-cai samples ranged from 3.24 to 4.23 and 3.49 to 6.50, respectively. The TA of the Jiang-shui samples varied from 1.9 to 8.7 g/L, whereas the Suan-cai samples had a higher TA (1.04–13.58 g/L). The salt concentrations (0.8–3.4 g/L) in the Jiang-shui samples were lower than those in the Suan-cai samples (4.1–22.7 g/L). The concentrations of sucrose, glucose and fructose were low (≤ 2.53 g/L) in all Jiang-shui and Suan-cai samples. In total, the contents of lactic (2.49–9.83 g/L), acetic (0.60–5.06 g/L) and oxalic acid (0.03–4.61 g/L) in Jiang-shui samples were lower than those in Suan-cai samples. J6 and S5 were the samples with the highest oxalic acid content in Jiang-shui and Suan-cai, respectively. J6 (8.39 g/L) and J9 (9.83 g/L) were the samples with the highest lactic acid content in Jiang-shui, whereas the two samples with the highest lactic acid content in Suan-cai were S6 (13.31 g/L) and S7 (12.21 g/L). Meanwhile, compared with other samples, the contents of fructose in J6 (2.24 g/L), J9 (1.45 g/L), S6 (2.53 g/L) and S7 (1.77 g/L) were higher. The results showed that no significant differences in pH values and salinity between the Jiang-shui samples and the Suan-cai samples ($p < 0.05$), whereas no significant differences were found in TA, lactic, acetic and oxalic acid (Fig. 1).

3.2. Phylogenetic composition and alpha-diversity

85,246 per samples in average, i.e. 1,704,922 reads in total, were obtained from the 20 samples. The rarefaction analysis based on OTUs at 97% similarity for these samples (Fig. 2A) indicated that our sequencing depth met the requirements for sequencing and analysis. Additionally, the species accumulation curves indicated that our samples were sufficient for OTUs test and could predict the species richness of samples (Fig. 2B).

Table 2 showed the OTU numbers and the values of alpha-diversity. ACE and Chao1 showed bacterial richness. Shannon index and Simpson index represented the bacterial community diversity. The values of ACE, Chao1, Simpson and Shannon varied among these samples. The values of good coverage per sample was > 0.99 , indicating that the

information was sufficient to reveal most bacterial community of samples. The *t*-tests also showed the significant differences ($p < 0.05$) between the Jiang-shui and the Suan-cai samples in all alpha-diversity estimators (Fig. 1). The *alpha*-diversity of the bacterial compositions in the Jiang-shui samples was significantly lower than that of the Suan-cai samples (Fig. 1).

3.3. Bacterial community diversity

The 16S rRNA gene sequencing showed that the microbial communities of all samples covered 25 phyla, 60 classes, 112 orders, 220 families, 440 genera and 255 species.

At the phylum level (Fig. 3.A), *Firmicutes* was the most dominant phylum in both Jiang-shui and Suan-cai samples, except for one sample (S6), whose dominant phylum was *Proteobacteria*. The relative abundance of *Firmicutes* in the Jiang-shui (87.7–97.6%, with the mean average of 95.1%) was higher than that (23.5–87.7%, with the mean average of 67.5%) in the Suan-cai. On the contrary, the relative abundance of *Proteobacteria* in the Jiang-shui samples (1.2–10.2%, with the mean average of 2.6%) was lower than that (11.4–70.4%, with mean average of 23.0%) in Suan-cai samples.

At the family level (Fig. 3.B), 64.8% to 97.6% bacterial in the samples belonged to the top 10 families (*Lactobacillaceae*, *Planococcaceae*, *Enterobacteriaceae*, *Moraxellaceae*, *Xanthomonadaceae*, *Acetobacteraceae*, *Lachnospiraceae*, *Microbacteriaceae*, *Leuconostocaceae* and *Rhizobiaceae*). Overall, *Lactobacillaceae* was generally the most abundance family in all samples except for S6 and S10. *Enterobacteriaceae* was the most dominant phylum in S6, while *Planococcaceae* was the most dominant phylum in S10. The proportions of *Lactobacillaceae* (87.4–97.1%) in the most Jiang-shui samples were higher than those (16.7–87.2%) in the Suan-cai samples. However, the average relative abundances of other top four families in top ten, i.e. *Planococcaceae*, *Enterobacteriaceae*, *Moraxellaceae* and *Xanthomonadaceae* were 0.05%, 0.1%, 0.05% and 0.01% in the Jiang-shui samples were lower than those in the Suan-cai samples (7.3%, 10.0%, 2.3% and 4.0%).

At the genera level, *Lactobacillus* was the most abundant in all samples (14.8–96.5%) except for the samples of J6, S6 and S10. The other genera with high abundance, included *Pediococcus* (0.4–57.3%), *Sporosarcina* (0.02–48.3%), *Citrobacter* (0–44.3%), *Psychrobacillus*

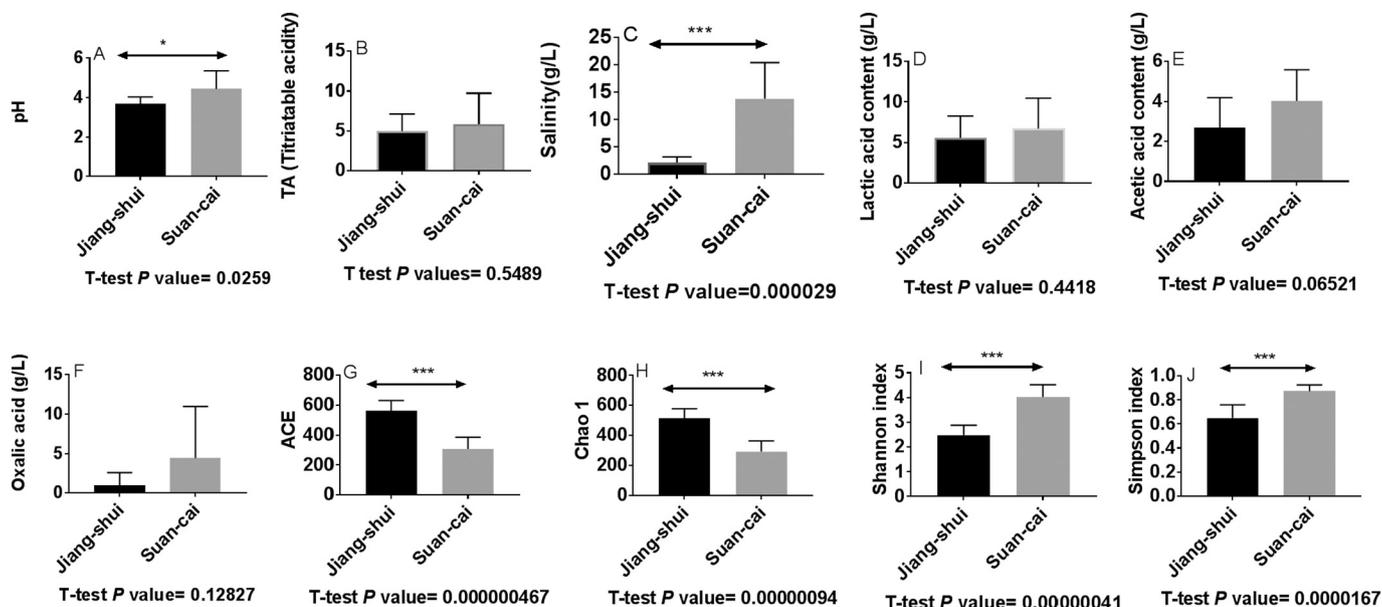


Fig. 1. Comparison of physicochemical properties and diversity indices between Jiang-shui and Suan-cai samples. (A) pH; (B) TA; (C) Salinity; (D) Lactic acid content; (E) Acetic acid content; (F) Oxalic acid; (G) ACE; (H) Chao 1; (I) Shannon index; (J) Simpson. *t*-tests were performed for each pairwise comparison. **P* < 0.05, ***P* < 0.01, ****P* < 0.001. *P* values refer to Multi performed between two groups.

(0–21.5%), *Acinetobacter* (0.05–16.03%), *Stenotrophomonas* (0–10.41%), *Acetobacter* (0–9.24%), *Serratia* (0.01–9.18%) and *Lachnoclostridium* (0–4.89%), respectively. We also observe a few exceptions, e.g. the samples of J6, S10 and S6. The dominant genera in these samples were *Pediococcus* (57.3%), *Acinetobacter* (50.1%) and *Sporosarcina* (44.3%), respectively, rather than *Lactobacillus*. In general, *Lactobacillus* was more abundant in the Jiang-shui samples than in the Suan-cai samples, whereas *Pediococcus* in the Jiang-shui samples was less than in Suan-cai samples.

The top ten species in all samples included *L. fermentum*, *L. amylolyticus*, *L. pontis*, *Pediococcus parvulus*, *L. coryniformis*, *L. pentosus*, *L. parabrevis*, *Acetobacter lovaniensis*, *Bradyrhizobium elkanii* and *L. reuteri* (Table 3). The relative abundance of *L. amylolyticus* in samples J2, J4 and J8 were much higher than that in other samples. The relative abundance of *Pediococcus parvulus* in sample J7 was the highest,

whereas the relative abundance of *L. coryniformis* and *L. pentosus* in sample S5 was the highest and the relative abundance of *L. parabrevis* in samples S1 was the highest. *L. amylolyticus*, *L. fermentum* and *L. pontis* had higher relative abundance than the other bacterial species in Jiang-shui samples, while the Suan-cai samples harbor a high relative abundance of *Pediococcus parvulus*, *L. coryniformis*, *L. pentosus* and *L. parabrevis*.

3.4. Comparison between Jiang-shui and Suan-cai samples

The PcoA (Fig. 4), based on the weighted and unweighted UniFrac distances, suggested an obvious separation of the bacterial communities between Jiang-shui and Suan-cai samples. Based on the weighted, the first (PC1) and second (PC2) axes showed values of cumulative percentage variance of species equal to 71.51% and 15.21%. In addition,

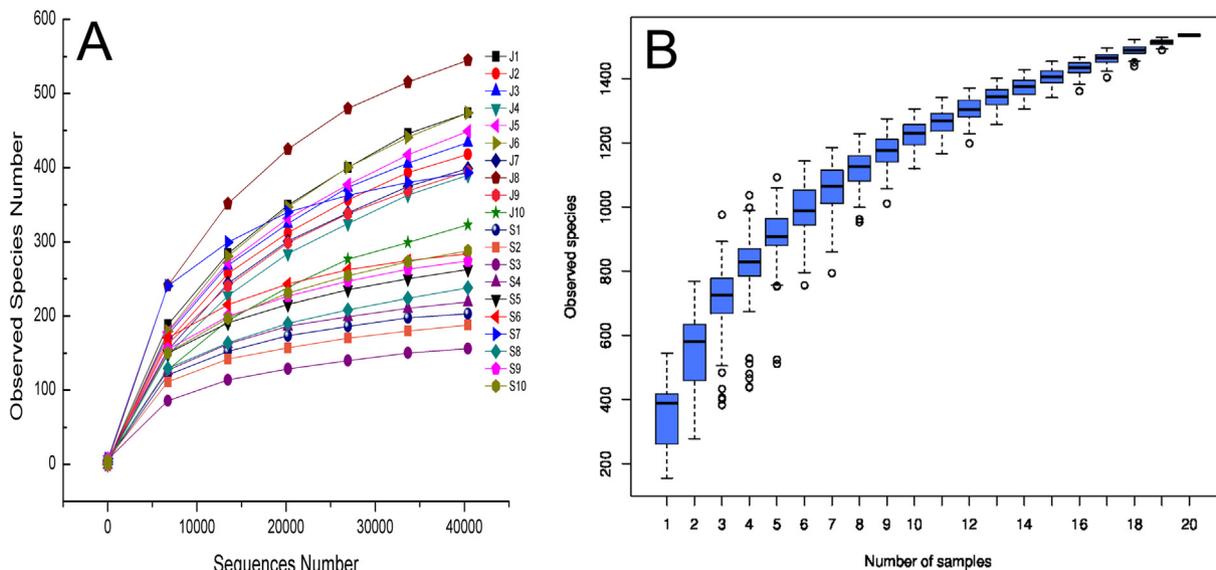


Fig. 2. Rarefaction curves and species accumulation curves. (A) Rarefaction curves based on V3-V4 of 16S rRNA gene. (B) Species accumulation curves.

Table 2
Total read number, alpha-diversity index and Good's coverage for bacteria per sample.

Samples	Total reads	Average length (bp)	Observed species	Ace	chao1	Shannon	Simpson	Good's coverage
Jiang-shui								
J1	89,321	430	474	630	579	1.84	0.442	0.996
J2	83,842	430	421	520	481	2.55	0.661	0.997
J3	99,542	430	448	567	511	2.89	0.765	0.997
J4	92,563	430	398	548	494	2.19	0.625	0.997
J5	87,263	430	449	601	542	1.94	0.498	0.997
J6	85,513	430	453	627	555	2.87	0.658	0.996
J7	82,125	430	408	530	483	2.58	0.737	0.997
J8	83,266	430	553	664	625	2.64	0.680	0.997
J9	85,348	430	390	501	463	2.28	0.654	0.997
J10	76,355	430	322	432	398	2.98	0.786	0.997
Suan-cai								
S1	92,007	430	215	240	230	3.51	0.826	0.999
S2	80,911	430	214	356	324	4.03	0.902	0.998
S3	77,933	430	153	181	176	3.65	0.892	0.999
S4	65,580	430	224	248	243	4.00	0.900	0.999
S5	77,286	430	253	302	292	4.27	0.880	0.999
S6	87,705	430	284	321	310	3.82	0.786	0.999
S7	84,097	430	399	469	445	4.92	0.929	0.998
S8	91,352	430	244	320	292	3.96	0.884	0.998
S9	90,696	430	278	303	289	4.70	0.938	0.999
S10	92,217	430	286	347	243	3.38	0.797	0.997

J means Jiang-shui; S means Suan-cai.

according to the unweighted UniFrac distances, PC1 and PC2 accounted for 32.95% and 15.66% of the variance in the microbiota of samples, respectively.

The microbial community heat map analysis and Cluster-tree (unweighted FastUniFrac) were used to compare the differences and similarity of the bacterial community structures among samples (Fig. 5). As shown in Fig. 5A, the proportion of *Pediococcus*, *Sporosarcina*, *Psychrobacillus*, *Psychrobacter*, *Halomonas*, *Marinomonas*, *Pseudoalteromonas*, *Weissella*, *Alcaligenes*, *Stenotrophomonas*, *Rhizobium*, *Lachnoclostridium*, *Thermus*, *Pseudomonas*, *Pseudochrobactrum*, *Pantoea*, *Ochrobactrum*, *Serratia*, *Providencia*, *Citrobacter*, *Leucobacter*, *Shewanella* and *Ochrobactrum* were more abundant in Suan-cai samples, whereas bacteria such as *Lactobacillus*, *Acetobacter*, *Nesterenkonia*, *Streptococcus* and *Leuconostoc* were more abundant in Jiang-shui samples. All samples were divided into 2 clusters (Fig. 5B). The samples J1, J2, J3, J4, J5, J6, J7, J8, J9 and J10 formed the first group, suggesting a similarity of the bacterial community among Jiang-shui samples; the remaining samples (S1, S2, S3, S4, S5, S6, S7, S8, S9 and S10) formed the second group, indicating a similar community structure among Suan-cai samples. These two groups also showed an obvious difference of microbial community between Jiang-shui and Suan-cai groups.

Linear Discriminant Analysis Effect Size (LEfSe) analysis was used to determine the significant differences between Jiang-shui and Suan-cai of the bacterial communities (Fig. 6A and B). The relative abundances of *L. parafarraginis*, *L. fermentum*, *L. amylolyticus*, *Firmicutes*, *Bacilli*, *Lactobacillales*, *Lactobacillaceae*, *Lactobacillus* and *L. delbrueckii* were significantly higher in Jiang-shui, while *Proteobacteria*, *Gammaproteobacteria*, *Enterobacteriales*, *Enterobacteriaceae*, *Citrobacter*, *L. coryniformis*, *L. pentosus*, *L. parabrevis*, *L. similis*, *Xanthomonadaceae*, *Stenotrophomonas*, *Xanthomonadales*, *Pediococcus*, *Pediococcus parvulus*, *Serratia*, *L. malefermentans*, *Rhizobiales*, *Pseudomonadales*, *Serratia marcescens* and *Moraxellaceae* were higher in Suan-cai.

4. Discussion

This study aimed to reveal the composition and diversity of bacterial community in the Jiang-shui and Suan-cai samples and compare their differences.

TA (Titratable acidity) and pH are two basic characteristics of fermented vegetables, which not only influence bacterial communities but also represent the quality of fermented vegetable, e.g. maturity or

spoilage in fermented vegetables (Lee et al., 2017; Zhang et al., 2016). Most of our Jiang-shui and Suan-cai samples had been already mature, because their pH values and TA were generally below 4 and above 0.3 g/100 g that corresponds the features of mature Chinese fermented vegetables (Zhang et al., 2016, 2018). A few samples, especially S6, with the abnormal high pH and low TA were probably spoiled contaminated during production, because the spoilage of fermented vegetables lead to the increase in pH value and the decrease in TA (Medina et al., 2016).

The salinity of Jiang-shui in this study was less 4 g/1000 g, consistent with previous study that Jiang-shui is salt-free fermented vegetables (Jun et al., 2018). Meanwhile, the salinity of our Suan-cai samples ranged 4.1–22.7 g/1000 g that meet the quality standard of low-salt fermented vegetables (salinity < 50 g/1000 g) (Zhang et al., 2016; Zhao and Ding, 2008).

Alpha-diversity represents the richness and the diversity of microbial community in fermented vegetables (Cao et al., 2017). Alpha-diversity indices were used to evaluate the community richness and bacterial biodiversity (Fig. 2). The results showed that the most Suan-cai samples harbor much higher bacterial diversity than that in the Jiang-shui samples.

In this work, we found that the two most abundant phyla in our samples were the *Firmicutes* and *Proteobacteria* in both Jiang-shui samples and Suan-cai samples. Meanwhile, *Lactobacillaceae* was found to be the most abundant family, consistent with the previous studies in fermented vegetables (Cao et al., 2017; Chen et al., 2016a).

Lactobacillus was the most dominant genus in the most samples, which was consistent with the results of previous studies on Chinese fermented vegetables (Cao et al., 2017; Wu et al., 2015). However, they were quite different from another famous Asian fermented vegetable, Korean kimchi, whose microbial communities are dominated by *Leuconostoc*, *Lactobacillus* and *Weissella* (Kim and Chun, 2005; Park et al., 2012). Actually, *Leuconostoc* and *Weissella* were not the main genera in our samples. Moreover, the relative abundance of *Lactobacillus* in the Jiang-shui samples was generally higher than in the Suan-cai samples. Meanwhile, compared to Jiang-shui samples, higher *Pediococcus* was discovered in Suan-cai samples. These differences in bacterial community may be caused by the production process, raw materials and geographical distribution (Jeong et al., 2013; Lee et al., 2017). It is notable that the microbial communities of S6 and S10 are significantly different from the other samples. We detected highly *Citrobacter* in

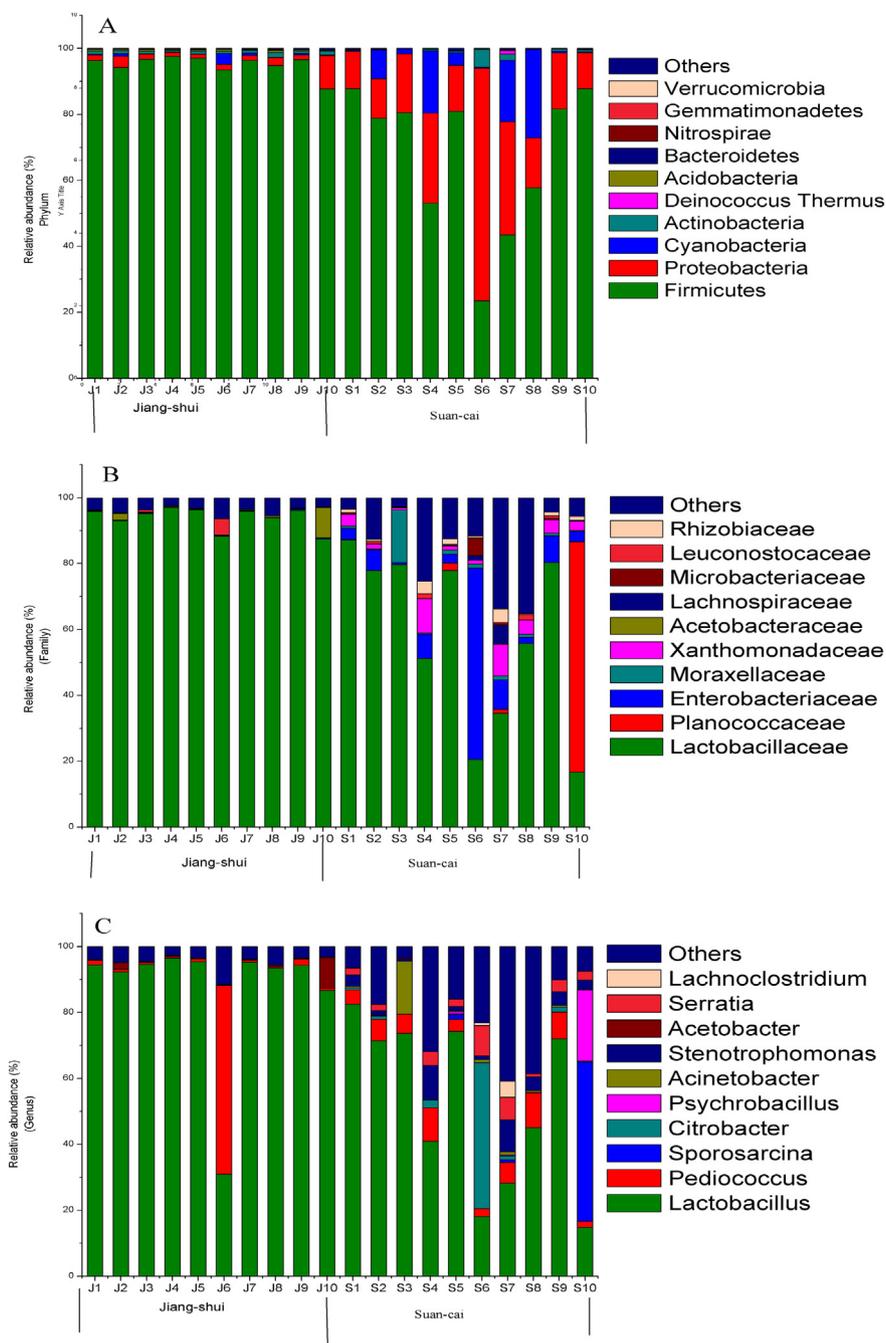


Fig. 3. Relative abundance of bacterial community compositions at Phylum (A), Family (B) or Genus (C) levels in Jiang-shui and Suan-cai Samples.

Sample S6 and Sporosarcina in S10 rather than Lactobacillus. That might explain the high pH value and low acidity of these two samples.

L. plantarum was the most common species of *Lactobacillus* in Chinese fermented vegetables (Wu et al., 2015; Xiong et al., 2012). However, it was not the major bacterial species in this study. Our results revealed that *L. fermentum* and *L. parabrevis* were the dominant species in Jiang-shui samples, whereas *L. parabrevis*, *L. coryniformis*, *L. pentosus* and *Pediococcus parvulus* were the major strains in Suan-cai samples, showing a significant difference in bacterial species between Jiang-shui and Suan-cai. In addition, another famous Chinese fermented vegetables, Sichuan paocai has the dominant species of *Lactobacillus acetotolerans* (Cao et al., 2017). These previous studies and our results indicated that the dominant species varied in different fermented vegetables.

In this study, we confirmed that the great diversity of lactic acid

bacteria (LAB) in Jiang-shui, which is also reported by the previous study (Li et al., 2014). However, our study suggests that analysis of microbial community may be impacted by the applied methods. For example, we found that *Lactobacillus* was the major genus in Jiang-shui samples, which was slightly different from previous reports that the dominant bacterial genera were *Lactobacillus* and *Weissella* by culture-independent denaturing gradient gel electrophoresis (DGGE) analysis (Jun et al., 2018) or *Lactobacillus* and *Pediococcus* by culture-independent HTS (Chen et al., 2016a) in Jiang-shui. Moreover, the highest level of species in our Jiang-shui samples was *L. fermentum* followed by *L. amylolyticus*, which some previous reports indicated that *L. brevis*, *L. plantarum* and *L. parabuchneri* were the major species in Jiang-shui samples (Jun et al., 2018). In addition to the variation in analytical methods, manufacturing processes, starter cultures, raw materials, sampling locations, etc. can also lead to the differences

Table 3
Abundance of the top ten species in fermented vegetables.

Samples	<i>L. fermentum</i>	<i>L. amylolyticus</i>	<i>L. pontis</i>	<i>Ped. parvulus</i>	<i>L. coryniformis</i>	<i>L. pentosus</i>	<i>L. parabrevis</i>	<i>Ace. lovaniensis</i>	<i>Bra. elkanii</i>	<i>L. reuteri</i>	Others
J1	10.1%	5.6%	2.5%	1.4%	0.4%	0.1%	0.1%	0.1%	0.1%	0.1%	79.4%
J2	20.2%	53.3%	0.4%	0.7%	2.4%	2.1%	0.2%	2.0%	0.1%	ND	18.5%
J3	28.3%	2.3%	0.3%	0.6%	16.1%	0.7%	0.1%	0.1%	0.1%	ND	51.4%
J4	5.3%	56.6%	12.7%	0.5%	0.5%	0.1%	0.1%	0.2%	ND	0.9%	23.0%
J5	16.3%	4.4%	0.3%	0.8%	0.6%	0.5%	0.1%	0.1%	0.1%	0.1%	76.6%
J6	3.4%	4.2%	0.6%	57.3%	5.1%	1.6%	8.3%	0.1%	0.1%	ND	19.3%
J7	33.1%	22.9%	0.3%	0.7%	1.9%	2.1%	0.2%	0.2%	0.1%	ND	38.6%
J8	8.6%	47.3%	2.2%	0.4%	0.3%	0.0%	0.1%	0.3%	0.1%	0.5%	40.2%
J9	36.9%	3.5%	0.3%	1.8%	5.1%	0.2%	0.2%	0.1%	ND	ND	51.9%
J10	6.3%	5.0%	0.6%	0.7%	0.5%	0.1%	0.1%	8.8%	ND	ND	77.8%
S1	ND	ND	ND	4.3%	3.7%	11.1%	37.4%	ND	ND	ND	43.5%
S2	0.1%	ND	ND	6.4%	3.3%	7.9%	25.4%	ND	ND	ND	57.0%
S3	0.0%	ND	ND	8.0%	11.8%	14.4%	11.1%	ND	ND	ND	54.7%
S4	0.3%	ND	ND	11.7%	2.9%	5.6%	10.9%	ND	ND	ND	68.6%
S5	0.1%	ND	ND	3.5%	27.0%	18.2%	7.9%	ND	ND	ND	43.3%
S6	ND	ND	ND	2.4%	2.4%	2.3%	3.3%	ND	ND	ND	89.6%
S7	0.2%	ND	ND	6.3%	10.9%	1.6%	2.2%	ND	ND	ND	79.1%
S8	ND	ND	ND	10.5%	6.7%	4.0%	12.7%	ND	ND	ND	66.0%
S9	0.8%	ND	ND	8.1%	13.1%	5.1%	16.1%	ND	ND	ND	56.9%
S10	0.2%	ND	ND	1.8%	2.3%	0.6%	3.9%	ND	ND	ND	91.2%

J means Jiang-shui; S means Suan-cai.

among the studies (Lee et al., 2017; Liu and Tong, 2017). Notably, the species of S6 and S10 significantly differed from the other samples. We detected highly relative abundance of *Citrobacter gillenii* in Sample S6 and *Psychrobacillus psychrodurans* in S10 rather than *Ped. parvulus*, *L. coryniformis*, *L. pentosus* and *L. parabrevis*, which might result in high pH value and low acidity in these two samples.

Suan-cai is very popular in Northeast China. In our study, we observed that *Lactobacillus* (*L. parabrevis*, *L. coryniformis* and *L. pentosus*) and *Pediococcus* (*Pediococcus parvulus*) were the major genera, which differs from previous reports that *Leuconostoc* (*Leuconostoc* sp.), *Bacillus* (*Lysinibacillus* sp), *Pseudomonas* (*Pseudomonas* sp.) and *Lactobacillus* (*L. minor*, *L. taiwanensis*, *L. acidophilus*, *L. fermentum*, *Lactobacillus* sp, *L. gasseri*, *L. plantarum*, *L. brevis*, *L. jensenii*, *L. curvatus* and *L. coryniformis*) were the most common genera by culture-dependent method and DGGE in Suan-cai (Wu et al., 2015) or that *Lactobacillus*, *Leuconostoc*, *Enterobacter*, *Accumulibacter*, *Thermotoga*, *Pseudomonas*, *Clostridium*, *Rahnella* and *Citrobacter* were predominant microorganisms by DGGE in Suan-cai (Zhou et al., 2018). The difference of bacterial composition profiling may be due to microbial detection methods, geographical environment, fermentation conditions, etc. (Jeong et al., 2013; Lee

et al., 2017; Park et al., 2018). Moreover, compared to other samples of Suan-cai, HTS could provide more comprehensive microbial information than culture-pendent method or DGGE (Jeong et al., 2013). To some extent, these results also indicate a wide variation in the bacterial diversity and community in Suan-cai.

The bacterial community of the two products is also significantly different from each other at the species level. The prevalent species in the Jiang-shui samples, e.g. *L. fermentum*, *L. amylolyticus*, *L. pontis*, *Acetobacter lovaniensis*, *Bradyrhizobium elkanii* and *L. reuteri* were very low in the Suan-cai samples. On the other hand, the Suan-cai samples had a high level of *Pediococcus parvulus*, *L. coryniformis*, *L. pentosus* and *L. parabrevis*, which were very low in the Jiang-shui samples. These results indicated that the bacterial community in Jiang-shui is distinct from that in Suan-cai.

As mentioned above, the TA, salt and organic acid (lactic, acetic and oxalic acid) concentration in the Suan-cai samples were generally higher than in the Jiang-shui samples. Previous studies have reported Jiang-shui is a saltless fermented vegetable (Jun et al., 2018) and Suan-cai is a salted fermented vegetable (Zhou et al., 2018), which was consistent with the results of the present work that the salt

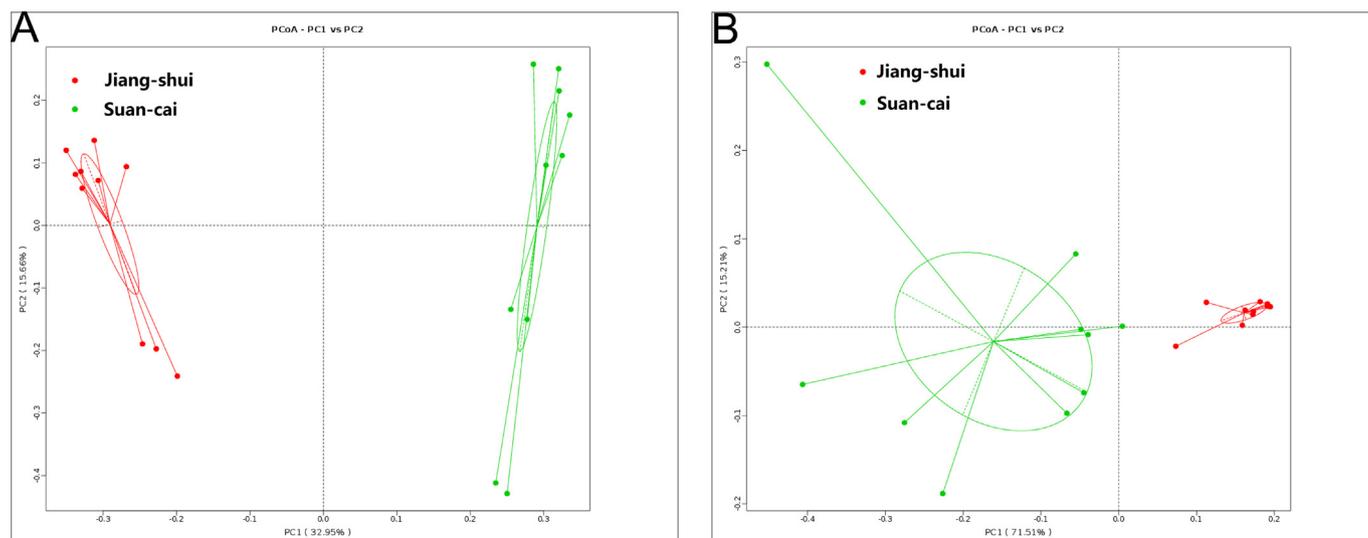


Fig. 4. UniFrac unweighted (A) and weighted (B) principal coordinate analysis (PCoA) scores plot based on principal components 1, 2.

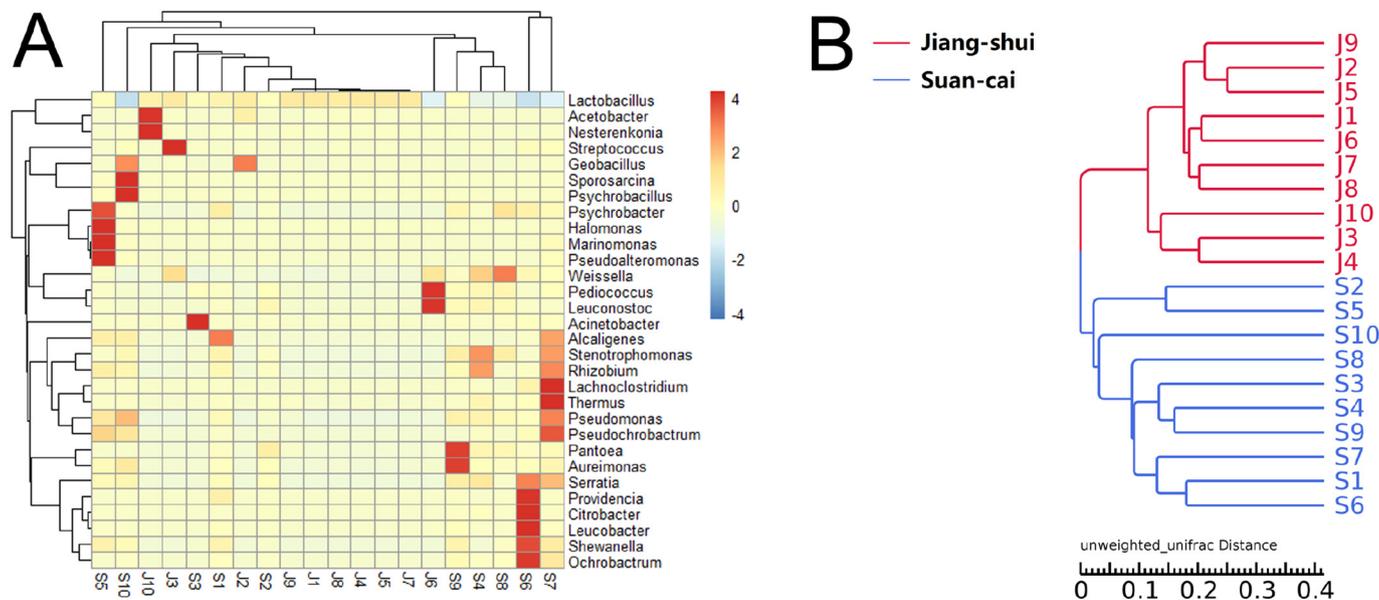


Fig. 5. Bacterial community heatmap analysis (A) and multiple samples similarity tree (B).

concentration in Suan-cai samples was much higher than in the Jiang-shui samples. In addition, we hypothesized that the increasing osmotic pressure caused by high salt concentration might lead to the oxalic acid leaking out from the plant cells. The differences in acid concentration are also influenced by the fermentation time (Park et al., 2018). The fermentation time of Jiang-shui often lasts 4–10 days (Q.Q. Li et al., 2017), while the production of Suan-cai is much longer, around 40 days (Wu et al., 2015; Zhou et al., 2018). Therefore, we hypothesized that the contents of lactic acid and acetic acid in Suan-cai were higher than

those in Jiang-shui due to the accumulation of lactic acid and acetic acid with a long period of time in Suan-cai samples, which caused the acid concentrations in the Jiang-shui differed from that in the Suan-cai. Some previous studies attempted to establish the correlation between the dominant species and the acid indices in fermented vegetables (Cao et al., 2017). Thus, the variation in acid concentrations may be another reason of the different microbial communities in the two fermented vegetables.

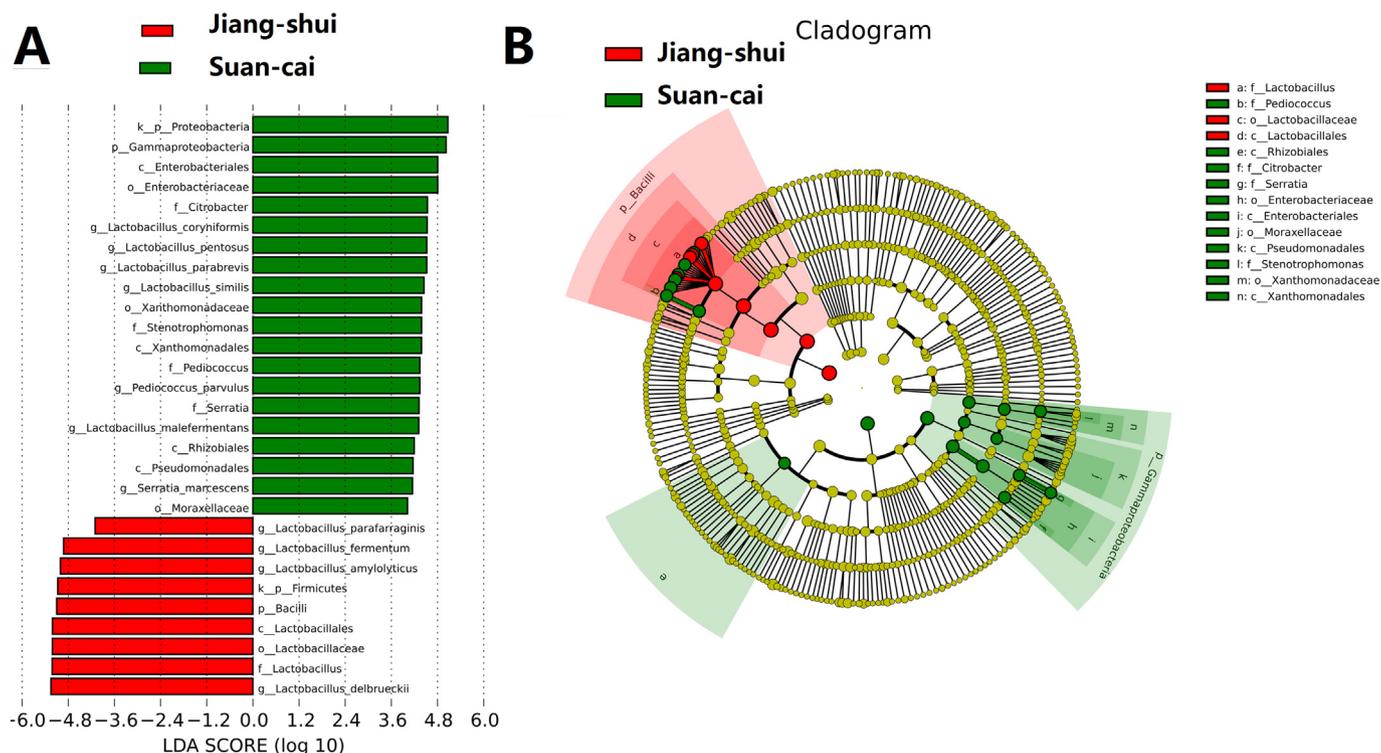


Fig. 6. LEfSe analyses of Jiang-shui and Suan-cai. Red: Jiang-shui; Green: Suan-cai. p -values < 0.05 considered significant. (A) Histogram of the microbiota of Jiang-shui and Suan-cai with a threshold value of 4 (Jiang-shui, $n = 10$; Suan-cai, $n = 10$); p values < 0.05 considered significant. (B) Cladogram representing the abundance of those taxa in the Jiang-shui and Suan-cai. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

5. Conclusions

We used the HTS technique to identify the bacterial communities in the Jiang-shui and the Suan-cai samples, and compared their microbial and physicochemical properties. 25 phyla, 440 genera and 255 species were identified in all samples. Despite the variances of bacterial community across the different samples within the group, the bacterial composition of the Suan-cai differed from Jiang-shui's. Jiang-shui was predominated by *Lactobacillus*, while the Suan-cai was predominated by *Lactobacillus* and *Pediococcus*. The major species in Jiang-shui were *L. amylolyticus*, *L. fermentum* and *L. pontis*, whereas the dominant species in Suan-cai were *Pediococcus parvulus*, *L. coryniformis*, *L. pentosus* and *L. parabrevis*. These results provided a basic understanding of the microbial composition and the significant difference of bacterial profiles in traditional Jiang-shui and Suan-cai. However, the roles of these species in these two kinds of food are still unknown, further research is needed to investigate the roles of these species in these two fermented vegetables.

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