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Comparison of bacterial diversity in traditionally homemade paocai and Chinese spicy cabbage

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ABSTRACT

This study aimed to investigate bacterial diversity in paocai and Chinese spicy cabbage and compare the microbial communities using high-throughput sequencing. Bacteria representing 26 phyla, 480 genera and 338 species were observed in these Chinese fermented vegetables. *Firmicutes* and *Proteobacteria* were the main phyla observed in both paocai and Chinese spicy cabbage. Additionally, *Lactobacillus*, *Pediococcus*, *Serratia*, *Stenotrophomonas* and *Weissella* were the major genera observed in both paocai and Chinese spicy cabbage. Overall, the relative abundances of *Lactobacillus*, *Pediococcus* and *Weissella* in Chinese spicy cabbage were much higher than those in paocai, but the proportions of *Stenotrophomonas* and *Serratia* in Chinese spicy cabbage were less than those in paocai. The results showed that the composition of the microbial community in Chinese spicy cabbage was positively correlated with total titratable acidity (TA), lactic acid and acetic acid contents but was negatively correlated with salinity. In contrast, the composition of the microbial community in paocai was negatively correlated with TA, lactic acid and acetic acid contents but was positively correlated with salinity. This study provides insights into the relationship between bacterial profiles and environmental factors in Chinese spicy cabbage and paocai, and its findings will aid in guiding future research on fermented vegetables.

1. Introduction

Chinese fermented vegetables are popular foods that date back to the Zhou Dynasty (Xiong et al., 2016). Similarly to other traditional fermented foods, such as sourdough, ripened cheeses and wine (Brysch-Herzberg and Seidel, 2015; Garofalo et al., 2015; Lhomme et al., 2016; Nalepa and Markiewicz, 2017), fermented vegetables are usually prepared under non-sterile fermentation conditions, and a number of microorganisms participate in the fermentation process. Meanwhile, the microbial composition and population will be altered by various factors, such as geographical and climatic conditions, the parameters of fermentation process, and the kinds of vegetables used for fermentation (Cao et al., 2017; Li et al., 2017a,b; Liu and Tong, 2017; Park et al., 2012).

Previous studies on Chinese sauerkraut and spicy cabbage focused mainly on lactic acid bacteria. For example, bacteria from the genera *Lactobacillus* and *Leuconostoc* (*Leu*) were frequently detected in paocai and included *Lactobacillus fermentum*, *L. delbrueckii*, *L. oris*, *L. paracasei*, *L. plantarum*, *L. casei*, *L. zeae* and *Leu. mesenteroides* subsp. *mesenteroides* (Liu et al., 2017; Xiong et al., 2012). In contrast, Chinese spicy cabbage (also known as “Chinese kimchi”), which is similar to Korean kimchi, is a fermented vegetable that is made from Chinese cabbage fermented in the presence of various species (Jeong et al., 2013); this kind of fermented vegetable was predominated by bacteria from the genera *Lactobacillus*, *Leuconostoc*, and *Weissella*, including *L. sakei*, *L. curvatus, Leu. citreum* and *Weissella confuse* (Jeong et al., 2013; Lee et al., 2005). However, the results of these previous studies do not reflect the true microbial composition of paocai and Chinese spicy cabbage. Moreover, a large-scale investigation of the bacterial communities present in paocai and Chinese spicy cabbage in China has not been performed, and few studies have reported the differences in the microbial communities in these two kinds of food.

Molecular tools based on 16S rRNA (16S rDNA) sequencing have been used to analyse bacterial diversity (Cho et al., 2009; Doulgeraki et al., 2012) and gather comprehensive and detailed knowledge of the microbial flora present in fermented vegetables. For example, polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) has been used to study the microbial composition of suan-cai and kimchi (Lee et al., 2005). Meanwhile, random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR), repetitive

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element PCR and species-specific PCR techniques have provided extensive and detailed descriptions of the microbial structures present in fermented vegetables from the Eastern Himalayas (Nguyen et al., 2013). However, these methods cannot completely reveal the bacterial community structure and diversity in fermented vegetables due to their limitations. High-throughput sequencing technology (HTS), also known as "next generation" sequencing technology, is characterized by the ability to sequence hundreds of thousands to millions of DNA molecules in parallel at one time and with short reading lengths (Xu et al., 2017). Compared with other methods, HTS provides a comprehensive and accurate description of bacterial communities and diversity in food, and has been applied to investigations of fermented food due to its speed, accuracy, high effectiveness during analysis, lower cost, and greater comprehensiveness (Li et al., 2017a,b; Tian et al., 2017). HTS techniques have recently been used to investigate bacterial communities in 12 types of French cheese (Dugat-Bony et al., 2016) and bacterial diversity in Spain wines (Portillo and Mas, 2016), to monitor bacterial changes in traditional Jiaozi and sourdough (Li et al., 2017a,b), and to analyse the bacterial communities present in Chinese fermented vegetables (Liu and Tong, 2017).

It is known that fermented vegetables are predominated by bacteria from the genus *Lactobacillus*. However, information regarding the bacterial communities present in Chinese paocai and spicy cabbage and the differences between them are lacking; this information is a basis for better understanding of Chinese fermented vegetables. The present investigation thus aimed to investigate and compare the bacterial communities present in paocai and Chinese spicy cabbage from different provinces in China. We collected paocai and Chinese spicy cabbage in Southwest and Northeast China, respectively, and analysed their bacterial diversity using HTS.

2. Materials and methods

2.1. Materials

Forty-five different traditionally fermented vegetables, including 27 mature paocai samples from Southwest China and 18 mature Chinese spicy cabbage samples from Northeast China, were collected from households and stored at 4 °C in the laboratory.

The steps in the Chinese spicy cabbage manufacturing process are as follows: first, the outer leaves and wilted leaves are removed and the remaining leaves are washed, cut into 1.5 cm-wide strips, and put into a pot and salted for 8–15 h with 2–3% salt; next, the salted cabbage is squeezed to remove excess moisture and then put into the pot again and mixed with red pepper powder (1–2%), scallion (2–4%), ginger (0.5–1%), garlic (1–2%), and fermented anchovy sauce (0.5–1.5%); finally, the prepared Chinese spicy cabbage is incubated at 10 °C or below for 10–20 or 20–30 days, respectively (Pu, 1984; Zeng, 2002). Chinese spicy cabbages are usually fermented in winter or spring in Northeast China. The paocai manufacturing process is as follows: fresh cabbages were cut into small pieces (3–8 cm), washed, drained and put into 2.5–15 L jars with seasoning mixtures that included crystal sugar (0–2%), hot red pepper (2–4%), garlic (1–3%), ginger (1–2%), Chinese prickly ash (0–1.5%), anise (0–1%), and salt (2–10%); subsequently, the jars were usually water-sealed and stored at room temperature (20–25 °C) for 4–7 days (Xiong et al., 2012). Paocai is usually fermented year-round in Southwest China. Generally, the fermentation temperature of paocai is higher than that of Chinese spicy cabbages, while the fermentation time of paocai is shorter than that of Chinese spicy cabbages.

2.2. Physicochemical parameters

The pH level of the brine samples was measured with a pH meter (PHS-25; Shanghai Precision Scientific Instruments Company, China). Using titration up to pH 8.2 ± 0.2 with 0.1 N NaOH, the TA values were calculated (Chuah et al., 2016) and interpreted as grams of lactic acid per 100 mL of brine. The lactic acid and acetic acid concentrations were monitored using the method by (Xiong et al., 2014a,b). The salt concentration was measured with a salinity meter (SA287; Qingdao Tlead International Co., Ltd., China). The nitrite contents in paocai and Chinese spicy cabbage were determined using the N-(1-naphthyl)-ethylenediamine dihydrochloride spectrophotometric method (Ding et al., 2018).

2.3. DNA extraction and PCR amplification for 16S rRNA sequence

Genomic DNA was extracted using the rapid bacterial genomic DNA isolation kit (Sangon Biotech, Shanghai, China). The following universal primers were used: forward primer 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and reverse primer 806R (5′-GGACTACHVGGGTWTCTAAT-3′) (Li et al., 2017a,b). The following PCR conditions were used: an initial denaturation at 94 °C for 5 min, 30 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 50 s and extension at 72 °C for 30 s, and a final extension at 72 °C for 5 min. The PCR products were visualized using 2% agarose gel electrophoresis and recovered using a gel extraction kit (Qiagen, Germany). The amplicon library was prepared using a sample preparation kit (Illumina, USA), and HTS was performed using an Illumina HiSeq platform (Beijing Novogene Bioinformation Science and Technology Co., Ltd., China).

2.4. Sequence data analysis

After sequencing, the sequence reads were filtered using the Illumina software to remove low quality sequences. The sequence data was merged using FLASH software (Version 1.2.7; Magoc and Salzberg, 2011) and filtered using QIIME software (Version 1.9.1; Caporaso et al., 2010). Sequences were removed if the sequence length was shorter than 200 bp or when the average quality score was less than 25. Chimeric sequences were detected and removed using UCHIME (Edgar et al., 2011). Afterward, OTUs defined by a 97% similarity were selected using UPARSE (version 7.0.1001, http://drive5.com/uparse/; Edgar, 2013). The α- and β-diversity indices of the 16S rRNA gene sequences were analysed using Qiime software (version 1.9.1) and R software (version 2.15.3). Rarefaction curves, the Shannon diversity index, Ace indices, Chao 1 richness, and Simpson and Good's coverage index were calculated to evaluate the alpha diversity (Cao et al., 2017). Hierarchical clustering was performed to assess the similarity of the bacterial communities present among the 45 samples (Tian et al., 2017), and canonical correlation analysis (CCA) was used to analyse the relationships between the fermented vegetable communities and environmental factors (Xiao et al., 2017). Spearman's correlation analysis was used to estimate the correlations among species in terms of richness (Cao et al., 2017). The linear discriminant analysis (LDA) effect size (LEfSe) algorithm was used to analyse the different taxa found in the paocai and Chinese spicy cabbage groups (Tian et al., 2017). LDA values > 4 were considered to indicate statistical significance. Non-metric multidimensional scaling (NMDS) was used to reveal differences between groups and within groups of samples (Portillo Mdel et al., 2016).

2.5. Statistical analysis

Physiochemical properties and α-diversity indices for each group were analysed and expressed as the mean ± standard deviation (implemented in SPSS Statistics 20). Significant differences in physicochemical characteristics and α-diversity indices between the Paocai and Chinese spicy cabbage groups were analysed using *t*-test with PRISM 7 (Graphpad Software). The parameters of CCA and Spearman's correlation were determined by R software (version 3.4.4). The results with p-values of less than 0.05 were considered to be statistically significant.

Table 1
Physicochemical properties of the Paocai samples and Chinese spicy cabbage samples.

Group	Sample name	Sampling area	Sampling location	Salinity (g/100 ml)	pH	Titratable acidity (g/100 ml)	Lactic acid content (mM/L)	Acetic acid content (mM/L)	Nitrite Content (mg/kg)
Paocai	PC1	Yunnan Province	Naxi	3.18	3.36	1.69	145.56	93.10	5.64
	PC2	Yunnan Province	Lijiang	2.28	3.32	1.70	338.21	65.55	2.05
	PC3	Yunnan Province	Dali	2.04	3.94	1.10	170.47	57.18	2.56
	PC4	Yunnan Province	Mangshi	3.21	3.85	1.70	416.89	42.54	5.12
	PC5	Yunnan Province	Chuxiong	3.33	4.92	0.87	130.80	142.21	6.66
	PC6	Yunnan Province	Xishuangbanna	1.62	3.67	1.69	160.02	85.94	4.61
	PC7	Yunnan Province	Simao	2.1	3.59	1.32	188.77	40.75	6.66
	PC8	Yunnan Province	Kunming	0.67	4.55	0.85	104.77	64.11	5.12
	PC9	Yunnan Province	Qujing	4.2	3.75	0.70	105.93	35.01	3.07
	PC10	Yunnan Province	Zhaotong	0.43	3.84	0.96	138.50	37.69	5.12
	PC11	Guizhou Province	Anshun	2.25	3.7	1.25	320.56	101.82	4.10
	PC12	Guizhou Province	Guiyang	0.32	3.64	0.85	159.60	94.82	2.56
	PC13	Guizhou Province	Qiannan	4.18	4	0.56	58.74	20.96	7.17
	PC14	Guizhou Province	Kaili	1.91	4	0.44	61.13	44.85	0.00
	PC15	Guizhou Province	Qiandongnan	0.24	3.87	0.47	68.36	16.44	6.15
	PC16	Guizhou Province	Liupanshui	2.96	4	1.22	182.60	47.41	4.10
	PC17	Guizhou Province	Bijie	5.62	4	0.44	149.74	28.59	5.64
	PC18	Guizhou Province	Zunyi	4.12	3	1.32	187.97	36.29	4.61
	PC19	Sichuan Province	Xichang	3.38	3.59	0.56	93.99	13.59	5.64
	PC20	Sichuan Province	Leshan	3.78	3.84	0.57	83.56	15.82	29.71
	PC21	Sichuan Province	Zigong	8.91	3.99	0.50	65.56	18.82	2.05
	PC22	Sichuan Province	Yibing	5.82	3.19	0.90	158.82	12.17	3.07
	PC23	Sichuan Province	Neijiang	7.39	3.79	0.75	130.55	35.61	7.68
	PC24	Sichuan Province	Ziyang	8.62	4.45	0.38	46.28	25.99	6.15
	PC25	Sichuan Province	Deyang	4.87	3.72	1.10	253.56	81.60	13.83
	PC26	Sichuan Province	Mianyang	2.85	4.26	1.05	103.52	97.66	3.57
	PC27	Sichuan Province	Chongqing	8.79	3.99	0.58	123.47	102.62	5.64
			Mean + SD[a]	3.67 ± 2.5[a]	3.85 ± 0.40[a]	0.95 ± 0.43[a]	153.63 ± 89.20[a]	54.04 ± 34.69[a]	5.86 ± 5.39[a]
Chinese spicy cabbage	CK1	Jilin Province	Hunchun	2.36	4.02	1.56	182.69	116.58	7.97
	CK2	Jilin Province	Hunchun	2.59	3.64	1.95	220.45	193.64	1.99
	CK3	Jilin Province	Hunchun	2.81	3.98	2.29	392.96	130.22	10.46
	CK4	Jilin Province	Hunchun	2.13	3.76	1.63	193.95	137.99	4.48
	CK5	Jilin Province	Hunchun	1.93	3.97	1.53	291.71	217.45	8.96
	CK6	Jilin Province	Tumen	2.13	3.78	1.78	241.80	177.52	6.47
	CK7	Jilin Province	Tumen	2.34	3.6	2.82	372.45	171.98	7.97
	CK8	Jilin Province	Yanji	2.48	3.86	1.29	170.14	123.21	1.49
	CK9	Jilin Province	Yanji	2.04	3.8	2.16	271.72	140.63	6.97
	CK10	Jilin Province	Yanji	2.53	3.86	2.47	357.28	172.37	3.98
	CK11	Jilin Province	Longjing	2.58	4.01	1.83	299.46	136.17	5.98
	CK12	Jilin Province	Longjing	2.25	4	1.83	298.82	147.62	3.49
	CK13	Liaoning Province	Dandong	1.75	3.63	2.25	329.38	194.54	0.00
	CK14	Jilin Province	Helong	1.87	4.28	2.42	201.32	176.16	4.98
	CK15	Jilin Province	Helong	2.6	3.75	2.25	313.28	152.66	1.49
	CK16	Jilin Province	Tumen	2.12	4.08	2.90	313.82	151.96	1.49
	CK17	Jiin Province	Antu	2.25	3.87	2.13	264.65	241.11	0.00
	CK18	Jilin Province	Antu	1.88	3.91	2.25	289.79	201.23	4.48
			Mean + SD[a]	2.26 ± 0.30[a]	3.88 ± 0.17[a]	2.07 ± 0.44[a]	278.09 ± 65.67[a]	165.72 ± 34.30[a]	4.59 ± 3.13[a]

Yunnan Province, Guizhou Province, and Sichuan Province belong to the southwest of China; Jilin Province and Liaoning Province belong to the northeast of China. PC means Paocai; CK means Chinese spicy cabbage.

[a] Mean ± standard deviation.

3. Results

3.1. Physicochemical features of the brines

As shown in Table 1, the salt concentration ranged from 0.24 g/100 mL to 8.91 g/100 mL, with an average of 3.10 g/100 mL. The pH value ranged between 3 and 4.92, with an average of 3.86. The TA ranged from 0.38 g/100 mL to 2.90 g/100 mL, with an average of 1.39 g/100 mL. The TA of paocai and Chinese spicy cabbage ranged from 0.38 g/100 mL to 1.70 g/100 mL and 1.53 g/100 mL to 2.90 g/100 mL, respectively. The concentrations of lactic acid and acetic acid ranged from 46.28 mM/L to 416.89 mM/L and 12.17 mM/L to 142.20 mM/L, respectively, in paocai and from 170.14 mM/L to 392.96 mM/L and 98.71 mM/L to 241.11 mM/L, respectively, in Chinese spicy cabbage. Lactic acid concentration in the samples was positively correlated ($p < 0.05$) with TA and acetic acid concentration and negatively correlated with salt concentration ($p < 0.05$; Fig. 7). The mean TA, lactic acid, and acetic acid concentrations in Chinese spicy cabbage were 2.07 ± 0.44 g/100 mL, 278.09 ± 65.67 mM/L, and 165.72 ± 34.30 mM/L, respectively, while in paocai they were lower (0.95 ± 0.43 g/100 mL, 153.63 ± 89.20 mM/L, and 54.04 ± 34.69 mM/L, respectively). As shown in Fig. 1A, the salt content was significantly higher in the paocai groups than in the Chinese spicy cabbage groups. In contrast, the titratable acidity, lactic acid level, and acetic acid level in the paocai group were significantly lower than in the Chinese spicy cabbage group ($p < 0.01$; Fig. 1C, D and 1E), while no significant differences in pH were observed between the paocai group and the Chinese spicy cabbage group (Fig. 1B). The nitrite concentrations in paocai and Chinese spicy cabbage were 5.86 ± 5.39 and 4.59 ± 3 .13 mg/kg, respectively (Table 1).

3.2. Comparison of diversity indices among samples

A total of 3,958,347 reads (an average of 86,529 reads for the paocai samples and 90,114 reads for the Chinese spicy cabbage samples) were generated from 45 Chinese fermented vegetable samples (Tables 2 and 3). The bacterial α-diversity indices (Shannon, Chao 1, observed species, Good's coverage index, Simpson, and ACE indices) for each sample and group were calculated (Tables 2 and 3). Based on 97% similarity, the Good's coverage index for each sample was above 0.99 (Table 2), which implied that the sequencing provided adequate coverage in terms of classification diversity. As shown in Table 2, the microbial α-diversity indices showed differences among the samples. The Shannon diversity for samples from different sites are shown in Tables 2 and 3; the diversity index obtained for paocai (3.38 ± 0.71) were lower than that obtained for Chinese spicy cabbage (3.60 ± 0.49). As shown in Fig. 1F, G and 1H, there were no significant differences in the Shannon, Chao1, and observed species indices between the paocai and Chinese spicy cabbage groups, while the Simpson index was significantly lower in paocai than in Chinese spicy cabbage (Fig. 1I).

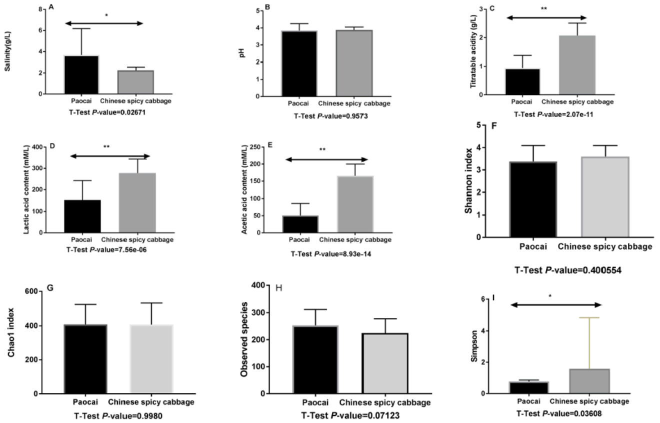

Fig. 1. Comparison of physicochemical characteristics and α diversity indices between Paocai and Chinese spicy cabbage samples. *$P < 0.05$, **$P < 0.01$. T-test P values refer to tests carried out between the Paocai and Chinese spicy cabbage groups.

3.3. Microbial structure

The sequences comprising the total reads corresponded to 26 phyla, 186 families, and 480 genera (data not shown). As shown in Fig. 2A and B, the major phyla were *Firmicutes* and *Proteobacteria*, followed by *Deinococcus-Thermus*, *Cyanobacteria*, *Actinobacteria*, *Acidobacteria*, *Bacteroidetes*, *Fusobacteria*, *Spirochaetes*, and *Saccharibacteria*. The phyla distribution percentages varied among the samples (Fig. 2A). Among the phyla, *Firmicutes* was the most abundant, ranging from 12.21% to 95.29% of flora in the paocai samples and from 72.04% to 95.61% in the Chinese spicy cabbage samples. In addition to *Firmicutes* (average abundance of 53.95% in the paocai group and 85.83% in the Chinese spicy cabbage group), *Proteobacteria* was another major phylum that was observed in both groups and accounted for 39.83% and 12.47% of flora in paocai and spicy cabbage, respectively (Fig. 2B). Among the samples, *Lactobacillaceae* was the major family observed, followed by *Enterobacteriaceae* and *Xanthomonadaceae*. Meanwhile, the phylogenetic classification of different samples showed differences between the paocai and Chinese spicy cabbage samples in terms of 10 main bacterial families, including *Lactobacillaceae*, *Enterobacteriaceae*, *Xanthomonadaceae*, *Leuconostocaceae*, *Moraxellaceae*, *Thermaceae*, *Bacillaceae*, *Microbacteriaceae, Methylobacteriaceae, and Rubrobacteriaceae* (Fig. 2B and D). The relative richness of *Enterobacteriaceae* and *Xanthomonadaceae* was lower in Chinese spicy cabbage than in paocai, while that of *Lactobacillaceae* was higher in Chinese spicy cabbage than in paocai. The most representative genera were *Lactobacillus, Serratia, Stenotrophomonas, Pediococcus, Weissella, Acinetobacter, Thermus, Psychrobacter, Escherichia, and Methlobacterium* (Fig. 2E, F, and Fig 3A). The relative abundances of *Lactobacillus* (67.44%), *Pediococcus* (13.69%) and *Weissella* (3.34%) were higher in Chinese spicy cabbage than in paocai (47.22%, 3.16% and 1.4%, respectively). Meanwhile, the relative abundances of *Serratia* (3.37%) and *Stenotrophomonas* (4.67%) were lower in Chinese spicy cabbage than in paocai (17.15% and 15.32%, respectively). As shown in Fig. 3B, the species *L. coryniformis, P. parvulus, L. parabrevis, and L. pentosus* were more abundant in the Chinese spicy cabbage group than in the paocai group. In contrast, the relative abundances of *L. acetotolerans* and *S. marcescens* were lower in the Chinese spicy cabbage group than in the paocai group.

3.4. Community comparison

NMDS analysis results revealed the separation of the bacterial communities in the two groups of samples. The bacterial communities in the Chinese spicy cabbage samples were more similar to each other than to those in the paocai samples (Fig. 4), indicating that the differences in bacterial communities were less significant ($p < 0.05$) in the Chinese spicy cabbage group than in the paocai group.

3.5. Bacterial cluster analysis

UPGMA cluster analysis of the 45 fermented vegetable samples (27 mature paocai and 18 mature Chinese spicy cabbage samples) was performed based on the identified OTUs (Fig. 5). Two different clusters at the OTU level were observed in the UPGMA tree: Group 1, PC27, and Group 2, CK (Chinese spicy cabbage) and PC (paocai) except for PC27. This finding was consistent with the results showing that the samples in PC27 were significantly different from the Chinese spicy cabbage samples and other paocai samples (Fig. 2). Group 2 showed relatively tight clustering, which suggested the presence of a high similarity among paocai samples observed at relatively low salinity (PC8, PC3,

Table 2
Alpha diversity of Paocai and Chinese spicy cabbage samples in China.

Sample name	Sampling area	Sampling location	Total reads	Shannon index	Chao1 index	Observed species	Good's coverage	Simpson	Ace
PC1	Yunnan Province	Naxi	84,019	3.09	395	227	0.998	0.725	322
PC2	Yunnan Province	Lijiang	77,085	2.66	224	161	0.999	0.674	190
PC3	Yunnan Province	Dali	85,719	3.66	364	211	0.998	0.844	298
PC4	Yunnan Province	Mangshi	85,645	2.92	355	221	0.998	0.726	273
PC5	Yunnan Province	Chuxiong	79,357	3.35	321	214	0.999	0.817	275
PC6	Yunnan Province	Xishuangbanna	82,361	2.93	317	228	0.998	0.722	290
PC7	Yunnan Province	Simao	88,513	3.22	411	257	0.998	0.769	354
PC8	Yunnan Province	Kunming	91,068	5.30	488	370	0.999	0.933	410
PC9	Yunnan Province	Qujing	85,325	3.53	247	197	0.999	0.821	231
PC10	Yunnan Province	Zhaotong	96,657	3.13	467	244	0.998	0.749	349
PC11	Guizhou Province	Anshun	97,837	3.55	426	190	0.998	0.852	255
PC12	Guizhou Province	Guiyang	90,135	4.39	673	375	0.998	0.897	469
PC13	Guizhou Province	Qiannan	82,662	2.98	428	243	0.998	0.726	320
PC14	Guizhou Province	Kaili	87,254	3.05	410	256	0.998	0.764	338
PC15	Guizhou Province	Qiandongnan	85,457	4.26	365	239	0.998	0.904	301
PC16	Guizhou Province	Liupanshui	89,840	3.20	522	324	0.998	0.664	395
PC17	Guizhou Province	Bijie	91,882	3.21	632	354	0.997	0.706	511
PC18	Guizhou Province	Zunyi	81,561	2.65	377	223	0.998	0.687	318
PC19	Sichuan Province	Xichang	78,526	2.33	281	220	0.999	0.551	258
PC20	Sichuan Province	Leshan	88,103	3.28	361	245	0.998	0.813	319
PC21	Sichuan Province	Zigong	91,650	2.66	352	230	0.998	0.747	272
PC22	Sichuan Province	Yibing	80,037	3.89	308	222	0.999	0.889	271
PC23	Sichuan Province	Neijiang	94,431	3.01	477	288	0.998	0.756	354
PC24	Sichuan Province	Ziyang	81,461	3.31	693	300	0.997	0.769	453
PC25	Sichuan Province	Deyang	86,656	3.40	459	366	0.998	0.761	447
PC26	Sichuan Province	Mianyang	88,960	3.06	310	189	0.999	0.795	247
PC27	Sichuan Province	Chongqing	84,083	5.25	370	252	0.999	0.938	312
CK1	Jilin Province	Hunchun	88,688	4.32	382	281	0.998	0.874	339
CK2	Jilin Province	Hunchun	95,811	4.02	350	212	0.999	0.902	268
CK3	Jilin Province	Hunchun	80,076	3.25	744	154	0.998	0.827	230
CK4	Jilin Province	Hunchun	95,251	2.67	139	95	0.999	0.730	117
CK5	Jilin Province	Hunchun	83,076	3.62	355	219	0.998	0.854	272
CK6	Jilin Province	Tumen	94,009	3.25	425	267	0.998	0.778	343
CK7	Jilin Province	Tumen	87,536	3.60	414	216	0.998	0.870	311
CK8	Jilin Province	Yanji	86,364	3.72	405	221	0.999	0.878	254
CK9	Jilin Province	Yanji	91,891	3.18	413	218	0.998	0.787	274
CK10	Jilin Province	Yanji	96,291	3.18	207	153	0.999	0.766	191
CK11	Jilin Province	Longjing	91,122	3.88	506	209	0.999	0.896	257
CK12	Jilin Province	Longjing	89,421	3.06	335	255	0.999	0.764	298
CK13	Liaoning Province	Dandong	94,950	3.52	395	231	0.998	0.836	298
CK14	Jilin Province	Helong	91,199	4.07	477	292	0.998	0.850	385
CK15	Jilin Province	Helong	86,265	3.98	427	285	0.998	0.848	346
CK16	Jilin Province	Tumen	86,454	3.93	349	199	0.999	0.900	259
CK17	Jilin Province	Antu	97,327	3.09	525	250	0.998	0.771	326
CK18	Jilin Province	Antu	86,332	4.51	488	293	0.998	0.914	358

Yunnan Province, Guizhou Province and Sichuan Province belong to the southwest of China; Jilin Province and Liaoning Province belong to the northeast of China. PC means Paocai; CK means Chinese spicy cabbage.

PC5, PC10, PC6, PC13, PC4, PC18, PC26, PC7, PC14 and PC21). Meanwhile, a significant difference in microbial composition was observed in the Chinese spicy cabbage samples and the high-salinity paocai samples. UPGMA cluster analysis found a high similarity between the Chinese spicy cabbage samples and the paocai samples with the highest salinity (PC19, PC20, PC22, PC23, PC24 and PC25). These results indicated that the microbiota in the Chinese spicy cabbage samples were more similar to each other than to those present in the paocai samples, which was consistent with the results of the NMDS analysis.

3.6. Linear discriminate analysis (LDA) effect size (LEfSe) analysis

To determine the differences in relative microbial abundance between the paocai and Chinese spicy cabbage groups, the LEfSe algorithm (LDA log score threshold ≥ 4) was used at the OTU level (Fig. 6).

Table 3
Number of total reads, observed diversity richness (OTUs), estimated OTU richness (Chao 1), Shannon, observed species, and Ace of Paocai and Chinese spicy cabbage group.

Group	Total reads	Shannon index	Chao1 index	Observed species	Good's coverage	Simpson	Ace
Paocai	2336284	91.27	11,033	6846	26.952	20.999	8832
Mean ± SD[a] (paocai)	86,529 ± 5375[a]	3.38 ± 0.71[a]	408 ± 117[a]	254 ± 59[a]	0.998 ± 0.0006[a]	0.778 ± 0.090[a]	327 ± 78[a]
Chinese spicy cabbage	1622063	64.85	7336	4050	17.971	15.045	5126
Mean ± SD[a](Chinese spicy cabbage)	90,114 ± 4905[a]	3.60 ± 0.49[a]	408 ± 127[a]	225 ± 53[a]	0.998 ± 0.0005[a]	0.836 ± 0.057[a]	284 ± 65[a]

[a] SD, standard deviation.

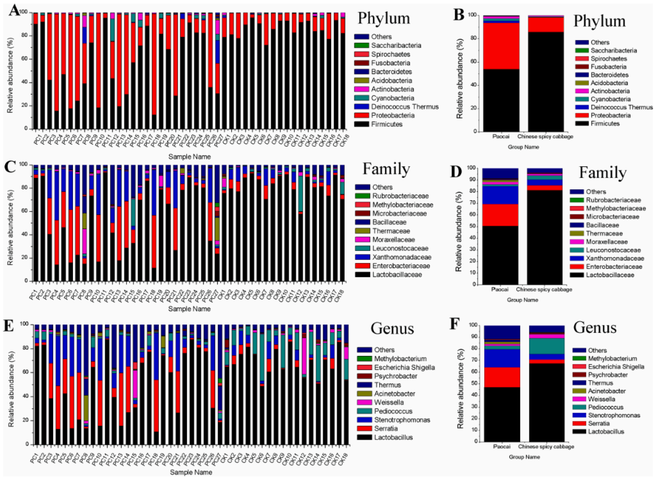

Fig. 2. Relative abundance of bacteria community in Paocai samples and Chinese spicy cabbage samples at phylum (A,B),family (C,D) and genus (E,F) level. PC means Paocai; CK means Chinese spicy cabbage.

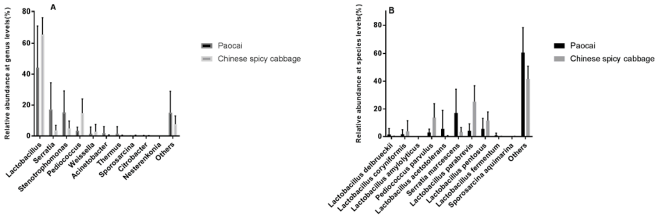

Fig. 3. Comparison of bacterial community in Paocai and Chinese spicy cabbage groups at genus levels (A) and species levels (B).

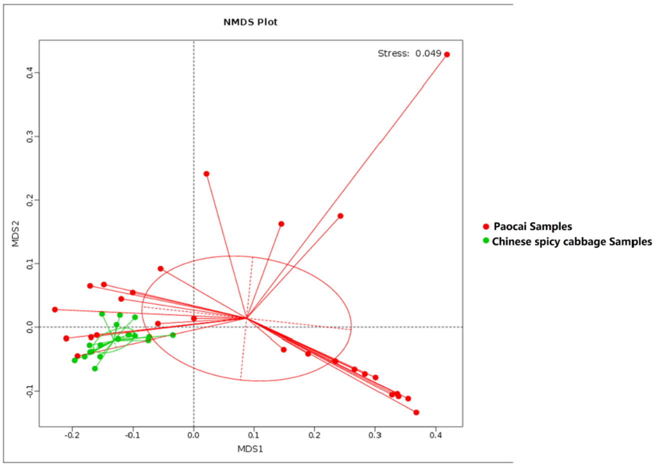

Fig. 4. Non-metric multi-dimensional scaling (NMDS) of the data set (45 samples). Red: Paocai; Green: Chinese spicy cabbage.

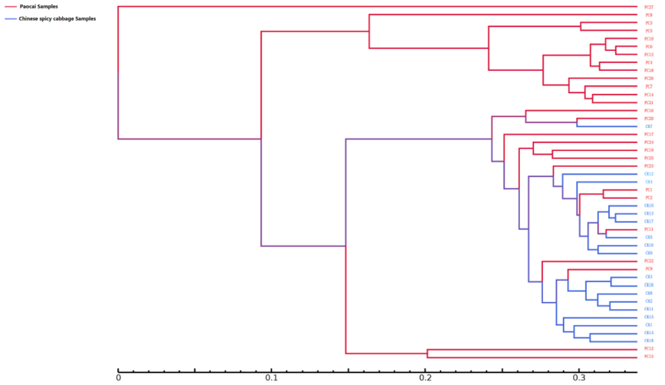

Fig. 5. Cluster-tree of bacterial communities of Paocai samples and Chinese spicy cabbage. Red: PC (Paocai); Blue: CK (Chinese spicy cabbage).

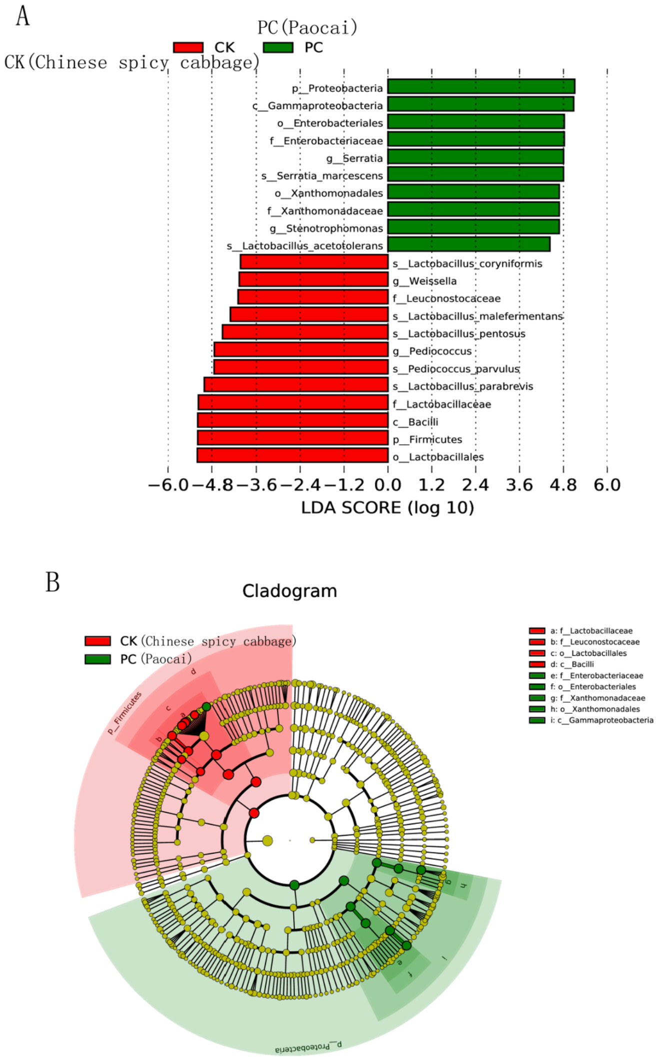

Fig. 6. LEfSe analyses of paocai group and Chinese spicy cabbage group. Red: PC (Paocai); Green: CK (Chinese spicy cabbage). (A) Histogram of the results of LEfSe paocai group and Chinese spicy cabbage group and their respective effect sizes; p-values < 0.05 considered significant. (B) Cladogram showed taxonomic representation of differences between PC (Paocai) and CK (Chinese spicy cabbage).

As shown in Fig. 6, there were 10 taxa in the paocai group and 12 taxa in the Chinese spicy cabbage group. The paocai group had greater proportions of *Enterobacteriaceae* and *Xanthomonadaceae* and members of the phylum *Proteobacteria*, whereas the Chinese spicy cabbage group had greater relative proportions of *Lactobacillaceae* and *Leuconostocaceae*, which belong to *Firmicutes*. At the genus level, bacteria from *Serratia* and *Stenotrophomonas* were the most differentially abundant in the paocai group, while bacteria from *Lactobacillus*, *Weissella* and *Pediococcus* were the most abundant in the Chinese spicy cabbage group. These results were in agreement with those shown in Fig. 3A. *Serratia*, which represents pathogenic microorganisms, was found mainly in the paocai group. This could be attributed to many factors, such as temperature, the storage device used, the production process, raw materials, dimensions and longitude, that contributed to a failure to effectively inhibit growth of pathogenic bacteria in the paocai group. In addition, *L. acetotolerans* was abundant in the paocai group. However, *L. coryniformis*, *L. malefermentans*, and *L. parabrevis* were enriched in the Chinese spicy cabbage group, which is in agreement with the present result (Fig. 3B). These key phylotypes contributed to the differences in microbiota composition between the paocai and Chinese spicy cabbage groups.

3.7. Relationships between microbial community structures and environmental parameters

The relationships between the microbial community structures and environmental factors (Table 2) were evaluated using the ordination diagram according to canonical correlation analysis (CCA; Fig. 7). Axes 1 and 2 accounted for 49.62% and 24.79% of the total constrained variation, respectively. Axis 1 (horizontal) was positively correlated with TA, lactic acid and acetic acid contents, while axis 2 (vertical) was strongly correlated with salinity. Based on the length of the connecting wire, acetic acid had the greatest influence on the fermented vegetable flora, followed by lactic acid, TA and salinity. However, pH had no significant effect on microbial community structure. The results showed that the microbial community in the Chinese spicy cabbage group was positively correlated with TA, lactic acid and acetic acid contents. Meanwhile, the microbial community structure in the paocai group was negatively correlated with TA, lactic acid and acetic acid contents. In contrast, the microbial community structure in the paocai group was positively correlated with salinity, whereas the bacterial composition in the Chinese spicy cabbage group had a negative relationship with salinity. Based on the Mantel test results, salinity ($r = 0.321$, $p = 0.001$) had the strongest correlation with the bacterial community of OTU species, followed by acetic acid ($r = 0.2373$, $p = 0.001$) and lactic acid ($r = 0.1395$, $p = 0.004$). However, pH ($r = 0.0483$, $p = 0.242$) and TA ($r = 0.1069$, $p = 0.021$) had weak correlations with the bacterial communities in this study.

3.8. Relationship between microbial abundance and environmental parameters

As shown in Fig. 8, the abundances of *Lactobacillus parabrevis* ($p < 0.01$), *Ped. parvulus* ($p < 0.01$), *L. pentosus* ($p < 0.01$), *L. coryniformis* ($p < 0.05$), *L. malefermentans* ($p < 0.01$), and *Solanum torvum* ($p < 0.01$) were positively correlated with TA, lactic acid and acetic acid contents. In contrast, the abundances of *L. acetotolerans* ($p < 0.05$) and *Thermus scotoductus* ($p < 0.05$) showed a negative correlation with TA, lactic acid and acetic acid contents. Among these species, only *L. malefermentans* was negatively correlated with salinity in terms of abundance ($p < 0.01$), whereas the abundance of *L. acetotolerans* ($p < 0.05$) was positively correlated with salinity. The abundance of *Serratia marcescens* was negatively correlated with acetic acid content ($p < 0.01$), which explained the low abundance of this species in the Chinese spicy cabbage group. However, pH showed no significant correlation with microbial abundance, which was consistent with the CCA results.

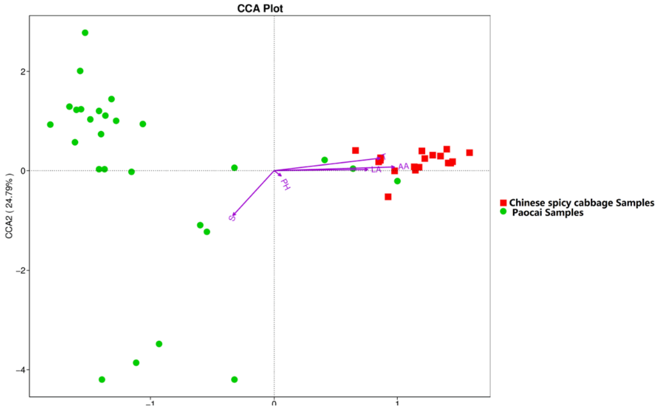

Fig. 7. Canonical correlation analysis (CCA) of the Chinese fermented vegetable communities in China. Green indicates Paocai samples; red indicates Chinese spicy cabbage samples. TA, LA, AA and S represent total acid, lactic acid, acetic acid and salt, respectively.

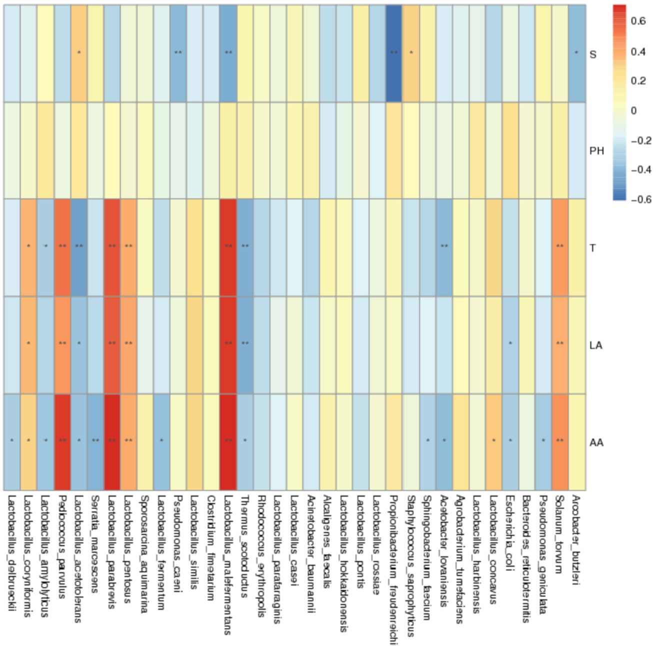

Fig. 8. Spearman rank correlation analysis to study the environmental factors and dominant species in Chinese fermented vegetable, including paocai and Chinese spicy cabbage. The Spearman correlation coefficient r ranges from −0.5 to 0.5; $r < 0$ is negative correlation, $r > 0$ is positive correlation. $^{*}P < 0.05$, $^{**}P < 0.01$.

4. Discussion

The objective of this study was to investigate the bacterial profiles in paocai and Chinese spicy cabbage samples and compare the bacterial communities using HTS. This study also explored the relationship between microbial abundance and environmental parameters in these samples.

The dominant bacteria in paocai and Chinese spicy cabbage may vary between different sampling locations, but *Lactobacillus* was the most dominant genus in fermented vegetables, which was in agreement with the results of previous studies on Chinese sauerkraut (Cao et al., 2017; Liu and Tong, 2017; Wu et al., 2015; Xiong et al., 2012). However, this result is quite different from that found for another famous Asian fermented vegetable, Korean kimchi, in which *Leuconostoc*, *Lactobacillus* and *Weissella* predominated (Cho et al., 2006; Jeong et al., 2013). *Weissella* and *Leuconostoc* were not the predominant genera in this study, but these two genera have been frequently found in most of the Chinese spicy cabbages at a low level and at levels much higher than those found in paocai in this study (data not shown). These differences may be related to several factors, including fermentation temperature, production process, raw materials and geographical distribution. *Pediococcus* has been reported to be the second-most dominant genus in paocai (Cao et al., 2017), but it was detected at a very low level in our paocai samples. However, *Pediococcus* was the second-most dominant genus observed in the Chinese spicy cabbage samples, and the relative proportion of *Pediococcus* was much higher in the Chinese spicy cabbage samples than in the paocai samples. Moreover, this genus has rarely been identified in other studies (Cho et al., 2006; Haque et al., 2015; Jeong et al., 2013; Liu et al., 2017; Xiong et al., 2012). Thus, this is the first finding suggesting that *Pediococcus* is the dominant bacteria in Chinese spicy cabbage, and may play a role in the microbial community in Chinese spicy cabbage. The relative abundance of *Lactobacillales* was much higher in the Chinese spicy cabbage group than in the paocai group. This distinction is further highlighted by the fact that the TA, lactic acid and acetic acid contents were higher in the Chinese spicy cabbage group than in the paocai group. This difference could be due to seasonal changes, inappropriate culture conditions, raw materials, the preparation process and some limitations present in previous reports (Cho et al., 2006; Jeong et al., 2013; Kim and Chun, 2005; Liu and Tong, 2017; Xiong et al., 2016).

According to the results, *Proteobacteria* was the second-most predominant bacteria and showed a negative correlation with *Firmicutes*, which was similar to that observed in previous studies (Cao et al., 2017; Lee et al., 2005). Compared with the paocai samples, the Chinese spicy cabbage samples contained lower abundances of *Proteobacteria*. This may be because *Proteobacteria* was negatively correlated with *Firmicutes*.

In China, the maximum nitrite level in pickled vegetables should not exceed 20 mg/kg, as described previously (Hou et al., 2013). In this study, only a single sample within the paocai and Chinese spicy cabbage samples (2%) had a nitrite level that was > 20 mg/kg. Therefore, in China, low levels of nitrite are widely present in pickled vegetables, which is consistent with a previous report (Hou et al., 2013).

Serratia and *Stenotrophomonas* were commonly found in all our samples. These two genera are comprised of opportunistic pathogens that can cause urinary tract infections, endocarditis, intravenous catheter-associated infections, wound infections, pneumonia, bloodstream infections and ocular infections (Abraham et al., 2016; Devi et al., 2018). The relative abundances of *Serratia* and *Stenotrophomonas* in the paocai group were much higher than those in the Chinese spicy cabbage group. Contamination by bacteria from the genera *Serratia* and *Stenotrophomonas* has been reported in paocai and other fermented vegetables (Cao et al., 2017; Park et al., 2012)., but few studies have reported that *Serratia* is the second-most dominant genus and *Stenotrophomonas* is the third-most prevalent genus in paocai. This is alarming and indicates that fermentation conditions for homemade paocai are poorly regulated. These results and previous studies imply that there is a hidden microbial safety risk and potential spoilage in homemade paocai and Chinese spicy cabbage. Therefore, it is necessary to control the growth of potentially pathogenic bacteria and other bacterial contaminants in fermented vegetables.

The LEfSe analysis revealed significant differences in the microbiological structures in paocai and Chinese spicy cabbage. Paocai had high abundances of *Serratia*, *Stenotrophomonas* and *Lactobacillus acetotolerans*. This result was consistent with a previous study on paocai (Cao et al., 2017) showing that paocai was dominated by *L. acetotolerans*. Meanwhile, HTS analysis revealed that *Weissella* in Chinese spicy cabbage was very active (data not shown), which was in agreement with the results of previous reports (Cho et al., 2006; Lee et al., 2005). Moreover, *L. coryniformis*, *L. malefermentans*, *L. parabrevis*, and *Ped. parvulus* species were much higher in abundance in the Chinese spicy cabbage samples than in the paocai samples; this is the first study to report that Chinese spicy cabbage has a high abundance of *Pediococcus*. These bacterial differences may be related to the raw materials, raw material treatment, fermentation time and temperature, regions, seasons, and vegetable varieties used.

TA appears to be one of the most important factors that influences the bacterial community, which is in agreement with a previous study reporting that the bacterial diversity in fermented vegetables was primarily related to TA (Cao et al., 2017). In addition, the increase in acidity was related to the accumulation of lactic acid and acetic acid (Xiong et al., 2012). This study also showed that TA was positively correlated to lactic acid and acetic acid contents. Both lactate and acetate are the major organic acids produced by LAB in paocai and Chinese spicy cabbage. Lactate has been previously reported to be the main fermentation product in Sichuan paocai (Cao et al., 2017; Xiong et al., 2012) and Korean kimchi (Jeong et al., 2013). Another major organic acid, acetic acid, was positively related to Chinese spicy cabbage. Acetic acid is mainly produced by heterofermentative LAB (Xiong et al., 2014a,b). This result showed that the acetic acid content in Chinese spicy cabbage samples was much higher than that in paocai, which is consistent with the finding that the proportions of heterofermentative LAB were relatively higher in the Chinese spicy cabbage samples than in the paocai samples. Therefore, further studies are required to investigate the influence of lactate and acetate on bacterial succession during the fermentation process of paocai and Chinese spicy cabbage. Paocai and Korean kimchi are salted, fermented vegetables (Jeong et al., 2013; Xiong et al., 2012). Salt has an important effect on the microbial communities in pickled vegetables (Xiong et al., 2016). Our results demonstrated that the microbial communities in paocai and Chinese spicy cabbage were affected by the salt content, especially in paocai. Overall, the salt level in the paocai group was much higher than that in the Chinese spicy cabbage. We speculated that high salt content may inhibit the growth of some lactic acid bacteria, which would cause the relative abundance of lactic acid bacteria to be much lower in the paocai samples than in the Chinese spicy cabbage. Therefore, salinity is also an important factor that influences bacterial composition in fermented vegetables.

Most bacteria previously found in fermented vegetables belonged to *Firmicutes* and *Proteobacteria* (Jeong et al., 2013; Li et al., 2017a,b; Liu and Tong, 2017), which was consistent with our results. The abundances of most species belonging to *Firmicutes*, such as *L. parabrevis*, *Ped. parvulus*, *L. pentosus*, and *L. coryniformis*, were positively correlated with TA, lactic acid and acetic acid contents in the paocai and Chinese spicy cabbage samples. However, the abundances of most species belonging to *Proteobacteria*, including *Thermus scotoductus*, and *Serratia marcescens*, were negatively correlated with TA, lactic acid and acetic acid contents in this study, which was in agreement with the findings of a previous study on paocai (Cao et al., 2017). The abundance of another species of *Firmicutes*, *L. acetotolerans*, showed a negative correlation with TA, lactic acid and acetic acid contents in this study; this result was unexpected because a previous study has shown that the abundance of *L. acetotolerans* was positively correlated with TA, lactic acid and acetic acid contents (Cao et al., 2017), which is clearly different from our results. Both our previous study (Xiong et al., 2016) and this study showed that salt has an important effect on pathogens and is negatively correlated with the abundance of *Pseudomonas caeni,* indicating the sensitivity of pathogens to salt. Therefore, future studies should explore the effects of bacterial succession, metabolites, environmental changes, and the interaction of microbial community composition and environmental parameters on vegetable fermentation.

5. Conclusions

The differences in microbiota composition and physicochemical parameters in paocai and Chinese spicy cabbage were investigated in this study. *Lactobacillus*, *Serratia* and *Stenotrophomonas* were the major genera observed in paocai, whereas *Lactobacillus* and *Pediococcus* were the major genera observed in Chinese spicy cabbage. Overall, the TA, lactic acid and acetic acid contents were much higher in Chinese spicy cabbage than in paocai, while the salt content was lower in Chinese spicy cabbage than in paocai. The community structure showed a significant correlation with physicochemical parameters, such as TA, salt, lactic acid and acetic acid. The present study provides insights into understanding of the microbes found in Chinese fermented vegetables and are useful for the production of these vegetables. Further research should explore the role of the dominant *Lactobacillus* species and their effects on the flavour and sensory experience of fermented vegetables. Moreover, several potentially pathogenic bacterial species should be controlled in fermented vegetable products.

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