

Physicochemical characteristics and bacterial communities of *meigan cai* and *zao cai*, two home-made fermented vegetables

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Abstract

Meigan cai and *zao cai* are two important traditional fermented vegetables in Fujian Province, China. However, there is little information on the bacterial community of these two fermented vegetables. In the present work, we evaluated and compared the physicochemical characteristics and bacterial communities of *meigan cai* and *zao cai*. Results revealed that Firmicutes and Proteobacteria were the main dominant phyla, and *Lactobacillus* and *Serratia* were the main genera. Principal coordinate and significance analysis showed that the bacterial communities were similar between *meigan cai* and *zao cai*. Except for glucose, there was no significant difference in the physicochemical characteristics between *meigan cai* and *zao cai*. Our study provided information on the bacterial communities of *meigan cai* and *zao cai*, and a guide for the production of these two fermented vegetables.

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Introduction

Fermented vegetables date back to 3,000 years ago in China (Xiong *et al.*, 2016). Due to the unique flavour, texture, and various functions of fermented vegetables (Zhao *et al.*, 2007; Zhang *et al.*, 2016), the study of Chinese fermented vegetables such as *pao cai* (Cao *et al.*, 2017), serofluid dish (Chen *et al.*, 2016), *suan cai* (Wu *et al.*, 2015), and *zha cai* (Liang *et al.*, 2018) have attracted considerable attention. *Meigan cai* and *zao cai* are widely consumed in large provinces in Southern China, especially in the Fujian Province of China. These two fermented vegetables are typically steamed or stir-fried with chicken, fish, or beans (Ye, 2013; Shen *et al.*, 2018). During the production of *meigan cai*, fresh mustards are washed and dehydrated in the sun for one to two days. After the addition of salt, the half-dried vegetable is cut into small pieces (3 - 6 cm by 1 - 3 cm), and transferred into a 5 - 10 L or bigger fermentation container, without any spices, for approximately 20 days of fermentation. On the other hand, the production of *zao cai* processing consists of the following: (1) the fresh vegetable is washed and dehydrated in the sun for one to two days, and (2) the half-dried vegetable

is transferred into a jar with some red lees to allow fermentation for two to three months.

Over the past decade, the bacterial communities in Chinese fermented vegetables have been studied in many reports (Cao *et al.*, 2017; Liu and Tong, 2017). *Leuconostoc*, *Bacillus*, *Lactobacillus*, *Pseudomonas*, *Staphylococcus*, *Enterobacter*, *Weissella*, *Sphingomonas*, *Psychrobacter*, *Tetragenococcus*, and *Corynebacterium* are predominant in Chinese fermented vegetables (Wu *et al.*, 2015; Zhou *et al.*, 2018; Shang *et al.*, 2019). Despite the growing knowledge on the microbial ecology in Chinese fermented vegetables (Xiong *et al.*, 2012; Chen *et al.*, 2016; Zhang *et al.*, 2018b), there is little information on the bacterial communities of *meigan cai* and *zao cai*, which restricts the upgrading and upscaling of these food products.

In the present work, we investigated and compared the bacterial communities of home-made *meigan cai* and *zao cai*. The aim was to acquire information on the bacterial ecology of *meigan cai* and *zao cai*, and to provide a guide for the production of these two Chinese fermented vegetables.

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Materials and methods

Materials

We obtained *zao cai* ($n = 9$) and *meigan cai* ($n = 7$) from local families in Fujian Province, China. All samples were stored at $-80\text{ }^{\circ}\text{C}$ in our laboratory.

Physicochemical properties

The pH value and the titratable acidity (TA) of the 16 samples were measured according to Chuah *et al.* (2016). The TA was interpreted as gram of lactic acid per kilogram. The salt concentration was determined using a salinity meter. The contents of sucrose (Sur), glucose (Glu), fructose (Fru), lactic acid (LA), acetic acid (AA), and oxalic acid (OA) were determined using HPLC as described in our previous study (Liu *et al.*, 2019a). The nitrite (NI) content was measured as previously reported (Ding *et al.*, 2018).

DNA extraction and Illumina HiSeq sequencing

The DNA was extracted from the samples using a rapid bacterial genomic DNA isolation kit (Sangon Biotech, Shanghai, China). The 16S rRNA genes were amplified by PCR using forward primers 338F: (5'-ACTCCTACGGGAGGCAGCA-3') and reverse primer 806R: (5'-GGACTACHVGGGTWTCTAAT-3') (Liu *et al.*, 2019c). The amplification program consisted of an initial denaturation step at $98\text{ }^{\circ}\text{C}$ for 1 min, followed by 30 cycles of denaturation at $98\text{ }^{\circ}\text{C}$ for 10 s, primer annealing at $50\text{ }^{\circ}\text{C}$ for 30 s, and extension at $72\text{ }^{\circ}\text{C}$ for 30 s, with a final elongation step performed at $72\text{ }^{\circ}\text{C}$ for 5 min. The amplification products were sequenced using Illumina HiSeq 2500 at Novogene (Beijing, China).

Sequence analysis

Raw reads were merged using FLASH (Version 1.2.7), and then quality-filtered using the QIIME software (Version 1.9.1) (Caporaso *et al.*, 2011). Based on the UCHIME algorithm, chimera sequences were identified and removed using USEARCH software (Edgar *et al.*, 2011). Based on the 97% similarity, the sequence with high quality with were clustered into operational taxonomic units (OTUs) (Caporaso *et al.*, 2010).

Rarefaction curves, species accumulation boxplot, observed species, Shannon, Simpson, Chao1 index, and Good's coverage were performed to estimate the alpha diversity (within-sample species

richness) of each sample (Liu and Tong, 2017). Canonical correspondence analyses (CCA) were used to determine the correlations between physicochemical parameters and fermented vegetables (Liu *et al.*, 2019a). For the network analysis, Spearman's rank correlation coefficients (ρ) were calculated between genera occurring in fermented vegetables using the "Hmisc" R package (Huang *et al.*, 2020). Subsequently, the Spearman's rank correlation coefficient between the dominant genera within co-occurrence modules (p value < 0.001 and robust [$|\rho| > 0.6$]) were visualised using the "igraph" package in R software (Zhong *et al.*, 2020). The obtained data were deposited in NCBI Sequence Read Archive under accession no. SRA:SRP471933.

Statistical analysis

Differences in the physicochemical characteristics and bacterial diversity between *meigan cai* and *zao cai* groups were estimated using the *t*-test (GraphPad Prism 7). *p*-values less than 0.05 were considered statistically significant. Unweighted pair-group method with arithmetic means (UPGMA) and principal coordinate analysis (PCoA) were used to compare the bacterial communities between *meigan cai* and *zao cai* (Liu *et al.*, 2019a; 2019c). The graphs were generated using the GraphPad Prism 7 (GraphPad Software).

Results

Physicochemical features of *meigan cai* and *zao cai*

Table 1 shows that the pH value was 3.82 - 4.53 in *meigan cai*, and 3.73 - 5.16 in *zao cai*. The TA of *meigan cai* and *zao cai* was 1.77 - 22.85 g/kg and 2.86 - 20.98 g/kg, respectively. The salt content was lower in *meigan cai* (14 - 66 g/kg) than in *zao cai* (24 - 61 g/kg). The nitrite content of *meigan cai* and *zao cai* varied from 3.42 to 19.64 and 2.56 to 45.83 mg/kg, respectively. Among sucrose, fructose and glucose, the concentration of sucrose was the highest, followed by fructose and glucose, in both *meigan cai* and *zao cai*. In total, the contents of oxalic acid (0 - 23.68 g/kg), lactic acid (0 - 5.14 g/kg), and acetic acid (0 - 18.43 g/kg) were higher in *meigan cai* than in *zao cai*. Among these three organic acids, the concentration of oxalic acid was the highest, followed by lactic and acetic acids, in both samples. There was a significant difference ($p < 0.05$) in glucose between *meigan cai* and *zao cai*, but there were no significant

Table 1. Physicochemical properties of *meigan cai* and *zao cai*.

Group	Sample	Vegetable	Sampling area	pH	TA (g/kg)	S (g/kg)	NI (mg/kg)	Sur (g/kg)	Glu (g/kg)	Fru (g/kg)	OA (g/kg)	LA (g/kg)	AA (g/kg)	Lng	Lat
<i>Meigan cai</i>	MGC1	Mustard	Wuyishan, Fujian	4.24	3.55	41.00	6.26	12.38	0.00	0.00	0.00	0.00	0.05	117.01	25.12
	MGC2	Mustard	Wuyishan, Fujian	4.25	8.47	44.00	10.53	13.50	0.00	0.00	5.97	0.00	0.05	117.01	25.12
	MGC3	Mustard	Longyan, Fujian	3.70	11.72	14.00	3.42	1.22	0.00	1.26	10.34	0.00	0.46	117.01	25.12
	MGC4	Mustard	Longyan, Fujian	3.82	8.96	18.00	19.64	4.45	0.00	1.31	18.68	0.72	0.05	119.52	26.65
	MGC5	Mustard	Longyan, Fujian	4.18	22.85	62.00	8.25	9.06	0.00	1.70	4.22	5.14	0.74	117.01	25.12
	MGC6	Mustard	Longyan, Fujian	4.35	4.63	66.00	17.65	19.75	0.02	0.06	23.68	0.00	0.73	118.03	27.77
	MGC7	Mustard	Ningde, Fujian	4.53	1.77	47.00	0.85	7.37	0.00	0.00	0.00	0.00	0.43	118.03	27.77
<i>Zao cai</i>	ZC1	Mustard	Fuzhou, Fujian	3.96	6.80	48.00	13.66	10.18	0.92	0.41	18.43	3.16	0.05	118.17	26.65
	ZC2	Mustard	Fuzhou, Fujian	3.85	18.71	42.00	14.52	12.36	0.89	1.81	12.41	1.44	0.05	118.17	26.65
	ZC3	Mustard	Nanping, Fujian	3.73	12.80	42.00	2.56	8.54	0.00	0.00	0.00	2.88	0.40	119.39	25.73
	ZC4	Mustard	Nanping, Fujian	3.83	19.11	32.00	16.79	8.51	0.22	1.40	4.30	1.74	0.05	117.36	26.13
	ZC5	Mustard	Fuqing, Fujian	5.16	4.04	34.00	2.56	10.62	0.00	0.00	0.27	0.00	0.05	119.30	26.08
	ZC6	Mustard	Sanming, Fujian	3.74	13.99	59.00	17.65	15.69	0.00	0.74	10.83	0.26	0.05	117.36	26.13
	ZC7	Mustard	Fuqing, Fujian	4.15	20.98	61.00	0.00	12.99	0.34	0.00	17.08	2.60	0.44	117.36	26.13
	ZC8	Mustard	Sanming, Fujian	4.47	2.86	43.00	8.25	8.79	0.00	0.00	0.00	0.00	0.66	119.30	26.08
	ZC9	Mustard	Sanming, Fujian	4.04	6.26	24.00	43.83	5.23	0.00	0.00	0.65	0.00	0.05	117.36	26.13

differences in other physicochemical parameters (pH, TA, salt, nitrite, sucrose, fructose, oxalic acid, lactic acid, or acetic acid) between *meigan cai* and *zao cai* (Figure 1).

Total read number, OTUs, and alpha diversity of *meigan cai* and *zao cai*

Table 2 shows that the 16 fermented vegetables had a total of 1,430,408 reads with an average of 89,401 reads per sample. The reads in each sample ranged from 83,008 to 99,085. The rarefaction curve, species accumulation boxplot, and good coverage indices (> 0.99) revealed that the sample numbers, sequence depth, abundance, and evenness of the species well met the requirements of the sequencing

and analysis for all samples. Both Ace indices (191 - 1218) and Chao indices (177 - 1264) of the fermented foods indicated that the bacterial richness among these samples were significantly different. Sample ZC5 displayed the highest Shannon (4.834) and Simpson indices (0.897), whereas sample MGC5 had the lowest Shannon (1.647) and Simpson indices (0.374). In general, the numbers of observed species (272 ± 113), Ace indices (414 ± 174), Chao indices (380 ± 159), Shannon indices (3.34 ± 0.86), and Simpson indices (0.78 ± 0.18) were lower in *meigan cai* than in *zao cai*. However, there was no significant differences ($p > 0.05$) in the Ace, Chao, Shannon, or Simpson indices found between *meigan cai* and *zao cai* based on the *t*-test results (Figure 1).

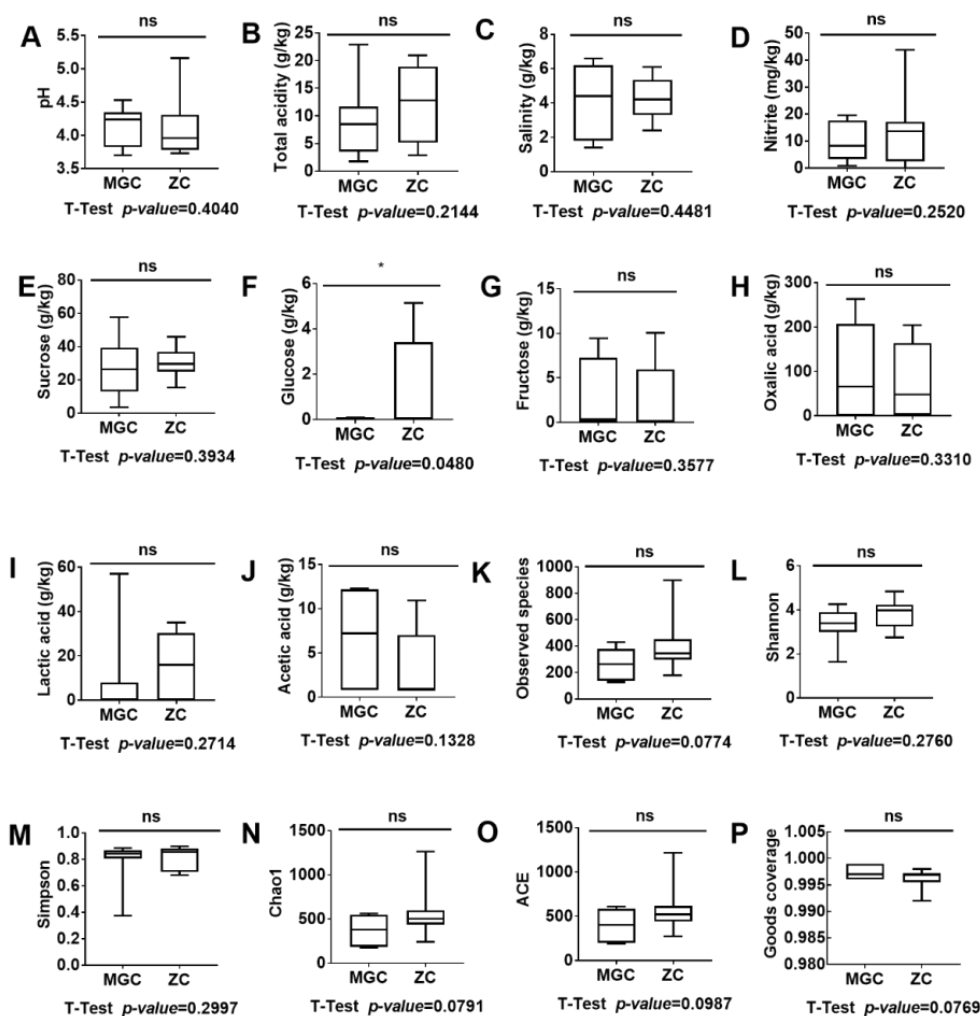


Figure 1. Comparison of physicochemical properties and diversity indices between *meigan cai* and *zao cai*. (A) pH; (B) TA; (C) salinity; (D) nitrite; (E) sucrose; (F) glucose; (G) fructose; (H) oxalic acid; (I) lactic acid; (J) acetic acid; (K) observed species; (L) Shannon; (M) Simpson; (N) Chao 1; (O) ACE; and (P) Goods coverage. * $p < 0.05$, and ** $p < 0.01$. MGC: *meigan cai*; ZC: *zao cai*; and ns: no significant difference.

Table 2. Sample description, number of total reads, and alpha diversity of fermented vegetables.

Group	Sample	Vegetable	Sampling area	Total reads	Observed species	Ace	Chao1	Goods coverage	Shannon	Simpson
<i>Meigan cai</i>	MGC1	Mustard	Wuyishan, Fujian	92,912	264	366	338	0.998	3.38	0.817
	MGC2	Mustard	Wuyishan, Fujian	97,893	429	585	563	0.996	4.255	0.886
	MGC3	Mustard	Longyan, Fujian	83,008	378	608	550	0.996	3.846	0.869
	MGC4	Mustard	Longyan, Fujian	85,380	318	545	467	0.997	3.902	0.841
	MGC5	Mustard	Longyan, Fujian	85,675	139	191	184	0.999	1.647	0.374
	MGC6	Mustard	Longyan, Fujian	94,209	128	200	177	0.999	2.987	0.805
	MGC7	Mustard	Ningde, Fujian	84,107	248	402	382	0.997	3.334	0.845
<i>Zao cai</i>	ZC1	Mustard	Fuzhou, Fujian	95,201	332	556	506	0.996	3.285	0.71
	ZC2	Mustard	Fuzhou, Fujian	86,370	312	524	503	0.997	3.967	0.867
	ZC3	Mustard	Nanping, Fujian	83,447	440	681	652	0.995	4.188	0.899
	ZC4	Mustard	Nanping, Fujian	99,085	466	539	547	0.997	4.292	0.867
	ZC5	Mustard	Fuqing, Fujian	81,334	898	1218	1264	0.992	4.834	0.897
	ZC6	Mustard	Sanming, Fujian	87,344	286	439	452	0.997	2.751	0.681
	ZC7	Mustard	Fuqing, Fujian	92,223	356	473	454	0.997	4.122	0.855
	ZC8	Mustard	Sanming, Fujian	93,508	345	444	424	0.997	3.244	0.701
	ZC9	Mustard	Sanming, Fujian	88,712	180	271	242	0.998	3.224	0.844

Bacterial composition

The microorganisms in these fermented vegetables were classified into 24 phyla, 41 classes, 107 orders, 216 families, 556 genera, and 418 species. Figure 2 shows the top ten phyla, families, and genera. As shown in Figure 2A, the top ten phyla among these samples consisted of Firmicutes (15.6 to 92.2%), Proteobacteria (5.9 to 84.1%), Cyanobacteria (0 to 18.8%), *Deinococcus Thermus* (0 to 15.3%), Actinobacteria (0.2 to 5.0%), Acidobacteria (0 to 0.7%), Fusobacteria (0 to 0.6%), Gemmatimonadetes (0 to 0.6%), Bacteroidetes (0 to 0.5%), and Chloroflexi (0 to 0.2%). Figure 3B shows that the relative abundance of Firmicutes was higher in *meigan cai* (56%) than in *zao cai* (49.1%), whereas the percentage of Proteobacteria was lower in *meigan cai* (39.6%) than in *zao cai* (43.7%).

At the family level (Figures 2C and 2D), the top ten families among these samples had Lactobacillaceae (10.7 to 70.4%), Enterobacteriaceae (3.5 to 79.3%), unidentified Cyanobacteria (0.0 to 8.8%), Moraxellaceae (0.3 to 23.4%), Leuconostocaceae (0.2 to 22.9%), Xanthomonadaceae (0.3 to 4.9%), Burkholderiaceae (0.1 to 10.4%), Bacillaceae (0.1 to 25.2%), Streptococcaceae (0 to 6.7%), and Deinococcaceae (0 to 15.3%), with an average of 41.5, 30.9, 3.4, 5.9, 4.6, 1.7, 1.3, 2.2, 0.5, and 1.0%, respectively. Sample ZC6 had the highest prevalence of Lactobacillaceae, while sample MGC5 had the highest prevalence of Enterobacteriaceae. The relative abundance of Lactobacillaceae was higher in *meigan cai* (46%) than that in *zao cai* (37.9%), while the percentage of Enterobacteriaceae was lower in *meigan cai* (33.8%) than that in *zao cai* (28.6%). At genus level (Figures 2E and 2F), the top ten genera were *Lactobacillus* (5.1 to 69.8%), *Serratia* (2.2 to 79.0%), unidentified Cyanobacteria (0.1 to 15.7%), *Pectobacterium* (0 to 26.4%), *Psychrobacter* (0 to 22.6%), *Acinetobacter* (0.2 to 20.8%), *Pediococcus* (0 to 37.9%), *Weissella* (0.1 to 22.3%), *Bacillus* (0 to 24.9%), and *Deinococcus* (0 to 15.3%), with an average of 36.5, 27.1, 3.4, 2.3, 2.1, 3.8, 5.0, 4.3, 1.9, and 1.0%, respectively. The top ten species in all samples were the *Lactobacillus pentosus* (5.6 to 28.3%), *Pediococcus pentosaceus* (0 to 12.1%), *L. sakei* (2.4 to 34.4%), *L. alimentarius* (0.1 to 246%), *Bacillus anthracis* (0 to 24.5%), *Psychrobacter celer* (0 to 20.3%), *Weissella viridescens* (0 to 19.5%), *Boechera gunnisoniana* (0 to 18.7%), *Acinetobacter baumannii*

(0 to 18.2%), and *Deinococcus geothermalis* (0 to 15.3%) (Table 3).

Based on the weighted and unweighted UniFrac distances, PCoA results revealed that *meigan cai* and *zao cai* had similar bacterial communities (Figures 3A and 3B). Based on the unweighted UniFrac distances, the first (PC1) and second (PC2) axes showed values of cumulative percentage variance of species equal to 21.27 and 14.3%, respectively. In addition, based on the weighted UniFrac distances, PC1 and PC2 accounted for 73.83 and 9.65% of the variance in the microbiota of samples, respectively. Based on the Unifrac distance, most examples formed a cluster, except sample ZC5. In contrast, the Cluster-tree (weighted Fast Unifrac), showed that all samples were divided into two clusters (Figure 3C). Samples MGC1, MGC3, MGC4, ZC5, ZC6, and ZC9 formed the first cluster, and samples MGC2, MGC5, MGC6, MGC7, ZC1, ZC2, ZC3, ZC4, ZC7, and ZC8 formed the second cluster (Figure 3D). Therefore, both unweighted Fast Unifrac distance and weighted Fast Unifrac distance indicated a similar microbial structure between *meigan cai* and *zao cai* samples, consistent with the results of PCoA results.

Relationships between bacterial communities and environmental factors

The relationships between the bacterial community structures and environmental factors (Table 2) were evaluated using the ordination diagram based on canonical correlation analysis (CCA) (Figure 3E). Axes 1 and 2 accounted for 37.2 and 22.81% of the total constrained variation, respectively.

Network analyses of bacterial communities

Based on the genera of *meigan cai* and *zao cai*, the spearman's rank correlation coefficient between the dominant genera and the abundance of the top 50 OTUs was calculated [strong ($\rho > 0.6$) and significant ($p < 0.05$) correlations]. The resulting network consisted of 48 dominant OTU genera in *meigan cai* and 50 dominant OTU genera in *zao cai*. The modularities of *meigan cai* and *zao cai* were 0.556 and 0.43, respectively, indicating that the network had a modular structure. The genera in the co-occurrence networks of *meigan cai* and *zao cai* were classed into six phyla, and the main phyla were Firmicutes and Proteobacteria. Compared to *zao cai*,

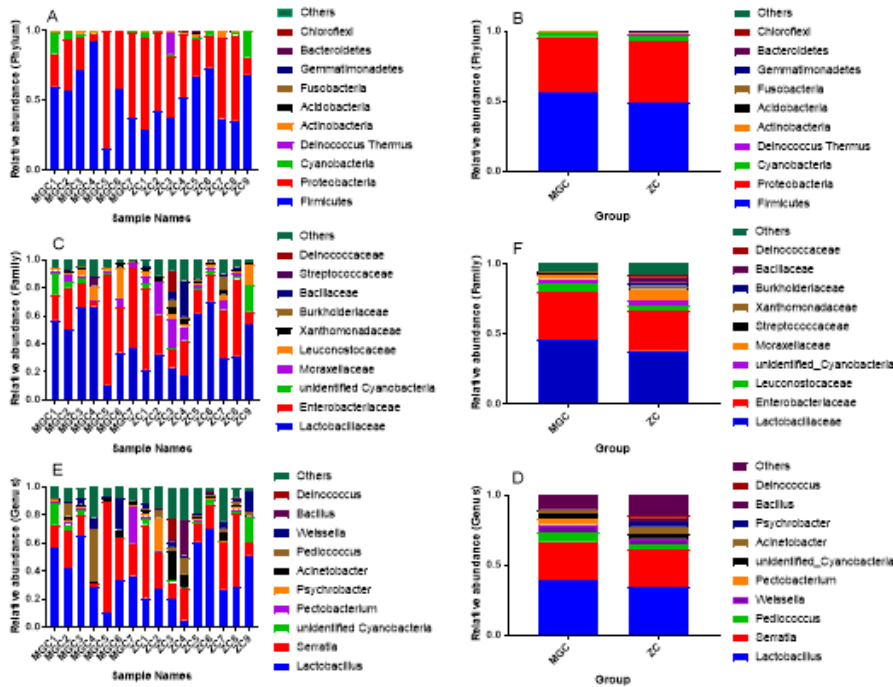


Figure 2. Bacterial composition of *meigan cai* and *zao cai* based on OTUs (3% distance). Relative bacterial abundances at phylum (A and B), family (C and D) and genus (E and F) levels. MGC: *meigan cai*; ZC: *zao cai*.

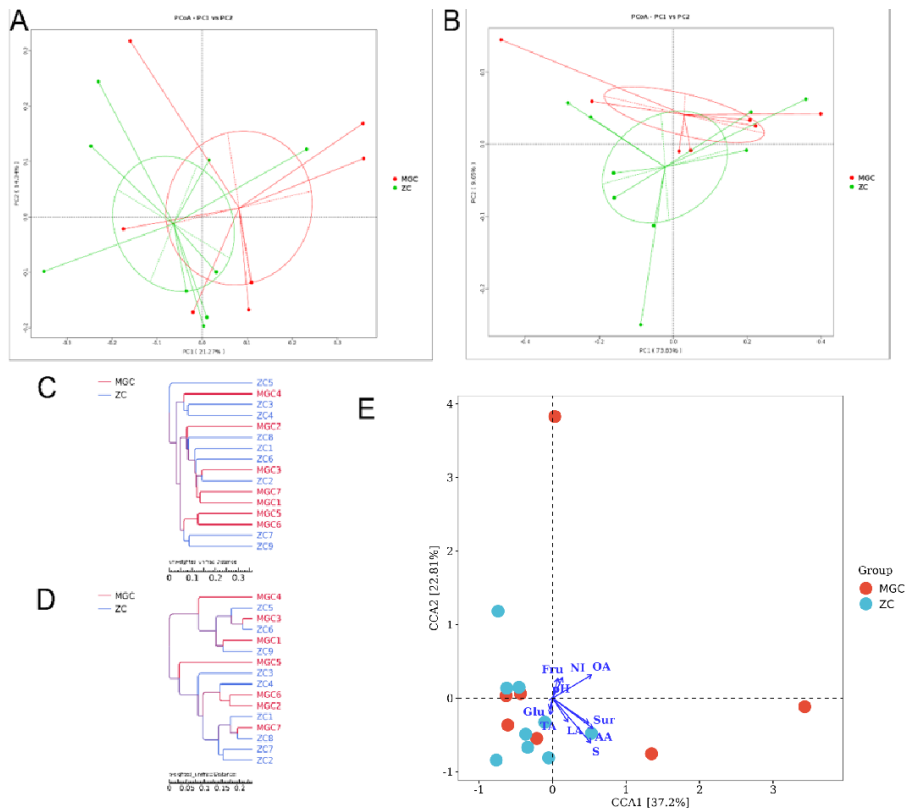


Figure 3. Principal coordinate analysis (PcoA) of unweighted UniFrac distances (A) and weighted UniFrac distances (B), cluster-tree analysis of unweighted UniFrac distances (C) and weighted UniFrac distances (D), and CCA (canonical correlation analysis) ordination between bacterial communities and physicochemical variables (environmental variables) in fermented vegetables (E). MGC: *meigan cai*; ZC: *zao cai*; TA: Titratable acids; S: salinity; LA: lactic acid; AA: acetic acid; OA: oxalic acid; Glu: glucose; Sur: sucrose; Fru: fructose; NI: nitrite; Lng: longitude; and Lat: latitude.

Table 3. Relative abundance (%) of top ten species in dominant bacteria in *meigan cai* and *zao cai*.

Group	Sample	<i>L. pentosus</i>	<i>Ped. pentosaceus</i>	<i>L. sakei</i>	<i>L. alimentarius</i>	<i>Bac. anthracis</i>	<i>Psy. celer</i>	<i>Wei. viridescens</i>	<i>Boe. gunnisoniana</i>	<i>Aci. baumannii</i>	<i>Dei. geothermalis</i>	Other
<i>Meigan cai</i>	MGC1	5.6	0.0	34.4	1.5	0.0	0.5	0.0	15.3	0.6	0.0	42.0
	MGC2	14.2	2.6	4.0	3.0	0.0	1.1	0.0	1.6	0.6	0.0	72.7
	MGC3	19.1	0.4	22.2	6.5	0.1	0.7	0.1	3.0	0.5	0.0	47.5
	MGC4	8.5	36.3	2.4	2.3	0.1	0.0	0.1	0.0	0.2	0.0	50.0
	MGC5	3.9	0.0	3.0	0.1	0.0	0.0	0.9	0.1	1.7	0.0	90.2
	MGC6	24.8	0.0	5.2	0.5	0.0	0.0	19.5	0.0	2.7	0.0	47.2
	MGC7	10.5	0.0	4.5	1.2	0.0	0.7	0.0	0.1	2.7	0.0	80.2
M ± SD ^a	12.4 ± 7.5	5.6 ± 13.6	10.8 ± 12.5	2.2 ± 2.2	0.0 ± 0.0	0.4 ± 0.4	2.9 ± 7.3	2.9 ± 5.6	1.3 ± 1.1	0.0 ± 0.0	0.0 ± 0.0	61.4 ± 19.2
<i>Zao cai</i>	ZC1	9.6	0.2	3.9	2.6	0.0	1.0	2.8	2.6	1.1	0.0	76.3
	ZC2	11.8	4.8	2.8	2.1	0.3	20.3	0.0	0.1	0.6	0.0	57.1
	ZC3	14.7	2.9	2.4	0.1	1.1	0.0	0.2	1.4	18.2	15.3	43.8
	ZC4	1.8	12.1	0.5	0.2	24.5	0.0	0.0	0.1	5.8	0.2	54.8
	ZC5	11.7	0.0	5.6	24.6	0.1	0.6	0.0	0.7	0.4	0.0	56.2
	ZC6	52.8	0.1	6.5	2.0	0.0	0.6	0.0	2.4	1.7	0.1	33.7
	ZC7	9.7	3.0	3.9	5.9	0.3	0.1	0.4	0.2	1.6	0.2	74.8
	ZC8	11.5	2.4	3.8	1.6	0.0	0.8	0.0	1.3	1.5	0.0	77.1
	ZC9	28.3	2.6	5.0	4.6	0.1	0.0	0.1	18.7	0.1	0.0	40.6
M ± SD ^a	16.9 ± 15.2	3.1 ± 3.7	3.8 ± 1.8	4.9 ± 7.6	2.9 ± 8.1	2.6 ± 6.7	0.4 ± 0.9	3.1 ± 5.9	3.5 ± 5.8	1.8 ± 5.1	0.0 ± 0.0	57.2 ± 16.2

(^a) mean ± standard deviation. *L. pentosus*, *Ped. pentosaceus*, *L. sakei*, *L. alimentarius*, *Bac. anthracis*, *Psy. celer*, *Wei. viridescens*, *Boe. gunnisoniana*, *Aci. baumannii*, and *Dei. geothermalis* represented *Lactobacillus pentosus*, *Pediococcus pentosaceus*, *Lactobacillus sakei*, *Lactobacillus alimentarius*, *Bacillus anthracis*, *Psychrobacter celer*, *Weissella viridescens*, *Boechera gunnisoniana*, *Acinetobacter baumannii*, and

meigan cai showed higher modularity and lower average degree, which indicated more significant niche difference in *meigan cai* than that in *zao cai*. Therefore, the biofilm systems of bacterial communities might become more specific in *meigan cai* than in *zao cai*.

Discussion

Bacterial communities play a critical role in fermented vegetables (Wu *et al.*, 2015; Xiao *et al.*, 2018), hence, their information is very important. Although *meigan cai* and *zao cai* are commonly consumed in Fujian Province of China, few studies have investigated the bacterial communities on these two fermented vegetables. In the present work, we investigated and compared the bacterial communities between *meigan cai* and *zao cai*.

In general, TA was > 0.3 g/100 g, which agreed with the TA levels in most fermented vegetables (Zhang *et al.*, 2016; 2018a). In addition, similar to other low salt fermented vegetables (Zhao and Ding, 2008; Zhang *et al.*, 2016; Liu *et al.*, 2019b), the salinity of most *meigan cai* and *zao cai* samples in the present work was < 50 g/kg, suggesting that these two fermented vegetables are low-salt fermented vegetables.

In accordance with other Chinese fermented vegetables, such as *pao cai* (Cao *et al.*, 2017), *zha cai* (Liang *et al.*, 2018), and serofluid dish (Chen *et al.*, 2016), *meigan cai* and *zao cai* were also mainly composed by Firmicutes, followed by Proteobacteria. At the genus level, the present and previous works showed that *Lactobacillus* was the main genus in Chinese fermented vegetables (Chen *et al.*, 2016; Cao *et al.*, 2017; Li *et al.*, 2017; Liu and Tong, 2017), indicating that *Lactobacillus* plays a crucial role in Chinese fermented vegetables. However, the results differed from the results of Korean kimchi, a famous fermented vegetable in Asian, which contains mainly *Leuconostoc*, *Lactobacillus* and *Weissella*. Moreover, *Leuconostoc* and *Weissella* were not the main genera found in the present work.

As the second most dominant genus in the present work, *Serratia* was commonly found in most samples, which was quite different from previous studies which reported that *Weissella*, *Leuconostoc*, or *Pediococcus* existed in Chinese fermented vegetables as the second most dominant genus (Liang

et al., 2018; Zhang *et al.*, 2018a; Zhou *et al.*, 2018). We speculate that these differences might have been related to several factors, such as production process, raw materials and geographical distributions, salinity, and fermentation temperatures (Jeong *et al.*, 2013; Lee *et al.*, 2017; 2018; Park *et al.*, 2018). *Serratia* is opportunistic pathogen which could cause tract infections, intravenous catheter-associated infections, urinary tract infections (UTIs), pneumonia, septicaemia, meningitis, endocarditis, and wound infections (Devi *et al.*, 2018). The percentage of *Serratia* was very high in *meigan cai* and *zao cai*. Although previous work have reported that many fermented vegetables such as *pao cai* (Cao *et al.*, 2017) and Korean kimchi (Park *et al.*, 2012) were contaminated by *Serratia*, but the present work is the first report to say that *Serratia* was the second dominant genus in both *meigan cai* and *zao cai*. Therefore, the result of the present work indicated that *meigan cai* and *zao cai* were easily contaminated by *Serratia*, thus warranting its control in the fermentation of home-made *meigan cai* and *zao cai* to avoid infection and spoilage.

In total, the average relative abundance of *L. pentosus* was lower in *meigan cai* than in *zao cai*, whereas the average relative abundance of *P. pentosaceus* and *L. sakei* was higher in *meigan cai* group than in *zao cai*. The relative abundance of *L. Pentosus*, *P. pentosaceus*, and *L. sakei* was the highest in samples ZC6, MGC4, and MGC1, respectively. Compared with other samples, samples ZC7 and MGC4 had much higher relative abundance of *L. pentosus*. Samples ZC4 and ZC5 had the highest relative abundance of *L. alimentarius* and *B. anthracis*, respectively. In addition, the highest percentage of *W. viridescens*, *B. gunnisoniana*, and *A. baumannii* were in the samples ZC2, MGC6, and ZC9, respectively. Our results showed that *L. pentosus*, *P. pentosaceus*, *L. sakei*, and *L. alimentarius* were the most prevalent species in both *meigan cai* and *zao cai*, which differed from the previous studies that reported *L. plantarum* was present in many kinds of fermented vegetables, such as *jiang shui*, *pao cai*, and *suan cai* (Xiong *et al.*, 2012; Wu *et al.*, 2015; Zhang *et al.*, 2018b). Therefore, these results indicated that the *Lactobacillus* species in different fermented vegetables were different (Cao *et al.*, 2017; Zhang *et al.*, 2019; Guan *et al.*, 2020).

The most dominant genus *Lactobacillus* showed a positive connection with *Psychrobacter*, *Arthrobacter*, *Pseudomonas*, *Sphingomonas*, and *Massilia* in *meigan cai*, and a negative connection with *Stenotrophomonas*, *Acinetobacter*, *Staphylococcus*, *Anaeroplasma*, *Brevibacterium*, and *Streptococcus* in *zao cai*. As the second most dominant genus, *Serratia* showed a negative connection with other genera (*Bacillus*, *Enterococcus*, *Brevibacterium*, *Microbacterium*, and *Streptococcus*) in *meigan cai*, and a positive connection with other genera (*Psychrobacter* and unidentified *Enterobacteriaceae*) in *zao cai*. *Pediococcus* and *Weissella*, belonging to the Lactobacillaceae family, two of the most abundant species in fermented vegetables, are also found in the present work. *Pediococcus* in *meigan cai* and *zao cai* was associated with eight and three genera, respectively. *Weissella* in *meigan cai* and *zao cai* was associated with three and four genera, respectively. *Pediococcus* was associated with *Acetobacter*, *Lactococcus*, *Rhodococcus*, *Enterococcus*, *Brevibacterium*, *Streptococcus*, and *Cutibacterium* in *meigan cai*, but not in *zao cai*. In turn, *Pediococcus* was associated with *Staphylococcus* in *zao cai*, but not in *meigan cai*. In addition, *Weissella* showed a negative association with *Raoultella*, *Enterococcus*, and *Dyella* in *zao cai*, while there were both positive and negative correlations between *Weissella* and other genera in *meigan cai*. These variances indicated that the correlations among genera in various fermented varied, underscoring the likelihood that differences in microbial symbiotic relationships were linked to environmental factors (Huang *et al.*, 2020; Zhong *et al.*, 2020).

Conclusion

The present work revealed the dominant bacteria in different fermented vegetables. A wide biodiversity of bacterial communities was found among 16 home-made fermented vegetables samples. We found that the bacterial communities were similar between *meigan cai* and *zao cai*. To the best of our knowledge, this is the first study that investigated the microbial diversity of *meigan cai* and *zao cai* in Fujian Province, China. The network analysis revealed different associations among the bacterial communities of *meigan cai* and *zao cai*, and a guide to produce these fermented vegetables. Future studies should clarify the similarities in bacterial composition

between *meigan cai* and *zao cai*, and the associations among bacteria in these two fermented vegetables.

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