Original Article
Food Science and Technology

ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)

DOI: https://doi.org/10.1590/fst.104821

Unraveling the effects of *Lactobacillus sakei* inoculation on the microbial quality and bacterial community diversity of chili sauce by high-throughput sequencing

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Abstract

In this study, the effects of the starter culture *Lactobacillus sakei* on microbial quality and the bacterial community diversity of chili sauce were investigated. In starter culture inoculated chili sauce (CPN sample), *Lactobacillus* spp. was predominant bacteria with 86.08% of the relative abundance on the 30th day of fermentation. Meanwhile, the spoilage organisms and pathogenic bacteria, including *Staphylococcus* spp., *Enterobacter* spp., *Pseudomonas* spp. and *Neorhizobium* spp. were nearly undetectable in starter culture inoculated chili sauce. In contrast, *Lactobacillus* spp. and *Weissella* spp. were predominant bacteria, and trace *Pseudomonas* spp. and *Neorhizobium* spp. were still detectable at the end of fermentation in the spontaneous fermentation (CPS sample). These results revealed that *L. sakei* could be used as a potential starter culture for the microbial quality improvement.

Keywords: bacterial community diversity; chili sauce; *Lactobacillus sakei*; microbial quality.

Practical Application: Chili sauce as a Chinese typical fermented condiment product is well preferred by most Chinese consumers. However, the hazards of microorganisms a in fermented foods had attracted researchers to attention. With the development of sequencing technology, the structure and succession of bacteria community would be analyzed by high-throughput technology in fermented foods. In order to investigate the effects of starter culture on the microbial quality and the structure and succession of bacteria community in chili sauce, we systematically screened *Lactobacillus sakei* as starter culture. The results showed that starter culture can improve microbial quality by inhibiting the growth of native microflora originated from raw and supplemental materials, such as *Staphylococcus* spp., *Lonsdalea* spp., *Pseudomonas* spp., *Neorhizobium* spp., *Enterobacter* spp.. These results provide valuable information for further studies of looking for relations with microorganism in chili sauce.

1 Introduction

Chili sauce as the Chinese traditional fermented condiment has been well preferred by Chinese consumers, especially in Sichuan province in China. The deterioration in sensory properties of chili sauce by spontaneous fermentation are mainly caused by foodborne spoilage microorganisms and pathogenic bacteria, resulting in the chili sauce that is not homogenous or one that is of inferior quality with safety standards that cannot be guaranteed. It has been reported that the gas-forming spoilage bacteria, mainly identified as *Bacillus licheniformis, Lactobacillus acidipiscis,* and *Lactobacillus alimentarius*, have been isolated from chili sauce (Niu et al., 2020). Furthermore, pathogenic bacteria, such as *Escherichia coli*, *Yersinia enterocolitica,* and *Bacillus thuringiensis*, have been detected in chili sauce (Niu et al., 2020; Estrada-Garcia et al., 2002). Thus, microbial quality during fermentation is one of the key factors in determining the sensory, flavor, and safety profiles of chili sauce.

Recently, the starter cultures have been applied into fermented foods to improve their nutritional quality and prolong shelf life. These starter cultures could induce the production of bacteriocin or acidic byproducts, which could inhibit the growth of foodborne pathogens (Kim et al., 2018). It is noteworthy that *L. sakei* can thrive in high-salt, low-water, low-temperature, and low-pH conditions (Chiaramonte et al., 2009), and has an ability to produce bacteriocins to inhibit pathogenic bacteria (Aasen et al., 2000). It has been reported that *L. sakei* inoculation in fermented food could inhibit the growth of Enterobacteriaceae and *Listeria monocytogenes* (Wang et al., 2015a, b).

Although the value of the starter culture is well known, its contribution to microbial quality in Chili sauce has not been fully described. Therefore, it is interest to further characterize the microbial quality of Chili sauce fermented by the starter culture inoculation. Microbial quality has been mainly assessed using culture dependent method, which has the drawback of detecting only cultivable bacteria, potentially only a small portion of the true microbial population (Wang et al., 2018a; Syahbanu et al., 2020). Thus, there is a high risk of misidentification of the microbial ecology using culture dependent method. In recent years, molecular biology-based techniques that are culture independent have been widely used. These techniques include denaturing gradient gel electrophoresis (DGGE), restriction fragment length polymorphism analysis (RFLP), and random amplified polymorphic DNA fingerprinting analysis (RAPD).

Received 02 Oct., 2021
Accepted 17 Nov., 2021
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Nowadays, the emergence of high throughput sequencing (HTS) technology has widened the scope of microbial analysis in nature, and can provide additional detailed information compared with RFLP, RAPD, and DGGE. Notably, HTS can improve the quality and speed of sequencing. Recently, HTS was used to investigate the diversity of the microbial community in sausage (Wang et al., 2018b) and douchi (Yang et al., 2016; Gaviao et al., 2021).

In this study, the starter culture of *L. sakei* was inoculated in chili sauce. Comparative analysis of bacterial communities by HTS technology in chili sauce between starter culture inoculation and spontaneous fermentation were performed to gain insight regarding the improvement of microbial quality.

2 Materials and methods

2.1 Starter culture

L. sakei used as a starter culture in this study was successfully isolated and purified from Sichuan pickles by Meat-Processing Application Key Lab of Sichuan Province, China, which was cultured to a final concentration of 10^7–10^8 CFU/g and then immediately prepared into freeze-drying powder.

2.2 Chili sauce preparation and sampling

Fresh chili peppers (*Capsicum frutescens L.*) were purchased from a local market (Sichuan, China) and kept at 4°C prior to use, which variety was Nanchong Chao Tian La, which is cultivated in Nachong city, Sichuan Province, China. The fresh chili peppers were washed with distilled water and then crushed for 15 s by a crusher. After crushing, the r crushed pepper was mixed with other ingredients. Chili sauce was manufactured according to the following recipe described by Niu et al. (2020) with a minor modification to yield a kilogram of the final product: chili (760 g), salt (60 g), garlic (80 g), Chinese prickly ash (3 g), Allium macrostemon Bunge (25 g), Litsea pungens Hemsl (50 g), and other ingredients (20 g). The mixture was inoculated with 0.05% (w/w) of the starter culture labeled as CPN, and the mixture without the starter culture inoculation was labeled as CPS (as the control). After inoculation, mixture was transferred into 5 liter ceramic jars and fermented anaerobically at 25 °C with a humidity level of 85-90%. The chili sauce (100g) was periodically sampled in triplicate from fermentation on day 0, 5, 10, 15, 20, 25, 30, 35, and 40 for physicochemical properties and microbial quality analyses.

2.3 Bacterial counts

Microbial quality in the chili sauce samples was monitored by culture-dependent method based on plate counts. Total viable counts (TVC), lactic acid bacteria (LAB) counts, *Pseudomonas* spp. counts, *Bronchothrix* spp. counts and *Enterobacteriaceae* counts were performed according to the methods described as Wang et al. (2018a). Briefly, 5 g of sample was removed aseptically from each group to sterile bags containing 225 mL of 0.85% saline (Chen et al., 2019; Boeno et al., 2019). Then the mixture was homogenized for 2 min using a sterile homogenizer (Scientz-11L, China). Thereafter, serial decimal dilutions were undertaken using 0.85% for microbiological analysis.

TVC and LAB counts were enumerated on plate count agar (PCA, Sangon Biotech Co. Ltd, Shanghai, China) and De Man, Rogosa, Sharpe Agar (MRS, Sangon Biotech Co. Ltd, Shanghai, China), respectively, which were all incubated at 37 °C for 48 h (Ramos, et al., 2021). *Pseudomonas* selective Agar (Sangon Biotech Co. Ltd, Shanghai, China) and Steptomycin Thallous Acetate Agar (STAA, Shandong Tuopu Biological Engineering Co., Ltd., Shandong, China) were applied for *Pseudomonas* spp. counts and *Bronchothrix* spp. counts, respectively, which were all incubated at 25 °C for 48 h. *Enterobacteriaceae* was determined on Violet Red Bile Glucose Agar (VRBGA, Shandong Tuopu Biological Engineering Co., Ltd., Shandong, China) with incubation at 37 °C 24 h. The results were expressed as $\log_{10}$ CFU/g sample.

2.4 Bacterial community analysis and succession analyses by high throughput sequencing

DNA extraction

The E.Z.N.A™ Mag-Bind Soil DNA Kit (OMEGA, USA) was used to extract the microbial DNA of samples. DNA quality was assessed using agarose gel electrophoresis according to the method described by Wang et al. (2018a). The DNA concentration was determined according to the guarantee values of OD_{260}/OD_{280} and OD_{260}/OD_{230} above 1.8 and 2.0, respectively. The DNA quality was checked on a 1.5% agarose gel according to the methods as reported by Wang et al. (2018a).

Illumina high-throughput sequencing

The DNA was extracted to use as the template and the V4 region of bacterial 16S rRNA was amplified using PCR with the universal bacterial primers 515F and 806R (Wang et al., 2019). The PCR amplification consisted of a two-step PCR technology. The first PCR step used untagged primers to work 25 cycles, whereas the second PCR step used tagged primers with the products from the first step as a template to work 5 cycles, according to the methods reported by Wang et al. (2021). Then the sequencing reaction was carried out on a MiSeq Illumina instrument (Illumina, USA) at Sangon Biotech Co., Ltd (Shanghai, China).

Data analysis

The Operational taxonomic unit (OTU) at an identity threshold of 97% using UPARSE software (Edgar, 2010) was used and set to 97% sequence similarity to use RDP to calculate estimator values of the Shannon index and Chao1 (Minoche et al., 2011). Alpha diversity was evaluated using Good's coverage, Simpson, Shannon diversity indices and Chao 1 richness (Grice et al., 2009). Principal component analysis (PCA) depicted the composition of the bacterial communities in chili samples. The similarities and differences in bacterial community among all samples are depicted using a Venn diagram (Liu et al., 2018).

Statistical analysis

Data were given as mean values accompanied with the standard deviation. Duncan's multiple range test (significance

p<0.05) was employed for the independence of error terms using the SPSS Statistics software (IBM, Chicago, Ill., U.S.A.).

3 Results and discussion

3.1 Effect of the starter culture on microbial quality of chili sauce

Five principal microbiological indicators, namely bacterial TVC counts, LAB counts, *Brochothrix* spp. counts, *Pseudomonas* spp. counts and *Enterobacteriaceae* counts, were used to evaluate the microbial quality. The results of bacterial enumeration of all samples during fermentation are shown in Figure 1. The *Enterobacteriaceae* and *Brochothrix* spp. were not detected in all samples through the fermentation ($\log_{10}$ CFU/g < 1). As shown in Figure 1A, the initial bacterial TVC counts were 3.65 $\log_{10}$ CFU/g and 4.85 $\log_{10}$ CFU/g in CPS and CPN samples, respectively. As fermentation time extended, the bacterial TVC counts in CPN sample markedly increased and to 7.8 $\log_{10}$ CFU/g on the 20th day, then the bacterial TVC counts reduced rapidly and reduced to 4.25 $\log_{10}$ CFU/g at the end of fermentation. Correspondingly, the bacterial TVC counts in CPS sample were significantly lower than that of CPN sample. As shown in Figure 1B, the LAB counts both in CPS and CPN samples exhibited a similar change with the bacterial TVC counts. Both the bacterial TVC counts and LAB counts in CPN samples were higher than that of CPS samples because of inoculation of *L. sakei*. Moreover, at the beginning of fermentation, trace *Pseudomonas* spp. which have been considered as the spoilage bacteria. were detected both in CPS and CPN samples. At the end of fermentation, trace *Pseudomonas* spp. were still detected in CPS sample, while *Pseudomonas* spp. were not detected in CPN sample. The results revealed that the growth of *Pseudomonas* spp. in chili sauce could be inhibited by inoculation of *L. sakei* through interspecific competition, which are conducive to hygiene levels improvement in chili sauce during fermentation.

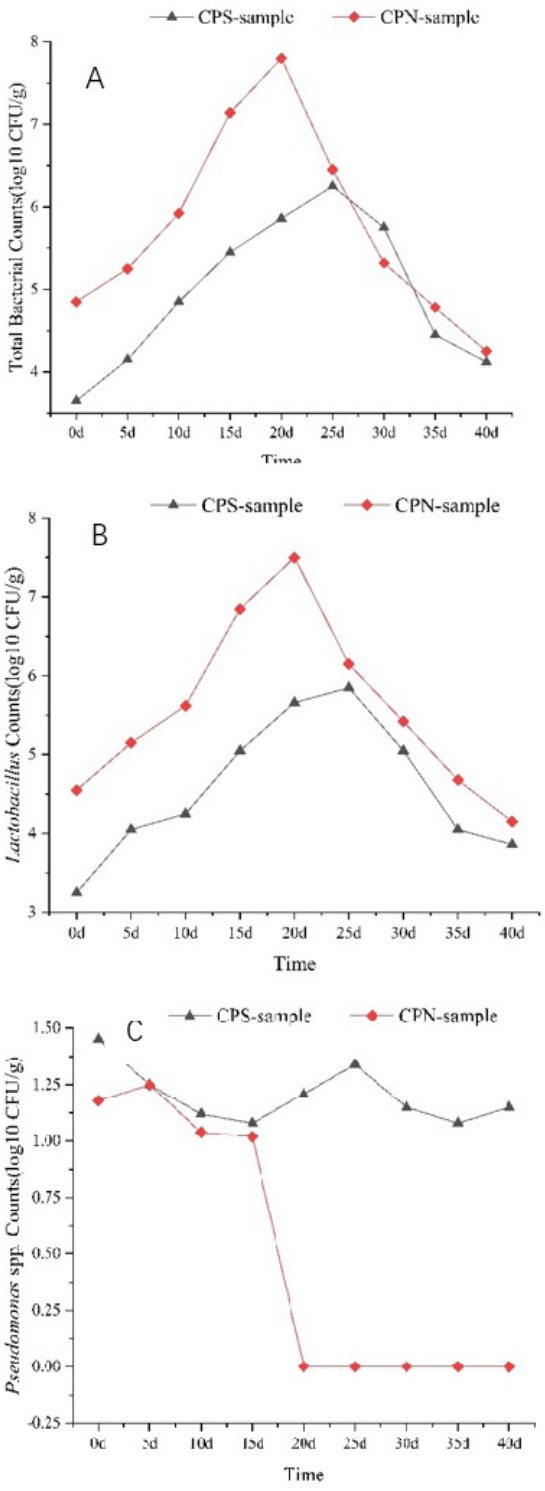

Figure 1. The Effect of *Lactobacillus sakei* inoculation on bacterial TVC counts (A), LAB counts (B) and *Pseudomonas* spp. counts (C) in chili sauce during fermentation.

3.2 Effect of the starter culture on bacterial communities

Comparison of alpha diversity in the CPS sample and CPN sample

The bacterial alpha diversities of CPS and CPN samples over different fermentation time are shown in Table 1. The ACE index, Chao 1, Shannon and Simpson indices were used to compare the diversity and abundance of bacterial communities in chili sauce. All high-quality bacterial tags of the CPS and CPN samples were 72,513 and 101,146, respectively, which were generated from 18 examined samples sets, across the entire chili-sauce fermentation process, namely CPS-1 (0 d), CPS-2 (5 d), CPS-3 (10 d), CPS-4 (15 d), CPS-5 (20 d), CPS-6 (25 d), CPS-7 (30 d), CPS-8 (35 d), CPS-9 (40 d), CPN-1 (0 d), CPN-2 (5 d), CPN-3 (10 d), CPN-4 (15 d), CPN-5 (20 d), CPN-6 (25 d), CPN-7 (30 d), CPN-8 (35 d), and CPN-9 (40 d). All sequences were clustered with a 97% sequence identity level cut-off, in which the OTUs ranged from 118 to 203 in the CPN samples, and from 119 to 198 in the CPS sample. A total of 15 OTUs and 10 OTUs were shared by the CPN and CPS samples, respectively, as shown in Figure 2. These results indicated that the succession of bacterial diversity communities of the CPN sample has a low level changed than that of the CPS sample during the fermentation. Good's coverage values ranged from

Table 1. The Operational Taxonomic Units (OTUs) for CPN and CPS samples.

Time (d)	Reads		Observed OTUs		ACE Index		Chao 1 Index		Good's coverage		Shannon Index		Simpson	
	CPS	CPN	CPS	CPN	CPS	CPN	CPS	CPN	CPS	CPN	CPS	CPN	CPS	CPN
0	6000	6570	140	137	413.93	280.85	269.55	231.55	0.99	0.99	1.41	1.47	0.50	0.48
5	5925	7871	141	147	321.90	330.07	252.71	237.23	0.99	0.99	1.43	1.86	0.54	0.29
10	6374	8274	148	170	429.48	518.00	254.73	398.00	0.99	0.99	1.67	1.58	0.43	0.38
15	7651	5919	198	154	530.61	372.09	367.45	271.04	0.99	0.99	2.00	1.97	0.26	0.31
20	7337	6910	180	187	356.45	567.23	280.64	340.03	0.99	0.99	2.04	1.95	0.25	0.29
25	6395	7943	193	203	316.81	541.50	300.33	381.36	0.99	0.99	1.80	1.59	0.38	0.39
30	10160	17521	124	127	316.70	403.02	238.38	265.06	0.99	1.00	1.48	0.75	0.46	0.73
35	11406	22599	139	128	321.18	307.11	216.54	211.18	0.99	1.00	1.32	0.73	0.52	0.75
40	11265	17539	119	118	349.85	267.83	223.12	182.57	0.99	1.00	1.13	0.82	0.59	0.72

CPS: control sample; CPN: sample inoculated with the starter culture.

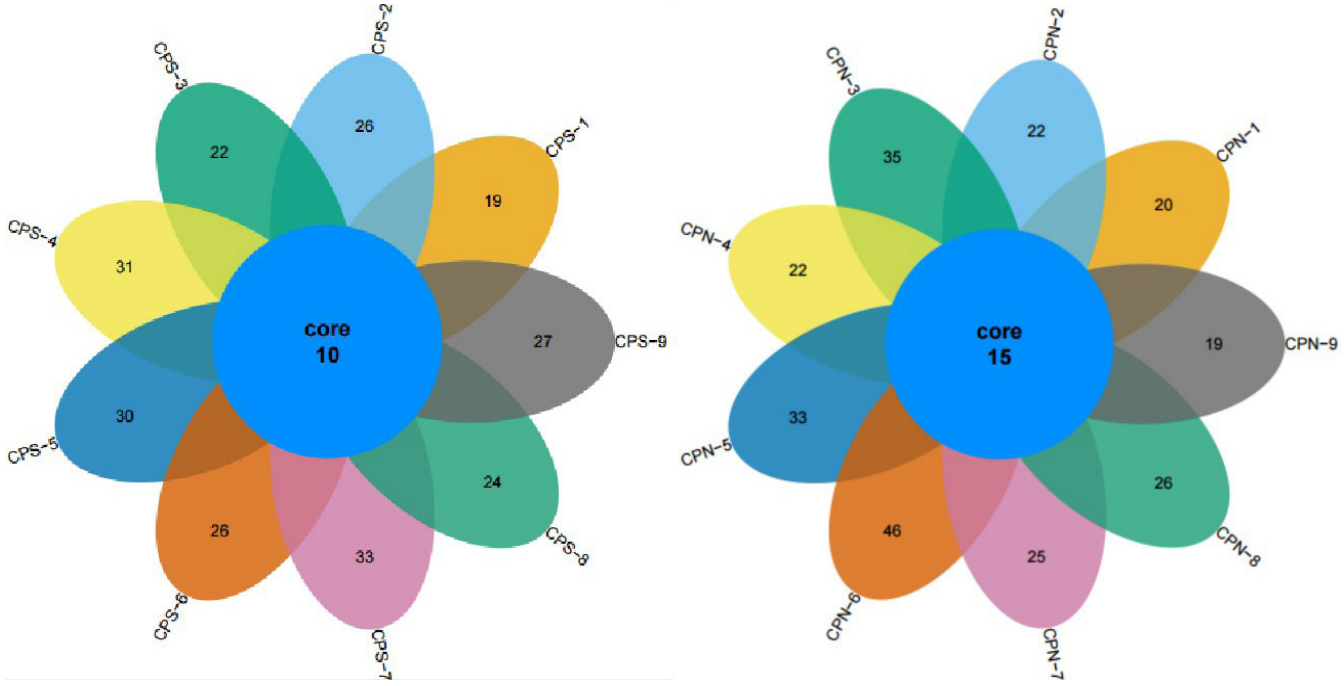

Figure 2. CPS-1: control sample fermented for 0 d, CPS-2: control sample fermented for 5 d, CPS-3: control sample fermented for 10 d, CPS-4: control sample fermented for 15 d, CPS-5: control sample fermented for 20 d, CPS-6: control sample fermented for 25 d, CPS-7: control sample fermented for 30 d, CPS-8: control sample fermented for 35 d, CPS-5: control sample fermented for 40 d, CPN-1: sample inoculated with starter culture and fermented for 0 d, CPN-2: sample inoculated with starter culture and fermented for 5 d, CPN-3: sample inoculated with starter culture and fermented for 10 d, CPN-4: sample inoculated with starter culture and fermented for 15 d, CPN-5: sample inoculated with starter culture and fermented for 20 d, CPN-6: sample inoculated with starter culture and fermented for 25 d, CPN-7: sample inoculated with starter culture and fermented for 30 d, CPN-8: sample inoculated with starter culture and fermented for 35 d, CPN-9: sample inoculated with starter culture and fermented for 40 d.CPS: control sample; CPN: sample inoculated with the starter culture.

99-100% for bacterial OTUs in all samples, which suggested that the major bacterial OTUs were captured (Grice et al., 2009). The overall trend of the bacterial Chao 1 richness estimate increased from 0 to 15 d fermentation with a range from 269.55 to 367.45 in the CPS sample, then decreased from 15 d to 40 d, and eventually reached 223.12 on the 40th day. Accordingly, the bacterial Chao 1 richness estimate of CPN samples fluctuated during the entire fermentation process. The CPN sample richness estimator indicated a change from 231.55 to 398.00 from 0 to 10 d, then decreased slightly from 10 to 15 d, reached 381.36 on the 25th day, and finally achieved its minimum value of 182.57 on the 40th day. In addition, the Shannon index for bacterial diversity of the CPS and CPN samples was 1.13 and 0.82 at the end of the fermentation, respectively. The Shannon index of the CPN sample was lower than that of the control sample, suggesting the complexity of the bacterial structure and abundance during spontaneous fermentation. Accordingly, the starter culture (*L. sakei*) as the dominant bacteria can inhibit other bacteria in the fermentation environment as well as in the raw material.

Effect of starter cultures on the composition of the bacterial community

The effect of *L. sakei* on the bacterial community during the fermentation of chili sauce is shown in Figure 3 and Figure 4. In the chili sauce inoculated with the starter culture (CPN sample), the shared bacterial OTUs mainly belonged to Firmicutes and Proteobacteria at the phylum level, and *Lactobacillus* spp., *Weissella* spp. and *Pediococcus* spp. at the genus level. Moreover, in the control sample, the shared bacterial OTUs mainly belonged to Firmicutes and Proteobacteria at the phylum level, and *Weissella* spp., *Enterobacter* spp., *Lactobacillus* spp., and *Pediococcus* spp. at the genus level. The levels of the Proteobacteria, Bacteroidetes, and Actinobacteria in chili sauce inoculated with *L. sakei* were lower than that of the spontaneous fermentation at the end of the fermentation. Firmicutes was the dominant phylum at all fermentation stages, and its abundance increased from 9.39% to 92.58% from the first day to the 35th day in the CPN sample. Subsequently, a slight drop was observed in the relative abundance of bacteria on the 40th day.

During the initial fermentation, *Enterobacter* spp. was the most abundant bacterial genus in the CPS sample, accounting for 8.17% of bacteria. Furthermore, the proportion of other spoilage organisms and pathogens in the CPS sample, such as *Staphylococcus* spp., *Lonsdalea* spp., *Pseudomonas* spp. and *Neorhizobium* spp. was 0.12%, 0.90%, 2.93%, and 1.00%, respectively. However, *Lactobacillus* spp., *Pediococcus* spp. and *Weissella* spp. were barely detected in the CPS sample at the initial fermentation. In the CPS sample, the proportion of

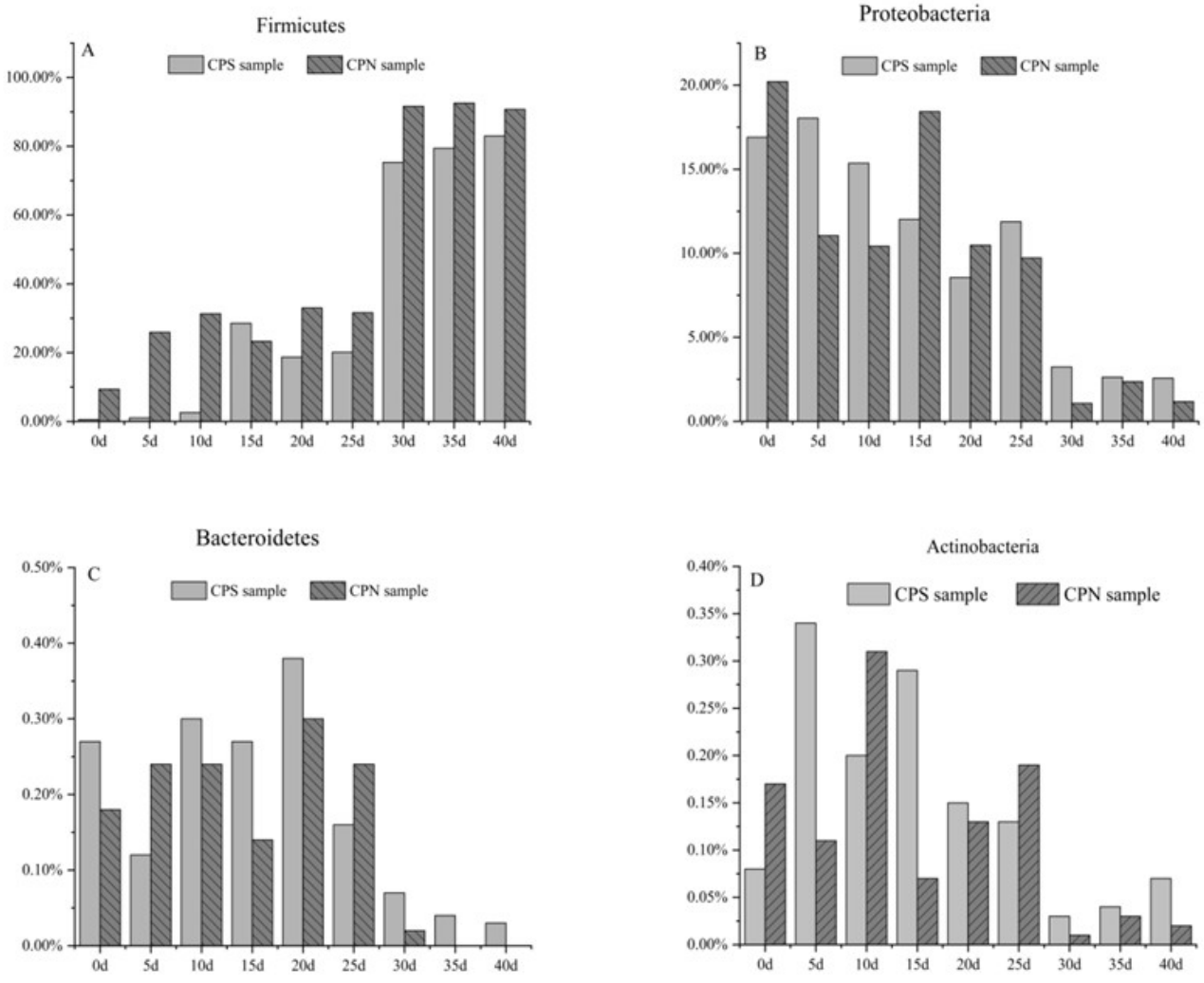

Figure 3. Relative abundance of the bacterial communities at the phylum level. Taxonomic classification of 97% sequence identity clusters demonstrated at genera classification levels. A: Comparison of Firmicutes in the control sample (CPS sample) and sample inoculated with the starter culture (CPN sample) during fermentation, B: Comparison of Proteobacteria in the control sample (CPS sample) and sample inoculated with the starter culture (CPN sample) during fermentation, C: Comparison of Bacteroidetes in the control sample (CPS sample) and sample inoculated with the starter culture (CPN sample) during fermentation, D: Comparison of Actinobacteria in the control sample (CPS sample) and sample inoculated with the starter culture (CPN sample) during fermentation.

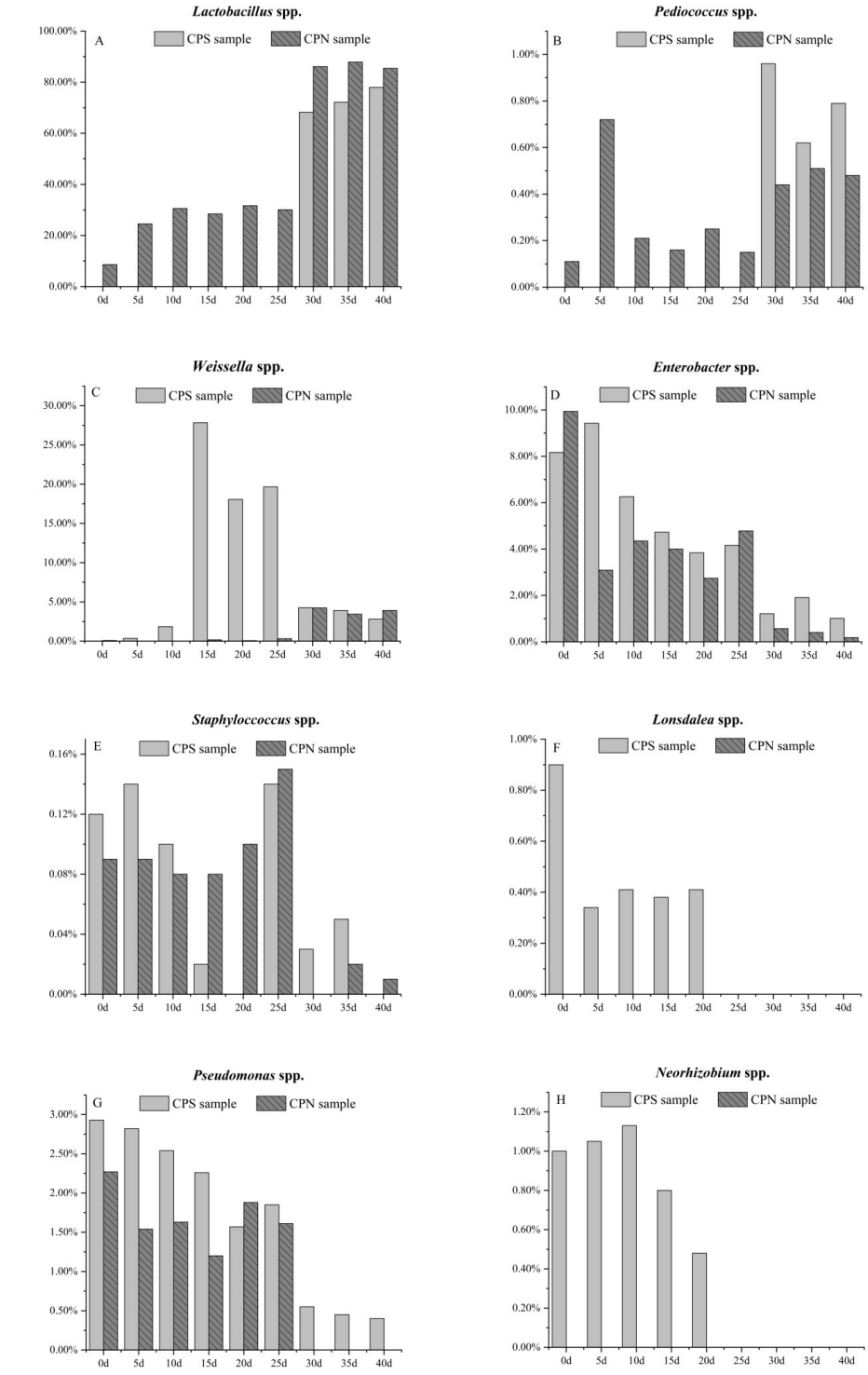

Figure 4. Relative abundance of the bacterial communities proportions at the genus level. Taxonomic classification of 97% sequence identity clusters demonstrated at genera classification levels. A: Comparison of *Lactobacillus* spp. in the control sample (CPS sample) and sample inoculated with the starter culture (CPN sample) during fermentation, B: Comparison of *Pediococcus* spp. in the control sample (CPS sample) and sample inoculated with the starter culture (CPN sample) during fermentation, C: Comparison of *Weissella* spp. in the control sample (CPS sample) and sample inoculated with the starter culture (CPN sample) during fermentation, D: Comparison of *Enterobacter* spp. in the control sample (CPS sample) and sample inoculated with the starter culture (CPN sample) during fermentation, E: Comparison of *Staphylococcus* spp. in the control sample (CPS sample) and sample inoculated with starter culture (CPN sample) during fermentation, F: Comparison of *Lonsdalea* spp. in the control sample (CPS sample) and sample inoculated with the starter culture (CPN sample) during fermentation, G: Comparison of *Pseudomonas* spp. in the control sample (CPS sample) and sample inoculated with the starter culture (CPN sample) during fermentation, H: Comparison of *Neorhizobium* spp. in the control sample (CPS sample) and sample inoculated with the starter culture (CPN sample) during fermentation.

Enterobacter spp. increased from 8.17% to 9.43% during the first 5 days of fermentation, followed by a drop in the relative abundance of bacteria from the 5 d to 40 d, accounting for an abundance of 1.01% at the end of the fermentation. On the other hand, *Weissella* spp. underwent a sharp increase from 0.35% to 27.84% from the 5 d to 15 d, and decreased from 27.84% to 2.80% during the remaining 25 d. Unexpectedly, only *Pediococcus* spp. were detected during fermentation from the 30 d to 40 d. However, the relative abundance of *Lactobacillus* spp. rapidly increased from the 30 d to 40 d in the CPS sample during fermentation, and resulted in becoming the most dominant bacterial genus. As the chili sauce was fermented in a closed environment, the oxygen in the system was consumed over time. In this study, *Lactobacillus* spp. as facultative bacteria were detected to become the dominant bacterial community at the end of the fermentation of chili sauce, which were in well line with the results reported by Lee et al. (2006).

The spontaneous fermentation (CPS samples) showed the presence of undesirable bacteria such as *Enterobacter* spp. and *Staphylococcus* spp. *Enterobacter cloacae* could infect wounds and cause respiratory tract infections (Paterson, 2006), whereas *Staphylococcus aureus* could cause foodborne poisoning, nausea, and vomiting (Lowy, 1998). Moreover, *Pseudomonas* spp. are responsible for the decay of plants and are capable of infecting human wounds that can even result in sepsis (Tryfinopoulou et al., 2002). The presence of spoilage bacteria and pathogenic bacteria will result in a decline in the quality and safety of food. According to research reports, *Lactobacillus sakei* is one of starter cultures with great potential capacity to inhibit the growth of *Listeria monocytogenes*, which is mainly used in fermented meat products and rarely used in fermented vegetables (Takahashi et al., 2021). Although the industrial bacteriocins have antibacterial effect at present, it shows certain limitation which cannot completely inhibit gram-positive bacteria and pathogenic bacteria (Gao et al., 2010). However, *Lactobacillus sakei* can produce broad-spectrum bacteriocins and hydrogen peroxide. The Sakacin C2 produced by *Lactobacillus sakei* has inhibitory effects on many gram-positive and gram-negative pathogens bacteria and has antagonistic effects on intestinal pathogenic microorganisms (Gao et al., 2012). In addition, Sakacin C2 mainly acts on the outer membrane of harmful microorganisms, which is conductive to the entry of antimicrobial substances into the cell by enhancing the permeability of the outer membrane (Gao et al., 2010, 2012). Undesirable genera, such as *Enterobacter* spp. (9.94%), *Staphylococcus* spp. (0.09%), and *Pseudomonas* spp. (2.27%) were detected on the first day in the CPN sample. Subsequently, *Enterobacter* spp., *Staphylococcus* spp. and *Pseudomonas* spp. were all almost undetectable at the end of the fermentation. Unexpectedly, *Lonsdalea* spp. and *Neorhizobium* spp. were not detected during the entire fermentation process of the CPN sample. Collectively, these results indicated that *L. sakei* could effectively inhibit the growth of spoilage organisms and pathogens during fermentation of chili sauce.

Effect of the starter culture on bacterial succession

The similarities in bacterial communities of the chili sauce inoculated with *L. sakei* (CPN sample) and the spontaneous fermentation (CPS sample) based on PCA are shown in Figure 5. These results showed that bacterial communities obviously separated among eighteen different samples. The first, second, and third axes showed values of cumulative percentage variance of species equal to 62%, 35%, and 2%, respectively. In total, 99% variances of species were explained based on the three axes. Using PCA, the bacterial communities in spontaneous fermentation (CPS sample) were found to widely vary during fermentation. Correspondingly, the chili sauce inoculated with *L. sakei* was found to be relatively stable.

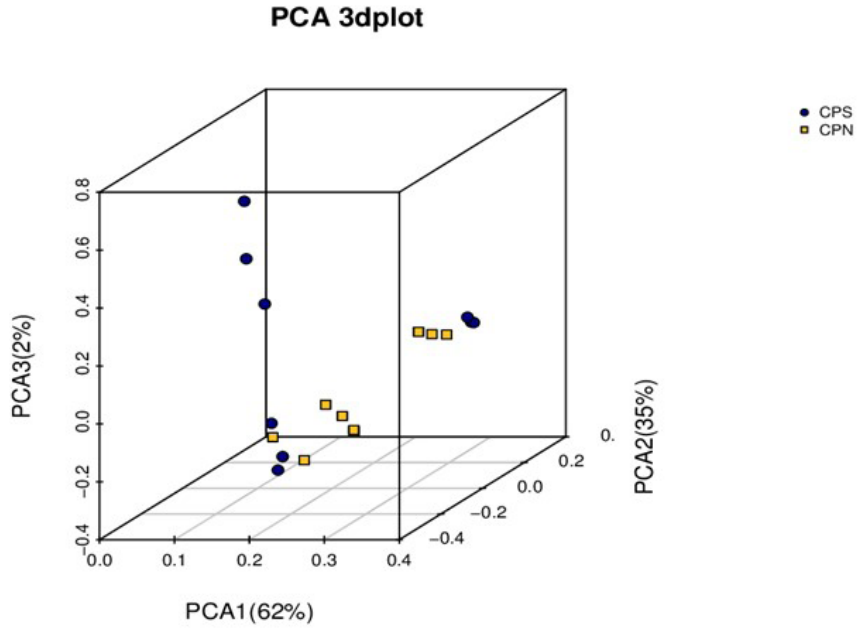

Figure 5. PCA of samples according to bacterial diversity.

4 Conclusions

The microorganisms involved in fermentation play key roles in the flavor development of chili sauce as well as in rendering its safety for consumption. *L. sakei* was used as a starter culture for fermentation because it improves the safety profile of fermented foods by producing organic acids and bacteriocin that acts as natural preservative. These results revealed that the bacterial structure of the CPN samples was better than that of the CPS sample. The growth of *Staphylococcus* spp., *Lonsdalea* spp., *Pseudomonas* spp. and *Neorhizobium* spp. were inhibited in the chili sauce inoculated with *L. sakei*, which were conducive to improving the microbial qualityand optimizing the bacterial structure in the fermentation environment.

Acknowledgements

The research was supported by Yibin University Solid State Fermentation Resources Utilization Sichuan Key Laboratory Open Fund (2020GTJ004, 2019GTJ010) and Sichuan Science and Technology Plan Application Foundation Project (2018JY0217).

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