Original paper

Dominant Microorganisms during the Spontaneous Fermentation of Suan Cai, a Chinese Fermented Vegetable

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Received March 17, 2014; Accepted May 12, 2014

Microbial dynamics and biochemical processes during suan cai fermentation were investigated. The pH decreased from 7.3 to 4.0 at day 12 of suan cai fermentation, and then remained at approximately 4.1. Water-soluble carbohydrate decreased from 15.1% to 4.5% of dry matter at day 18 of fermentation. Nitrite content comprised 54.6 mg kg⁻¹ of fresh matter at day 6, and then decreased dramatically. Lactic acid, acetic acid and ethanol were the main volatile products identified, and concentrations were 6.8 g L⁻¹, 0.78 g L⁻¹ and 32.2 g L⁻¹ at the end of fermentation, respectively. 16S rDNA clone libraries showed that bacteria during the fermentation included Acinetobacter sp., Pseudomonas fragi, Klebsiella sp., Citrobacter sp., Betaproteobacteria sp. and lactic acid bacteria, which included Leuconostoc mesenteroides, Lactobacillus curvatus, Lact. plantarum and Lact. oligofermentans. At day 6, LAB comprised 63.8% of the bacterial population. On day 30, all bacteria were LAB. Lact. curvatus dominated the fermentation. Lact. oligofermentans was found to be involved in the vegetable fermentation for the first time. 26S rDNA D1/D2 clone libraries revealed that eukaryotic microorganisms included Stramenopile sp. and soil fungus at day 12. Candida sake, Cystofilobasidium infirmominutatum, Claclosporium sp. and Tilletiopsis washingtonensis were also detected until day 30 of fermentation. This is the first study to focus on eukaryotic diversity during suan cai fermentation. These results provide a comprehensive understanding of the spontaneous suan cai fermentation process and a foundation for controlling suan cai fermentation and quality.

Keywords: suan cai, fermentation, dynamics, microbial succession, bacteria, eukaryote

Introduction

Suan cai is a traditional sauerkraut in Northeast China. It is generally made from Chinese cabbage. After washing the leaves, Chinese cabbage is soaked in a ceramic jar with salt and pressed under a stone. After one-month fermentation, it becomes edible. In the past, the northeastern part of China was too cold to cultivate leaf vegetables during the winter, so people used this method to preserve vegetables. Now, suan cai has become an essential food in Northeast China during the winter. Suan cai is increasingly available at the market, and its production scale is expanding. However, almost all commercial suan cai producers use a spontaneous fermentation method. The long fermentation period and variable quality are common problems in suan cai marketization (Ni et al., 2011).

Many physical and chemical changes occur during vegetable fermentation. These changes directly influence the quality of the final product. It is generally considered that microorganisms cause these changes (Fleming et al., 1995; Nguyen et al., 2013). Correctly understanding the microbial dynamics that occur during fermentation is essential to creating a final product that has good flavor and quality. To facilitate the successful transition from spontaneous to artificial fermentation, the microflora present during spontaneous suan cai fermentation must be investigated (Hansen, 2002; Holzapfel, 2002).
Investigations of the microflora during suan cai fermentation began in the 1990s. *Lactobacillus plantarum*, *Lact. brevis*, *Lact. minor*, *Lact. fermentum*, *Lact. harbinensis* and *Pediococcus acidilactici* were commonly isolated (Miyamoto et al., 2005; Xiao et al., 2006; Zhou et al., 2006). Recent results have shown that *Lactobacillus*, *Pediococcus*, *Leuconostoc* and *Weissella* species can be detected at the later stages of fermentation. The *Lactobacillus* species during the fermentation included *Lact. plantarum*, *Lact. brevis*, *Lact. reuteri* and *Lact. sakei* (Yan et al., 2009; Zhang and Meng, 2010). Investigations of the bacterial dynamics from beginning to end of fermentation are scarce. Furthermore, traditional suan cai fermentation is an open-air fermentation process that involves eukaryotic microorganisms (Zhong and Guo, 1995), but it is generally believed that the role of these eukaryotic microorganisms is minimal. Therefore, the investigation of eukaryotic microorganisms in this process has been neglected, and to the best of our knowledge, eukaryotic diversity during suan cai fermentation has not been examined at all. In this study, the traditional suan cai fermentation process was investigated. Bacterial and eukaryotic dynamics during the fermentation process were evaluated, and biochemical analyses were performed. The information from this study provides new insights into suan cai fermentation and should become a basis for the development of suitable starter cultures for suan cai fermentation.

**Material and Methods**

**Suan-cai fermentation** Fermentation of Chinese cabbages (*Beassica pekinensis*) was performed according to traditional methods (Fig. 1). Briefly, Chinese cabbages were harvested, air-dried for two days, trimmed and then soaked in a 750 L jar. The proportion of salt to Chinese cabbage was 1% (w/w). One stone was put on the top of the jar filled with the Chinese cabbages. Three jars were fermented for 30 days. The temperature was measured throughout fermentation using a thermometer. The thermometer probe extended 10 cm below the surface. The temperature was read at 7:00 am each day. Sampling was performed at days 0, 6, 12, 18, 24 and 30 of fermentation.

**Sampling and sample preparation** At each fermentation time point, the samples were collected. For analyzing eukaryotic diversity, fermented liquid (15 mL) was collected at the surface of each jar. After centrifugation at 5,800 x g for 10 min at 4°C, the pellet was resuspended with 0.5 mL of extraction buffer (10 mM Tris-HCl and 40 mM EDTA, pH 9.0). Three pellets from 3 jars were collected in one tube, which was stored at -20°C for DNA extraction. For bacterial analyses, the fermented juice of Chinese cabbages in the third layer under the surface was pressed out of the cabbage, and 15 mL of fermented juice was collected. After centrifugation and dilution, the pellets were stored at -20°C for DNA extraction. Supernatant (0.7 mL) was added to 0.3 mL of acetonitrile (high performance liquid chromatography (HPLC) grade, DIKMA, Lake Forestry, USA). After vortexing, standing for 10 min, centrifuging 10 min at 10,800 x g and filtration with a 0.22 μm filter, the supernatant was stored at -20°C for high-performance liquid chromatography (HPLC) analyses. Another 0.5 mL of fermented juice was used for microbiological evaluation. The second-layer leaves were homogenized for nitrite measurement. The third-layer leaves were oven-dried at 105°C for

![Fig. 1. The process of the traditional spontaneous suan cai fermentation. ①, Filling in the jar; ②, fermentation at day 0; ③, fermentation at 12 d; ④, fermentation at 30 d.](image-url)
Microbial enumeration and chemical analyses LAB and other bacteria were enumerated by culturing on agar plates. LAB were cultured on MRS agar (De Man et al., 1972) for two days at 30°C. Other bacteria were cultured in nutrient agar (Johnson and Case, 1992) for two days at 37°C. Nine plates were counted for each jar. The pH measurements were taken using a pH meter (B-212, Horiba, Kyoto, Japan). Dry matter (DM) was determined by oven drying for three hours at 105°C. The WSC contents of the samples were determined by colorimetry after reaction with an anthrone reagent (Thomas, 1977). The suan cai nitrite content was determined using the colorimetric nitrite assay described by Ito et al. (Ito et al., 1979). An HPLC system (Waters, USA) with a dual λ absorbance detector (Waters 2487) was used to determine volatile products (VPs). Chromatographic separation was performed using a high affinity cation-exchange column (Aminex HPX-87H, 300 × 7.8 mm, Bio-Rad, USA). The column temperature was 40°C. The flow rate was mL min⁻¹. The concentration of H₂SO₄ in the mobile phase was 5 mM. A 30 min run was used for all of the samples.

Statistical analyses using Duncan’s test were performed using SPSS 13.0 software. Differences were considered significant at P < 0.05 level.

DNA extraction and PCR amplification DNA was extracted from the fermentation products using the benzyl chloride method (Zhu et al., 1993). The extracted DNA was used as the template for PCR amplification after RNase digestion. The 50 μL PCR reactions contained 15 ng of template DNA, 1 × PCR buffer (Mg²⁺ free), 0.16 mM dNTPs, 1.5 mM MgCl₂, 0.45 μM of each primer and 1 U of Takara rTaq DNA polymerase (Takara, Japan). The amplification primers for 16S rDNA were 8F and 1492R (Table 1). The amplification primers for DGGE of eukaryotes 26S rDNA D1 region were NL1-GC and LS2 (Table 1). The amplification primers for cloning libraries of the 26S rDNA D1/D2 region were NL1 and NL4 (Table 1). The thermocycler program consisted of an initial DNA denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 93°C for 1 min, annealing at 50°C 1 min for bacteria and at 52°C 45 s for eukaryotes, elongation at 72°C for 1 min 30 s and ended with a final elongation step at 72°C 3 min 50 s.

Denaturing gradient gel electrophoresis (DGGE) and sequencing DGGE using to analyze the dynamics of eukaryotes was performed with the Dcode™ system (Bio-Rad Laboratories, Hercules, CA) (Muyzer et al., 1993). PCR products (13 μL) were applied to 1-mm thick, 6 – 12% (w/v) polyacrylamide gradient gels in a 0.5 × TAE electrophoresis buffer (20 mM Tris-HCl pH8.3, 10 mM acetic acid, 0.5 mM EDTA) with a 20 – 60% denaturant gradient (where 100% was defined as 7 M urea with 40% formamide). Electrophoresis was performed at a constant voltage of 200 V and a temperature of 61°C for 5 h. The bands on the gel were stained with SYBR Green I (Pedro et al., 2001) and photographed under 320-nm UV light using the Bio-Best-200 Imaging System (SIMON, USA). Bands at different positions were excised from the gel (Yang et al., 2008). The DNA was recovered and re-amplified with the primers NL1-GC and LS2. After another DGGE, the band at the same position with first DGGE was excised from the gel. After recovery and re-amplification with the primer NL1 and LS2, sequencing was conducted by Sangon Biotech (Shanghai, China).

Cloning libraries After purification using the Tiangen DNA Purification Kit (Tiangen, China), the PCR products were ligated into the pGEM-T Easy Vector (Promega, USA) according to the manufacturer’s protocol. After transformation into Escherichia coli JM 109 and blue-white screening, clone libraries were constructed. Colony PCR was performed to screen for clones with unique sequences. For bacteria, the 25 μL amplification reaction was incubated. The AluI (New England Biolabs, USA) restriction enzyme was used for the subsequent DNA digestion. The DNA digestion was performed at 37°C for 3 hours in a 20 μL mixture containing 10 U of AluI and 3 μL of PCR product. Restriction fragments were separated on a 1% (w/v) agarose gel containing 1.5 μg ml⁻¹ ethidium bromide. Bands were visualized using 302 nm UV transillumination and photographed. Representative clones for each fragment pattern were selected for sequencing. For eukaryotes, after the amplification with the primers NL1-GC and LS2, DGGE was used to screen the clones, and clones with different melting positions were selected for sequence analyses (Yang et al., 2008).

Phylogenetic analysis and nucleotide sequence accession

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>8F</td>
<td>5’-AGAGTTTGATCCTGGCTCAG-3’</td>
<td>(Yang et al., 2011)</td>
</tr>
<tr>
<td>1492R</td>
<td>5’-CGGTATACCTTGTAGCTTTG-3’</td>
<td>(Yang et al., 2011)</td>
</tr>
<tr>
<td>NL1-GC</td>
<td>5’-CGGCCCGCAGGCAGGGGCGGGGCGGGCCACGGGGGGGCATATCAATAAGCGGAGGAAAG-3’</td>
<td>(Baleiras Couto et al., 2005)</td>
</tr>
<tr>
<td>NL2</td>
<td>5’-ATCCCCAACAACATCGACTC-3’</td>
<td>(Baleiras Couto et al., 2005)</td>
</tr>
</tbody>
</table>

Table 1. Primers in this study
The sequences generated in this study were compared with those in GenBank (http://blast.ncbi.nlm.nih.gov/Blast.cgi), and sequences with ≥99% similarity to the partial 16S rDNA (1500bp) or 26S rDNA (600bp) were considered to belong to the same species. A neighbor-joining tree was constructed using MEGA 5.0 software (Tamura et al., 2011). The sequences were deposited in GenBank under the accession number KF018058 ~ KF018066 and KJ781298 ~ KJ781299 for bacteria and KF776395 ~ KF776400 for eukaryotes.

Results

Change in pH The pH dynamics of the fermentation system are shown in Fig. 2. As fermentation progressed, the pH exhibited an overall decreasing trend. A noticeable change in pH occurred primarily on day 12, decreasing from 7.3 to 4.0. Afterward, the pH was approximately 4.1.

Microbial enumeration and chemical analyses The results of microbial enumeration and chemical analyses are shown in Table 2. An increase in LAB and other bacteria primarily occurred on day 6. The log colony forming units (CFUs) mL⁻¹ of other bacteria began to decrease after 6 days of fermentation. On day 30, the log CFU mL⁻¹ of other bacteria was 2.4. However, the change in LAB numbers was different than for other bacteria. On day 6, the log CFU mL⁻¹ was 7.7. After day 6, the log CFU mL⁻¹ increased to 8.4 and did not change significantly ($P < 0.05$).

The initial WSC of the Chinese cabbage was 15.1% (w/w). Changes in WSC content primarily occurred on day 18, decreasing to 4.5%, and then did not change significantly ($P < 0.05$). The highest nitrite value was observed on day 6 (54.6 mg kg⁻¹ FM). On day 12, the nitrite content decreased to 23.0 mg kg⁻¹ FM and continued to decrease until the end of fermentation ($P < 0.05$). The highest proportion of bacteria (56.6%) at the end of fermentation time points is shown in Fig. 4.

Eukaryotic diversity and dynamics Eukaryotic dynamics during suan cai fermentation are shown in Fig. 5. The sequence information from fragments of approximately 200 bp in size indicated that Candida sake, Sinapis alba, Atibidopsis thaliana, Unculuted Ascomycota and Unculuted soil fungus were present in the fermentation. On day 6 of fermentation, only uncultured soil fungus was detectable. On day 12 of fermentation, Candida sake was also detected. As fermentation progressed, increasing amounts of eukaryotic microorganisms were detected. The highest levels of eukaryotic microorganisms were detected at 30 d of fermentation.

Bacterial diversity and dynamics In this study, the number of clones in each library was greater than 80 (data not shown). The phylogenetic analysis was based on the sequences of 16S rDNA cloning libraries (Fig. 3). The microorganisms detected included Acinetobacter sp., Pseudomonas fragi, Klebsiella sp., Citrobacter sp. and betaproteobacterium sp. The LAB included Leuconostoc mesenteroides, Lactobacillus curvatus, Lact. plantarum and Lact. oligofermentans.

The proportion of bacteria in the clone library at different fermentation time points is shown in Fig. 4. Leuconostoc mesenteroides numbers were highest (39.2%) on day 6. LAB represented 63.8% of the population. On day 12, the predominant species were Lact. curvatus (41.1%) and Lact. oligofermentans (21.1%). LAB comprised 74.4% of the total population. On day 18, the population contained 81.2% LAB. The proportion of Lact. plantarum was significantly increased compared with day 12. On day 30, the level of Lact. plantarum was 40.8%. At day 24, LAB comprised 96.1% of the population. Lact. curvatus accounted for the highest proportion of bacteria (56.6%). At the end of fermentation, all bacteria detected were LAB. Other bacteria were detected primarily on day 0 and 12.

Lactic acid, acetic acid and ethanol were detected. The accumulation of these three substances largely occurred during the first 12 days. On day 30, the lactic acid and acetic acid concentrations were 6.8 g L⁻¹ and 0.78 g L⁻¹, respectively. The amount of lactic acid was approximately 9-fold of that of acetic acid. After 18 d of fermentation, the amount of ethanol increased to 37.5 g L⁻¹. There were no significant changes in ethanol concentration after this ($P < 0.05$).
Fig. 3. Phylogenetic analysis based on the sequences of 16 S rDNA during suan cai fermentation using the neighbor-joining method. The bar represents 5% sequence divergence. The numbers in parentheses indicated GenBank accession number. LO, LC, LP, LE, AC, UC, β-P, PF, UK, CN1, and CN2-2, representative clones detected in this study.
Fig. 4. Microbial composition at different time point of fermentation. LO, LC, LP, LE, AC, UA, UC, β-P, PF, UK, CN1, and CN2-2, microorganisms detected in this study. The closest species were as follows respectively: LO, *Lactobacillus oligofermentans*; LC, *Lactobacillus curvatus*; LP, *Lact. plantarum*; LE, *Leuconostoc mesenteroides*; AC, *Acinetobacter* sp.; UC, Uncultured *Citrobacter*; β-P, beta proteobacterium; PF, *Pseudomonas fragi*; UK, Uncultured *Klebsiella*; CN1, Uncultured *Chryseobacterium*; CN2-2, Uncultured *Cyanobacterium*.

Fig. 5. DGGE profile during the traditional suan cai fermentation. Fermentation days are indicated by the following labels: 6d, 12d, 18d, 24d, and 30d. A, *Candida sake*; B, *Sinapis alba*; C, *Aibidopsis thaliana*; D, Uncultured *Ascomycota*; E, Uncultured soil fungus.
Fig. 6. Phylogenetic analysis based on the sequences of the 26 S rDNA D1/D2 region during suan cai fermentation using the neighbour-joining method. The bar represents 2% sequence divergence. The numbers in parentheses indicate GenBank accession numbers. CL, CS, CI, TW, St, and SF indicate representative clones detected in this study.
soil fungus, other eukaryotes included *Candida sake*, *Cystofilobasidium infirmominutatum*, *Cladosporium* sp. and *Tilletiopsis washingtonensis*.

**Discussion**

**Dynamics of pH and chemical analyses** Variations of microbial populations of several foods, such as American sauerkraut (Plengvidhya et al., 2007), kimchi (Lee et al., 2005), and Chinese paocai (Xiong et al., 2012) during the fermentation were well investigated. These variations resulted in chemical changes in fermented system. In this study, the decrease in pH primarily occurred in the early stages of fermentation (Fig. 2). Concurrently, the CFUs of LAB and other bacteria increased significantly (Table 2). The WSC concentration decreased dramatically during fermentation. These results indicate that the number of microorganisms increases rapidly during the early stages of fermentation. WSCs were consumed by the microorganisms, and VPs were concurrently produced. As fermentation progressed, VPs continued to accumulate. In this acidic environment, a gradual increase in LAB became stable, and the growth of other bacteria was inhibited. This inhibition of the growth of other bacteria may be due to acidity (Liu et al., 2011) or inhibitory substances (Cotter et al., 2005).

During vegetable fermentation, the accumulation of nitrite is a common problem (Yan et al., 2008). Many reports have shown that nitrite consumption is harmful to human health and is associated with increased incidence of cancer (Majumdar, 2003; Seel et al., 1994). During fermentation, nitrate in plant issues is converted into nitrite. The nitrite content of a fermentation system increases during early fermentation stages before decreasing rapidly (Prasad and Chetty, 2008; Yan et al., 2008). In this study, we measured changes in nitrite content during the suan cai fermentation process, and the observed trend was similar to that of other reports of vegetable fermentation (Hou et al., 2013; Park and Cheigh, 1992; Yan et al., 2008). Previous studies have shown that LAB decreases nitrite content in fermentation systems (Oh et al., 2004; Wang et al., 2013; Yan et al., 2008). In this study, nitrite levels peaked at day 6. The increase in LAB was detectable from 6 d fermentation onward ($P < 0.05$). The growth of LAB also resulted in the consumption of a large amount of nitrite (Oh et al., 2004); nitrite content decreased from day 6 to day 30 of fermentation.

**Bacteria during the fermentation** Fermented foods are subjected to the actions of microorganisms and enzymes to facilitate desirable biochemical changes (Liu et al., 2011). During spontaneous fermentation, the microbial composition can vary due to variable materials and fermentation conditions (McDonald et al., 1991). Previous studies on sauerkraut fermentation have focused on the cabbage material used. The fermented material and conditions were different in this study compared with previous research (Breidt et al., 1993; Chen et al., 2006; Plengvidhya et al., 2004). Previous reports on suan cai fermentation, focused on the microbial diversity at the latter stage of fermentation. Some probiotic bacteria were isolated (Yang et al., 2012; Zeng et al., 2009). There have been no reports on the bacteria dynamics during suan cai fermentation. In this study, 16S rDNA clone libraries were constructed to analyze the microbial dynamic during suan cai fermentation. Overall, LAB comprised the primary microorganisms. LAB increased dramatically at day 6, and the total
LAB comprised 63.8% of the population (Fig. 4). On day 30, the detected microorganisms were all LAB. The detected LAB species included Leuconostoc mesenteroides, Lactobacillus curvatus, Lact. plantarum, and Lact. oligofermentans. Leuconostoc mesenteroides was consistently detected until day 18 of fermentation. However, Leuconostoc mesenteroides was the dominant species on day 6 of fermentation. Therefore, the suan cai fermentation process can be divided into heterofermentative fermentation and homofermentative fermentation stages. The results were similar to previous research on fermented vegetables (Chao et al., 2009; Plengvidhya et al., 2007).

The microbial diversity results in this study revealed the involvement of Lact. oligofermentans in the vegetable fermentation process for the first time. Lact. oligofermentans levels peaked on day 12 of fermentation. Lact. oligofermentans was isolated from poultry products and identified as a novel species by Koort et al in 2005. This species produces acid from a few substrates, such as ribose, L-arabinose and D-xylose, but not from D-arabinose or L-xylose. The utilization of maltose, gluconate, D-glucose and N-acetylglucosamine is weak or delayed. Lact. oligofermentans grows well at 15℃ and more slowly at 4℃, but no growth was observed at 37℃ (Koort et al., 2005). In this study, low fermentation temperature possibly provided the proper conditions for the growth of Lact. oligofermentans. In our lab, this microorganism in suan cai fermentation has been isolated and the analyses of its concrete function are under way. The microbial succession results (Fig. 4) indicated that Lact. curvatus might play a crucial role in suan cai fermentation. Lact. curvatus has been detected in vacuum-packaged meat and kimchi in Korea (Dykes and von Holy, 1994; Lee et al., 2006). Its optimum growth temperature is 26℃ (Torriani et al., 1996). This result is potentially significant for screening efficient inoculants for controlling the suan-cai fermentation. Lact. plantarum was detected on day 12. On day 30 of fermentation, Lact. plantarum comprised 40.7% of the population. This result indicates the acid resistance of Lact. plantarum (McDonald et al., 1990) and also explains why Lact. plantarum is often a dominant microorganism at the latter stages of sauerkraut fermentation (Breidt et al., 1993; Plengvidhya et al., 2007).

### Table 2: Microbial enumeration and chemical analyses during suan cai fermentation

<table>
<thead>
<tr>
<th>Time</th>
<th>LAB*</th>
<th>Other bacteria</th>
<th>WSC</th>
<th>Nitrite</th>
<th>Lactic acid</th>
<th>Acetic acid</th>
<th>Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>d</td>
<td>Log CFU g⁻¹</td>
<td>% DM</td>
<td>mg kg⁻¹ FM</td>
<td>g L⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>-</td>
<td>15.1 ± 1.2a</td>
<td>8.0 ± 1.6d</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>7.7 ± 0.1b</td>
<td>10.0 ± 0.9b</td>
<td>54.6 ± 4.6a</td>
<td>3.8 ± 0.4c</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>8.4 ± 0.1a</td>
<td>7.4 ± 1.0c</td>
<td>23.0 ± 1.8b</td>
<td>6.0 ± 0.7b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>8.4 ± 0.1a</td>
<td>4.5 ± 0.3d</td>
<td>13.0 ± 2.9a</td>
<td>8.2 ± 0.3a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>8.5 ± 0.1a</td>
<td>3.8 ± 0.5d</td>
<td>7.9 ± 1.4b</td>
<td>8.0 ± 0.5a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>8.5 ± 0.1a</td>
<td>3.3 ± 0.4d</td>
<td>4.2 ± 0.8d</td>
<td>6.8 ± 0.1b</td>
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</tr>
</tbody>
</table>

*LAB, lactic acid bacteria; WSC, water-soluble carbohydrate; CFU, colony forming unit; DM, dry matter; FM, fresh matter; a, b, c, in one column indicate a significant difference (P < 0.05).
that can be used as biocontrol agents (Liu et al., 2011; Morales et al., 2008). The proportions of these yeasts were found to be 29% and 3%, respectively in this study. Overall, the yeasts accounted for 32% of all eukaryotic microorganisms. Heterofermentative lactic acid bacteria can ferment glucose to lactic acid and ethanol (Ganzele et al., 2007). Generally, the ethanol produced only by heterofermentative lactic acid bacteria is lower than the levels of lactic acid produces. In this study, the ethanol concentration detected was approximately 32.2 g L\(^{-1}\) in the fermented system, which was higher than the concentration of lactic acid (6.7 g L\(^{-1}\)). Therefore, the higher levels of ethanol might result from yeast fermentation. A small number of Cladosporium and Tilletiopsis washingtonensis were also detected. There have been no related reports on their role in vegetable fermentation. In the further study, the typical microorganisms needed to be isolated to clear relative functions during the fermentation. The results of this study will provide a new understanding for suan cai fermentation and will contribute future studies of fermentation control.

Acknowledgements This work was supported by the Project of National Basic Science Personnel Training Fund (J1210053) and the National Science Foundation of China (31301543).

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