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Analysis of Bacterial Communities in Fermented Traditional Food *Suan-cai* and *La-baicai* from Yanbian in China Using DGGE

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***Suan-cai* and *la-baicai* are traditional fermented food with Chinese cabbage and still popular in the northeast of China. The present study firstly compared the diversity of lactic acid bacteria involved in *suan-cai* and *la-baicai* using denaturing gradient gel electrophoresis (DGGE). The results indicated that Weissella sp., *Lactobacillus sakei* and uncultured *Leuconostoc gasicomitatum* present in the fermentation process of all *suan-cai* and *la-baica*, which were also the predominate microflroa in *la-baicai*. Rod shaped of *Lactobacillus plantarum* and *Lactobacillus brevis* were only found in *suan-cai* samples. Moreover, *Lactobacillus curvatus* and *Lactococcus lactis* were also found in some of the *suan-cai* and *la-baica* samples. It shows different lactic acid bacteria involved in the fermentation of two traditional products might be due to the method for making.**

Key words: Lactic acid bacteria, *suan-cai*; *la-baicai*, northeast of China, DGGE.

Food fermentation is one of the oldest food processing and preservation methods and dates back thousands of years in China[1]. *Suan-cai* and *la-baicai* are traditional fermented food made in the northeast of China both using Chinese cabbage. Chinese cabbage has been consumed for centuries in China, and fermented cabbage is one of effective way to preserve the plant in winter in the northeast of China. Between two fermented foods, *suan-cai* and *la-baicai* do have some similarities, e.g. using Chinese cabbage as material, addition of salt, and maintaining the low temperature, *etc*.. But *la-baicai* product also contains the spice of hot red pepper, and making process is different as well, which may be able to induce some major different culture of lactic acid bacteria (LAB).

In traditional fermented food, LAB always play a crucial role in forming the key flavour, texture and preservative qualities. Some studies have analyzed the population of LAB in *suan-cai* using culture dependent methods in China. Zhang et al. (2009) isolated 97 Lactobacillus strains and 2 exopolysaccharide (EPS)-producing strains *Lactobacillus casei* and *Lactobacillus plantarum* were selected[2]. Three high-acid-yield strains, *Lactobacillus brevis*, *Lactobacillus plantarum* and *Leuconostoc mesenteroides*, were separated from the juice of *suan-cai* fermented naturally and were identified bacterially using the classica1 classification[3]. But there was little study of bacteria involved in the fermentation of *la-baicai* in China. The production of *la-baicai* is similar as the kimchi,

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a traditional fermented cabbage food in Korea, for *la-baicai* is mainly made and consumed by Chinese Korean. Previous studies have reported that the population dynamics of LAB in kimchi[4]. Hence, more researches are needed to identify LAB in *suan-cai* and *la-baicai*.

Since it is difficult to culture most bacteria in samples from the environment, using only culturing methods is inadequate to evaluate the structure of bacterial communities[5]. Denaturing gradient gel electrophoresis (DGGE) is one of powerful culture-independent tools for analysis microbial biota in complex environment, including traditional fermented foods[6]. The present study aims to identify the bacteria community involved in traditional fermented *suan-cai* and *la-baicai* collected from Yanbian in Jilin province of China using culture-independent DGGE, which analyze 16S rRNA gene marker. For the best of our knowledge, it is the first report to reveal the diversity of LAB in *suan-cai* and *la-baicai* using DGGE.

MATERIALS AND METHODS

Sample collection

In this study, a total of 6 samples of *suan-cai* (3 samples) and *la-baicai* (3 samples) souse were collected from families of Yanbian in Jilin province. Yanbian is the colony of Chinese Korean people which located in the northeast of China, and *suan-cai* and *la-baicai* are both important food for local people. Samples were aseptically collected and then frozen immediately until getting to the laboratory for microbiological analysis.

DNA extraction using bead beating

The community DNA was extracted separately from 1mL volume of each sample of *suan-cai* souse using the bead-beating method with some improvements[7]. Sample of *suan-cai* souse was mixed with 5 mL PBS, after vortex for 30 s, sample was centrifuged at 12000 g for 5min. The suspension was removed to an 1.5 mL eppendorf tube with 0.5 g glass beads, following blended in a Bead-Beater (FastPrep-24, MP Biomedicals, Ohio, USA) for 3 times, and 30s for every time at the speed 6.0. SDS of 50µLwas added to the sample for incubation on ice bath for 10min. The supernatant was collected by centrifugation at 14,000 g for 10 min. Then 180 µLCTAB/NaCl were added, and also the same volume of phenol/chloroform/isoamyl alcohol (25:24:1, v/v) was added, following mixed for 1 min, centrifugation was performed at 12,000 g for 10min. The DNA was precipitated with ice-cold isopropanol, and washed with ethanol by centrifugation (8000 g, 3min), then resuspended in TE. The concentration was measured by optical density at 260 nm (OD_{260nm}).

Polymerase chain reaction and DGGE analysis

Primers V3F+GC (5'-CGCCCGCCGC GCGCGGCGGGCGGGGCGGGGGCACGGGGGCC TACGGGAGGCAGCAG-3') and V3R (5'-ATTACCGCGGCTGCTGG-3' were used in the study for amplification of V3 region of 16S rRNA gene of bacteria (8). Each reaction (25 µL) included 10 pmol of each primer, 200 ìL each dNTP, 10 mmol L^{-1} Tris-HCl (pH 9.0), 1.5 mmol L^{-1} $MgCl_2$, 2.5 U Taq polymerase, and 1 ìL DNA solution. The cycling conditions were set up as one cycle of 94°C for 2 min; 35 cycles at 94°C for 30 s, 55 °C for 45 s, and 72°C for 1 min; and a final step at 72°C for 7 min. PCR production was detected through 0.8% electrophoresis.

DGGE analysis was performed using DCode universal mutation detection system (Bio-Rad, Hercules, USA) in 16 cm × 16 cm × 1 mm of 8% (w/v) polyacrylamide gel acrylamide-bisacrylamide (37.5:1). The denaturing gradient ranged from 35 to 55% using urea plus formamide. The electrophoresis was performed at 120 V for 8 h in 0.5 × TAE buffer maintained at 60°C. Gels were stained with silver (9).

DGGE band sequencing

DGGE bands on gel were extracted and transferred to 100 ìL sterile water for washing 3 times by centrifugation. 50ìL sterile water was added before incubation the sample at 50°C for 2~3 h, and then maintained at 4°C for overnight (10). 1ìL supernatant containing DNA was re-amplified and sequenced by Shanghai Sangni Biosciences Corporation. The identification of sequences was performed by BLAST search of DNA Databank at Genbank.

RESULTS AND DISCUSSION

Sample collection

The present study collected 6 souse samples of home-made *suan-cai* (3 samples) and *la-baicai* (3 samples) from Yanbian province in

the northeast of China. All the chosen families have rich experience in making *suan-cai* and *la-baicai*, and living in house is able to keep a lower fermented temperature than in apartment with heater. The temperatures of samples were ranged from 10.3 to 14.9°C. The pH values of samples were also detected. As all of *suan-cai* and *la-baicai* samples were made for over 1 month, the pH values were ranged from 3.5 to 4.1. The decrease of pH value is probably due to the growth of LAB. In modern society, environmental pollution and urbanization promoting may influence the growth of LAB in nature. The high pH can induce the growth of pathogenic or spoilage bacteria in *suan-cai*. Recently, some LAB agents have been used in families in city to ferment *suan-cai* with good flavor and quality. Therefore, it is one of the reasons to identify LAB in naturally fermented *suan-cai* products.

DGGE fingerprinting

DGGE is a useful tool for revealing the microbial biota in foods because it allows the rapid detection of species and changes in the compositions based on the direct amplification of bacterial DNA involved[11]. The DGGE fingerprint of LAB in 6 samples of *suan-cai* and *la-baicai* souse are shown in Figure 1 and bacteria identification is listed in Table 1. Generally, although the sample is collected from same families, the DGGE fingerprint of bacteria community is different. Based on the analysis, nine bands in average were obtained from *suan-cai* sample, while six bands were found from *la-baicai* sample on gel. There were 3 common bands were identified in all of samples, and confirmed to correspond to *Weissella* sp. (band a), *Lactobacillus sakei* (band f) and uncultured *Leuconostoc gasicomitatum* (band h). However, bands b, d, e, i and k were only observed in *suan-cai* sample, whereas band c was found only in *la-baicai* sample. Among the special bands, bands b, d, e and k were identified as *Lactobacillus plantarum* (band b), *Lactobacillus brevis* (band d and e), uncultured bacterium clone (band k) and Pseudoalteromonas sp. (band c), respectively. Moreover, *Lactobacillus curvatus* (band g) was found in all *suan-cai* samples and on *la-baicai* sample. Band l was identified as *Lactococcus lactis* in two *suan-cai* samples and on *la-baicai* sample, and uncultured bacterium clone (band j) was also found in one *suan-cai* sample and one *la-baicai* sample.

Bands indicative of *Weissella* sp., *Lactobacillus sakei* and uncultured *Leuconostoc gasicomitatum* present in the fermentation process of all *suan-cai* and *la-baicai*, indicating their importance roles.

Weissella sp. would appear to be widely distributed and play an important role in fermented foods prepared from plants[12]. In kimich, *Weissella confusa* strains have been detected[13], and also in sugar caneÿcarrot juice[14], and a traditional Chinese vinegar "Shanxi aged vinegar"[15].

L. sakei and *L. curvatus* were both identified in the study, but only *L. sakei* was

Table 1. Identification of bands obtained from DGGE analysis of bacterial community in *suan-cai* and *la-baicai* samples

Bands	Species	Identification %	Closest relative (NCBI accession no.)
a	*Weissella* sp.	98	HQ452826.1
b	*Lactobacillus plantarum*	100	JN786879.1
c	*Pseudoalteromonas* sp.	88	HQ201959.1
d	*Lactobacillus brevis*	100	JN792500.1
e	*Lactobacillus brevis*	98	JN792500.1
f	*Lactobacillus sakei*	100	JN673548.1
g	*Lactobacillus curvatus*	100	JN673549.1
h	Uncultured *Leuconostoc gasicomitatum*	100	JF756325.1
i	Unidentified	-	-
j	Uncultured bacterium clone	97	GU548708.1
k	Uncultured bacterium clone	97	FJ370774.1
l	*Lactococcus lactis*	95	EU337106.2

identified in all collected samples. In the previous study of kimchi showed that *L. sakei* dominated the microbial population throughout kimchi fermentation perhaps for the consuming sucrose involved[16], which corresponding the present result that *L. sakei* was the major bacteria in *la-baicai*, indicating the similar between *la-baicai* and kimchi. Xiang Yang and Xiangchen Meng (2009) also found 10 Lactobacilli strains in s*uan-cai*, including *L. sakei*[17]. *Lactobacillus sakei* and *Lactobacillus curvatus* have always been found to be the main microbial flora in the later phase of the fermentation process when most fermented food turn sour for the ripening process[18]. Besides, although *L. sakei* and *L. curvatus* are closely related species, it is easy to separate using DGGE.

The specie of *Leuconostoc* sp. were always observed in the fermentation of food, e.g., *da-jiang* made in the northeast of China[19], kimichi in Korea[13], and meat products in India[20], etc.. In *suan-cai*, the isolation of *Leu. mesenteroides* has been reported[3]. *Leuconostoc gelidum* and *Leuconostoc gasicomitatum* were also identified in kimich through Amplified ribosomal DNA restriction analysis (ARDRA)[21]. Hence, *Leuconostoc* spp. would also seem to play an important role in plant food fermentations.

Furthermore, *Lactobacillus plantarum* and *Lactobacillus brevis* were only observed in *suan-cai* samples. The two species were widely distributed in fermented food in China, such as koumiss, fermented rice noodle, and fermented camel milk, and etc.[22,23,24]. In the previous study, *Lactobacillus plantarum* and *Lactobacillus brevis* have been found in *suan-cai*, accompanied with *Lactobacillus casei*, *Lactobacillus coryniformis*, *Lactobacillus sakei*[17]. In the study of Chao et al. (2009), *Lactobacillus plantarum* and *Lactobacillus brevis* were found became dominant in the *suan-tsai*, a traditional fermented mustard product in Taiwan[25]. Besides, *Lactobacillus brevis* has also been identified in wine and sourdough fermentation[26, 27]. The result is corresponding to the previous findings using traditional culture-dependent method.

The closest match to band l was indicated for *Lactococcus lactis*. *Lactococcus lactis* has not been identified in *suan-cai* and *la-baicai*. But in another traditional fermentation food made by Chinese cabbage, sichuan pickles, *L. lactis* was found with rapid growth[28]. Sichuan pickles is popular in the province of Sichuan in the Southwest of China. Because of the gentle temperature all the year, the production of sichuan pickles do not limited by seasons. Based on the previous analysis, *Leuconostoc mesenteroi*, *L. plantarum*, *Lactobacillus casei*, *Lactobacillus brevis*, and *Lacotococcus lactis* were the major LAB involved[29].

In addition, it is interesting to identify the *Pseudoalteromonas* sp. (band c) in *la-baicai* sample 3 which needed further study. Currently, the genus *Pseudoalteromonas* comprises 35 recognized species, with *Pseudoalteromonas haloplanktis* as the type species[30]. Many *Pseudoalteromonas* species have been isolated from the marine environment, and novel *Pseudoalteromonas* strain has been reported recently.

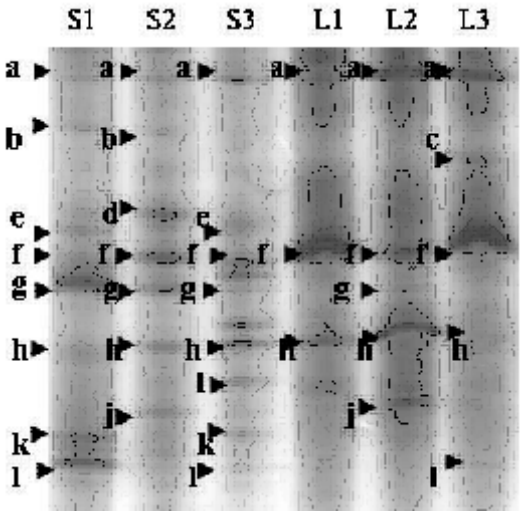

Fig. 1. DGGE proûles of PCR-ampliûed DNA from the bacterial population in *suan-cai* and *la-baicai* samples

CONCLUSION

After all, compared the LAB species involved in *suan-cai* and *la-baicai*, rod-shaped LAB of *Lactobacillus plantarum*, *Lactobacillus brevis*, *Lactobacillus sakei* and *Lactobacillus curvatus*, and cocci-shaped LAB of *Weissella* sp., uncultured *Leuconostoc gasicomitatum* and *Lactococcus lactis* were dominate in *suan-cai*,

whereas rod shaped of LAB of *Lactobacillus sakei* and *Lactobacillus curvatus*, and cocci shaped LAB of *Weissella* sp. and uncultured *Leuconostoc gasicomitatum* were the main fermented culture in *la-baicai*. DGGE is suitable for analysis of LAB community in traditional fermented food like *suan-cai* and *la-baicai*.

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