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Short communication

Microbiota dynamics related to environmental conditions during the fermentative production of *Fen-Daqu*, a Chinese industrial fermentation starter

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ABSTRACT

Chinese *Daqu* is used as a starter for liquor and vinegar fermentations. It is produced by solid state fermentation of cereal–pulse mixtures. A succession of fungi, lactic acid bacteria and *Bacillus* spp. was observed during the production of *Daqu*. Mesophilic bacteria followed by fungi, dominated the first phase of fermentation. Next, lactic acid bacteria increased in relative abundance, resulting in an increase of the acidity of *Daqu*. At the final stages of fermentation, *Bacillus* spp. and thermophilic fungi became the dominant groups, possibly due to their tolerance to low water activity and high temperature. Both culture-dependent and culture-independent analyses confirmed that *Bacillus* spp. were ubiquitous throughout the process. Yeast species such as *Wickerhamomyces anomalus*, *Saccharomycopsis fibuligera* and *Pichia kudriavzevii* were present throughout almost the entire fermentation process, but the zygomycetous fungus *Lichtheimia corymbifera* proliferated only during the final stages of fermentation. Canonical correspondence analysis (CCA) revealed the significance of acidity, moisture content and temperature in correlation with the composition of the microbial communities at different stages.

1. Introduction

Chinese liquor (a traditional distilled alcoholic beverage) and vinegar are important commercial fermented products in China with an annual production of about 12 million metric ton and 5 million metric ton in the country, respectively (Han, 2007). *Daqu* serves as a fermentation inoculum, and it makes a considerable contribution to the flavor of Chinese liquor and traditional Chinese vinegar (Wu et al., 2009). It is an intermediate natural fermentation product that contains metabolically active microorganisms and enzymes. It is an essential ingredient responsible for the release of fermentable sugars from sorghum starch. Generally, *Daqu* can be classified into four major types, i.e. light-flavor *Daqu*, strong-flavor *Daqu*, sauce-flavor *Daqu* and miscellaneous-flavor *Daqu* (Zheng et al., 2011). *Fen-Daqu* is a light-flavor *Daqu* that is prepared from barley and peas in five steps: (i) formulation of ingredients; (ii) grinding and mixing; (iii) shaping; (iv) incubation (about 1 month); and (v) maturation (about 6 months). The incubation stage, also called the fermentation stage, as described by Zheng et al. (2012) can be divided into seven stages according to the core temperature profile of *Daqu* during its production (Fig. 1).

Fen-Daqu is produced using traditional fermentation technology and contains microorganisms that are naturally present in the ingredients (i.e., barley and peas) and its production environment (i.e., tools, soil, air, and machines) (Lei, 2011). Recently, the microbial diversity in various types of *Daqu* has been investigated (Lei, 2011; Wang et al., 2011; Zheng et al., 2012). However, limited data have been reported on the microbial communities prevailing during *Daqu* production (Li et al., 2013). With their study, only a culture-independent cloning method was used, and the microbial dynamics in relation with the environmental conditions during *Daqu* fermentation processes has not been reported. Therefore, the objectives of this study were to analyze changes in temperature, acidity, moisture content and microbial communities during *Daqu* production and to understand the predominance and succession of microbes during its fermentation process as a function of dynamics of environmental conditions.

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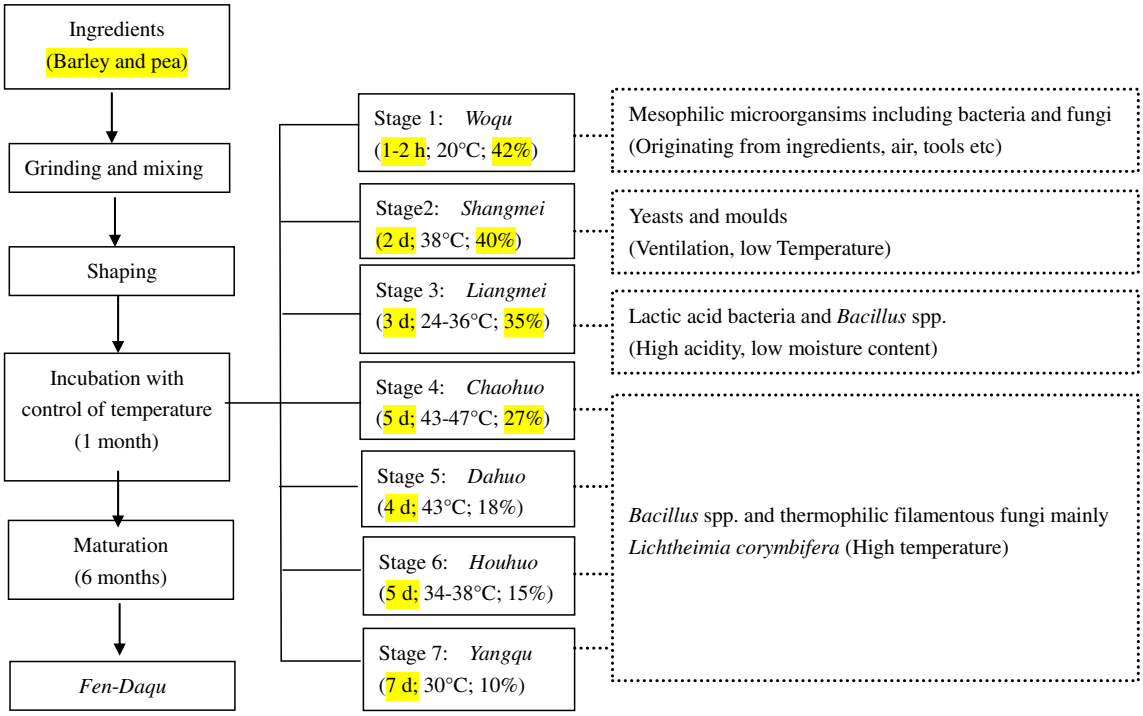

Fig. 1. Process diagram for the production of *Fen-Daqu*. Note: The text within the rectangle marked with black-dotted lines presents the dominant microorganisms at each stage. The content given in brackets presents the factors that determine microbial diversity at that stage. The percentage (%) represents the moisture content in the stages.

2. Materials and methods

2.1. Sampling

Fen-Daqu samples were obtained from Shanxi Xinghuacun Fenjiu Distillery Co. Ltd. (Fenyang, Shanxi, China) during the month of October 2009, which is the season of *Daqu* production. Samples were separately taken from four independent processes and collected at the seven production stages, as shown in Fig. 1. At each of the sampling events, approximately 100 g of *Daqu* was aseptically collected in triplicate (in order to obtain adequate representation, three blocks were randomly selected from upper, middle and lower layers), ground and pooled to provide an experimental *Daqu* powder sample. Samples were then collected into sterile Stomacher bags (Stomacher® Lab System, London, UK), and transported to the laboratory in a cooler box.

2.2. Microbiological analysis—culture-dependent methods

2.2.1. Enumeration and isolation

Samples from all 7 stages, each weighing 10 g were transferred to Stomacher bags and homogenized with 90 mL sterile PPS (peptone physiological salt) solution containing (g/L) peptone (Oxoid LP0034) 1, and NaCl 8.5, in a Stomacher Lab-blender 400. Portions (1 mL) of the diluted suspensions were plated on different selective agar media. Total aerobic bacteria and spores, lactic acid bacteria, Enterobacteriaceae and fungi were enumerated on Plate Count Agar (PCA; Oxoid CM035), MRSA (Oxoid CM0361), Violet Red Bile Glucose Agar (VRBGA; Oxoid CM0485) and Malt Extract Agar (MEA; Oxoid CM0059), respectively. These were incubated at different temperatures (30 °C, 37 °C and 55 °C) for the isolation and enumeration (by recording the number of CFU) of specific groups of micro-organisms (Zheng et al., 2012).

2.2.2. Extraction of DNA from pure culture

Single colony isolates for subsequent DNA extractions were obtained from the counted plates. The plates corresponding to a number of colonies less than or equal to 50, were selected to perform isolations. The square root of the total number of colonies was randomly picked up from both duplicate plates. After purification, isolates were grown under the same conditions as used previously for cultivation. Cells at stationary phase were collected by centrifugation at 9000 *g* for further extraction of total DNA. The genomic DNA of bacteria and fungi was extracted according to the method described by Zheng et al. (2012).

2.2.3. Nucleotide sequence accession numbers

The primers used in this study are listed in Table 1. The 16S rRNA gene sequences of the bacteria in this study were deposited in GenBank under the accession numbers KJ526822–KJ526949; the 26S rRNA and ITS gene sequences of the yeasts were deposited in GenBank under the accession numbers KJ526950–KJ526985 and KJ527033–KJ527069, respectively; the ITS gene sequences of the molds were deposited in GenBank under the accession numbers KJ527009–KJ527032.

2.3. Microbiological analysis—culture-independent methods

2.3.1. PCR-DGGE analysis and bands excision

Total DNA was extracted from *Daqu* powder by the same method used previously (Zheng et al., 2012). Two sets of universal primers were tested for the amplification of a fragment of the 16S rRNA gene and the 26S rRNA gene (Table 1). Amplifications, DGGE analysis and identification of bands of interest were performed as described by Zheng et al. (2012). The sequences of excised bands in this study were deposited in GenBank under the accession numbers KJ526994–KJ527008 for bacteria and the accession numbers KJ526986–KJ526993 for fungi.

2.4. Physicochemical analysis—determination of temperature and relative humidity

The surface temperature of *Daqu* was recorded with a calibrated mini infrared thermometer gun (UNI-T UT301A, Beijing, China) at the time of sampling. The relative humidity (RH) of the incubation room was recorded with a humidity/temperature logger (Testo 175-H2, Shanghai, China). The continuous online detection of the core temperature of *Daqu* and

Table 1
PCR primers used in this study.

Primer	Sequence (5′ → 3′)	Aims	Reference
B-for[a]	AGAGTTTGATCCTGGCTCAG	Amplification 16S rRNA gene and sequencing for bacteria	(Lima et al., 2012)
B-rev[a]	AAGGAGGTGATCCAGCCGCA		
NL1-for	GCATATCAATAAGCGGAGGAAAAG	D1 and D2 domains of 26S rRNA gene and sequencing for yeasts	(Zheng et al., 2012)
RLR3R-rev	GGTCCGTGTTTCAAGAC		
ITS5-for	GGAAGTAAAAGTCGTAACAAGG	Amplification of ITS1-5.8S-ITS2 gene and sequencing for yeasts	(Zheng et al., 2012)
ITS4-rev	TCCTCCGCTTATTGATATGC		
V9G-for	TTACGTCCCTGCCCTTTGTA	Amplification of ITS1-5.8S-ITS2 gene and sequencing for molds	(Zheng et al., 2012)
LS266-rev	GCATTCCCAAACAACTCGACTC		
EUB968GC-for	CGCCCGGGGCGCGCCCCGGGCGGGGCGGGGGCAGGGG AACGCGAAGAACCTTAC	Bacterial DGGE	(Zheng et al., 2012)
EUBL1401-rev	CGGTGTGTACAAGACCC		
EUB968-for	AACGCGAAGAACCTTAC	Sequencing of excised bands from bacterial DGGE	(Zheng et al., 2012)
EUBL1401-rev	CGGTGTGTACAAGACCC		
NL1GC-for	CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGG GCATATCAATAAGCGGAGGAAAAG	Fungal DGGE	(Zheng et al., 2012)
LS2-rev	ATT CCC AAA CAA CTCGAC TC		
NL1-for	GCATATCAATAAGCGGAGGAAAAG	Sequencing of excised bands from fungal DGGE	(Zheng et al., 2012)
LS2-rev	ATT CCC AAA CAA CTCGAC TC		

The GC clamp is underlined.
[a] for: forward; rev: reverse.

room temperature was performed with electronic temperature sensors (iButton, Maxim, USA) which were inserted into the center of the *Daqu* blocks (see Supplementary data, Fig. S1) and stuck on the wall of incubation room. For this purpose, during stage 1, three *Daqu* blocks were randomly selected from each incubation room and labeled. The i-buttons recorded data at hourly intervals until the end of stage 7. Means and standard deviations were calculated.

2.5. Statistical analysis

DGGE data and environmental data were analyzed by canonical correspondence analysis (CCA), using CANOCO 4.5 for Windows software (Biometris, The Netherlands). Significance was tested by the distribution-free Monte Carlo test using 199 random permutations.

3. Results

3.1. Changes in viable cell counts over time during Daqu fermentation

Changes in microbial counts were monitored during the seven stages of *Fen-Daqu* fermentation (see Table 2). The average bacterial counts of the various groups at the start of fermentation (stage 1) were rather low and varied between 3.4 log cfu/g and 6.2 log cfu/g with mesophilic bacteria dominant. After this stage, total counts increased over time, reaching values as high as 9–11 log cfu/g for mesophilic and thermophilic bacteria and bacterial spores, and 5–7 log cfu/g for Enterobacteriaceae and lactic acid bacteria. The levels of mesophilic and thermophilic bacteria and bacterial spores remained at the same level ($P < 0.05$) after stage 5 (Dahuo). In the case of lactic acid bacteria, the highest numbers were obtained at stage 3 which decreased thereafter (Table 2). With respect to fungi, average levels of 5.2 to 8.1 log cfu/g were observed. The number of fungi increased approximately 3 orders of magnitude during stage 2 (Shangmei) and showed a declining trend until the final stage 7 (Yangqu).

3.2. Microbial communities during fermentation

By combining data from culture-dependent and -independent analysis methods, evidence for the presence of 22 bacteria species and one uncultured bacterium was found in *Daqu*, representing the genera *Bacillus*, *Lactobacillus*, *Acetobacter*, *Lactococcus*, *Staphylococcus*, *Weissella*, *Pediococcus*, *Microbacterium*, *Georgenia* and the Enterobacteriaceae family (Table 3). Members of the genus *Bacillus* comprised approximately 50% of total bacterial isolates with *Bacillus licheniformis* as the most common species. DGGE analysis confirmed the high relative abundance of *Bacillus* spp. throughout the fermentation process. *Weissella cibaria* and *Weissella confusa* were dominant during *Daqu* fermentation and were present in different ratios during each fermentation stage. Apart

Table 2
Changes of viable microbial counts (log cfu/g), pH, and acidity during fermentation of *Fen-Daqu*.

	Fermentation stages*						
	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6	Stage 7
TMAB (30 °C)	6.2 ± 0.1^{a}	8.2 ± 0.3^{b}	9.6 ± 0.1^{c}	8.5 ± 0.1^{b}	10.8 ± 0.4^{d}	10.4 ± 0.9^{d}	10.4 ± 0.3^{d}
TTAB (55 °C)	3.5 ± 0.3^{a}	5.2 ± 1.3^{b}	5.3 ± 1.1^{b}	7.2 ± 1.0^{c}	8.1 ± 0.3^{cd}	8.6 ± 0.6^{d}	8.5 ± 0.5^{cd}
Mesophilic aerobic bacterial endospore (30 °C)	4.1 ± 0.5^{a}	5.9 ± 0.4^{b}	5.1 ± 0.2^{b}	7.3 ± 0.2^{c}	9.6 ± 1.0^{e}	8.7 ± 0.6^{d}	8.8 ± 0.6^{de}
Thermophilic aerobic bacterial endospore (55 °C)	3.7 ± 0.1^{a}	5.9 ± 0.4^{b}	5.9 ± 0.4^{b}	7.4 ± 1.1^{c}	8.4 ± 0.4^{cd}	8.8 ± 0.3^{d}	8.5 ± 0.4^{cd}
Lactic acid bacteria	4.9 ± 0.3^{a}	5.7 ± 0.2^{bc}	6.5 ± 0.1^{d}	6.3 ± 0.1^{cd}	6.1 ± 0.7^{cd}	6.0 ± 0.5^{cd}	5.3 ± 0.6^{ab}
Enterobacteriaceae	3.4 ± 0.2^{a}	3.8 ± 0.4^{ab}	4.2 ± 0.8^{bc}	5.0 ± 0.2^{d}	4.7 ± 0.2^{cd}	4.6 ± 0.5^{cd}	4.1 ± 0.5^{bc}
Fungi	5.2 ± 0.4^{a}	8.1 ± 0.4^{d}	7.7 ± 0.2^{cd}	7.3 ± 0.1^{bc}	7.4 ± 0.3^{bc}	7.4 ± 0.5^{bc}	7.2 ± 0.3^{b}
pH	4.3 ± 0.1^{a}	4.2 ± 0.1^{a}	4.4 ± 0.3^{a}	6.1 ± 0.6^{b}	6.8 ± 0.1^{c}	6.8 ± 0.1^{c}	6.9 ± 0.1^{c}
Acidity (g lactic acid/kg dry matter)	1.4 ± 0.2^{a}	4.6 ± 0.5^{d}	4.2 ± 0.3^{d}	3.7 ± 0.4^{c}	3.1 ± 0.7^{bc}	3.1 ± 0.4^{b}	3.3 ± 0.5^{bc}

TMAB: total mesophilic aerobic bacteria.
TTAB: total thermophilic aerobic bacteria.
Value represent means ± SD ($n = 4$). Means with different superscripts are significantly different horizontally (one-way ANOVA; $P < 0.05$).
* Stage 1: *Woqu*; stage 2: *Shangmei*; stage 3: *Liangmei*; stage 4: *Chaohuo*; stage 5: *Dahuo*; stage 6: *Houhuo*; stage 7: *Yangqu*.

Table 3
Microbiota composition at each stage of *Fen-Daqu* fermentation.

		Fen-Daqu fermentation stages[a]						
		Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6	Stage 7
Bacteria	*A. pasterianus*		[11.8]+(I)				[6.25]+(I)	
	A. tropicalis			[5]+(I)				
	B. anthracis/*B. cereus*	+(D)		[15]+(I)		+(D)	+(D)	+(D)
	B. amyloliquefaciens						[12.5]+(I)	
	B. circulans			[5]+(I)				
	B. licheniformis	[37.5]+(I/D)	[23.5]+(I/D)	[15]+(I/D)	[81.25]+(I/D)	[88.2]+(I/D)	[43.75]+(I/D)	[50]+(I/D)
	B. megaterium	+(D)		[15]+(I/D)		+(D)		+(D)
	B. pumilus	[6.25]+(I)	[11.8]+(I/D)	[10]+(I)			[25]+(I)	
	B. subtilis	+(D)	[23.5]+(I)	+(D)				[25]+(I/D)
	Enterobacter sp./*Escherichia* sp.	[25]+(I)	[23.5]+(I)	[15]+(I)				
	E. aerogenes	+(D)		+(D)	+(D)	+(D)		
	Georgenia sp.	[6.25]+(I)						
	Lb. curvatus/*Lb. sakei*		[5.8]+(I)					
	Lb. plantarum	+(D)		[10]+(I)	+(D)	+[11.8](I/D)	+(D)	+(D)
	Lb. sanfranciscensis		+(D)		+(D)	+(D)	+(D)	
	Lc. lactis	[12.5]+(I)						
	Microbacterium sp.			[5]+(I)				
	Ped. pentosaceus	[12.5]+(I)		[5]+(I)	[18.75]+(I)		[12.5]+(I)	[12.5]+(I)
	S. epidermidis							[12.5]+(I)
	S. saprophyticus	+(D)			+(D)	+(D)	+(D)	+(D)
	Uncultured bacterium		+(D)	+(D)		+(D)	+(D)	+(D)
	Ws. cibaria	+(D)	+(D)	+(D)	+(D)	+(D)	+(D)	+(D)
	Ws. confusa	+(D)	+(D)	+(D)	+(D)	+(D)	+(D)	+(D)
Yeasts	*D. hansenii*					+(D)	+(D)	
	P. kudriavzevii		[38.4]+(I/D)	[23.1]+(I/D)	[28.5]+(I/D)	[8.3]+(I/D)	[25]+(I/D)	[28.5]+(I/D)
	Pseudozyma sp.	[7.7]+(I)						
	Sm. fibuligera	+(D)	+(D)		+(D)	+(D)	+(D)	+(D)
	W. anomalus	[46.1]+(I/D)	[23]+(I/D)	[69.2]+(I/D)	[71.4]+(I/D)	[66.7]+(I/D)	[37.5]+(I/D)	[28.5]+(I/D)
Filamentous fungi	*Alternaria* sp.			[7.6]+(I)				[7.2]+(I)
	Asp. versicolor						[6.2]+(I)	
	Cladosporium sp.	[23.1]+(I)						
	L. corymbifera					[25]+(I)	[25]+(I)	[35.8]+(I)
	Penicillium sp.	[15.3]+(I)						
	Phoma sp.	[7.7]+(I)						
	Rhizomucor pusillus						[6.2]+(I)	
	R. stolonifer		[38.4]+(I)					

+ indicates species is detected at this stage; [I] indicates strain obtained only by culturing at this stage; [D] indicates strain obtained only by DGGE at this stage; [I/D] indicates strain obtained from both isolation and DGGE at this stage. *A*: *Acetobacter*; *B*: *Bacillus*; *E*: *Enterobacter*; *Lb*: *Lactobacillus*; *Lc*: *Lactococcus*; *Ped*: *Pediococcus*; *S*: *Staphylococcus*; *Ws*: *Weissella*; *D*: *Debaryomyces*; *P*: *Pichia*; *Sm*: *Saccharomycopsis*; *W*: *Wickerhamomyces*; *L*: *Lichtheimia*; *Asp*: *Aspergillus*; *R*: *Rhizopus*; [ ] indicates the frequency of presence (%) of each species within the groups of bacteria or within the groups of yeasts and molds at each stage of fermentation.

[a] Stage 1: *Woqu*; stage 2: *Shangmei*; stage 3: *Liangmei*; stage 4: *Chaohuo*; stage 5: *Dahuo*; stage 6: *Houhuo*; stage 7: *Yangqu*.

from *Bacillus* sp. and *Weissella* sp., *Pediococcus pentosaceus* was also found frequently.

A total of 5 yeast species were obtained of which *Pichia kudriavzevii*, *Saccharomycopsis fibuligera* and *Wickerhamomyces anomalus* were detected in six, six and seven stages, respectively (Table 3). Among the filamentous fungi, the zygomycetous species *Lichtheimia corymbifera* (formerly *Absidia corymbifera*) appeared during stage 5 and increased in relative abundance towards the end of the fermentation (Table 3).

3.3. Changes in environmental factors during fermentation

The pH of *Daqu* increased steadily after stage 3 of the fermentation process, the titratable acidity increased rapidly during stage 2, reaching the maximum value of approximately 5 g/kg at the end of this stage, followed by a decline (Table 2).

Changes in core and surface temperatures of *Daqu* blocks and of the incubation room, as well as relative humidity and moisture content that occurred during the *Daqu* fermentation process were monitored continuously (Fig. 2). The core temperature increased from 20 °C to 52 °C between stage 1 and stage 5, and thereafter decreased rapidly to ambient room temperature in stage 6. A gradual decrease of moisture content took place throughout the whole process, from 40 g water/100 g *Daqu* at the start to around 10 g water/100 g *Daqu* at the final stage. The changes in room temperature were less pronounced than those of the core of the *Daqu* blocks. With increasing temperature, the RH of incubation room dropped from approximately 100% to 20%.

3.4. Microbiota composition in relation to environmental variables

Canonical correspondence analysis (CCA) was carried out using abundant DGGE bands together with environmental variables. Species–environment correlations for both axes were higher than 0.99 (canonical coefficients), suggesting that microbial diversity was strongly correlated with environmental factors. In the CCA triplot (Fig. 3), the length of arrows indicated the relative importance of that environmental factor in explaining variation in microbial profiles, while the angle between the arrows (environmental factor–environmental factor or environmental factor–species) indicated the degree to which they were correlated. It shows that acidity, pH and surface temperature were the three most important environmental variables which could be correlated with the microbial diversity in different samples, since the lengths of these arrows are longer than those of others. Moisture content was the sole environmental factor that correlated with the microbial diversity at stages 3 and 7. Acidity correlated with the composition of the microbial community of stage 2, in which a relatively high abundance of *Ws. confusa*, *Ws. cibaria*, *Bacillus cereus*, *Bacillus pumilus* and *P. kudriavzevii* was found. Temperature and pH were found to be strongly correlated with the composition of the microbiota during the stages 4, 5 and 6.

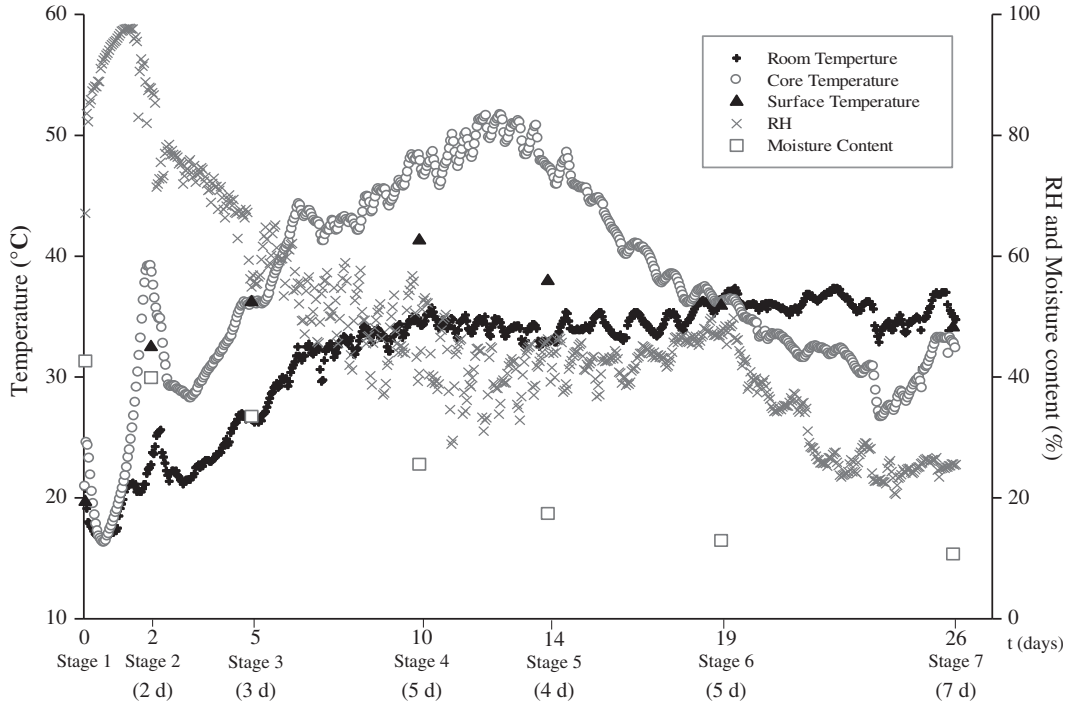

Fig. 2. Changes in temperature, RH, and moisture content during fermentation of *Fen-Daqu* (stage 1: *Woqu*; stage 2: *Shangmei*; stage 3: *Liangmei*; stage 4: *Chaohuo*; stage 5: *Dahuo*; stage 6: *Houhuo*; stage 7: *Yangqu*).

4. Discussion and conclusions

This study showed that species of the genus *Bacillus* are predominant during all stages of *Fen-Daqu* fermentation. By combining both culture-dependent and culture-independent data, stage 3 was identified as the stage with the highest diversity of *Bacillus* spp. This result is in agreement with the data of Yan et al. (2013). In our study, seven *Bacillus* species, namely *Bacillus anthracis*/*B. cereus*, *Bacillus amyloliquefaciens*, *Bacillus circulans*, *B. licheniformis*, *Bacillus megaterium*, *B. pumilus*, and *Bacillus subtilis*, were found during the whole *Daqu* fermentation process, whereas almost all these species were detected simultaneously at stage 3. Of these bacilli, only *B. licheniformis* and *B. subtilis* were found in the study of *Fen-Daqu* production by Li et al. (2013). This result emphasizes the importance of using two complementary methods because higher numbers of species could be obtained. *Bacillus* spp. can survive harsh environmental conditions such as drying (Deng et al., 2012). *Daqu* after stage 3 became dry due to evaporation, caused by the use of forced ventilation in order to control the incubation temperature during stage 3. This explains the decrease of both room temperature and core temperatures in Fig. 2. As a result, the moisture content of *Daqu* decreased. Zhao et al. (2011) reported that bacilli have a better ability to survive under low moisture conditions than other bacteria. Canonical correspondence analysis also revealed the significance of moisture content in correlation with the microbial communities at this stage (Fig. 3).

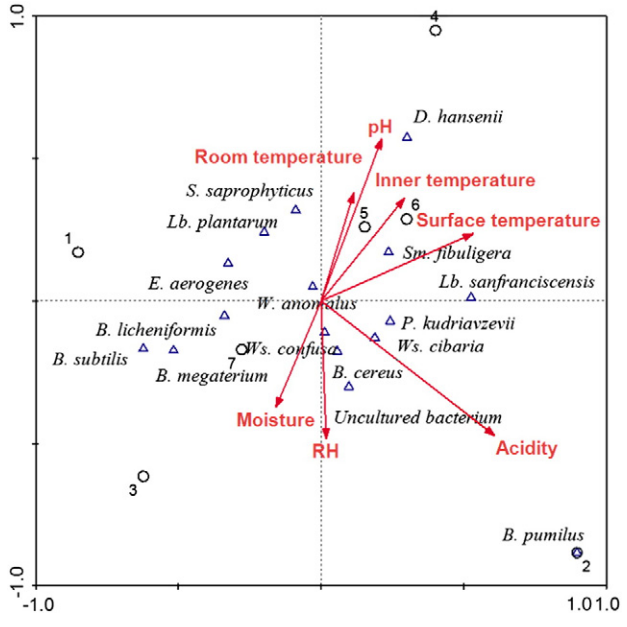

Fig. 3. Canonical correspondence analysis (CCA) of DGGE community profiles from *Daqu* samples from the 7 stages. Numbers refer to the different stages, arrows refer to different environmental factors, and triangles refer to the individual abundant microbial species.

The number of thermophilic bacteria and bacterial spores increased from 5.9 at stage 3 to 8.8 log cfu/g at stage 6 (Table 2), when the diversity of *Bacillus* spp. decreased. Correspondence analysis indicated that pH and temperature were the most important factors that correlated with the composition of the microbial community during the stages 4, 5 and 6. The core temperature during these three stages reached a maximum of about 52 °C between stages 4 and 5 (Fig. 2). Such high temperature is expected to have a selective effect, favoring thermotolerant, aerobic endospore-forming bacteria. Only *Bacillus* spp., such as *B. licheniformis* and *B. subtilis*, are able to grow between 50 and 60 °C (Burgess et al., 2010). This may be the reason why members of the genus *Bacillus* spp. were dominating the microbial community during the last four stages. This could also explain why *B. licheniformis* and *B. subtilis* were encountered frequently in strong or sauce flavor *Daqu* (Wang et al., 2011) in which fermentation temperatures are even higher than in *Fen-Daqu*.

LAB also play a role in the production of *Daqu* (Zheng et al., 2011), especially *Lactobacillus* spp. (Li et al., 2013). Acidity was recognized as the most significant factor that correlated with the composition of the microbial communities of stage 2 (Fig. 3). The increase in acidity correlates with the occurrence of high numbers of LAB and fungi, especially

Lactobacillus sanfranciscensis, *Ws. cibaria*, *Ws. confusa*, *W. anomalus* and *P. kudriavzevii*.

The fungal community associated with *Fen-Daqu* fermentation was found to be less diverse than the bacterial one. A succession of yeast species during fermentation could not be observed. Li et al. (2013) reported the predominance of *P. kudriavzevii* in *Fen-Daqu*; however, in our study two other yeasts species (*Sm. fibuligera* and *W. anomalus*) were predominant during *Daqu* fermentation, since they occurred during almost the entire production process. *Sm. fibuligera* has been reported to degrade and assimilate raw starch as a carbon source (Chi et al., 2009) so it may contribute to the formation of fermentable carbohydrates for the subsequent alcoholic fermentation. *W. anomalus* and *P. kudriavzevii* are known as ester-producing yeasts and a higher abundance of these organisms correlates with an elevated concentration of esters in liquor (Li et al., 2012; Wu et al., 2012).

Filamentous fungi are commonly used in solid state fermentations, because of their relatively high tolerance to low water activity and their production of hydrolytic enzymes (Rahardjo, 2005). The major molds associated with *Daqu* fermentation can be categorized into two groups. The first group belongs to the family of Mucoraceae. These are known as strong amylase producers in amylolytic Asian fermentation starters (Rahardjo, 2005). Of these fungal species, *L. corymbifera* occurred during stage 5 and persisted until the end of the fermentation. The relatively high core temperature of the *Daqu* above 45 °C during stages 4–5 allows only thermophilic or thermotolerant fungi to survive. This may be true for *L. corymbifera* that has been reported to grow at temperatures as high as 48–52 °C (Weitzman et al., 1995), and may explain that it was detected during the last three stages of *Daqu* fermentation. The second group comprises *Aspergillus* spp., which are used in solid state fermentations and also are known to produce extracellular proteolytic and saccharolytic enzymes (Rahardjo, 2005).

In this study, both culture-dependent and culture-independent approaches were combined to analyze the dynamics of microbial communities during *Daqu* fermentation. Although both techniques may provide different results due to the use of various sampling methods, they showed sufficient overlap of information on the dominant groups of microbial communities. Only 6 bacterial species, i.e. *B. cereus*, *B. licheniformis*, *B. megaterium*, *B. pumilus*, *B. subtilis* and *Lactobacillus plantarum* and 2 fungal species, i.e. *P. kudriavzevii* and *W. anomalus* could be detected by both approaches. By the culture independent method, no bands were identified as filamentous fungi. We assume that the absence of bands for filamentous fungi in our DGGE gel is mainly caused either by (1) inadequate DNA extraction of filamentous fungi from the complex food matrix of *Daqu*, or (ii) by the presence of high concentrations of competing DNA, such as that from yeasts in *Daqu*. These results reinforce the necessity of employing both culture-dependent and culture-independent approaches to uncover the microbial diversity of complex microbial ecosystems, such as *Daqu*.

Li et al. (2013) applied cloning methods to analyze the microbial communities during the production of *Fen-Daqu*, and they reported the predominance of lactobacilli and *P. kudriavzevii* in *Daqu*. However, our study showed the predominance of different groups of microorganisms at different stages of the fermentation. In conclusion, a succession in relative abundance of fungi, LAB and *Bacillus* spp. was observed during the *Daqu* fermentation process, which can be linked to changes in environmental conditions such as pH, temperature, acidity and moisture content (Fig. 1). Better knowledge of microbial succession driven by environmental changes may facilitate long-term technological developments and innovation that will benefit the liquor and vinegar industry.

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