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ORIGINAL PAPER

Characterisation of microbial communities in Chinese liquor fermentation starters *Daqu* using nested PCR-DGGE

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Abstract In this study, characterises of the microbial community structures of three typical Chinese liquor *Daqu*, as well as different kinds of light flavour *Daqu* were investigated using nested PCR-denaturing gradient gel electrophoresis (DGGE). The results showed that microbial diversity was considerably different, and the microfloral compositions were highly variable among various *Daqu*. Lactic acid bacteria, which accounted for 30.95 % of all identified bacteria, were dominant in all *Daqu* samples, whereas *Bacillus* species were also predominant in the *Luzhou* (14.8 %) and *Langjiu Daqu* (18.2 %). *Citrobacter* and *Burkholderia* were first identified in light flavour *Daqu*. *Aspergillus* was the dominant moulds, and the non-*Saccharomyces* yeast species, *Saccharomycopsis fibuligera*, *Wallemia sebi*, *Wallemia muriae*, and *Pichia subpelliculosa*, were the dominant yeasts. *Rasamsonia*, *Galactomyces*, *Geotrichum* and *Wallemia* were first identified using nested PCR-DGGE. Cluster analysis indicated that the microbial community structures of different *Daqu* samples exhibited some differences. These may be ascribed to the different peak production temperatures, raw material constituents and microhabitats around the liquor enterprises. The current study provides insights into the microbial community structures of three typical *Daqu* samples, and may facilitate the development of starter cultures for manufacturing Chinese liquor.

Keywords Fermentation starters · Bacterial communities · Fungal communities · Microbial diversity · Nested PCR-DGGE

Introduction

Daqu is an essential fermentation starter for the conventional oriental fermented foods, such as liquor (Gao et al. 2010; Liu et al. 2012; Wang et al. 2011) and vinegar (Wu et al. 2012). It is not only an important crude enzyme preparation and primary microbial sources, but also a portion of brewing materials that are closely related with the flavour characteristics of the products (Zhang et al. 2012). In general, *Daqu* is mainly categorised into three different types in Chinese conventional liquor manufacturing: light flavour, *Luzhou* flavour and soy sauce flavour. As is known, *Daqu* is produced through solid fermentation, which involves three phases: shaping, ripening and drying. During this procedure, numerous microorganisms, enzymes and flavour compounds are enriched. However, the manufacturing process employed, production temperature, raw material constituents and microhabitats around the liquor enterprises are different, producing different microbial compositions and various *Daqu* characteristics. Therefore, fully understanding the microbial compositions and the characteristics of different types of *Daqu* may contribute to the development of starter cultures and Chinese liquor manufacturing.

In the last decades, culture-dependent techniques have been employed to study the microbial compositions of *Daqu* (Shi et al. 2001; Wang et al. 2008b; Yang et al.

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2007). Wang et al. (2008b) explored the microbial community in *Maotai Daqu* based on a culture-dependent technique and suggested that *Bacillus* and *Aspergillus* strains are the dominant bacteria and moulds, and the most frequently isolated yeasts strains are from genus *Saccharomyces*. However, the cultivable microbes accounted for only 1–10 % of the microbes in the environment due to the culture conditions and competition from abundant microbial species, which led to inaccurate description of microbial compositions (Yen et al. 1992). Recently, culture-independent techniques such as PCR-denaturing gradient gel electrophoresis (DGGE; Wang et al. 2011) and restriction fragment-length polymorphism (RFLP; Diguta et al. 2011) have been widely used to study the microbial community in micro-ecological environments. Among these techniques, PCR-DGGE has also been conducted to investigate the microbial diversity of *Daqu* in the last decade (Liu et al. 2012; Wang et al. 2011; Zheng et al. 2012). For example, the microbial diversity of *Fen Daqu* was determined by combining culture-dependent techniques with culture-independent techniques, and several bacterial orders such as *Bacillales* and *Lactobacillales*, yeast families such as *Saccharomycopsidaceae* and *Dipodascaceae* and mould families such as *Thrichocomaceae* were identified (Shi et al. 2009; Zheng et al. 2012). By contrast, direct PCR-DGGE is insensitive to species with low copy numbers in complex microbial community from natural environments (Miletto et al. 2007). As an alternative method, nested PCR detects target DNA at concentrations several times lower than conventional PCR (Hafez et al. 2005) and it has been proved to be useful for minimising the difficulties associated with species with low copy numbers (Liu et al. 2012).

As is the main source of microbes in liquor manufacturing, the microbial community in *Daqu* needs to be investigated comprehensively and systematically to understand the characteristics of different typical *Daqu* and their contributions to liquor brewing. Therefore, this study aims to investigate and compare the microbial community structures in three typical Chinese liquor fermentation starters using nested PCR-DGGE.

Materials and methods

Sampling

Three typical types of *Daqu*, which were stored for 6 months to mature after ripening, were collected from *Fenjiu*, *Luzhou Laojiao* and *Langjiu* liquor brewing enterprises (Table 1). They are famous manufacturers of the representative light flavour (*Fenjiu* flavour), strong aromatic flavour (*Luzhou* flavour) and soy sauce flavour (*Maotai* flavour) liquor respectively in China. Considering the peak production temperature differs during the manufacturing process, "*Fen*" *Daqu* was further categorised into three different kinds of *Daqu*: *qing cha* (QC), *hou huo* (QH) and *hong xin* (QX). Three parallel samples were collected from the top, middle, and bottom of the storeroom, which were transferred into sterile bags and stored at −20 °C.

Table 1 Different *Daqu* samples

Sample no.	Sources	Peak production temperature (°C)
QC	*Fenjiu* Wine Factory Co., Ltd. (Xinghuacun, Shanxi Province)	45–46
QH	*Fenjiu* Wine Factory Co., Ltd. (Xinghuacun, Shanxi Province)	48–49
QX	*Fenjiu* Wine Factory Co., Ltd. (Xinghuacun, Shanxi Province)	46–47
LZ	*Luzhou Laojiao* Co., Ltd. (Luzhou City, Sichuan Province)	50–60
LJ	*Langjiu* Group Co., Ltd. (Guling County, Sichuan Province)	60–65

DNA extraction

Five grams of samples were suspended in 20 mL of sterile 0.1 mol/L phosphate-buffered saline (PBS, pH 8.0) along with three grams of sterile glass beads (Φ:2 mm), and were eddied at 200 rpm for 5 min at ambient temperature. The suspension was then centrifuged (800×*g*, 4 °C) for 10 min, and subsequently, the supernatants collected were centrifuged (10,000×*g*, 4 °C) for 10 min. The resulting sediments were washed 2–3 times with 10 mL of PBS, resuspended in 2.0 mL of PBS buffer and stored at −20 °C until DNA extraction. Genomic DNA was extracted according to a previously described method (Zhou et al. 1996). The extracted DNA was assessed via electrophoresis on 0.6 % (w/v) agarose gels and was used as templates for PCR amplification.

PCR amplification

Nested PCR was used to increase sensitivity and facilitate the DGGE analysis. To analyse bacterial diversity, the primer set 27f/1492r (Kim et al. 2010) was used to amplify the nearly completed 16S rRNA encoding gene during the first round of PCR. Subsequently, the initial PCR products were diluted and then used as templates for nested PCR that targets the V3 region using the specific primers 357f-GC/517r (Hu et al. 2009; Muyzer et al. 1993) to create a suitable DNA fragment for DGGE analysis. To analyse fungal diversity, the 26S rRNA gene was subjected to PCR

amplification using the universal primer set NL 1/NL 4 (Thanh et al. 2008), followed by nested PCR using the primer set NL 1-GC/LS 2 (Abe et al. 2008) to create a proper DNA fragment for DGGE analysis. The PCR products were examined via electrophoresis on 1 % agarose gels before DGGE analysis.

DGGE analysis

The PCR products were analysed via DGGE using a DCode™ Universal Mutation Detection System apparatus (Bio-Rad, USA). All nested PCR products were applied onto 8 % (w/v) polyacrylamide (acrylamide: bis-acrylamide 37.5:1) gels with linear denaturing gradient (100 % corresponded to 7 M urea and 40 % (v/v) formamide) increasing in the direction of electrophoresis in 1× TAE buffer (40 mM Tris–acetate, 1 mM EDTA). The optimal denaturant gradient was 30–60 % for bacteria (Muyzer et al. 1993), and 10–50 % for fungi (Wang et al. 2011). Electrophoresis was performed at a constant voltage of 150 V for 5 h at 60 °C. After electrophoresis, the gels were stained for 30 min with 30 ml of SYBR green I (1:10,000), and then documented with a Gel Doc™ XR + imaging system (Bio-Rad, USA).

Excision of DGGE bands and sequencing

Representative DGGE bands were excised from the gels using a sterile blade, washed with ethanol, and eluted overnight in 50 µl of Tris–EDTA (pH 8.0) at 4 °C to allow the DNA fragment to diffuse out of the gels. The solutions were then centrifuged at 10,000×*g* for 5 min and the supernatants were collected and used as templates for reamplification using the same primers without the GC-clamp on the 5′ ends. Subsequently, the reamplification products were purified using a Universal DNA Purification Kit (TIANGEN, Bejing, China), and then delivered to Sangon (Shanghai, China) for sequencing. The sequences obtained were compared with the 16S rRNA and 26S rRNA gene sequences available in GenBank (http://www.ncbi.nlm.nih.gov) to identify their closest phylogenetic relatives.

Statistical analysis

Statistical analysis was performed using the Quantity One software (Bio-Rad, USA), which was used to convert individual DGGE lanes to densitometric profiles. The intensity of each band was calculated. The microbial diversity indices were determined based on the number and relative intensity of the bands in each DGGE profile, and calculated using the PAST software (Palaeontology Statistics, http://folk.uio.no/ohammer/past/). The sample profiles were clustered using NTSYS-pc 2.10e software.

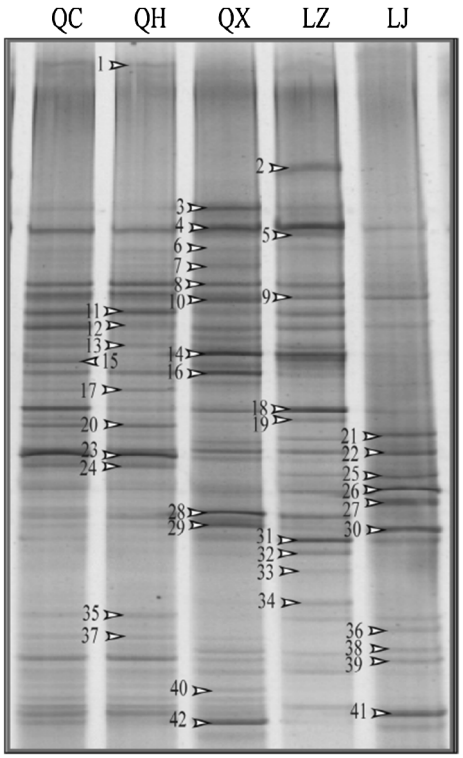

Fig. 1 PCR-DGGE profiles of the bacterial communities in three typical *Daqu* samples. QC, QH, QX, LZ and LJ represent the *Daqu* samples shown in Table 1. Numbers 1–42 represent the bands excised

Table 2 Microbial diversity indices calculated from the DGGE profiles of bacterial communities

Sample no.[a]	Species richness (*R*)	Shannon's index (*H*)	Dominance (*D*)
QZ	36	3.584	0.028
QH	35	3.555	0.029
QX	33	3.496	0.030
LZ	29	3.367	0.034
LJ	22	3.091	0.045

[a] *Daqu* samples shown in Table 1

Results

Bacterial community diversity

The DGGE profiles of the bacterial communities in different types of *Daqu* are shown in Fig. 1, and notable differences were observed. The bacterial diversity indices were calculated, as shown in Table 2. The diversity indices of the three different kinds of light flavour *Daqu* exhibited no significant differences. Furthermore, their species richness (*R*) and Shannon's indices (*H*) were higher than those

Table 3 Sequencing results of bacterial DGGE bands

Band no.[a]	Closest relatives[b]	Accession no.	Identity (%)[c]
1	*Lactobacillus sanfranciscensis* (HM162420.1)	KF432092	99
2	*Weissella confusa* (JX188072.1)	KF432093	99
3	*Staphylococcus aureus* (JQ958397.1)	KF432094	99
4	*Leuconostoc mesenteroides* (JQ658346.1)	KF432095	99
5	*Weissella confusa* (JX041943.1)	KF432096	99
6	*Staphylococcus lentus* (JX154084.1)	KF432097	99
7	*Weissella koreensis* (HQ896200.1)	KF432098	99
8	*Lactobacillus sanfrancisco* (M58830.1)	KF432099	99
9	*Staphylococcus saprophyticus* (JX415366.1)	KF432100	99
10	*Staphylococcus xylosus* (JX426123.1)	KF432101	99
11	*Lactobacillus fuchuensis* (AB063479.1)	KF432102	100
12	*Pediococcus pentosaceus* (FR873983.1)	KF432103	99
13	*Bacillus safensis* (HQ238893.1)	KF432104	99
14	*Lactobacillus lindneri* (GU138499.1)	KF432105	99
15	*Pediococcus acidilactici* (JQ806729.1)	KF432106	99
16	*Lactobacillus* sp. (HM352627.1)	KF432107	99
17	*Weissella* sp. (JX402126.1)	KF432108	99
18	*Staphylococcus kloosii* (HM209752.1)	KF432109	99
19	Uncultured *Staphylococcus* sp. (EU926958.1)	KF432110	99
20	*Enterobacter asburiae* (JX502182.1)	KF432111	99
21	*Lactobacillus* sp. (HM352624.1)	KF432112	99
22	*Virgibacillus* sp. (AB562922.1)	KF432113	99
23	*Lactobacillus sanfranciscensis* (GU138531.1)	KF432114	99
24	*Bacillus flexus* (JF939002.1)	KF432115	99
25	*Bacillus sporothermodurans* (FJ973531.1)	KF432116	99
26	*Bacillus licheniformis* (JX237852.1)	KF432117	99
27	*Bacillus ginsengihumi* (AB696801.1)	KF432118	99
28	*Enterobacter asburiae* (JX297470.1)	KF432119	99
29	*Citrobacter freundii* (AB741675.1)	KF432120	99
30	*Klebsiella oxytoca* (JQ312040.1)	KF432121	99
31	*Salibacillus* sp. (NR_043170.1)	KF432122	99
32	*Klebsiella pneumoniae* (AB680615.1)	KF432123	99
33	Enterobacteriaceae *bacterium* (EU341314.1)	KF432124	99
34	*Pantoea septica* (JQ638348.1)	KF432125	99
35	*Klebsiella variicola* (JQ280389.1)	KF432126	99
36	*Pantoea agglomerans* (JN835509.1)	KF432127	99
37	*Erwinia amylovora* (FJ594367.1)	KF432128	99
38	Uncultured *Klebsiella* sp. (GQ418068.1)	KF432129	99
39	*Pantoea dispersa* (JN835497.1)	KF432130	99
40	*Pantoea ananatis* (JN835526.1)	KF432131	99
41	Uncultured *Burkholderia* sp. (EU876657.1)	KF432132	95
42	*Enterobacter* sp. (HQ730479.1)	KF432133	99

[a] Bands are numbered according to Fig. 1

[b] Only the highest homology matches are presented

[c] Identity represents % similarity shared with the sequences in the GenBank databases

of the LZ and LJ samples, whereas their dominance indices (*D*) were lower than those of the LZ and LJ samples. The LZ *Daqu* had higher species richness (*R*) and Shannon's index (*H*) compared with the LJ *Daqu*, but it had a lower dominance index (*D*).

Representative bands of bacterial DGGE profiles were sequenced, and the results are shown in Table 3. The similarity of all sequences was higher than 99 % compared with those available in the GenBank database. The closest related microbes among all 16S rRNA gene sequences were affiliated with 13 genera (Table 3), and were classified into six families. Lactic acid bacteria (LAB), including *Lactobacillus*, *Pediococcus* and *Weissella*, accounted for 30.95 % of all bacteria identified, and were dominant in all *Daqu* samples. Five *Lactobacillus* species were identified in the *Daqu*, namely, *L. sanfrancisco*, *L. fuchuensis*, *L. lindneri*, *Lactobacillus* sp. and *L. sanfranciscensis*, whereas no *L. fuchuensis* was detected in LJ. For other LAB, *P. pentosaceus* was observed in all samples. However, *P. sacidilactici* was only detected in QC. *W. koreensis* was identified in QC, QH, QX and LZ, *Weissella* sp. was detected in QC, QH and LJ, whereas *W. confusa* was only found in the QX and LZ. Genus *Bacillus* was also predominant in both the LZ (14.8 %) and the LJ (18.2 %). *B. safensis* and *B. licheniformis* were detected in all *Daqu*, while the other three *Bacillus* species were only detected in several samples: *B. flexus* only presented in the QC and QH, whereas *B. sporothermodurans* and *B. ginsengihumi*

Table 4 Microbial diversity indices calculated from the DGGE profiles of fungal communities

Sample no.[a]	Species richness (*R*)	Shannon's index (*H*)	Dominance (*D*)
QC	23	3.006	0.057
QH	22	2.983	0.058
QX	19	2.821	0.069
LZ	16	2.600	0.088
LJ	21	2.974	0.059

[a] *Daqu* samples shown in Table 1

were identified in the LZ and the LJ. *Staphylococcus aureus* was absent in the LJ and *Staphylococcus xylosus* was absent in the LZ, whilst uncultured *Staphylococcus* sp. was only detected in the QC, QX and LZ. *Citrobacter freundii* and *Klebsiella variicola* were undetected in the LJ. Genus *Pantoea*, namely, *P. dispera* and *P. ananatis*, were detected in light flavour *Daqu*, whereas *P. septica* was only present in the QX and the LJ. *P. agglomerans* and *P. dispera* were also detected in the LJ. Genus *Ervinia* and *Burkholderia* were only identified in light flavour *Daqu*.

Fungal community diversity

Diversity indices were calculated (Table 4). No significant differences in diversity indices were observed between the QC and the QH in the three types of light flavour *Daqu*, but the species richness (*R*) and Shannon's index (*H*) of the QX were lower, whereas its dominance index (*D*) was higher. Light flavour *Daqu*, LZ *Daqu* and LJ *Daqu* significantly differed in diversity indices. The LZ sample had the lowest species richness (*R*) and Shannon's index (*H*), but the highest dominance index (*D*).

Representative bands of fungal DGGE profiles (Fig. 2) were excised, sequenced and identified. The results are shown in Table 5. The similarity of all band sequences was nearly >96 % compared with those available in the GenBank database. The closest related microbes among all 26S rRNA sequences were affiliated with 13 genera from 8 families. Among them, *Pichia*, *Wickerhamomyces*, *Saccharomycopsis*, *Aspergillus*, *Rasamsonia* and *Wallemia* were detected in all *Daqu* samples. For genus *Galactomyces*, *G. geotrichum* was detected in the QC, QH and LJ, whereas *G. candium* was detected in the LZ and LJ. For genus *Pichia*, no *P. kudriazevii*, *P. exigua* and uncultured *Pichia* sp. were detected in the LZ sample. *Endomyces fibuliger* was identified in the light and soy sauce flavour *Daqu*. *Pichia anomala*, classified under *Wickerhamomyces*, only presented in the LJ. No *Candida* and *Thrichomonascus* species were detected in the LZ sample, whereas *Candida silva* was merely detected in the

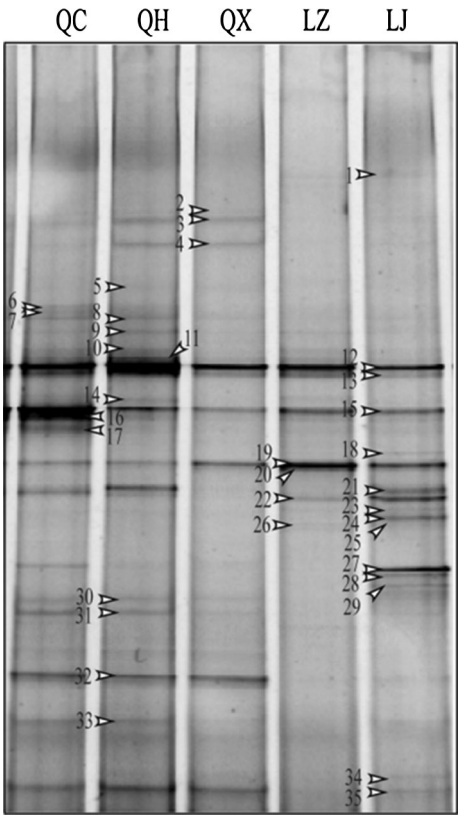

Fig. 2 PCR-DGGE profiles of fungal communities in three typical *Daqu* samples. QC, QH, QX, LZ and LJ represent the *Daqu* samples shown in Table 1. Numbers 1–35 represent the excised bands

QC and QH, and *Candida mucifera* was solely observed in the LJ. The other two species *Candida allociferrii* and *Candida catenulata* presented in the light and soy sauce flavour *Daqu*. Furthermore, genus *Debaryomyces* and *Thermoascus* were only identified in the LJ sample. The characteristics of dominant fungal species depended on the type of *Daqu*. *Saccharomycopsis fibuligera*, *Wallemia sebi*, *Wallemia muriae*, *Pichia subpellliculosa*, *P. kudriavzevii* and *Aspergillus* species were the predominant species in the light flavour *Daqu*. Aside from *Candida allociferrii*, which was the dominant species in the QC sample, *G. geotrichium* and *Saccharomyces cerevisiae* were also dominant in the QC and QH samples. *S. fibuligera*, *W. sebi*, *W. muriae*, *P. subpellliculosa*, and *Geotrichum* sp. were dominant in the LZ and LJ samples, whereas *G. geotrichum*, *Debaryomyces hansenii* and *C. allociferrii* were also the dominant fungal species in the LJ sample.

Cluster analysis of DGGE profiles

Cluster analysis was performed using bacterial and fungal DGGE data to estimate the order of relatedness among the different *Daqu* samples (Fig. 3). Cluster analysis of the

Table 5 Sequencing results of fungal DGGE bands

Band no.[a]	Closest relatives	Accession no.[b]	Identity (%)[c]
1	*Pichia subpelliculosa* (JQ419775.1)	KF432141	100
2	*Pichia kudriavzevii* (KC143811.1)	KF432142	100
3	*Uncultured Pichia* (JX409534.1)	KF432143	99
4	*Pichia exigua* (HE799658.1)	KF432144	99
5	*Issatchenkia orientalis* (EU543688.1)	KF432145	99
6,12	*Saccharomycopsis fibuligera* (JX645719.1)	KF432146 (67)	98
7	*Candida silvae* (FJ649188.1)	KF432147	99
8	*Endomyces fibuliger* (U09238.1)	KF432164	98
9	*Talaromyces emersonii*/ *Talaromyces byssochlamydoides* (DQ010015.1/EU021608.1)	KF432148	96
10,11	*Saccharomycopsis fibuligera* (KC222507.1)	KF432165 (66)	100
13,14	*Wallemia sebi* (JN938862.1)	KF432168 (69)	100
15,16	*Wallemia muriae* (DQ847517.1)	KF432170 (71)	98
17	*Issatchenkia orientalis* (EU543688.1)	KF432149	99
18,23	*Galactomyces candidum* (JN974271.1)	KF432150 (55)	99
19	*Pichia subpelliculosa* (JQ419894.1)	KF432151	100
20	*Pichia anomala* (FJ865436.1)	KF432152	99
21	*Galactomyces geotrichum* (JF262181.1)	KF432153	99
22	*Geotrichum* sp. (EU294127.1)	KF432154	99
24	*Debaryomyces hansenii* (KC111444.1)	KF432172	99
25	*Debaryomyces coudertii* (JN940508.1)	KF432156	99
26	*Issatchenkia orientalis* (FJ770560.1)	KF432157	99
27	*Candida allociferrii* (GU373789.1)	KF432173	100
28	*Candida mucifera* (AJ508572.1)	KF432174	100
29	*Candida catenulata* (FJ627977.1)	KF432158	99
30	*Candida catenulata* (FJ455109.1)	KF432159	100
31	*Saccharomyces cerevisiae* (JX103177.1)	KF432160	99
32	*Pichia kudriavzevii* (JX183970.1)	KF432161	99
33	*Issatchenkia occidentalis* (AB281316.1)	KF432162	99
34	*Thermoascus crustaceus* (JF922031.1)	KF432163	99
35	*Aspergillus affinis*/*Aspergillus cretensis* (JF805762.1/ EF661417.1)	KF432175	98

[a] Bands are numbered according to Fig. 2

[b] Only the highest homology matches are presented

[c] Identity represents the % similarity shared with the sequences in the GenBank database

bacterial communities showed that the *Daqu* samples QC and QH formed a group; samples QX, LZ and LJ were clustered into another group that was subdivided into two clusters, with the QX sample as a subgroup and the LZ and LJ samples clustered into another subgroup. In the fungal communities, the profiles were clustered into two groups, namely, group 1 (QC, QH and QX) and group 2 (LZ and LJ). Group 1 was subdivided into two subgroups, QC as a subgroup, and the other two were clustered into another subgroup.

Discussion

The microbial diversity of three representative types of *Daqu*, which involved three kinds of light flavour *Daqu*, was first investigated using nested PCR-DGGE. The results showed that the microbial community structures of the different *Daqu* samples were relatively complex and distinctly different. Thirteen identified bacterial genera were affiliated to six families, among which ten genera were previously detected using culture-independent methods (Liu et al. 2012; Shi et al. 2009; Tang et al. 2011; Wang et al. 2011; Zheng et al. 2012). *Pediococcus* was also detected using culture-dependent methods (Zheng et al. 2012). *Citrobacter freundii* and uncultured *Burkholderia* sp., with high intensity, were first detected using nested PCR-DGGE; these species secrete alkaline lipase, as well as hydrolyse triacylglycerols into glycerides, free fatty acids and glycerol (Gunasekaran et al. 2006; Liu et al. 2006). The genera *Citrobacter* and *Burkholderia* were first detected in the light flavour *Daqu*.

LAB are one of the dominant bacterial species in *Daqu* samples, which is consistent with previous studies (Liu et al. 2012; Wang et al. 2008b, 2011). Similar to other

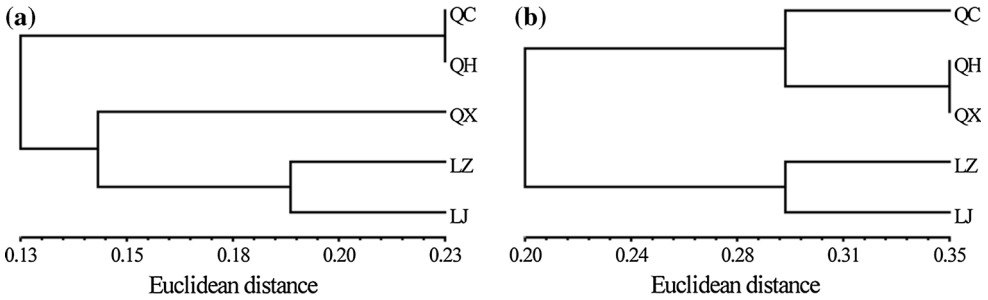

Fig. 3 Clustering analysis of microbial communities in *Daqu* samples. **a** Bacteria; **b** fungi. QC, QH, QX, LZ and LJ represent the *Daqu* samples shown in Table 1

fermentation processes, the lactic acid produced by LAB inhibit the propagation of other spoilage bacteria and provide substrates for forming lactate, which contributes to the specific flavour of Chinese liquor (Lv et al. 2012; Wang et al. 2008a). *Bacillus* species were dominant in the LZ and LJ *Daqu* samples, which have highest production temperatures slightly higher than that of the light flavour *Daqu*. The majority of *Bacillus* species secrete various hydrolytic enzymes and form heat-resistant spores. Previous studies indicated that the amylase and protease secreted by *Bacillus* convert starch and proteins into glucose and amino acids, thereby contributing to the formation of flavour compound precursors during fermentation (Wang et al. 2011; Zheng et al. 2012). Furthermore, *B. sporothermodurans* was also detected in the LZ and LJ *Daqu* samples, which form heat-resistant spores and was first detected in milk subjected to ultra-high temperature treatment (Pettersson et al. 1996). The functions of *B. sporothermodurans* in liquor manufacturing process are still unknown and need further investigation. In general, other bacteria such as *Staphylococcus* and *Enterobacter* occurred in low numbers. *S. xylosus*, which has a high intensity, produces aromatic compounds such as 3-methyl-1-butanol, diacetyl, 2-butanone and acetoin, which may play an important role in liquor manufacturing (Søndergaard and Stahnke 2002).

For the fungal communities, *Talaromyces emersonii*, *Talaromyces byssochlamydoides*, *W. sebi*, *W. muriae*, *G. candidum*, *G. geotrichum* and *Geotrichum* sp. were identified for the first time in *Daqu*. *T. emersonii* and *T. byssochlamydoides* produce xylanases and convert xylan polysaccharides into sugars, alcohols and other products (Gomes et al. 1993; McCarthy et al. 2005). The intense band (band 21), detected in the QH and the LJ samples, was closest phylogenetically to *G. geotrichum*. *Geotrichum* sp. and genus *Galactomyces*, which secrete proteases and lipases that degrade proteins and lipids into precursors of various volatile compounds, may contribute to liquor flavour (Phillips and Pretorius 1991; Sacristán et al. 2012). *Wallemia* is a halophilic fungal genus (Kunčič et al. 2010), but its functions in fermentation starters are still unknown and need further investigation.

Four genera of moulds were detected in the *Daqu* samples. *Aspergillus* species were the dominant moulds, which is consistent with previously obtained results (Wang et al. 2011; Zhang et al. 2007). Genus *Aspergillus* secretes various hydrolytic enzymes (Oda et al. 2006), and is considered as major producer of crude enzymes in *Daqu* (Lv et al. 2012; Wang et al. 2011). Eight genera of non-*Saccharomyces* yeasts and *S. cerevisiae* were identified in the *Daqu* samples. *S. fibuligera*, *W. sebi*, *W. muriae* and *P. subpelliculosa* were dominant in all *Daqu* samples. *S. fibuligera*, the main producer of amylase and glucoamylase, participates in degrading starch into glucose during the initial stages of alcohol fermentation (Lv et al. 2012; Wang et al. 2011; Zheng et al. 2012). According to previous reports (Kumar and Satyanarayana 2001), *P. subpelliculosa* produces glucoamylase and participates in converting starch into glucose. Although *S. cerevisiae* was detected in most *Daqu* samples, the band density was relatively weak, which indicated that it was not a dominant yeast, similar results were reported by Zheng et al. (2012).

The differences in microbial structures among different *Daqu* samples may be ascribed to differences in process parameters, especially peak production temperature and moisture, and microhabitats. Generally, production temperature and moisture influence the growth of microorganisms and the enrichment of different microbial species. In addition, the range of bacterial growth temperatures is relatively wider than that of fungi. Some bacterial species can grow at 45–65 °C or even at higher temperature, which would inhibit the growth of fungi or cause death. Hence, the slight difference in peak production temperature for each kind *Daqu* in light flavour types causes differences in microbial diversity indices although the processes are almost identical. Compared with the light flavour *Daqu*, the process parameters and raw material constituents of the LZ and LJ were also different. Therefore, distinct differences in microbial composition and diversity indices were observed among the three typical *Daqu* during the experiments.

In conclusion, notable differences in microbial community and diversity indices were observed among the three typical *Daqu*, as well as among the three kinds of

light flavour *Daqu* using nested PCR-DGGE. The results showed that the microbial composition and diversity indices of various types *Daqu* depended on the peak production temperature, raw constituents as well as on the microhabitats. *Citrobacter* and *Burkholderia* were first detected in the light flavour *Daqu*. LAB were dominant in all the *Daqu* samples, moreover *Bacillus* species were also predominant in the LZ and LJ. *Aspergillus* was the dominant moulds and the non-*Saccharomyces* yeasts including *S. fibuligera*, *W. sebi*, *W. muriae* and *P. subpelliculosa* were the dominant yeasts, *Rasamsonia*, *Galactomyces*, *Geotrichum* and *Wallemia* were identified in the *Daqu* samples for the first time. The present study provided new insights into the microbial community structures of three typical *Daqu* samples using nested PCR-DGGE, which may facilitate the development of better starter cultures for manufacturing Chinese liquor.

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