Original paper

Characterization of Microbial Community in *Daqu* by PLFA Method

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Received August 26, 2013 ; Accepted October 23, 2013

***Daqu*, as an essential saccharifying and fermentative agent during Chinese liquor manufacture, is closely related to the quality and yield of liquor. In order to explore the microbial community structure during the process of *Daqu* production, the phospholipid fatty acid (PLFA) patterns were analyzed. The results obtained by measuring the PLFA contents of pure mycelium and raw materials demonstrated that PLFA analysis was appropriate to explore the characteristics and shifts of the microbial community in *Daqu* sample. Analysis of the microbial community of *Daqu* by PLFA method suggested that fungi predominated the total amounts of biomass in *Daqu*. In addition, changes in contents of fungi and bacteria were also investigated during *Daqu*-making. From the shaped stage to the finished stage of *Daqu*, fungi dominated gradually, and a small amount of bacterial biomass were detected in all finished *Daqu* samples. In three finished *Daqu* samples, the fungal biomass accounted for 68.85% – 80.11% of total biomass. Furthermore, the contents of gram-positive bacteria were higher than that of gram-negative bacteria in all *Daqu* samples.**

Keywords: *Daqu*, phospholipid fatty acids, microbial community, fungal biomass

Introduction

Chinese liquor is one of the well-known distilled spirits in the world, and the manufacture technique is unique. *Daqu* is an essential starter culture during the production of Chinese liquor and has noteworthy impact on the flavor of the product (Xu *et al.*, 2010). Based on the aromas of distillate, several types of *Daqu* can be distinguished, such as light-flavor, strong-flavor and soy sauce-flavor, which depend on different manufacture techniques. *Daqu* is a complex substrate throughout the fermentation of grain via a natural inoculation of microbial communities originated from production environment. Therefore, microbial community and the metabolites are closely related to incubation conditions and environments (Zhang *et al.*, 2009). Although the production methods of various types of *Daqu* are slightly different, the process often involves three stages: material shaping, incubating (particularly, microbial community forming) and drying (decreased

to 13 – 14% of humidity by natural drying). The particular microbial community forming stage is the most important process for the production of various necessary enzymes and flavor compounds. For this reason, it is necessary to investigate the characteristic of microbial flora as well as their shifts to understand the relationship between microbial community and metabolites, and optimize the culture conditions (Wu *et al.*, 2009a). Recently, some cultivable studies on microbial community during the process of *Daqu* manufacture have been reported (Wang *et al.*, 2008). Whereas, it was not only time-consuming, but also difficult to obtain the microbial community structure effectively, since 80 – 99% of microorganisms were uncultured (Amann *et al.*, 1995). PCR-based fingerprinting technique, providing a higher resolution and more information about the changes in the whole microbial community structure, has been applied to investigate the *Daqu* microbial community (Wang *et al.*, 2011; Xiu *et al.*, 2012),

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short, 1 mL methanol-toluene solution (1:1) and 1 mL 0.2 M KOH-methanol were added, and the mixture were heated at 37°C for 30 min. After methylation, distilled water (2 mL) and acetic acid solution (1 M, 0.3 mL) were added. Fatty acid methyl esters were extracted in hexane (2 mL) twice and dried under N₂. Fatty acid methyl esters (FAMES) were analyzed by a gas chromatogram/mass spectrometry system (Thermo Fisher Corporation, USA). The GC system was equipped with a TR-5MS (30.0 m × 320 µm × 0.25 µm) capillary column. Identifications of FAMES were compared with the standards (C4-24, Accustandard, USA) and mass spectrum (NIST05). The methyl nonadecanoate fatty acid (19:0, Accustandard, USA) was added as an internal standard. To remove lipid contaminants, all glassware used was heated overnight at 500°C. Standard nomenclature is used to describe FAMES (Federle *et al.*, 2006; Frostegård and Bååth, 1996; Moore-Kucera and Dick, 2008; Olsson and Alström, 2000) and the PLFAs characteristic of Gram-positive bacteria (GM+), Gram-negative bacteria (GM-), and fungi were listed in Table 1. All values of PLFA concentrations were the mean of at least three independent extraction processes.

Statistical analysis Molar amounts [nmol/g (dry weight, dw)] of the individual PLFAs were used as an estimation of microbial biomass. The relative proportions of PLFAs expressed as arithmetic means ± standard deviations ($n = 3$) were used to describe the microbial community structure. Analysis of variance (One-way ANOVA) with Duncan's test ($P < 0.05$) was used to represent the significant differences of each PLFA data by SPSS 17.0 software (SPSS Inc. IL, USA).

Results

PLFAs content of mycelium Table 2 displayed the phospholipid fatty acids content of various fungal strains. As shown in Table 2, the profiles of PLFAs and their contents were significantly different (14.43 – 42.29 µmol/g dw) in the mycelium of various strains tested. The predominant PLFAs were 16:0, 18:2ω6,9 and 18:1ω9, and the latter two were often used as the indicator of fungal biomass (Klamer and Bååth, 2006). The ratios of the specific PLFAs to total PLFAs were different in all strains. As for *Aspergillus* (CGMCC3.324 and CGMCC3811), the ratios of 18:2ω6,9 were 39.2 mol% and 53.1 mol%, while the ratios

of 18:1ω9 were 26.9 mol% and 12.8 mol%, respectively. As for *Rhizopus* (SICC3.107 and SICC3.26), the ratios for 18:2ω6,9 were 37.5 mol% and 37.3 mol%, respectively. In addition, 18:3ω6,9,12 was only measured in *Mucoraceae*, and PLFA 18:2ω9,12 was only detected in *Mucor*. A detailed analysis of the results presented in Table 2 showed that similar proportions of fungi biomarkers (18:1ω9, 18:2ω6,9 and 18:3ω6,9,12) were observed for these strains. These results suggest that the analysis of specific biomarker (18:1ω9, 18:2ω6,9 and 18:3ω6,9,12) may be a feasible method to evaluate the biomass of fungal strains.

Comparison of PLFAs in the substrates before and after cultivation As shown in Table 3, eight PLFAs were detected in the substrate before cultivation, which consisted of five saturated, two monounsaturated and one polyunsaturated fatty acid, while only four PLFAs were detected in the substrate after cultivation. After cultivation, primary PLFAs were unsaturated such as 18:2ω6,9 and 18:1ω9, and the saturated PLFAs 14:0, 15:0 and 17:0 were reduced below the detection limit. In addition, the contents of 16:0 and 18:0 in cultured substrate were reduced by 40.5% and 50.0%, respectively. Although the content of total PLFAs was decreased, no obvious change was found in the proportions of PLFA 18:2ω6,9 and 18:1ω9 (63.0% and 67.2% for the proportions before and after cultivation, respectively).

PLFA profiles of *Daqu* samples Figure 2 showed the PLFA profiles of three different *Daqu* samples, and these samples exhibited similar profiles of PLFA. The dominant PLFAs of these *Daqu* samples were 16:0, 18:2ω6,9 and 18:1ω9, and these PLFAs amounted for more than 95% of total PLFAs during the shaped period of *Daqu* (Fig. 2). With the extension of incubation time, differences in PLFAs species and total amounts were observed. 9, 11, 15 PLFAs were detected during the ripening period in LJ, FG, and XF *Daqu* samples, respectively, and the total amounts of PLFA increased 2.40-, 2.51-, and 2.16-fold compared with that determined during the shaped period. Especially, the contents of 18:2ω6,9 and 18:1ω9 enhanced 2.95-, 2.96-, and 2.73-fold in LJ, FG, and XF *Daqu*, respectively. For finished *Daqu*, 10, 11 and 15 PLFAs were detected in LJ, FG, and XF *Daqu*, and the total PLFAs amount were 1645.21, 1904.91, and 1503.53 nmol/g (dw), respectively.

Table 1. PLFA marker used for taxonomic microbial groups

Taxonomic group	PLFA group	Specific PLFA markers	References
Bacteria	Multiple groups	a14:0, i16:0, 16:1ω9	(Frostegård and Bååth, 1996, Moore-Kucera and Dick, 2008)
Gram-positive bacteria	Branched PLFAs	i14:0, i15:0, i16:0, i17:0, a15:0, a17:0	(Moore-Kucera and Dick, 2008, Olsson and Alström, 2000)
Gram-negative bacteria	Cyclopropyl and monosaturated PLFAs	16:1ω9, 16:1ω5, 16:1ω7, cy17:0	(Moore-Kucera and Dick, 2008, Olsson and Alström, 2000)
Fungi	Polyunsaturated PLFAs	18:1ω9, 18:2ω6,9, 18:3ω6,9,12	(Federle <i>et al.</i> , 2006)

Table 2. Phospholipid fatty acids content of various fungal strains

Strain	PLFA content ¹ (μmol/g dw)											Total
	14:0	15:0	16:1ω7	16:0	17:0	18:3ω6,9,12	18:2ω9,12	18:2ω6,9	18:1ω9	18:0		
<i>Aspergillus niger</i>	ND ²	0.90 ± 0.19d	ND	12.15 ± 1.52b	0.2 ± 0.04	ND	ND	16.58 ± 4.24d	11.39 ± 1.96c	0.66 ± 0.23b		42.29 ± 3.95e
CGMCCC.324												
<i>Rhizopus</i> sp.	ND	0.09 ± 0.03ab	ND	5.98 ± 1.79a	ND	2.07 ± 0.19b	ND	9.60 ± 0.67c	7.32 ± 1.35b	0.54 ± 0.10ab		25.61 ± 3.85d
SICC3.107												
<i>Absidia corymbifera</i>	ND	ND	ND	6.45 ± 0.97a	ND	1.16 ± 0.16a	ND	3.73 ± 0.42ab	7.71 ± 0.82b	ND		19.05 ± 0.27bc
CQB43												
CGMCCC3811	ND	0.27 ± 0.05c	ND	4.66 ± 0.87a	ND	ND	ND	7.67 ± 0.45c	1.84 ± 0.12a	ND		14.43 ± 0.34a
<i>Rhizopus nigricans</i>	0.07 ± 0.01a	0.17 ± 0.03bc	0.13 ± 0.02	5.21 ± 0.61a	ND	1.29 ± 0.26ab	ND	6.51 ± 0.81bc	3.52 ± 0.38a	0.58 ± 0.13ab		17.47 ± 1.40ab
SICC3.26												
<i>Mucor</i>	0.80 ± 0.19b	ND	ND	4.35 ± 0.74a	ND	4.39 ± 0.49c	1.01 ± 0.12	1.36 ± 0.01a	10.00 ± 0.09c	0.35 ± 0.04a		22.28 ± 0.88cd
MZ1-24												

Different letters represented significant differences (ANOVA with Duncan's test, $P < 0.05$)

¹Mean ± SD, (n = 3)

²ND, not determined

Table 3. Comparison of fatty acids proportion in substrate before and after incubation.

	Fatty acid component (%)							
	14:0	15:0	16:1ω7	16:0	17:0	18:2ω6,9	18:1ω9	18:0
A ^a	0.15 ± 0.004	0.20 ± 0.009	0.12 ± 0.004	35.43 ± 3.04	0.15 ± 0.005	51.62 ± 4.03	11.37 ± 0.15	0.96 ± 0.08
B ^b	ND ^c	ND	ND	32.10 ± 3.03	ND	56.81 ± 4.16	10.36 ± 0.66	0.73 ± 0.06

^a substrate before incubation

^b substrate after incubation

^c ND, not determined

Microbial community structure of *Daqu* samples Changes in microbial community structure of *Daqu* sample during the process of *Daqu* manufacture were investigated (Fig. 3). No significant difference was observed in total biomass of all samples in the initial state of *Daqu*-making. The biomass increased from shaped period

Fig. 2. PLFAs composition of *Daqu* samples collected from different periods. (A) LJ *Daqu*; (B) FG *Daqu*; (C) XF *Daqu*. Error bars indicate standard deviations ($n = 3$).

to ripened period and then slightly decreased in finished phase. In the initial stage of *Daqu* production process, the PLFAs resulted from wheat cell and some endophytic fungus. With the extension of cultivation time, microorganisms originated from the environment propagated gradually and formed particular microbial community. During the ripened stage of cultivation, the fungi dominated in all of the *Daqu* samples, and the proportion of the bacteria PLFAs was slightly increased. As for finished *Daqu*, the fungi were still dominant in three kinds of *Daqu*, which accounted for 74.46%, 80.11% and 68.85% of total PLFAs amounts in LG, FG, and XF samples, respectively. In addition, in all samples, the contents of gram-positive bacteria were higher than that of gram-negative bacteria, which accounted for 4.72%, 3.80%, and 5.83% of total biomass in LG, FG, and XF *Daqu* samples, respectively.

Discussion

In the present study, microbial community structures in *Daqu* sample were determined by using PLFA method. Phospholipid fatty acid, the biomarker of living microorganisms, is not found in dead cell or storage compounds (Zelles, 1997). Certain PLFA biomarkers are related to specific groups of microorganism. Thus, PLFA method have effectively applied to characterize the shifts of the microbial community structure (Bäath *et al.*, 2005). However, some studies reported that the phospholipids component of cell membrane were rapidly metabolized following cell death, but this conclusion obtained by particular experimental conditions (Hill *et al.*, 2000). Furthermore, the PLFA biomarkers 18:1ω9, 18:2ω6,9 and 18:3ω3,6,9 commonly used as fungal biomarkers in soil have also been found in plant tissues (the raw materials of *Daqu*-making) (Laczkó *et al.*, 2003). Therefore, it is necessary to investigate the feasibility of PLFA method on determining the *Daqu* microbial community structure, as wheat and pea are main materials during *Daqu* manufacture.

6 fungal strains, which were common microorganisms during *Daqu* production, were selected for PLFA analysis. The specific PLFAs of fungi (18:1ω9 and 18:2ω6,9, and 18:3ω3,6,9) accounted for more than 90% of total PLFA excluding 16:0. The results suggested that specific biomarkers may be a possible method to determine the biomass of fungal strains. In addition, the fungal biomarkers PLFA 18:1ω9 and 18:2ω6,9 were detected in the substrate before and after cultivation, and the result indicated that the fungal biomass and percentage (mol%) may be overestimated by the sum of PLFA 18:1ω9, 18:2ω6,9 and 18:3ω3,6,9. Pre-treatments such as sieving and/or hand-picking were used to eliminate the biomarkers PLFA originated from plant cell contributed to soil samples (Kaiser *et al.*, 2010). But it was almost impossible to eliminate the interference of plants PLFAs to fungal PLFAs biomarkers by this manner since wheat and bran were essential substrates for *Daqu* manufacture. Nonetheless, the PLFAs concentration in culture mediums during the incubation process (incubated at 30°C for 72 h) had significant decrease (more than

37.5%, abiotic and/or biotic), which indicated that phospholipids were unstable in this process. The complete turn-over time for PLFAs in *Daqu* producing was not known, but previous study showed a short turn-over time for phospholipids in sediments (White *et al.*, 1979). And this degradation was thought to be fairly rapid in natural environments (King *et al.*, 1977; Klammer and Bååth, 2006). As for the long process (20 ~ 40 d) and the shift of temperature (30 ~ 65°C) during the *Daqu* production, the contribution of PLFAs from raw material to microorganism PLFAs biomarkers in *Daqu* could be neglected. There was other evidence from our previous

studies, which showed that the high correlation ($R = 0.987$, $P < 0.05$) between ergosterol (another fungal biomarker) and PLFA (18:2 ω 6,9) content in *Daqu* samples was obtained (Qin *et al.*, 2011). In addition, previous PLFAs analysis concerning decomposition of rice straw (Nakamura *et al.*, 2003) and the microbial communities of fermented grains (Zheng *et al.*, 2011), had shown that none/low concentration of PLFAs 18:2 ω 6,9 and 18:1 ω 9 was detected, although the great quantity of plant cell existed. According to these results, it was suggested that high concentration of PLFAs 18:2 ω 6,9 and 18:1 ω 9 in *Daqu* samples were accounted for fungal mycelium, and PLFA analysis could give more information of living microbes compared with other methods.

Changes in microbial community structure in the process of *Daqu* production were also investigated. The fungi of *Daqu* represent higher amounts of biomass than the bacteria. It should be realised however that since fungi biomass is larger than that of bacteria, this does not imply that fungi have a higher number or larger metabolic impact on *Daqu* (Zheng *et al.*, 2012b). During the shaped phase of *Daqu*, the PLFA biomarkers of both GM+ and GM- were not detected, and the fungal PLFAs may originate from the raw materials of *Daqu*. With the increase of incubation time, the biomass of fungi increased sharply, while the contents of bacteria increased gradually, and fungi still dominated the microflora. During the process of *Daqu*-making by solid-state fermentation, the temperature rose sharply due to the limitation of mass and heat transfer, and only the thermophile strains survived. Xiu *et al.* (Xiu *et al.*, 2012) determined the microbial diversity in *Maotai-Daqu*, and the results showed that *Bacillus* and lactic acid bacteria were the dominant bacteria during the ripening period of *Daqu*-making. *Bacillus* can survive in a low moisture content (13–14%) and high temperature (60–62°C) (Shi *et al.*, 2009). Lactic acid bacteria usually act as a regulator of microbial consortia diversity in a variety of food and may contribute to the formation of flavour compounds by producing a variety of enzymes (Liu *et al.*, 2011). During the finished period of *Daqu*-making, the percentage of PLFA biomarkers of bacteria in the finished *Daqu* was still very low compared with that of fungi. When the amounts of bacterial PLFA were converted into the number of bacterial cells, the average amount of bacterial PLFA was 1.4×10^{-17} mol/cell (Frostegård and Bååth, 1996). With this conversion factor, we could estimate that bacterial biomass in the finished *Daqu* samples were from 9.8×10^8 to 10.94×10^8 cells/g (dw). These results were a little higher than that detected from culture method ($6.3 \sim 21.8 \times 10^7$ cfu/g (dw)) (Wang *et al.*, 2008). This may be ascribed to the fact that some uncultured bacteria existed which could not be responded by culture-dependent method (Wang *et al.*, 2011). In addition, this high level of bacterial biomass in *Daqu* sample makes this starter different from other Asian traditional alcoholic fermentation starters such as Men and Ragi of which the bacterial loads of 2.6–6.2 Log cfu/g and 4.3–5.8 Log cfu/g, respectively, represented mainly lactic acid bacteria (Hesseltine *et al.*, 1988; Thanh *et al.*, 2008). Of

Fig. 3. Changes of microbial community structure during the process of *Daqu* production. Panels A, B, and C represent PLFAs concentration characterizing GM+, GM-, and fungi, respectively. LJ, FG, and XF present LJ *Daqu*, FG *Daqu*, and XF *Daqu*, respectively. Different letters indicate significant differences ($P < 0.05$, ANOVA, Duncan's test). Error bars indicate standard deviations ($n = 3$).

course, the different types of *Daqu* samples and the applicability of this conversion factor (calculated from soil bacteria) may also be explored further.

Acknowledgements This work was financially supported by the National Science Foundation of China (No. 31171742).

References

- Amann, R. I., Ludwig, W., and Schleifer, K. H. (1995). Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiol. Rev.* **59**, 143-169.
- Bäåth, E., Diaz-Ravina, M., and Bakken, L. (2005). Microbial biomass, community structure and metal tolerance of a naturally Pb-enriched forest soil. *Microbial Ecol.* **50**, 496-505.
- Blackwood, C. B., Hudleston, D., Zak, D. R., and Buyer, J. S. (2007). Interpreting ecological diversity indices applied to terminal restriction fragment length polymorphism data: Insights from simulated microbial communities. *Appl. Environ. Microb.* **73**, 5276-5283.
- Dowling, N. J. E., Widdel, F., and White, D. C. (1986). Phospholipid ester-linked fatty acid biomarkers of acetate-oxidizing sulphate-reducers and other sulphide-forming bacteria. *J. Gen. Microbiol.* **132**, 1815-1825.
- Farrell, M., Griffith, G. W., Hobbs, P. J., Perkins, W. T., and Jones, D. L. (2010). Microbial diversity and activity are increased by compost amendment of metal-contaminated soil. *FEMS Microbiol. Ecol.* **71**, 94-105.
- Federle, T. W., Dobbins, D. C., Thorntonmanning, J. R., and Jones, D. D. (2006). Microbial biomass, activity, and community structure in subsurface soils. *Ground Water*. **24**, 365-374.
- Frostegård, A. and Bäåth, E. (1996). The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. *Biol. Fert. Soils*. **22**, 59-65.
- Hesseltine, C., Rogers, R., and Winarno, F. (1988). Microbiological studies on amylolytic oriental fermentation starters. *Mycopathologia*. **101**, 141-155.
- Hill, G., Mitkowski, N., Aldrich-Wolfe, L., Emele, L., Jurkonie, D., Ficke, A., Maldonado-Ramirez, S., Lynch, S., and Nelson, E. (2000). Methods for assessing the composition and diversity of soil microbial communities. *Appl. Soil Ecol.* **15**, 25-36.
- Kaiser, C., Frank, A., Wild, B., Koranda, M., and Richter, A. (2010). Negligible contribution from roots to soil-borne phospholipid fatty acid fungal biomarkers 18: 2ω6, 9 and 18: 1ω9. *Soil Biol. Biochem.* **42**, 1650-1652.
- King, J. D., White, D. C., and Taylor, C. W. (1977). Use of lipid composition and metabolism to examine structure and activity of estuarine detrital microflora. *Appl. Environ. Microb.* **33**, 1177-1183.
- Klamer, M. and Bäåth, E. (2004). Estimation of conversion factors for fungal biomass determination in compost using ergosterol and PLFA 18: 2ω6, 9. *Soil Biol. Biochem.* **36**, 57-65.
- Klamer, M. and Bäåth, E. (2006). Microbial community dynamics during composting of straw material studied using phospholipid fatty acid analysis. *FEMS Microbiol. Ecol.* **27**, 9-20.
- Laczko, E., Boller, T., and Wiemken, V. (2003). Lipids in roots of *Pinus sylvestris* seedlings and in mycelia of *Pisolithus tinctorius* during ectomycorrhiza formation: Changes in fatty acid and sterol composition. *Plant Cell Environ.* **27**, 27-40.
- Liang, C., Fujinuma, R., and Balser, T. C. (2008). Comparing PLFA and amino sugars for microbial analysis in an upper michigan old growth forest. *Soil Biol. Biochem.* **40**, 2063-2065.
- Liu, S., Leathers, T. D., Copeland, A., Chertkov, O., Goodwin, L., and Mills, D. A. (2011). Complete genome sequence of *Lactobacillus buchneri* NRRL B-30929, a novel strain from a commercial ethanol plant. *J. Bacteriol.* **193**, 4019-4020.
- Müller, M., Handley, K., Lloyd, J., Pancost, R., and Mills, R. (2010). Biogeochemical controls on microbial diversity in seafloor sulphidic sediments. *Geobiology*. **8**, 309-326.
- Moore-Kucera, J. and Dick, R. P. (2008). PLFA profiling of microbial community structure and seasonal shifts in soils of a douglas-fir chronosequence. *Microbiol. Ecol.* **55**, 500-511.
- Nakamura, A., Tun, C. C., Asakawa, S., and Kimura, M. (2003). Microbial community responsible for the decomposition of rice straw in a paddy field: Estimation by phospholipid fatty acid analysis. *Biol. Fert. Soils*. **38**, 288-295.
- Olsson, S. and Alström, S. (2000). Characterisation of bacteria in soils under barley monoculture and crop rotation. *Soil Biol. Biochem.* **32**, 1443-1451.
- Qin, Z., Zheng, J., Peng, Y., Jin, Y., Huang, J., and Zhou, R. (2011). Characterization of microbial community structure in *Daqu* starter based on biomarkers. *Food Sci.* **32**, 165-170.
- Shi, J., Xiao, Y., Li, X., Ma, E., Du, X., and Quan, Z. (2009). Analyses of microbial consortia in the starter of *Fen* liquor. *Lett. Appl. Microbiol.* **48**, 478-485.
- Thanh, V. N., Mai, L. T., and Tuan, D. A. (2008). Microbial diversity of traditional Vietnamese alcohol fermentation starters (*banh men*) as determined by PCR-mediated DGGE. *Int. J. Food Microbiol.* **128**, 268-273.
- Wang, C., Shi, D., and Gong, G. (2008). Microorganisms in *daqu*: A starter culture of Chinese *Maotai-flavor* liquor. *World J. Microbiol. Biotechnol.* **24**, 2183-2190.
- Wang, H. Y., Gao, Y. B., Fan, Q. W., and Xu, Y. (2011). Characterization and comparison of microbial community of different typical Chinese liquor *Daqu*s by PCR-DGGE. *Lett. Appl. Microbiol.* **53**, 134-140.
- White, D., Davis, W., Nickels, J., King, J., and Bobbie, R. (1979). Determination of the sedimentary microbial biomass by extractable lipid phosphate. *Oecologia*. **40**, 51-62.
- Wu, X. H., Zheng, X. W., Han, B. Z., Vervoort, J., and Nout, M. J. R. (2009a). Characterization of Chinese liquor starter, "*Daqu*", by flavor type with ¹HNMR-based nontargeted analysis. *J. Agr. Food Chem.* **57**, 11354-11359.
- Wu, Y., Ding, N., Wang, G., Xu, J., Wu, J., and Brookes, P. C. (2009b). Effects of different soil weights, storage times and extraction methods on soil phospholipid fatty acid analyses. *Geoderma*. **150**, 171-178.
- Xiu, L., Kunliang, G., and Hongxun, Z. (2012). Determination of microbial

- diversity in *Daqu*, a fermentation starter culture of Maotai liquor, using nested PCR-denaturing gradient gel electrophoresis. *World J. Microbiol. Biotechnol.* **28**, 2375-2381.
- Xu, Y., Wang, D., Fan, W., Mu, X., and Chen, J. (2010). Traditional Chinese biotechnology. *Biotechnology in China II*. 189-233.
- Zelles, L. (1997). Phospholipid fatty acid profiles in selected members of soil microbial communities. *Chemosphere*. **35**, 275-294.
- Zhang, W. X., Wu, Z. Y., Zhang, Q. S., Wang, R., and Li, H. (2009). Combination of newly developed high quality Fuqu with traditional Daqu for Luzhou-flavor liquor brewing. *World J. Microbiol. Biotechnol.* **25**, 1721-1726.
- Zheng, J., Zhang, L., Shen, C., Zhang, S., Jin, Y., Zhao, J., and Zhou, R. (2011). Characteristics of microbial community structure in *Luzhou*-flavor fermentation pits. *Chinese J. Appl. Ecol.* **22**, 1020.
- Zheng, X., Yan, Z., Han, B., Zwietering, M. H., Samson, R. A., Boekhout, T., and Nout, M. (2012a). Complex microbiota of a Chinese “*Fen*” Liquor fermentation starter (*Fen-daqu*), revealed by culture-dependent and culture-independent methods. *Food Microbiol.* **31**, 293-300.
- Zheng, X. W., Tabrizi, M. R., Nout, M., and Han, B. Z. (2012b). *Daqu*--a traditional Chinese liquor fermentation starter. *J. Inst. Brew.* **117**, 82-90.