3 Biotech (2019) 9:89
https://doi.org/10.1007/s13205-019-1622-y

ORIGINAL ARTICLE

Contrasting bacterial community structure in artificial pit mud-starter cultures of different qualities: a complex biological mixture for Chinese strong-flavor Baijiu production

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Received: 26 April 2018 / Accepted: 8 February 2019 / Published online: 18 February 2019

Abstract

The complex starter culture for artificial pit mud (APMSC) hosts a wide variety of microbial communities that play a crucial role in Chinese strong-flavor Baijiu production. Based on its organoleptic properties, the quality of APMSC can be divided into normal and inferior quality grades. However, the relationship between the APMSC microbial community and APMSC quality is poorly understood. In this study, the bacterial community structure in normal and inferior APMSC derived from two different production batches was analyzed using denaturing gradient gel electrophoresis and Illumina MiSeq sequencing. Highly similar patterns of bacterial diversity and community structure were observed in the APMSC samples of the same quality, and a significant higher bacterial species diversity (Shannon index and Chao1) was detected in the normal compared to the inferior APMSC samples. Fifteen genera were detected in the APMSC samples, and seven (*Caproiciproducens, Clostridium, Lactobacillus, Bacillus, Pediococcus, Rummeliibacillus*, and *Sporolactobacillus*) were dominant, accounting for 92.12–99.89% of total abundance. Furthermore, the bacterial communities in the normal and inferior APMSC had significantly different structure and function. The normal APMSC was characterized by abundant *Caproiciproducens* and *Clostridium* and high caproic and butyric acid contents. In contrast, the inferior APMSC was overrepresented by *Lactobacillus* and *Bacillus* and lactic and acetic acids. This study may help clarify the key microbes sustaining APMSC ecosystem stability and functionality, and guide future improvements in APMSC production.

Keywords Baijiu · Artificial pit mud · Starter culture · Next generation sequencing

Introduction

Strong-flavor Baijiu (SFB) is a type of Chinese liquor that dominates the Chinese Baijiu industry and plays a crucial role in national economic development (Song 2016; Zheng and Han 2016). SFB is produced by distilling mixed, fermented grains (e.g., sorghum, wheat, and rice), and the fermentation occurs in cellars lined with pit mud (Zheng and Han 2016). The predecessor of pit mud is fresh soil. The pit mud matures over time, as it forms through microbial acclimation and domestication during long-term batch fermentation (Tao et al. 2014; Wang et al. 2014). In general, SFB quality is positively correlated with ethyl caproate content (Xu et al. 2009), but only mature pit mud can be used to brew a high quality SFB (Tao et al. 2014). Mature pit mud hosts a unique microbial community allowing for high quality SFB production. However, the long natural maturation period of at least 20 years (Tao et al. 2014) seriously restricts productivity of high quality SFB. Thus, the amount of natural mature pit mud cannot support the rapid development of the Chinese Baijiu industry.

Artificial pit mud has been widely used to construct cellars in the industry to increase productivity of high quality SFB, and this practice has a long history dating back to the 1960s (Ding et al. 2014). Artificial pit mud is a fermented product that uses fresh soil, wheat, and soybean

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s13205-019-1622-y) contains supplementary material, which is available to authorized users.

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meal incubated with a starter culture (Jing et al. 2010; Liu et al. 2017c). The starter culture for artificial pit mud (APMSC) is usually prepared by liquid state fermentation of mixed mature pit mud, Zaopei (fermented grain), and yellow water (a byproduct formed during Baijiu brewing) (Liu et al. 2017c). Previous studies have demonstrated that the microbial communities in the APMSC material are deeply diverse and include many unknown taxa (Li et al. 2015; Liu et al. 2017b; Sun et al. 2016). Based on its obvious enhancing effect on SFB quality (Liu et al. 2014; Wu et al. 2012, 2014a), we speculate that the APMSC hosts diverse adaptable communities that are responsible for high quality SFB production. Although Bacteroidetes, Clostridiales, and Lactobacillales are considered to be the dominant components of APMSC microbial communities as determined by denaturing gradient gel electrophoresis (DGGE) and colony count analyses (Ren et al. 2014; Yao et al. 2013; Zhang et al. 2015), the actual complexity and diversity of microbial communities within this complex system remains poorly understood.

The quality of APMSC is divided into two grades based on its organoleptic properties. Normal APMSC is characterized by a golden yellow color, strong fermentation gases, and a rich aroma, whereas inferior APMSC is characterized by a dull black color, little fermentation gas, and no or little aroma (Table S1). The key difference between the two APMSC states is that the normal APMSC allows for high quality SFB production, while the inferior APMSC does not. Producing a complex starter culture is a challenging task because the quality of the final product is unpredictable, as microbial composition is vulnerable to environmental fluctuations (Smid et al. 2014; Smid and Lacroix 2013). Understanding the microbial community structure and the correlation with APMSC quality is important to APMSC production. Detailed information on the key microbes sustaining ecosystem stability and eventually steering its function is important.

Various molecular methods have been employed to characterize the microbial community of natural and artificial pit muds to avoid isolating and culturing microbes (Ding et al. 2014; Liu et al. 2017b; Sun et al. 2017; Tao et al. 2014; Zhang et al. 2015). In particular, next generation sequencing (NGS) strategies have significantly increased our understanding of the SFB brewing microecosystem (Liu et al. 2017b; Sun et al. 2017; Tao et al. 2014). As a traditional fingerprint method, DGGE has been demonstrated to be a reliable technique for analyzing relative community structure (Liu et al. 2017a; Neilson et al. 2013). In this study, an extensive survey of the bacterial communities was employed to characterize normal and inferior APMSC by DGGE and MiSeq sequencing (NGS from Illumina), combined with an investigation of the chemical properties, including pH, as well as caproic, butyric, acetic, and lactic acid contents. The objectives of this study were to determine the abundance, taxonomic diversity, and composition of the bacterial communities in normal and inferior APMSC and to determine the relationships between the chemical properties and bacterial community composition in APMSC.

Materials and methods

Materials

APMSC was produced by fermenting a liquid mixture (50 L) composed of mature pit mud in use for 50 years (10%, w/v), 3% (w/v) Zaopei, 2% (v/v) yellow water, 2% (v/v) SFB, 0.5% (w/v) CH_3COONa, 0.1% (w/v) yeast extract, 0.05% (w/v) $(NH_4)_2SO_4$, 0.04% (w/v) KH_2PO_4, and 1% (w/v) $CaCO_3$ (Wanke, Chengdu, China) at 35 ± 2 °C for 15 days. The raw materials, mature pit mud, Zaopei, yellow water, and SFB were purchased from a famous Baijiu company (Luzhou, Sichuan Province, China). In total, nine APMSCs, including three inferior (A1, A2, and B3) and six normal (A4, A5, A6, B7, B8, and B9) samples were used in this study. The terms A and B represent the APMSC derived from two different production batches. The raw materials and the fermentation processes used to produce the APMSCs in the two different production batches were the same.

Chemical properties

The pH of the APMSC was measured using a PB10 pH meter (Sartorius, Gottingen, Germany). To quantify organic acids, APMSC supernatants were obtained by centrifugation at 5000 r/min for 10 min at 4 °C and then passed through a 0.45 μm pore size Millex GS filter unit (Sangon, Shanghai, China). The concentrations of caproic, butyric, and acetic acid were quantified using gas chromatography (Clarus 500; PerkinElmer, Waltham, MA, USA) equipped with a flame ionization detector and a CP-Wax 57 CB acidic column (50 m × 0.25 mm × 0.2 μm), as described in our previous study (Liu et al. 2017b). Lactic acid was quantified by a liquid chromatograph (Ultimate 3000, Thermo Fisher Scientific, Waltham, MA, USA) equipped with a UV–Vis detector and an Acclaim™ column (C_{18}, 4.6 × 250 mm, 5 μm). Lactic acid was detected at λ 215 nm. KH_2PO_4 (0.01 M) was used as the solvent at a flow rate of 0.8 mL/min. The column temperature and injection volume were 25 °C and 20 μL, respectively.

DNA extraction

Ten milliliters of APMSC sample was centrifuged at 5000 r/min for 10 min at 4 °C to extract the DNA. Then, the DNA was extracted from the precipitates using the EZNATM soil

DNA kit (Omega, Norcross, GA, USA), according to the manufacturer's instructions.

DGGE analysis

The V3 region of 16S rRNA was amplified. The PRBA338F primer (5′-ACT CCT ACG GGA GGC AGC AG-3′) with a GC clamp and the PRUN518R primer (5′-ATT ACC GCG GCT GCT GG-3′) were used (Lapara et al. 2000). The polymerase chain reaction (PCR) mixtures (50 µL) contained 1× buffer (1.5 mM Mg^{2+}), dNTP mixture (250 µM of each deoxynucleoside triphosphate), 1 µM of each primer, 1 U LATaq DNA polymerase (TaKaRa, Dalian, Liaoning, China). The amplification was performed using a S1000 thermo cycler (Bio-Rad, Hercules, CA, USA) as follows: 5 min at 94 °C; 30 cycles of 30 s at 92 °C, 30 s at 55 °C, 30 s at 72 °C; final extension at 72 °C for 7 min and cooling at 4 °C. The DGGE analysis (30–60% gradient) was performed using a DGGE-K2401-220 system (C.B.S. Scientific, Delmar, CA, USA), as described previously (Liu et al. 2015b).

Illumina MiSeq sequencing analysis

The V3, V4, and V5 regions of the 16S rRNA were amplified using a set of primers designed by GENEWIZ Inc. (Suzhou, Jiangsu, China). The V3 and V4 regions were amplified using forward primers containing the sequence "CCT ACG GRR BGC ASC AGK VRV GAA T" and reverse primers containing the sequence "GGA CTA CNV GGG TWT CTA ATC C". The V4 and V5 regions were amplified using forward primers containing the sequence "GTG YCA GCM GCC GCG GTA A" and reverse primers containing the sequence "CTT GTG CGG KCC CCC GYC AAT TC". PCR amplification, preparation of the NGS library, and Illumina MiSeq sequencing were conducted as reported in our previous study (Liu et al. 2017b).

Data analysis

The DGGE profiles were analyzed using Quantity One 4.2 software (Bio-Rad). The Shannon index was determined based on the relative quantities of the DGGE bands (Liu et al. 2015b). The QIIME data analysis package was used for the Illumina MiSeq sequencing data analysis (version 1.9.1; http://qiime.org/tutorials/tutorial.html). The forward and reverse reads were joined and assigned to samples based on the barcode sequences and then truncated by removing the barcode and primer sequences. Quality filtering of the joined sequences was performed, and sequences that did not fulfill the following criteria were discarded: sequence length < 200 bp, no ambiguous bases, and mean quality score ≥ 20. Qualified sequences were grouped into operational taxonomic units (OTUs) at a 97% identity level using VSEARCH (version 1.9.6) against the Silva 119 database (https://www.arb-silva.de/) (Quast et al. 2012). The Ribosomal Database Program Classifier was used to classify the OTUs into taxonomic categories at a confidence level of 80% (Wang et al. 2007). The richness index (Chao1) and diversity index (Shannon) within each individual sample were calculated using Mothur (v.1.30.1; http://www.mothur.org/wiki/) (Schloss et al. 2009). For the comparative bacterial community analysis, a principal coordinate analysis (PCA) was performed based on the DGGE and Illumina MiSeq sequencing data using CANOCO for Windows (http://www.canoco5.com/). A heatmap was prepared using Hemi software (version1.0; http://hemi.biocuckoo.org/) (Deng et al. 2014). A redundancy analysis (RDA) was carried out using CANOCO to examine the relationship between relative abundances of dominant genera and the chemical properties. Pearson's correlation coefficient analysis was performed between dominant genera and the chemical properties using SPSS 13.0 software for Windows (SPSS Inc., Chicago, IL, USA). Differences in the chemical properties, diversity indices, and relative abundance of the bacterial communities between groups were tested by the independent samples *t* test.

Accession number

The Illumina MiSeq sequencing data were submitted to the Sequence Read Archive of the NCBI database under the BioProject ID PRJNA449898 (Accession numbers SAMN08924515–SAMN08924523).

Results

APMSC chemical properties

The chemical properties of the APMSC samples are summarized in Table 1. The pH value in the inferior APMSC (5.05 ± 0.26, mean ± standard deviation) was numerical lower than that of normal APMSC (5.50 ± 0.38) ($p > 0.05$). However, organic acids showed a completely opposite pattern between normal and inferior APMSC. All normal APMSC samples had higher concentrations of caproic acid (1.75 ± 0.41 g/L) and butyric acid (1.20 ± 0.13 g/L), followed by acetic acid (0.64 ± 0.43 g/L) and lactic acid (0.06 ± 0.03 g/L). In contrast, high concentrations of acetic acid (1.00 ± 0.07 g/L) and lactic acid (0.83 ± 0.18 g/L) were measured in the inferior APMSC samples, whereas low concentrations of butyric acid (0.60 ± 0.18 g/L) and caproic acid (0.46 ± 0.16 g/L) were detected. No significant differences in the chemical properties were observed between the normal APMSC samples from the two different batches, except caproic acid (Table S2).

Table 1 The chemical properties, denaturing gradient gel electrophoresis (DGGE), and Illumina MiSeq sequencing data for the artificial pit mud-starter culture (APMSC) samples

	Inferior APMSC samples			Normal APMSC samples					
	A1	A2	B3	A4	A5	A6	B7	B8	B9
Chemical properties									
pH	5.04	5.32	4.79	5.06	5.48	5.66	5.97	5.77	5.03
Caproic acid (g/L)	0.34	0.64	0.41	1.61	1.53	1.17	2.39	1.85	1.95
Butyric acid (g/L)	0.82	0.49	0.51	1.20	1.17	1.01	1.32	1.17	1.36
Acetic acid (g/L)	0.94	1.07	1.00	0.35	0.39	0.38	0.47	0.82	1.45
Lactic acid (g/L)	0.85	0.65	1.00	0.00	0.07	0.08	0.08	0.07	0.03
DGGE									
Shannon	3.09	2.94	2.92	3.36	3.25	3.28	3.25	3.17	3.21
Illumina MiSeq									
Qualified sequences	59,408	62,179	55,261	58,137	60,579	49,737	67,750	57,109	58,478
Sequences length (bp)	431.14	431.94	430.33	428.01	429.12	428.44	428.32	427.64	427.16
Coverage (%)[a]	100	100	100	100	100	100	100	100	100
Chao1[a]	40.20	45.00	50.00	59.00	59.25	57.50	62.50	63.00	56.00
Shannon[a]	1.27	1.47	1.27	4.02	3.63	3.51	3.52	2.51	2.90

[a]The indices were calculated based on a cutoff of 97% similarity of 16S rRNA gene sequences and 46,937 sequences per sample

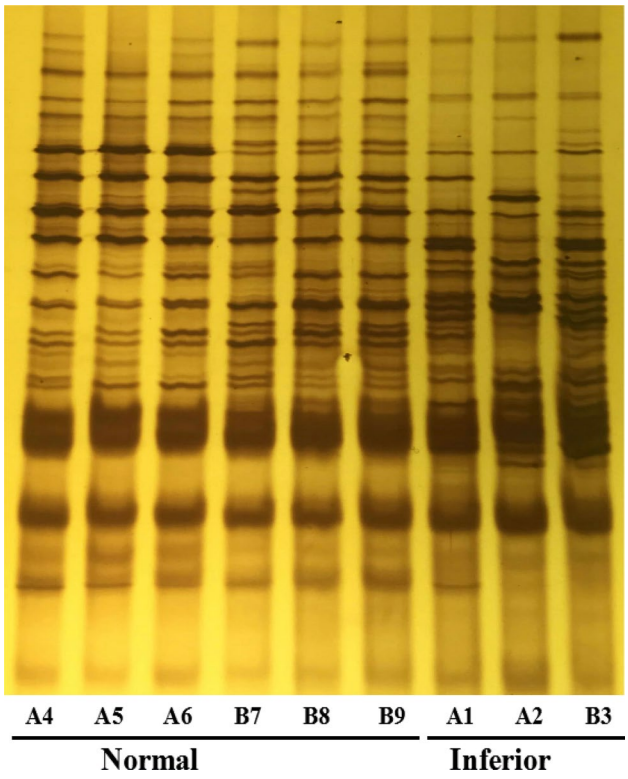

Fig. 1 Denaturing gradient gel electrophoresis (DGGE) profile of the bacterial communities in the normal and inferior artificial pit mud-starter culture (APMSC) samples

Bacterial community diversity revealed by DGGE

The DGGE profiles of the APMSC samples displayed large differences in band positions among the nine lanes (Fig. 1). In total, 31 distinct bands were observed, indicating a rich bacterial community. The Shannon index, which provides an estimate of bacterial diversity in a sample, decreased significantly from the normal (3.25 ± 0.06) to the inferior APMSC samples (2.98 ± 0.09) ($p < 0.05$) (Table 1). A PCA was conducted using the Unifrac approach to evaluate similarity among the bacterial communities (Fig. 2a). Axes 1 and 2 explained 55.36% and 17.23% of the total bacterial community variations among the samples, respectively. The results showed significantly different patterns of community structure between the normal and inferior APMSC samples.

Bacterial community diversity revealed by Illumina MiSeq sequencing

Sequencing of the APMSC resulted in 528,638 qualified sequences with an average length of 425.72 bp for all samples, and each sample contained 49,737–67,750 sequences (Table 1). Overall, 67 different OTUs (97% identity cutoff) with a sampling coverage of 100% were obtained based on 46,937 sequences per sample. Coverage indicated that all bacterial communities were well represented. Significant differences ($p < 0.05$) were observed in bacterial richness and

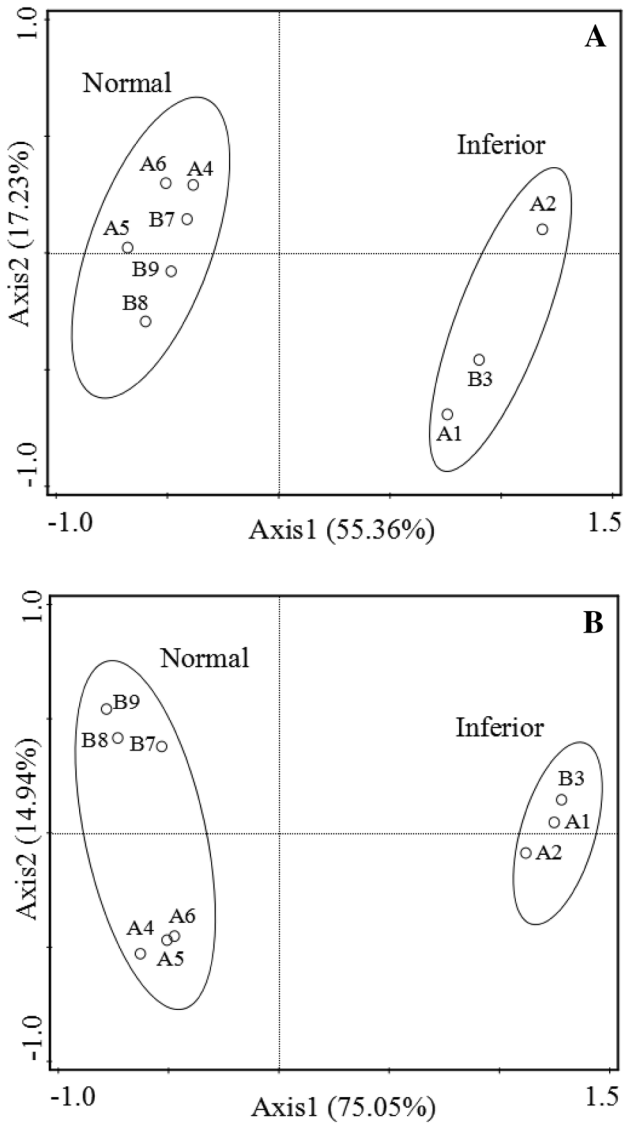

Fig. 2 Principal coordinate analysis (PCA) of the bacterial communities in the normal and inferior artificial pit mud-starter culture (APMSC) samples, calculated based on denaturing gradient gel electrophoresis (DGGE) (**a**) and Illumina MiSeq sequencing (**b**)

diversity according to the Chao1 and Shannon indices in the normal and inferior APMSC samples. The normal APMSC samples had a higher Chao1 richness index (59.54 ± 2.75) and a higher Shannon diversity index (3.35 ± 0.55) compared to the inferior APMSC samples (45.07 ± 4.90 for Chao1, 1.34 ± 0.11 for Shannon). This result was supported by the DGGE analysis, revealing less community diversity in the inferior APMSC than that in the normal APMSC. Additionally, no significant differences in bacterial richness or diversity were observed in the normal APMSC samples between the two different batches as determined by both methods ($p > 0.05$) (Table S3).

A PCA was performed to compare the differences in Illumina MiSeq bacterial sequencing (Fig. 2B). Axes 1 and 2 explained 75.05% and 14.94% of the total bacterial community variations among the samples, respectively. Interestingly, these results differed from the DGGE-based PCA profile; the results showed that the normal APMSC from the two different batches were separated along axis 2, indicating slight batch-dependent variation. However, both PCA methods produced a similar result that the bacterial community composition shifted greatly between the normal and inferior APMSC samples along axis 1, indicating that they harbored significantly different bacterial community structures.

Phylogenetic structure of the bacterial community by Illumina MiSeq sequencing

The taxonomic classifications of the APMSC samples are summarized in Table S4. Three phyla (Firmicutes, Proteobacteria, and Bacteroidetes) were detected, and the vast majority of sequences were assigned to Firmicutes, representing 99.95–100% of the bacterial communities in each APMSC sample. At the class level, 99.96–100% of the bacterial sequences were assigned to Bacilli and Clostridia. The remaining sequences belonged to the rare classes of Gammaproteobacteria, Alphaproteobacteria, and Bacteroidia. Of the two major class, Bacilli was the predominant class in the inferior APMSC (95.58 ± 2.13%), whereas Clostridia was the predominant class in the normal APMSC (77.06 ± 15.57%).

The sequences from all APMSC samples represented 15 different genera. We defined dominant genera as those shared by all APMSC samples and with average relative abundances > 1%. Seven genera (*Caproiciproducens, Clostridium, Lactobacillus, Bacillus, Pediococcus, Rummeliibacillus*, and *Sporolactobacillus*) constituted 92.12–99.89% of total abundance (Fig. 3, Table S4). The heatmap showed that the distribution of bacterial genera varied significantly between the normal and inferior APMSC samples (Fig. 3). Of these, *Lactobacillus* and *Bacillus* accounted for 62.14 ± 9.28% and 31.69 ± 8.17% of the total abundance in the inferior APMSC samples, respectively, whereas they were only 0.28 ± 0.22% and 13.95 ± 12.63% in the normal APMSC, respectively. In contrast, *Clostridium* and *Caproiciproducens* comprised 55.18 ± 10.92% and 18.35 ± 4.55% of total abundance in the normal APMSC samples, respectively, whereas the corresponding data were 3.37 ± 2.22% and 0.92 ± 0.47% in the inferior APMSC.

Among these groups, the abundance of Bacilli, Clostridia, *Caproiciproducens, Clostridium*, and *Lactobacillus* was significantly different between the normal and inferior APMSC samples ($p < 0.05$). Although the abundance of *Caproiciproducens, Bacillus*, and *Sporolactobacillus* was also different

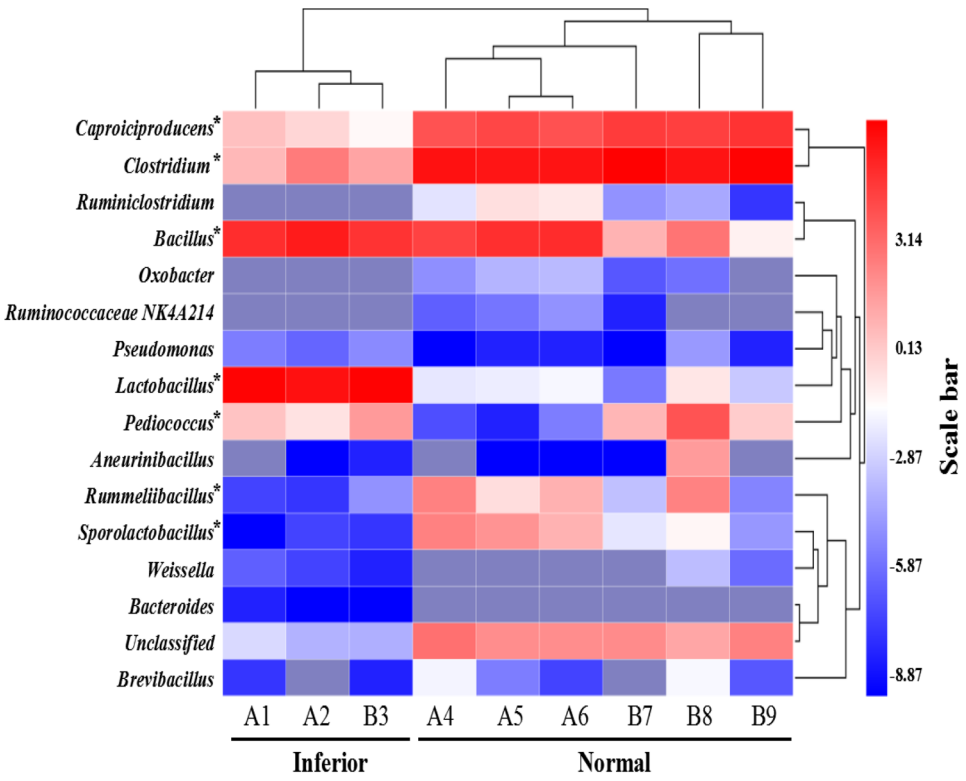

Fig. 3 Relative abundance of bacterial genera in the normal and inferior artificial pit mud-starter culture (APMSC) samples, calculated based on Illumina MiSeq sequencing. The *scale bar* shows the variation range of the log2 scale-based abundance of the genera. The symbol "*" is used to indicate genera shared by all APMSC samples and with average relative abundances > 1%

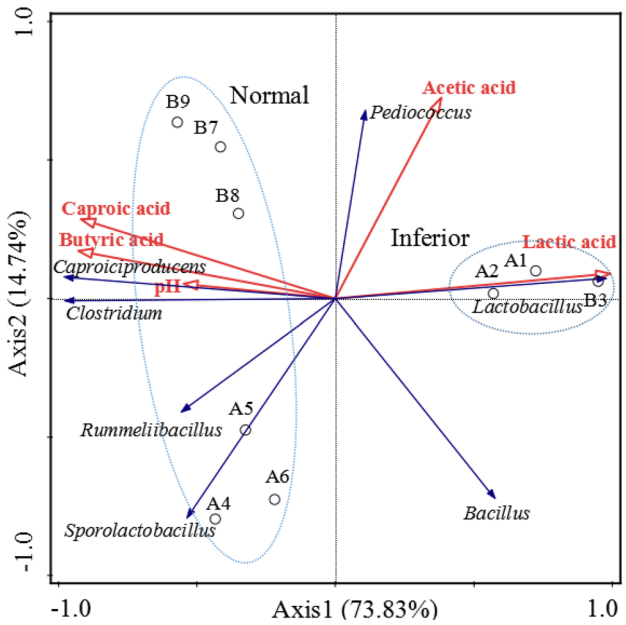

Fig. 4 Redundancy analysis (RDA) of bacterial communities as obtained by Illumina MiSeq sequencing and the chemical properties. *Arrows* indicate the direction and magnitude of chemical properties and dominant genera associated with the artificial pit mud-starter culture (APMSC) bacterial community structure

between the two different batches within normal APMSC samples ($p < 0.05$) (Table S5), a highly similar pattern of community structure was observed between batches on the heatmap.

Relationships between chemical properties and the bacterial community

The chemical properties and the dominant bacterial genera in the APMSC samples were selected to determine their RDA correlation (Fig. 4). Overall, the first two axes explained 88.57% of the variation in bacterial composition, suggesting a significant correlation between the bacterial community and chemical properties. Lactic acid was strongly and positively correlated with bacterial composition in the inferior APMSC samples, whereas it was negatively correlated with bacterial composition in the normal APMSC samples. However, pH, caproic acid, and butyric acid showed contrasting correlations. A Pearson's correlation analysis revealed that caproic and butyric acid were significantly positively correlated with the relative abundance of *Caproiciproducens* and *Clostridium*, but negatively correlated with the abundance of *Lactobacillus* and *Bacillus* (Table 2). In contrast, lactic acid levels were correlated positively with *Lactobacillus* but negatively correlated with *Caproiciproducens* and *Clostridium* ($p < 0.01$). Acetic acid levels correlated negatively with *Rummeliibacillus* and *Sporolactobacillus* ($p < 0.05$).

Table 2 Pearson's correlation coefficients between chemical properties and dominant bacterial genera in the artificial pit mud-starter culture (APMSC) samples

Taxa	Chemical properties				
	Caproic acid	Butyric acid	Acetic acid	Lactic acid	pH
Caproiciproducens	0.930**	0.933**	−0.133	−0.900**	0.507
Clostridium	0.885**	0.888**	−0.207	−0.933**	0.500
Lactobacillus	−0.891**	−0.881**	0.449	0.993**	−0.596
Bacillus	−0.790*	−0.771*	−0.153	0.562	−0.267
Pediococcus	0.454	0.243	−0.143	−0.057	0.484
Rummeliibacillus	0.540	0.480	−0.690*	−0.500	0.348
Sporolactobacillus	0.237	0.360	−0.725*	−0.513	−0.005

**Correlation is significant at $p < 0.01$. *Significant at $p < 0.05$

Discussion

A comprehensive investigation of the composition of the APMSC microbial ecosystem is important to understand and eventually guide its functionality. This study was an extensive examination of bacterial diversity in APMSC using DGGE and Illumina MiSeq sequencing. Small differences were observed in bacterial composition after comparing the results of the two techniques. For example, the PCA indicated that the formed cluster in the normal APMSC shown by DGGE differed from the formed separate clusters in the normal APMSC as shown by Illumina MiSeq sequencing. The PCA also indicated a cluster for inferior APMSC, as shown by Illumina MiSeq sequencing, whereas the inferior APMSC samples were highly dispersed, as shown by DGGE. These conflicting results may due to many factors, particularly primer specificity. Previous studies have demonstrated that the microbial community pattern can be greatly affected by the choice of primers (Derakhshani et al. 2016; Liu et al. 2015a). However, most of the DGGE results were in agreement with those found by Illumina MiSeq sequencing. Thus, the DGGE data are complementary and support the results obtained by Illumina MiSeq sequencing when comparing variations in bacterial community structure between the samples.

As expected, the PCAs revealed that bacterial community structure was significantly associated with APMSC quality by both methods. Further statistical analysis confirmed that the microbial diversity indices (Shannon and Chao1) and relative abundance of Bacilli, Clostridia, *Caproiciproducens, Clostridium*, and *Lactobacillus* were significantly different between the normal and inferior APMSC samples. The functional impact of the change in bacterial diversity was shown by the opposite organic acid patterns between the normal and inferior APMSC. This finding indicates that specific microbial and chemical parameters may play an essential role discriminating quality. For example, the 16S rRNA gene copies of *Clostridium* and *Lactobacillus* have been applied to evaluate the maturity level of pit mud (Zhang et al. 2017). It is well known that batch production of mixed cultures is not efficient for maintaining bacterial composition, and leads to variations in activity and quality of the final product due to complex microbial interactions (Smid et al. 2014; Smid and Lacroix 2013). In this study, the abundance of *Caproiciproducens, Bacillus*, and *Sporolactobacillus* was significantly different between the two batches within the normal APMSC group. Our PCA based on Illumina MiSeq sequencing also suggested that the APMSC bacterial community structure may be correlated with the batch. Although this batch-dependent effect was small, these observations suggest that combined multiple indicators, such as microbial, chemical, and organoleptic parameters, may allow for a more accurate reflection of APMSC quality.

According to a flavor analysis, the esters ethyl caproate, ethyl butyrate, ethyl acetate, and ethyl lactate are typical aromatic compounds in SFB (Fan and Qian 2006; Zheng et al. 2014). In particular, ethyl caproate, produced from esterification of caproic acid and ethanol, has an important effect on SFB quality and higher levels of ethyl caproate enhance the quality of SFB (Xu et al. 2009). Among the dominant genera identified, the relative abundance of *Caproiciproducens* and *Clostridium* was significantly and positively correlated with caproic and butyric acid contents, perhaps because these two genera produce caproic and butyric acid as major end-products of carbohydrate fermentation (Hu et al. 2015; Kim et al. 2015). There is no doubt that a high level of caproic acid in artificial pit mud enhances SFB quality. Meanwhile, the butyric acid that forms during fermentation is converted to ethyl butyrate and is a substrate for elongating the carbon chain to caproic acid (Ding et al. 2010). Our previous study demonstrated that *Caproiciproducens* and *Clostridium* are most highly represented in natural mature pit mud (Liu et al. 2017b). These two genera dominated the communities in normal APMSC samples, which may explain why this APMSC is important for high quality SFB production.

In contrast, *Lactobacillus* and *Bacillus*, which were negatively correlated with caproic acid content, were dominant in the inferior APMSC samples, mainly because both produce lactic acid as the major end-product during fermentation

(Michelson et al. 2006). It has been previously reported that lactic acid-producing bacteria limit caproic acid production (Yao et al. 2010). This finding was consistent with the trend of the change in caproic acid content (from 5.79 to 15.2 g/kg) across the relative abundance of *Lactobacillus* in pit mud with different usage times (from 62.28 to 4.23%) (Tao et al. 2014). Notably, lactic acid has positive and negative effects on SFB production. The positive interaction between lactic acid and SFB production is that moderate amounts of lactic acid facilitate formation of the aromatic ester. However, excessive concentrations of lactic acid are usually associated with the accumulation of calcium lactate, which can degrade pit mud (Wu et al. 2014b). Previous studies have shown that *Lactobacillus* is dominant in degraded pit mud (Hu et al. 2016; Sun et al. 2017). The RDA analysis in this study revealed that lactic acid was positively correlated with axis 1 and was strongly linked to the overall diversity and composition of the microbial community. Previous studies carried out on pit mud have suggested that any significant deviation in lactic acid affects the overall microbial community by altering pH (Hu et al. 2016; Tao et al. 2014). This is consistent with our finding that a relatively low pH was present in the inferior APMSC samples with high lactic acid concentrations. Although our analysis did not reveal a significant correlation between pH and relative abundance of the dominant bacterial genera, it is fair to propose that lactic acid played a key role shaping the overall community structure.

Conclusion

This study revealed the bacterial community structure and diversity in the APMSC for Chinese SFB production using DGGE and Illumina MiSeq sequencing. Our results demonstrate for the first time that very complex functional populations of bacteria inhabit the APMSC ecosystem, and that the bacterial community structure was significantly associated with APMSC quality and functioning. Significant variations in the levels of *Caproiciproducens, Clostridium, Lactobacillus, Bacillus*, and their products within APMSC may have the potential to predict the status of APMSC. These findings provide novel insight into the APMSC ecosystem and contribute to optimizing APMSC production and improving the quality of Chinese Baijiu.

Acknowledgements This study was supported by the National Natural Science Foundation of China (Grant number 31801534), the Sichuan Science and Technology Program (Grant number 2017JY0282), and the Foundation of the Thesis Program of Sichuan Academy of Agricultural Sciences (Grant number 2018LWJJ016).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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