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Filamentous fungal diversity and community structure associated with the solid state fermentation of Chinese *Maotai*-flavor liquor

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ABSTRACT

Maotai-flavor liquor is produced by simultaneous saccharification and fermentation (SSF) process under solid state conditions, including *Daqu* (starter) making, stacking fermentation and alcohol fermentation stages. Filamentous fungi produce many enzymes to degrade the starch material into fermentable sugar during liquor fermentation. This study investigated the filamentous fungal community associated with liquor making process. Eight and seven different fungal species were identified by using culture-dependent and -independent method (PCR-denaturing gradient gel electrophoresis, DGGE) analyses, respectively. The traditional enumeration method showed that *Daqu* provided 7 fungal species for stacking fermentation. The total population of filamentous fungi increased from 3.4×10^3 cfu/g to 1.28×10^4 cfu/g in the first 3 days of stacking fermentation, and then decreased till the end. In alcohol fermentation in pits, the population continuously decreased and few fungal species survived (lower than 1×10^3 cfu/g) after 10 days. Therefore, stacking fermentation is an essential stage for the growth of filamentous fungi. *Paecilomyces variotii*, *Aspergillus oryzae* and *Aspergillus terreus* were detected by both methods, and *P. variotii* and *A. oryzae* were the predominant species. Meanwhile, *P. variotii* possessed the highest glucoamylase (3252 ± 526 U/g) and *A. oryzae* exhibited the highest α-amylase (1491 ± 324 U/g) activity among the cultivable fungal species. Furthermore, the variation of starch and reducing sugar content was consistent with the growth of *P. variotii* and *A. oryzae* in *Zaopei* (fermented grains) during stacking fermentation, which implied that the two filamentous fungi played an important role in producing amylase for hydrolyzing the starch.

1. Introduction

Simultaneous saccharification and fermentation (SSF) process under solid state conditions is cost-effective and environment-friendly, and it has attracted considerable research interest in recent decades (Mohagheghi et al., 1992; Pandey, 2003). East Asian countries have adopted this method for food and beverage fermentation for centuries (Kim et al., 2009; Xu et al., 2012). This is different from most Western alcoholic beverages or liquors such as wine, beer and whisky, which separate hydrolysis and fermentation (Farías et al., 2010; Van Beek and Priest, 2002). During the SSF process, filamentous fungi secrete various enzymes to hydrolyze starch and protein, and increase the digestibility and probiotics content in foods and beverages (Song et al., 2010; Yu et al., 2012). Many fungal species such as *Aspergillus* sp., *Rhizopus* sp., *Mucor* sp. and *Penicillium* sp., have been reported to be involved in fermenting cheese, rice wine, soy sauce, etc. (Feng et al., 2013; Huang et al., 2012; Le Dréan et al., 2010). Furthermore, they contribute to the organoleptic properties of fermented products by synthesizing flavor components through lipase, 3-ketoacid decarboxylase and so on (Kinsella et al., 1976). It is important to study the filamentous fungal community associated with the fermentation process to assess the role that filamentous fungi play in the fermentation.

Maotai-flavor liquor, which has been widely consumed in China for centuries, is produced by a solid state fermentation process (Xu et al., 2012; Zheng et al., 2011). Its manufacture can be categorized into three stages: the *Daqu* making, stacking fermentation on the ground and alcohol fermentation in pits. *Daqu* is made of ground wheat with water and shaped into blocks, which are spontaneously fermented for 30 days and then stored for another 90 days to mature. The mature *Daqu* is mixed with 9-fold weight of sorghum, which has been soaked and steamed. Then the mixture (*Zaopei* in Chinese) is stacked as a cone on the ground for 2–7 days (Wu et al., 2012). After the stacking fermentation, the stacked grains are put into pits and sealed for alcohol fermentation for about 30 days (Xu et al., 2012).

Starch in sorghum cannot be directly utilized by most of the yeasts and bacteria, so it needs to be hydrolyzed into fermentable sugars through α-amylase and glucoamylase, which are produced by filamentous fungi (Fan et al., 2007; Wang et al., 2008; Zheng et al., 2011).

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Previous studies using a culture dependent method have identified *Aspergillus* sp., *Mucor* sp. and *Rhizopus* sp. in mature *Maotai*-flavor *Daqu* (Wang et al., 2008). Using a PCR-denaturing gradient gel electrophoresis (DGGE) technique, Xiu et al. (2012) also detected *Mucor racemosus* and *Thermoascus crustaceus* in *Maotai*-flavor *Daqu*. However, these studies of the fungal community only focused on the *Daqu* making process, and very little is known about the succession of filamentous fungi during the liquor fermentation of *Maotai*-flavor liquor. As the filamentous fungi play an important role in liquor fermentation, it is necessary to systematically study the fungal community in the later liquor fermentation stages.

The aim of this study was to investigate the filamentous fungal community during *Maotai*-flavor fermentation process. The fungal community was analyzed by using culture-dependent and -independent methods. In addition, the glucoamylase and α-amylase activity of the isolated species were determined to identify the potential functional fungal species. This study revealed the importance of filamentous fungi on the fermentation of *Maotai*-flavor liquor, which may help improve the liquor making techniques.

2. Materials and methods

2.1. Sample collection

Daqu and *Zaopei* (fermented grains) were obtained from one distillery factory, located in Guizhou Province, China. They were used in two independent liquor making processes in the summer of 2012. Samples of *Daqu* powder were collected before they were used for stacking fermentation. Stacking fermentation samples were obtained at the top and the middle of the cone, and alcohol fermentation samples in pits were collected from the upper layer as previously described (Wu et al., 2013). Each sample was divided into two parts after collection and immediately stored at 4 °C and −40 °C, for cultural and DNA analysis respectively.

2.2. Isolation and enumeration of filamentous fungi

Each sample (10 g) was homogenized in 90 ml of 0.9% NaCl solution in a 4 °C shaking incubator for 30 min at 200 rpm. Samples (1.0 ml) of the homogenate were serially diluted 10-fold in saline-peptone water, from which aliquots (0.1 ml) were plated on Rose Bengal Agar medium which was incubated at 28 °C for 3–7 days. Filamentous fungi were isolated and enumerated on the basis of different colony morphologies (diameter, reverse side color, surface color and spores) (Huang et al., 2012). The isolated colonies were streaked on new potato dextrose agar medium and purified, then maintained at 4 °C.

2.3. Genomic DNA extraction and conventional PCR amplification

Genomic DNA of the single isolated filamentous fungus was extracted according to the instruction of the Plant Genomic DNA Kit (TIANGEN, China). The extracted DNA showed absorbance ratios ranging from 1.7 to 1.9 at OD_{260}/OD_{280}. The ITS region was amplified by PCR using universal fungal primers ITS1/ITS4 (White et al., 1990). PCR reactions were performed in 50 μL of 1.5 mM $MgCl_2$, 0.5 μM of each primer, 0.2 mM dNTPs, 1 U Taq polymerase (Takara, Japan) and 10–30 ng of fungal DNA. PCR amplification reaction conditions were as previously described (White et al., 1990).

The samples were stored at −40 °C before total DNA extraction for DGGE and qPCR analysis. Prior to extraction, ddH_2O was used to wash the samples twice to reduce the effects of substances such as ethanol. The DNA was directly extracted according to the method as previously described (Li et al., 2011). The concentration of extracted DNA was determined by using a NanoDrop 8000 (Thermo Scientific, USA).

2.4. Sequencing and strain identification

The PCR products of the ITS region of 221 isolates were purified and sent to Sangon Biotech (Shanghai, China) for sequencing. The resultant sequences were aligned with those available in GenBank/NCBI database by using BLAST program (Huang et al., 2012).

2.5. DGGE analysis

For analysis of fungal diversity, PCR amplification of 18S rRNA gene was performed as described previously using the primer set of NS1 and GC-fung (May et al., 2001). The PCR products were analyzed by DGGE via a Bio-Rad DCode Universal Mutation Detection System (Bio-Rad, USA), as previously described (Du et al., 2013; Wang et al., 2011). Optimal separation was achieved with a 10–30% urea–formamide denaturing gradient. Sequences were aligned in GenBank by using the Blast Program for identification purposes. A quantitative analysis of DGGE fingerprints was carried out with Quantity One software (Bio-Rad, USA) as described Schauer et al. (2000).

2.6. Comparison of enzyme-producing ability of fungal isolates

To examine the ability of the isolated filamentous fungal species to produce α-amylase and glucoamylase, steamed wheat bran was used as the solid substrate. It was inoculated with a spore suspension of test species at a level of 10^6 spores/g, and incubated for 3 days at 30 °C (Huang et al., 2012). Glucoamylase activity was determined using the DNS as described by Bernfeld (1955). An enzyme activity unit (U) was defined as the amount of enzyme that liberated 1 mg of glucose per hour under assay conditions. The method to determine α-amylase activity used iodine reagent as previously described (Yoo et al., 1987). One unit (U) of α-amylase activity was defined as the quantity of enzyme required to hydrolyze 1 mg starch in 10 min at 40 °C. All the experiments were run in triplicate.

2.7. Chemical and quantitative analysis of Zaopei in the stacking fermentation stage

Samples (10 g) were mixed with 90 mL distilled water, ultrasonicated at 0 °C for 30 min, centrifuged at 4 °C for 5 min, then starch and reducing sugar were determined as described previously (Wu et al., 2013).

Two real-time qPCR methods were also used to monitor the growth of *Paecilomyces variotii* and *Aspergillus oryzae* in the stacking fermentation stage. Specific primers used for *P. variotii* were *Pv*For (5′-TGTCCTGACACCTGTTGCT-3′)/*Pv*Rev (5′-CTCAGACGGCAACCTTCCAG-3′), which targeted the ITS region and generated an amplicon of 120 bp. The primer *Ao*For/*Ao*Rev for *A. oryzae* which also targeted the ITS region, was obtained from previous study (Sardiñas et al., 2011). Each qPCR reaction was performed according to the description of SsoFast EvaGreen Supermix (Bio-Rad). The amplification conditions were as follows: preheating at 98 °C for 2 min and then 40 cycles of 98 °C for 5 s, 60 °C (for *P. variotii*) or 57 °C (for *A. oryzae*) for 5 s, and melting curve from 70 °C to 95 °C by increasing every 0.5 °C for 5 s (Bio-Rad CFX96, China).

3. Results and discussion

3.1. Microbiological analysis and identification based on the ITS region

A total of 221 fungal isolates were screened from the whole liquor making process and identified by sequencing the ITS rDNA region. All the isolates were assigned to 8 species, i.e., *P. variotii*, *A. oryzae*, *Penicillium namyslowskii*, *Rhizopus microsporus*, *Microascus cirrosus*, *Monascus purpureus*, *Penicillium chrysogenum* and *Aspergillus terreus* (Table 1).

The filamentous fungal community changed dramatically during the whole liquor making process. Seven fungal species were presented in

Table 1
The population of different filamentous fungal species during the entire liquor making process ($\times 10^3$ cfu/g).

Species	*Daqu*	Stacking fermentation stage								Alcohol fermentation stage						
		0d	1d	2d	3d	4d	5d	6d	7d	0d	5d	10d	15d	20d	25d	30d
P. variotii	340.0	3.1	3.7	4.2	12.4	7	4.3	6.3	5.7	5.5	1.0	0.5	0.4	0.3	0.3	0.7
A. oryzae	3	0.1	–[a]	0.5	–	0.1	0.6	–	0.1	–	–	–	–	–	–	–
P. namyslowskii	8	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
R. microsporus	7	–	–	–	0.4	–	–	–	–	–	–	–	–	–	–	–
M. cirrosus	22	–	–	–	–	–	–	–	–	–	–	0.3	–	–	–	–
M. purpureus	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
P. chrysogenum	16	–	–	–	–	–	–	–	–	0.6	–	–	0.4	–	0.6	–
A. terreus	–	0.2	0.2	0.1	–	0.1	–	–	–	–	–	–	–	–	–	–
Total [b]	398.0	3.4	3.9	4.8	12.8	7.2	4.9	6.3	5.8	6.1	1.2	0.8	0.8	0.3	0.9	0.7

[a] Filamentous fungal species were undetectable.
[b] The total population was obtained from the counts of all the filamentous fungal species.

the *Daqu* and the total population was 3.98×10^5 cfu/g (Table 1). The population of *P. variotii* was 3.1×10^5 cfu/g, which accounted for more than 85% of the total. At the same time, the population of *A. oryzae* was only 3.0×10^3 cfu/g.

In the stacking fermentation, the total population increased from 3.4×10^3 cfu/g to the peak of 1.28×10^4 cfu/g on the 3rd day, and then decreased to 5.8×10^3 cfu/g in the end. Only *P. variotii*, *A. oryzae*, *A. terreus* and *R. microsporus* were detected at this period, and *P. variotii* was still the predominant species, accounting for 90% of the total population. However, the total population of *P. variotii* at the beginning was lower than that detected in the *Daqu*, because *Daqu* powder was mixed with 9-fold (w/w) of steamed sorghum (Wu et al., 2013).

During the alcohol fermentation in the pits, the total population further decreased to less than 1×10^3 cfu/g after 10 days fermentation. *P. variotii* was the only filamentous fungus found consistently. Other species such as *M. cirrosus* and *P. chrysogenum* were found only sporadically.

3.2. PCR-DGGE analysis of the filamentous fungal succession

The DGGE fingerprints of the fungal community during the entire liquor making process based on the partial 18S rRNA genes are shown in Fig. 1 and the results of the sequencing of the highlighted bands are listed in Table 2.

The similarity of all highlighted band sequences was ≥98% compared with those available in the GenBank database. A total of 10 fungal species were detected by the nested PCR-DGGE method (Table 2), i.e., *P. variotii*, *A. oryzae*, *Penicilliopsis clavariiformis*, *Penicillium decumbens*, *Geosmithia argillacea*, *Monascus ruber* and *A. terreus*, including three

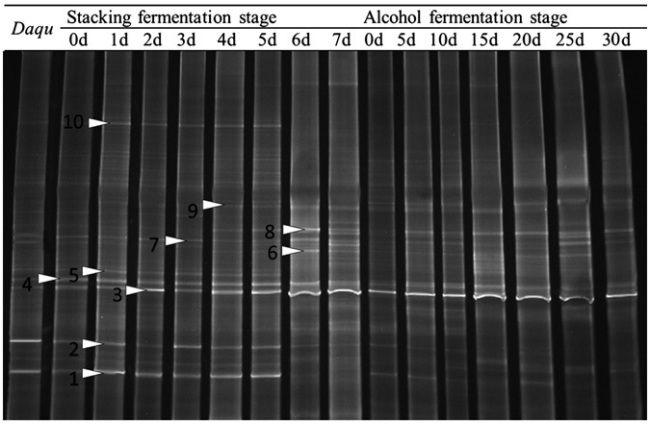

Fig. 1. DGGE profiles of fungal partial 18S rDNA gene fragments obtained with primers NS1/GC-fung. Lanes corresponding to different fermentation times are marked at the top. A 10 to 30% denaturing gradient was used.

yeast species, *Saccharomyces cerevisiae* (band 3), *Zygosaccharomyces bailii* (band 4) and *Torulaspora delbrueckii* (band 5).

In *Maotai*-flavor *Daqu*, a total of 7 fungal species were detected. *P. variotii* and *A. oryzae* were the dominant filamentous fungal species, and the relative abundance was 27.1% and 38.2%, respectively. Other filamentous fungi such as *M. ruber*, *A. terreus*, *P. decumbens* and *G. argillacea* were also encountered, but the abundance of each species was lower than 2.9%.

During stacking fermentation, all the fungal species from *Daqu* were detected, and *P. variotii* and *A. oryzae* were still the predominant species. The relative abundance of *P. variotii* showed a 2-fold increase during the stacking fermentation and *A. oryzae* increased from 8.4% to 16.5% before decreasing. Other fungi were less abundant.

During the alcohol fermentation in pits, the abundance of filamentous fungi decreased till the end. Numerous studies have reported changes in nutrient, temperature, organic acids and oxygen concentration all of which could affect the microbial community structure (Giovanna et al., 2012; Le Dréan et al., 2010; Li et al., 2011; Thanh et al., 2008; Wu et al., 2012). The lack of oxygen and increasing content of organic acid and alcohol in the pits are inhibitory to filamentous fungi, resulting in the loss of bioactivity and cell death (Huang et al., 2012).

Compared with culture dependent method, DGGE analysis detected additional fungal species, *G. argillacea*, *P. decumbens* and *M. ruber* during the whole fermentation process. This may be explained by the difficulty of culturing some fungal species (Handelsman et al., 1998). However, the culture dependent method detected *P. chrysogenum*, *P. namyslowskii*, *R. microsporus*, *M. purpureus* and *M. cirrosus* when compared with DGGE method. Directly extracting filamentous fungal DNA from complex solid-state substrates is more difficult than from liquid substrates, resulting in inadequate DNA for DGGE analysis. We assume that this could cause detection limits for the DGGE method, so that some fungal species were not detected by the PCR-DGGE method (Rantsiou et al., 2005; Zheng et al., 2012).

However, both methods detected *P. variotii*, *A. oryzae* and *A. terreus* during the liquor making process. Furthermore, *P. variotii* and *A. oryzae* were the predominant filamentous fungal species. Both methods showed growth of filamentous fungi during stacking fermentation and a continuous decrease during alcohol fermentation. These results indicated the utility of simultaneous use of culture-dependent and -independent techniques for studying filamentous fungal community in Chinese liquor making process.

3.3. The enzyme-producing properties of different fungal species

As glucoamylase and α-amylase are the enzymes important for degrading starch in liquor fermentation, and filamentous fungi were the main producers, the ability of different fungal species to produce these two enzymes was determined in this study. Table 3 shows that the glucoamylase and α-amylase activity of *P. variotii* reached

Table 2
The relative abundance (%) of filamentous fungi during the entire fermentation process quantified by PCR-DGGE.

Band no.[a]	Species	*Daqu*	Stacking fermentation stage								Alcohol fermentation stage						
			0d	1d	2d	3d	4d	5d	6d	7d	0d	5d	10d	15d	20d	25d	32d
1	*P. variotii*	27.1	10.5	22.5	17.0	12.8	16.5	19.0	2.7	2.4	7.52	5.6	1.9	2.2	4.7	1.6	–
2	*A. oryzae*	38.2	8.4	12.2	14.7	16.5	13.1	10.5	7.2	5.2	5.2	2.3	1.8	1.7	1.5	–	–
6	*P. clavariiformis*	1.3	4.4	2.5	2.2	4.0	1.9	2.2	10.3	7.2	–[b]	4.6	2.4	4.3	4.6	4.3	4.1
7	*P. decumbens*	2.0	8.3	4.6	5.5	8.1	3.3	2.9	8.1	7.9	–	4.5	4.8	2.6	2.3	2.3	3.6
8	*G. argillacea*	2.1	2.8	2.2	1.5	1.9	3.1	4.8	12.9	8.0	12.6	6.3	8.5	11.3	9.8	8.0	–
9	*M. ruber*	0.6	2.2	2.5	2.4	2.6	3.3	3.7	7.1	6.9	4.1	5.4	3.9	2.7	3.3	1.5	3.2
10	*A. terreus*	2.9	4.2	7.7	5.9	6.6	5.6	6.2	–	–	–	–	–	4.9	–	–	–

[a] Bands are numbered according to Fig. 1. Band 3, 4 and 5 were identified to be yeasts and were not listed in this table. The DGGE bands of yeasts have been accounted in the relative abundance analysis.
[b] Filamentous fungi were undetectable.

3252 ± 526 U/g and 1417 ± 430 U/g, respectively. A previous report showed that the glucoamylase and α-amylase activities of *P. variotii* obtained from *Hong Qu* (a starter for rice wine) were 2613.72 ± 206.48 U/g and 1718.91 ± 484.43 U/g, respectively (Huang et al., 2012). *P. variotii* possesses high enzymatic activity and is a fast-growing thermotolerant fungus, able to grow at low oxygen (Houbraken et al., 2008). These properties enabled it to survive through the high temperature of *Daqu* making and to be active in the stacking fermentation (Zheng et al., 2011). It also produced tannase, which could degrade the condensed tannins (Madeira et al., 2011, 2012). Sorghum, the main material of *Maotai*-flavor liquor contains about 2%–3% (w/w) condensed tannins in the sorghum bran (Awika et al., 2005; Xu et al., 2012). The condensed tannins could interact with starch (especially amylose), protein and digestive enzyme (α-amylase, protease), resulting in lower digestibility of ingredients and the stuck fermentation (Barros et al., 2012; Davis and Hoseney, 1979). The predominance of *P. variotii* during the whole fermentation process implied an important hydrolyzing function in *Maotai*-flavor liquor making.

Table 3 also shows that the glucoamylase and α-amylase activities of *A. oryzae* were 2463 ± 343 U/g and 1491 ± 324 U/g, respectively. *A. oryzae* has been used for thousands of years in Asia and is important in the production of Japanese soy sauce and sake (Yu et al., 2012; Zhao et al., 2013). Previous studies showed that *A. oryzae* possessed high proteolytic and saccharolytic enzyme activity, and it can also impart special flavors to the fermented products (Feng et al., 2013; Zhao et al., 2013). This study highlighted the presence of *A. oryzae* in the liquor making process, indicating that *A. oryzae* also plays an important role in Chinese *Maotai*-flavor liquor making process.

Monascus sp. can produce glucoamylase (1658 ± 219 U/g) and esterase, which can esterify acids and ethanol and contribute to the flavor of liquor (Weimin et al., 2012). *R. microsporus* is a strong α-amylase (1217 ± 216 U/g) producer. Lv et al. (2012) and Huang et al. (2012) reported that *M. ruber*, *M. purpureus* and *R. microspores* existed in the fermentation starter of *Hong Qu*, and showed high enzymatic activity. Their existence in the *Maotai*-flavor liquor process indicated their unique function in the liquor fermentation. Other species such as *Penicillium* sp., *Penicilliopsis* sp. and *Geosmithia* sp. with low enzymatic activity were considered as contaminants from the brewing environment due to the open operation of the manufacture, and they were not considered to be relevant to the liquor fermentation (Zheng et al., 2011).

Table 3
The enzyme producing ability of different fungal species obtained from the liquor fermentation process [a].

Species	Glucoamylase activity (U/g)	α-Amylase activity (U/g)
P. variotii	3252 ± 526	1417 ± 430
A. oryzae	2463 ± 343	1491 ± 324
P. namyslowskii	753.3 ± 76	172 ± 73
R. microsporus	1217 ± 216	240 ± 47
M. cirrosus	414 ± 35	–[b]
M. purpureus	1658 ± 219	292 ± 45
P. chrysogenum	842 ± 133	145.1 ± 59
A. terreus	1325 ± 244	356 ± 47

[a] The filamentous fungal species were cultured with bran for 72 h before determining the enzymes activity, and mean values were three independent experiments.
[b] Enzyme activity was undetectable.

3.4. Chemical and quantitative analysis of Zaopei in stacking fermentation stage

Stacking fermentation was an important process for filamentous fungi growth, and a large number of hydrolytic enzymes were accumulated during this stage. *P. variotii* and *A. oryzae* were the predominant fungi during the liquor making process. In order to more accurately analyze the growth of the two fungal species in stacking fermentation, real-time qPCR method was used. As shown in Fig. 2, *P. variotii* and *A. oryzae* grew consistently to the peak of 1.44×10^4 spores/g and 6.15×10^3 spores/g, and then decreased to 9.25×10^3 spores/g and 3.25×10^3 spores/g at the end of the fermentation, respectively.

A previous study showed that the saccharifying and liquefying enzyme activities of *Zaopei* both increased from about 30 U/g to more than 90 U/g during the stacking fermentation (Zhang et al., 2010a, b). Due to the low hydrolytic enzyme producing ability of bacteria and yeasts (Li et al., 2013), filamentous fungi were the main hydrolytic enzyme producers. Even though the filamentous fungi decreased slightly at the end of stacking fermentation, the presence of *A. oryzae* and *P. variotii* was consistent with the variation of the decreasing starch content and the increasing reducing sugar content in samples. The high amylase activity of *A. oryzae* and *P. variotii* indicated that they played an important role in the hydrolysis of starch.

During alcohol fermentation in pits, as the oxygen was exhausted and the content of ethanol and organic acid continuously increased, the filamentous fungi and enzymes gradually lost their bioactivity (Li

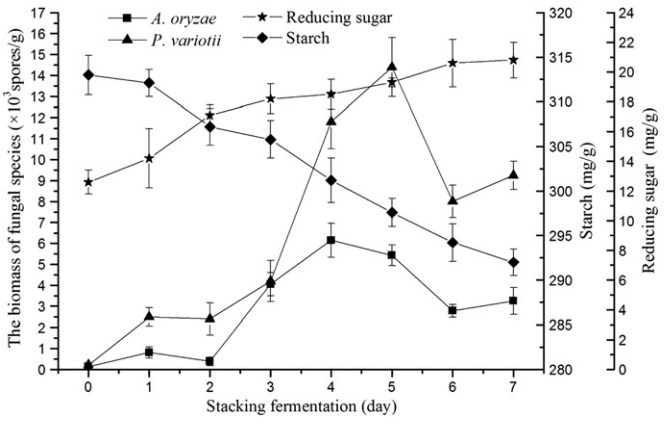

Fig. 2. Profile of the starch and reducing sugar content of *Zaopei* in stacking fermentation stage, and the growth of *P. variotii* and *A. oryzae* in this process. Error bars represented the standard deviation of the triplicate experimental data of the same position from two representative fermentation processes.

et al., 2011; Wu et al., 2013). After 10 days fermentation, the starch content remained stable while sugar content began to decrease because of the utilization by yeasts and bacteria (Wu et al., 2013). This report indirectly illustrates that the hydrolytic enzymes for liquor fermentation are mainly accumulated in the stacking fermentation stage and shows the importance of filamentous fungi for producing hydrolytic enzymes during this stage.

4. Conclusion

This work gained an insight into the filamentous fungal community during the whole Chinese *Maotai*-flavor liquor making process. A complex filamentous fungal community was observed as 11 filamentous fungi were detected in the liquor making process, and the stacking fermentation was an essential process for development of filamentous fungi. *A. oryzae* and *P. variotii* were the predominant fungal species and possessed high glucoamylase and α-amylase activity. The variation of reducing sugar and starch content in the samples was consistent with the growth of *P. variotii* and *A. oryzae*, which indicated their functional roles during SSF process under solid state conditions. This is different from Japanese *sake* fermentation, and will help us further understand the fermentative mechanism of Chinese *Maotai*-flavor liquor.

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