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Identification of yeast population dynamics of spontaneous fermentation in Beijing wine region, China

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Abstract – The aim of this study was (i) to investigate changes occurring in the yeast population profile during spontaneous fermentation of grape juice; (ii) to assess the proliferation of commercial yeast starter culture strains in vineyards; and (iii) to identify indigenous wine strains for future development of starter strains that better reflect the yeast biodiversity of China's grape-growing regions. To achieve this, yeasts were isolated at four different stages during fermentation of both hand-pressed and winery-sourced must samples of *Vitis vinifera* L. cv. Roussanne and Merlot. A total of 1600 yeast colonies were isolated and then grouped according to macroscopic and microscopic characteristics. A selection of 291 colonies from the different groups was subjected to species identification using the internal transcribed spacer regions of the 5.8S rRNA gene (ITS1-5.8S-ITS2 region) and the inter-delta (δ) sequence of the 26S rRNA D1/D2 region. In addition, 104 *Saccharomyces cerevisiae* colonies were subjected to strain identification. Twelve species belonging to nine different genera were found amongst the isolates. During the early stages of fermentation, it was found that *Hanseniaspora uvarum* and *Candida stellata* numerically dominated the four to six yeast species present, including a region-specific yeast, *Sporobolomyces beijingensis*. Two *S. cerevisiae* strains were isolated from the final stage of fermentation. These two indigenous strains, which were found to be different from the nine commercial yeast strains previously used as starter cultures in this particular Beijing-based winery, might possess potentially important region-specific oenological characteristics. This study provides the first essential step towards the preservation and exploitation of the hidden oenological potential of the untapped wealth of yeast biodiversity in China's wine-producing regions.

Key words: Beijing wine-producing region; spontaneous wine fermentation; yeast population profiles.

INTRODUCTION

Converting grape must into wine requires complex biochemical processes involving a select group of yeasts and bacteria. Yeast is key to alcohol formation in wine fermentations and multiple species are involved (Fleet *et al*., 1984; Pretorius *et al*., 1999; Delneri *et al*., 2003; Querol *et al.*, 2003; Jolly *et al*., 2006). In the initial stage of spontaneous fermentation, apiculate yeasts from the species *Hanseniaspora uvarum* (also called *Kloeckera apiculate*) are dominant (Masoud *et al.*, 2004; Hierro *et al.*, 2006). To a lesser extent, other non-*Saccharomyces* species of *Candida*, *Pichia*, *Rhodotorula*, *Hansenula* and *Kluyveromyces* can be detected (Mills *et al.*, 2002; Combina *et al.*, 2005; Di Maro *et al.*, 2007; Nisiotou *et al.*, 2007). As fermentation continues, and ethanol becomes increasingly concentrated, *Saccharomyces* (mostly *S. cerevisiae*) dominates and is responsible for fermentation. The presence of non-*Saccharomyces* species might be important in the production of secondary metabolites that could contribute to the taste and flavour of wine (Lambrechts and Pretorius, 2000; Fleet, 2003; Jolly *et al*., 2003a, 2003b, 2003c; Renouf *et al.*, 2006).

The role of *Saccharomyces* yeasts in winemaking has long been acknowledged, and commercial strains are commonly used as starter cultures to control the quality in vinification and to prevent spoilage of wine. Although spontaneous fermentations is much less reliable and reproducible than inoculated ferments, spontaneous fermentation is chosen by a small number of wineries in different wine regions based on the belief that mixed populations of indigenous yeasts yield wines with distinct sensorial quality, often described as wine with a fuller, rounder palate structure (Pretorius, 2000; Jolly *et al*., 2006). Therefore, understanding the population dynamics and evolution of indigenous yeast microflora in vineyards and wineries can help winemakers preserve indigenous strains, harness spontaneous fermentation, and better reveal the unique wine characters from a specific region (Pretorius, 2000).

One of the most effective approaches to identifying microorganisms within mixed populations, such as those participating

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in spontaneous wine fermentations, is the comparative DNA analysis of certain conserved regions within their genomes. The internal transcribed spacer regions of the 5.8S ribosomal RNA (rRNA) gene (ITS-5.8S rDNA) are key polymorphic markers to differentiate between yeast species, and polymerase chain reaction (PCR) methods are widely used in this regard (White *et al*., 1990). Restriction Fragment Length Polymorphism (RFLP) based on the ITS region is often used for rapid identification of yeast species during spontaneous wine fermentation (Guillamon *et al*., 1998). The sequences of the large subunit (26S) rRNA gene D1/D2 domain are available for almost all recognised yeast species (Fell *et al.*, 2000). Comparative sequence analysis of this domain is commonly used for yeast species identification. The δ-sequence was used to differentiate yeast species with the primer pair δ1/δ2 on the genus of *Saccharomyces* (Ness *et al*., 1993), and the primer pair δ12/δ21 were used for interdelta sequence analysis that showed clear discrimination of 53 commercial and laboratory *S. cerevisiae* strains (Legras and Karst, 2003).

Indigenous grape and wine-related yeasts were studied and described in many parts of both the so-called 'Old World' and 'New World' wine-growing regions, including Spain (Longo *et al*., 1991; De La Torre *et al*., 1999; Beltran *et al*., 2002), Portugal (Schuller *et al*., 2007), Italy (Agnolucci *et al.*, 2007; Domizio *et al.*, 2007), France (Fleet *et al*., 1984; Versavaud *et al.*, 1995; Demuyter*, et al.*, 2004; Valero *et al*., 2005, Masneuf-Pomarede *et al.*, 2007), Hungary (Sipiczki *et al*., 2004), the United States (Egli *et al.*, 1998: van Keulen *et al.*, 2003), Australia (Yap *et al*., 2000), South Africa (Khan et al., 2000; van der Westhuizen *et al*., 2000a, 2000b; Jolly *et al*., 2003a, 2003b, 2003c), Argentina (Combina *et al*., 2005), and Chile (Martinez *et al*., 2007). However, to the best of our knowledge, there are no documented reports on the indigenous vineyard and winery-associated yeast profiles in any region of China's rapidly expanding grape-growing regions.

The history of modern winemaking and wine research is relatively short in China. Though its wine production reached 4.5 million hl in 2006 (representing about 85% of consumption in the domestic market), the character, style and quality of Chinese wine and the underpinning winemaking technology are far from been fully identified, developed and optimised. For fermentation, a limited number of imported commercial yeast strains are used by most of China's wineries; however, spontaneous fermentation is also used in some of the smaller scale wineries.

The aim of the present study was (i) to investigate the evolution of yeast population profiles in spontaneous fermentation in must samples from a winery outside Beijing, which represents the climate, viticulture and vinification practices of that region with its typical north China continental climate; (ii) to investigate the possible proliferation of commercial yeast starter culture strains often used in this area through the surrounding vineyards and winery environments; and (iii) to identify indigenous wine strains for future development of starter strains that better reflect the yeast biodiversity of the China's grape-growing regions. By investigating the ecology and oenological potential of indigenous yeast strains associated with China's grape and wine production regions, this study opens up the possibility that, in the future, some winemakers might prefer to use individual or mixtures of indigenous yeasts as starter culture strains to reflect the regionality in Chinese wines. Such an approach could promote wine's most enthralling and fascinating characteristic, namely its diversity of style.

MATERIALS AND METHODS

Grape berry and reference yeast strains. *Vitis vinifera* L. (cv. Roussanne and Merlot) clusters were manually picked during harvest from 8-year-old vines (2006) at the Sino-French Viticulture and Enology Demonstration Farm (SFVEDF) and used for winemaking in the farm's winery. At the same time, a representative sample of 6-8 kg grapes of each variety were collected in sterile plastic bags and used in laboratory-scale winemaking experiments.

Standard strains of eight non-*Saccharomyces* yeast species were obtained from the China General Microbiological Culture Collection Center (CGMCC; Institute of Microbiology, Chinese Academy of Sciences) and nine commercial *Saccharomyces* strains, which have previously been used in the SFVEDF winery, were provided by that winery (Table 1).

TABLE 1 – Standard reference strains used in the study

Item No.*	Yeast strain designation	Yeast species	Source
SF1	EC1118	*Saccharomyces bayanus*	Lalvin
SF2	QA 23	*Saccharomyces bayanus*	Lalvin
SF3	Excellence XR Leure	*Saccharomyces cerevisiae*	Lamothe Abiet
SF4	BDX	*Saccharomyces cerevisiae*	Enoferm
SF5	DV10 selection champenoise	*Saccharomyces bayanus*	Lalvin
SF6	R-HST Riesling heiligenstein	*Saccharomyces cerevisiae*	Lalvin
SF7	ICV D21	*Saccharomyces cerevisiae*	Lalvin
SF8	Leuvre B 2006	*Saccharomyces cerevisiae*	Lamothe Abiet
SF9	Excellence SP	*Saccharomyces cerevisiae*	Lamothe Abiet
STD1	2.1706/04.4	*Pichia fermentans*	CGMCC
STD2	2.1593/91.6	*Hanseniaspora uvarum*	CGMCC
STD3	2.1440/03.2	*Kluyveromyces marxianus*	CGMCC
STD4	2.1602/91.6	*Torulaspora delbrueckii*	CGMCC
STD5	2.2188/01.1	*Candida stellata*	CGMCC
STD6	2.1465/85.7	*Issatchenkia orientalis*	CGMCC
STD7	2.1914/97.8	*Zygosaccharomyces rouxii*	CGMCC
STD8	2.2193/01.1	*Rhodotorula pinicola*	CGMCC

* SF: Sino-French Demonstration Farm ever used yeast strain; STD: standard strains from China's General Microbiological Culture Collection Center, Institute of Microbiology, Chinese Academy of Sciences.

Pressing and fermentation. In the winery, the grapes underwent standard winemaking procedures for white and red wines. For sampling, 5 litres of Roussane juice was obtained after pressing with an airbag presser (Babbri, France) and designated as RAP (Roussanne airbag pressed). Five litres of Merlot free-run juice was collected after de-stemming and crushing (Babbri, France) and designated as MAP. Under the standard winemaking protocols of the SFVEDF winery, 50 mg/l sulphur dioxide was automatically added after de-stemming and crushing; thus the sampled juice contained 50 mg/l sulphur dioxide.

In the laboratory, grapes of each variety were de-stemmed, crushed and pressed by hand in a sterile environment to avoid any possible contamination. Sulphur dioxide (50 mg/l) was also added to the extracted juice so that both the hand-pressed juice and the juice used in the winery trials contained the same amount of added sulphur dioxide. The two laboratory samples were designated as RHP (Roussanne hand pressed) and MHP (Merlot hand pressed), respectively. Spontaneous fermentation of the four juice samples were carried out in 3-litre glass bottles at 20 °C. Fermentation progress was recorded daily by monitoring the CO_2 release via measuring the sample weights.

Colony collection and grouping. Samples were taken at four different stages of fermentation, i.e. still (stage 1), start (stage 2), vigorous (stage 3) and final (stage 4) stage. These are designated RAP1-4, RHP1-4, MAP1-4, and MHP1-4, respectively. Aliquots (0.1 ml each) of a serial decimal dilution (from 10^{-1}-10^{-6}) of must samples from each stage were spread on agar plates of YPD (1% yeast extract, 2% peptone, 2% glucose, 2% agar) medium (Sangon, China). The plates were incubated at 28 °C for 3 days. One hundred yeast colonies were randomly selected from 10 replication Petri dishes for each sample, thus 1600 colonies in total were picked from RAP1-4, RHP1-4, MAP1-4, and MHP1-4. These selected colonies were preliminary grouped based on their macroscopic (texture, surface, margin, elevation and colour) and microscopic features.

Yeast identification. The stage 1 and 2 fermentation samples did not contain inhibitory alcohol levels and therefore it was expected that these samples would display a wider and more diverse range of yeast species. Thirty colonies were randomly subjected to DNA species identification when any group consisted of more than 30 colonies after the primary morphological identification; otherwise all the colonies of a group underwent DNA species identification. For stage 3 and 4 fermentation samples, 20 colonies from each group with more than 30 members were selected for DNA species identification. In total, 291 colonies from the different groups were subjected to species identification.

Strain identification was only done with *S. cerevisiae* colonies. In RAP1-4, RHP1-4, MAP1-4, and MHP1-4 samples, when more than 20 colonies were DNA identified as *S. cerevisiae* in a morphological group, 20 colonies were randomly selected for strain identification; otherwise all the *S. cerevisiae* colonies underwent strain identification. In total 104 *S. cerevisiae* colonies were subjected to identification at strain level.

The genomic DNA of yeast was isolated according to Makimura *et al*. (1994). The ITS region of each colony was amplified with the universal primer pairs ITS1 (5′-TCC GTA GGT GAA CCT GCG G-3′) and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) to identify yeast genera and species. The PCR products were digested with restriction endonucleases *Hae*III and *Hinf*I (Takara, Dalian, China) (White *et al*., 1990). The 26S rRNA gene D1/D2 domain was amplified with the following pair of primers: NL1 (5′-GCA TAT CAA TAA GCG GAG GAA AAG-3′) and NL4 (5′-GGT CCG TGT TTC AAG ACG G-3′). This amplification was carried out with the genomic DNA of yeast species where previous identification was doubtful or unclear (Fell *et al*., 2000). Yeast strain differentiation was based on delta sequence patterns of the genomic DNA after PCR amplification with the primer pair δ12 (5′-TCA ACA ATG GAA TCC CAA C-3′) and δ21 (5′-CAC TTA ACA CCG TAT ATG A-3′) according to Legras and Karst (2003).

PCR products and restriction fragments were separated on 1 and 2% agarose (Biowest, Shanghai, China) gels stained with GoldenView. A 100-bp DNA ladder marker (Tiangen, Beijing, China) served as the size standard. Gel images were processed with AlphaEase FC software (Alpha Innotech Corp., San Leandro, CA, USA). After purification, the PCR products of the ITS-5.8S rRNA and 26S rRNA D1/D2 regions were directly submitted for DNA sequencing by an ABI PRISM 3700 capillary sequencer (Applied Biosystems, Foster City, CA, USA). Sequence comparisons were performed using the basic local alignment search tool (Blast) within the NCBI database (National Center for Biotechnology Information).

RESULTS AND DISCUSSION

Spontaneous fermentation and yeast population profiles

The spontaneous fermentation process of the four samples is shown in Fig. 1. A total of 1600 colonies were isolated from four stages of the Roussanne and Merlot samples. The identification results and the percentage of colonies corresponding to each species at each fermentation stage are shown in Tables 2 and 3. The species *Candida stellata*, *Candida zemplinina*, *Cryptococcus flavescens*, *Hanseniaspora uvarum*, *Issatchenkia occidentalis*, *Issatchenkia orientalis*, *Metschnikowia fructicola*, *Pseudozyma aphidis, Rhodotorula mucilaginosa* and *Saccharomyces cerevisiae* were isolated at frequencies higher than 2%. *Sporidiobolus pararoseus* and *Sporobolomyces beijingensis*, both showing very low frequencies, were considered as sporadic species according to the standard of Esteve-Zarzoso *et al*. (2001).

Several previous studies conducted in a number of wine-producing countries reported yeasts belonging to genera such as *Candida*, *Hansenula*, *Kluyveromyces*, *Metschnikowia*, *Pichia*, *Rhodotorula*, *Torulaspora* during the initial stages of fermentation (Querol *et al.*, 1990, Longo *et al.*, 1991, Schütz and Gafner, 1994). In the present study, no yeasts belonging to the genera *Pichia* and *Hansenula* were isolated, while a few unusual apiculate yeasts, such as *Issatchenkia occidentalis*, *Issatchenkia orientalis* and *Sporobolomyces beijingensis*, were found. The absence of the former species and the presence of the latter might be explained by the different conditions in this Beijing-based winery because it is known that the microflora of grapes vary according to grape variety, to temperature, rainfall and other climatic influences, to soil, fertilization, irrigation and viticulture practices (e.g. vine canopy management), and to the development stage at which grapes are examined and fungicides applied to vineyards (Pretorius *et al*., 1999).

The climate of the Beijing wine region differs substantially from that of the world's main wine regions. Located at longitude 115°16′-115°58′ and latitude 40°4′-40°35′, the area is of typical continental monsoon climate. The cold and dry winters see temperatures sometimes drop below -20 °C and less than 1.3-3% of the area's 400 mm annual rainfall. The summer temperatures usually rise above 35 °C and more than 80% of the yearly precipitation is received during summer. Consequently, viticulture in Beijing differs dramatically from elsewhere. The other reason is that as a new wine region, the grape and wine-related microbial

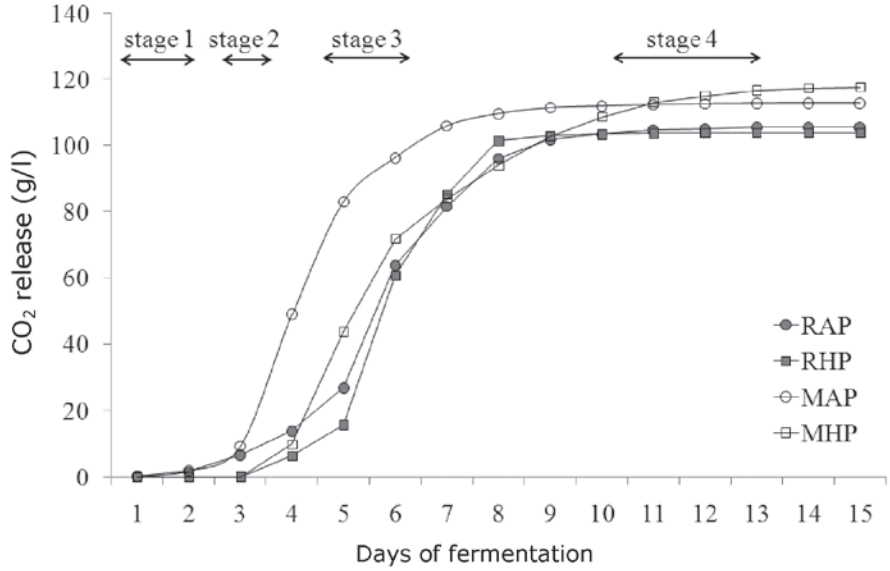

FIG. 1 - Spontaneous fermentation curves of different grape must sample with indication of the four fermentation stages according to the CO_2 releasing. The double arrows indicated sampling stages for yeast population separation and identification. Fermentation progress was recorded daily by monitoring the CO_2 release via measuring the sample weights.

TABLE 2 - Evolution of yeast species during spontaneous fermentation of Roussanne samples

Species	Colony frequency (%) in:							
	RHP				RAP			
	Stage 1	Stage 2	Stage 3	Stage 4	Stage 1	Stage 2	Stage 3	Stage 4
Saccharomyces cerevisiae		63.0	80.7	100	21.3	93.7	100	100
Hanseniaspora uvarum	43.4	4.5	19.3		21.8			
Candida stellata	4.2	10.1			28.3			
Candida zemplinina	13.3							
Issatchenkia occidentalis	21.2	22.4			22.7	2.1		
Issatchenkia orientalis	11.5							
Metschnikowia fructicola	6.4							
Pseudozyma aphidis					4.2	4.2		
Sporobolomyces beijingensis					1.7			

TABLE 3 - Evolution of yeast species during spontaneous fermentation of Merlot samples

Species	Colony frequency (%)							
	MHP				MAP			
	Stage 1	Stage 2	Stage 3	Stage 4	Stage 1	Stage 2	Stage 3	Stage 4
Saccharomyces cerevisiae		47.9	80.7	100	40.7	97.2	91.6	100
Hanseniaspora uvarum	56.6	52.1	15.7		11.5			
Candida stellata					29.3			
Candida zemplinina					8.4			
Issatchenkia occidentalis					8.4	2.8	8.4	
Rhodotorula mucilaginosa	9.2							
Cryptococcus flavescens	30.0							
Sporidiobolus pararoseus					1.7			
Sporobolomyces beijingensis	4.2		3.6					

populations will differ from those regions with a long history of grape growing and wine production. For example, a lower level of yeast biodiversity in newly established wineries was reported in other wine regions (Constanti *et al*., 1997). Finally, the addition of 50 mg/l SO_2 in the samples, which is standard practice in most Chinese wineries, could adversely affect the isolation of SO_2-sensitive yeast species such as *Hansenula* and *Pichia* (Gutierrez *et al*., 1999; Lopes *et al*., 2002),

Sporobolomyces yeasts are most commonly detected from natural samples, such as leaves and fruits. *S. beijingensis* was initially isolated and identified from leaves in Beijing's mountainous area (Wang and Bai, 2004), and it has never been reported in any other domestic or international regions. The morphology of the *Sporobolomyces* yeasts isolated during the study appeared be identical to those described by Wang and Bai (2004); the homology of the ITS sequences is up to 100%. The genus *Sporobolomyces* has been reported only in maturing grapes, and as unlikely to have a relationship to vintage and variety or to participate in fermentation (De La Torre *et al*., 1999).

As fermentation progressed and the alcohol concentration rose, the number of yeast species decreased. In the first stage of all four samples, there were four to six species present. During the second stage, only two to three species were isolated and *S. cerevisiae* was present in all the samples. The third stage was similar to the second, as *S. cerevisiae* became dominant and controlled the fermentation process. In the last stage, only *S. cerevisiae* was isolated.

In the present study it was found that only a few apiculate yeast species numerically dominated the microbial population during the initial stages of fermentation. However, they disappeared soon after the onset of the vigorous stage during which the alcohol concentrations rose sharply. *Hanseniaspora uvarum* and *Issatchenkia occidentalis* survived longer in the must, whereas the other non-*Saccharomyces* yeasts were present only for a short period of time.

Yeast diversity from different samples

Eleven non-*Saccharomyces* and one *Saccharomyces* yeast species were found in the spontaneously fermented Roussanne and Merlot must samples. In the hand-pressed Roussanne samples, seven yeast species were found, whereas in the corresponding Merlot samples, only five yeast species were isolated. *Issatchenkia orientalis*, *Metschnikowia fructicola* and *Pseudozyma aphidis* were only found in the Roussanne samples while *Cryptococcus flavescens*, *Rhodotorula mucilaginosa* and *Sporidiobolus pararoseus* were only found in the Merlot samples. The discrepancies between the yeast populations in the Roussanne and Merlot samples are not unusual; such differences were also reported by other researchers (De La Torre *et al*., 1999).

In the hand-pressed samples, *H. uvarum* was present until the final stage of fermentation, while in the winery-sourced samples it was only detectable during the first two stages. *Candida stellata* was absent in the MHP sample, but present in the MAP1 sample as the dominant species.

The stages of fermentation during which *S. cerevisiae* were detected, were different for the hand-pressed and winery-sourced samples. *Saccharomyces cerevisiae* was not isolated during the first stage of the two hand-pressed must samples, whereas the two corresponding winery-prepared samples showed a rather high frequency of *S. cerevisiae*. The earlier presence of *S. cerevisiae* in the latter samples is likely to be related to the winery environment. The source could include transport containers, press equipment and airborne matter.

***Saccharomyces cerevisiae* strain differentiation and their resources**

Only one member (i.e. *S. cerevisiae*) of the six species of the *Saccharomyces sensu stricto* group (Delneri *et al*., 2003) was isolated in the present study. The genomic sequences of 104 isolated strains of *S. cerevisiae,* identified by ITS-5.8S rRNA and 26S rRNA sequence comparisons, were amplified with the primers delta12/delta21 to further differentiate them and establish the genetic variation within the natural populations. The results demonstrated that about 20 individual colonies from the same fermentation sample showed the same amplified DNA fragment pattern and thus indicated they are likely to belong to a single strain. Two distinct *S. cerevisiae* strains were isolated. One was from the two hand-pressed samples (i.e. RHP and MHP), while the other was from the two winery gained samples (i.e. RAP and MAP) (Fig. 2).

Several *S. cerevisiae* strains could be isolated from the middle and/or final stages of the spontaneous fermentation samples (Gutierrez *et al*., 1999, Lopes *et al*., 2002). The relatively low number of *S. cerevisiae* strains was in line with the equally low number of yeast species detected in these trials. The limited

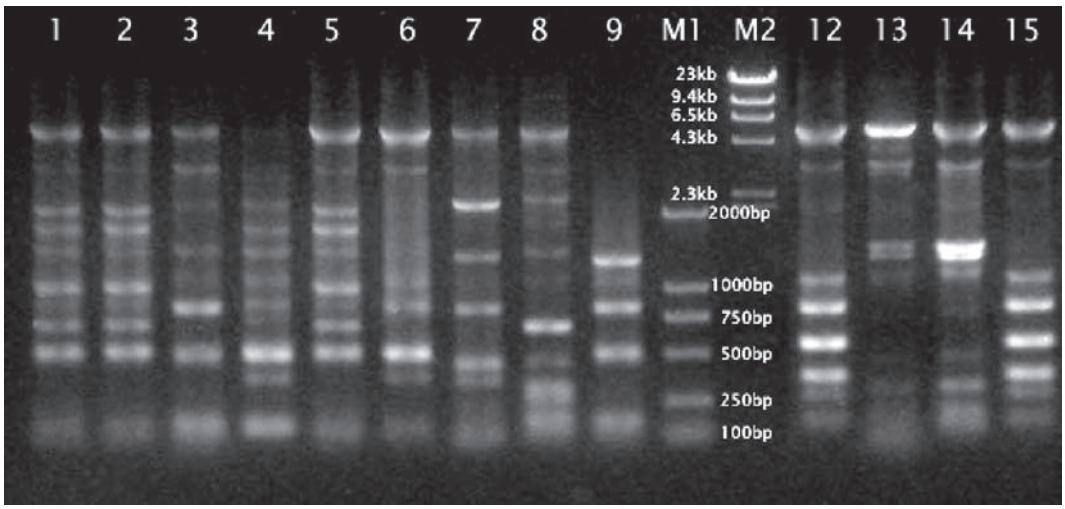

FIG. 2 - Amplification band of the commercial and isolated *Saccharomyces* strains by using delta12-delta21 primers. Lanes 1-9: the commercial strains of SF1-SF9 which have been applied in the history of the winery, lane M1: DNA marker DL2000, lane M2: λDNA/*Hae*III marker, lanes 12-15: yeast strains from MHP, MAP, RAP and RHP.

diversity of yeast species and *S. cerevisiae* strains detected here in northern China's wine-producing regions was also found with similar studies in three other wine regions, i.e. Ningxia, Gansu and Shandong (data not published).

Nine different commercial yeast strains of *S. cerevisiae* and *S. bayanus* have been used as inoculated starter cultures since the establishment of the SFVEDF winery with its first vintage in 2003. In the present study, none of these strains were found in any of the must samples - neither from grapes harvested from the adjacent vineyard or from the must samples prepared in the SFVEDF winery.

During winemaking and pomace treatment, commercial yeasts might spread into the vineyard and winery (Valero *et al.*, 2005; Schuller *et al.*, 2007). The two isolated *S. cerevisiae* strains in our study showed a distinct difference from all the nine commercial strains which have been used in the SFVEDF winery. This indicates that, under the present climate and winemaking conditions, the vineyard and winery proliferation of the commercial strains was fairly limited.

This finding is in line with the belief that *S. cerevisiae* is present on berry surfaces in very small numbers (10-100 CFU/g) and can be isolated only via enrichment culture techniques (Mannazzu *et al*., 2002). However, there are other researchers who believe that *S. cerevisiae* strains in wineries primarily come from alcoholic products or contaminated winery equipment rather than from the vineyard (Mortimer and Polsinelli, 1999; Querol *et al*., 2003). In the present study, *S. cerevisiae* was not found in the RHP1 and MHP1 samples, but was present at increasing numbers from the second stage of fermentation and onwards. As those grape clusters were processed in an aseptic environment after their harvest from the vineyard, our results support the belief that there is indeed a low number of *S. cerevisiae* present on the grape berries and that the grape-derived population of *S. cerevisiae* is enriched as the fermentation progresses.

In conclusion, it can be stated that this study forms part of a larger taxonomic survey within the ecological framework of the grape-growing regions of China, which is significantly different from most other wine-producing regions of the world in terms of *terroir* and culture. Since there is a growing need amongst leading winemakers for yeast starter culture strains that are better adapted to the different wine-producing regions of the world with their respective grape varietals, viticultural practices, winemaking techniques and styles of wine, this study provides an important step towards the preservation and exploitation of the hidden oenological potential of the untapped wealth of yeast biodiversity in the China's grape-growing regions.

Future work will focus on the seasonal variation of indigenous yeast profiles in China's expanding vineyards and the development of starter strains that will enhance the regionality of Chinese wines.

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