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ORIGINAL PAPER

Selection of indigenous *Saccharomyces cerevisiae* strains in Shanshan County (Xinjiang, China) for winemaking and their aroma-producing characteristics

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Abstract In order to select potential indigenous *Saccharomyces* strains, diversity of indigenous *Saccharomyces* strains in Shanshan County (Xinjiang, China) was preliminarily analyzed. Twenty-one genotypes were found through interdelta fingerprinting analysis. According to this result, representatives of each genotype were chosen to test the enological criteria. After tests of fermentation characteristics and growth ability, eight strains were finally selected as starters to further fermentation of Merlot must for aroma analysis and sensory evaluation at the same testing conditions, with one commercial strain F15 as control. Each strain of *Saccharomyces cerevisiae* produced individual volatiles in different concentrations and combinations which significantly influenced resulting wine flavour. Except of LFP522, all indigenous isolates produced more concentration of esters than F15. Higher concentrations of linalool, β-damascenone and citral, associated with *S. cerevisiae* LFE1809, considerably distinguished this strain from the others. Sensory evaluation present the Merlot wine fermented by LFE1225 isolated from Merlot, had the highest sensory score.

Ning Liu and Yi Qin have contributed equally to this work.

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Introduction

Yeasts play a fundamental role in winemaking, which convert sugar into ethanol and contribute for wine flavor as the result of metabolism. Recently, the use of commercial *Saccharomyces cerevisiae* strains is becoming a common practice in winemaking, due to their advantage to make the wine with stable quality and avoid the risk of unpleasant compounds by spoiled yeast (Scacco et al. 2012). However, the use of commercial *S. cerevisiae* strains may reduce the biodiversity of yeast strains that perform spontaneous fermentation, and consequently, to reduce the resulting wine complexity (Frezier and Dubourdieu 1992). So local autochthonous selected strains of *S. cerevisiae* as starters are rather advisable (Ortiz et al. 2013), since these yeasts are better acclimated to micro-area conditions of the wine production region (Martini and Martini 1990), and finally contribute to the sensory characteristics of wine (Le Jeune et al. 2006).

Recent molecular bio-techniques provide benefic tools for population dynamics studies on *S. cerevisiae*, and also proved extremely beneficial for yeast laboratories testing strains for their enological properties in order to optimize wild-strain isolates collection. The use of a rapid PCR-based protocol on the amplification of interdelta regions was initially proposed in 1993 (Ness et al. 1993). Delta elements form the LTR flanking retrotransposons TY1 and TY2 in yeast; about 300 such delta elements are described in the genome of S288C and are therefore good candidate targets for identification of polymorphisms (Legras and Karst 2003). In the intraspecific diversity studies of *S.*

cerevisiae strains in wine fermentation, interdelta analysis is one of the methods often used (Sun et al. 2009; Wang and Liu 2013).

For indigenous *S. cerevisiae* strains selection, many important oenological criteria should be considered, such as low volatile acidity production, low hydrogen sulfide production, high tolerance to alcohol, exhaustion of sugar potential and high fermentation activity, resistance to sulfur dioxide, resistance to low pH, etc. (Regodón et al. 1997). Li et al. (2011) preselected forty-six *S. cerevisiae* isolates according to their resistance to high ethanol concentration, low pH, high sulfur dioxide concentration and fermentation potential, and 12 strains with prominent fermenting properties were obtained. In order to choose autochthonous fermentative yeast starters, which are able to maintain typicity in Sherry wine, Rodriguez-Palero et al. (2013) chose five strains for physiological analysis: fermentative power, ethanol production, sugar consumption, acidity, volatile compound production, sensory quality, killer phenotype, desiccation, and sulfur dioxide tolerance, and one indigenous *Saccharomyces* strain, named J4 was finally selected for industrial fermentation. Recently, yeast selection has involved in the improvement of wine flavor, color, structure and other technological properties (Suárez-Lepe and Morata 2012).

Due to the market developing trend and the importance of yeast for wine flavor, studies on the isolation of native yeast and the potential of them to conduct wine fermentation are of great value for wine industries. The studies on the selection and evaluation of desirable indigenous wine yeast strains have been conducted in many viticulture regions, such as Sicily (Italy), Teramo (Italy), Frontera (Spain) and León (Spain) (Álvarez-Pérez et al. 2012; Carrascosa et al. 2012; Scacco et al. 2012; Suzzi et al. 2012). However, the yeast ecology was only studied in a few vineyards of major viticultural regions in China (Li et al. 2010, 2011; Wang and Liu 2013). Also, there are limited studies on the selection of indigenous wine yeasts and indigenous *S. cerevisiae* strains feasibility of inoculating the grape must in China (Liang et al. 2013). The objectives of this study were to investigate the diversity of the indigenous *S. cerevisiae* strains and to select the potential indigenous strains for high aroma quality in Shanshan County, Xinjiang Province, China. During spontaneous fermentation of six grape musts in this region, indigenous *Saccharomyces* isolates were selected by using growth morphology on Wallersterin Laboratory nutrient (WLN) agar and Lysine medium in this study; then genetic diversity of the *S. cerevisiae* isolatiton was analyzed by Interdelta sequence analysis; the representative isolates of each genotype were chosen and tested for the enological criteria, and finally some isolates were selected for Merlot must fermentation and their aroma-producing characteristics were also evaluated.

Materials and methods

Sample collection and yeast isolation

Samples were taken from six different spontaneous fermentation processes in Shanshan County from the vintage 2008.

Shanshan County is located in Turpan Depression surrounded by mountains, and lies in the northern part of Xinjiang Province, western part of China. It belongs to typical arid continental monsoon climate of warm temperate zone, with active accumulated temperature up to 4500–5000 °C and an annual rainfall of 20–50 mm.

Two different fermentations with *Vitis vinifera* varieties (Merlot and 'Mixed red grape'), three different fermentations by raisin grape varieties (Small-berry Thompson Seedless, Big-berry Thompson Seedless and 'Mixed white grape') and one by table grape variety (Red Globe) were sampled. For "Mixed red grape", it was the mix of Cabernet Gernischet, Cabernet Sauvignon and Cabernet Franc (1:1:1, m/m/m), "Mixed white grape" was the mix of Big-berry Thompson Seedless and Small-berry Thompson Seedless (1:1, m/m). All spontaneous fermentation processes were carried out according to Li et al. (2011). Based on WLN agar (Pallmann et al. 2001) and Lysine medium, 59 *Saccharomyces* isolates were selected in this study.

Strain typing of *S. cerevisiae* isolates

Interdelta sequence amplification of all *S. cerevisiae* strains was carried out with delta12 and delta21 primers (delta12:5′-TCAACAATGGAATCCCAAC-3′; delta21:5′-CATCTTAACACCGTATATGA-3′) according to Legras and Karst (2003). PCR products were separated by electrophoresis on 2.0 % agarose gel submitted to 100 V for 2.5 h in 1× TAE buffer and photographed under UV light.

Enological criteria

In order to confirm the inoculation and growth of the representative isolates of each genotype in grape musts, their growth abilities with three levels of ethanol (10, 13, 16 % v/v), three levels of pH (2.0, 2.5 and 3.5) and the tolerance to various concentrations of sulfur dioxide (100, 200, 250 mg/l) were determined following by Parish and Carroll (1987).

These wine yeasts were also inoculated in 200 ml "Triple M" synthetic grape juice to evaluate their fermentation characteristics. "Triple M" medium was prepared using the methods reported by Giudici et al. (1993) with some modifications, and contained the following (per

litre): glucose, 100 g; fructose, 100 g; L-tartaric acid 6 g; L-malic acid 3 g; citric acid 0.5 g; $(NH_4)_2SO_4$, 5 g; KH_2PO4, 0.85 g; K_2HPO_4, 0.15 g; $MgSO_4{\cdot}7H_2O$, 0.5 g; NaCl, 0.1 g; $CaCl_2$, 0.2 g; inositol, 6 mg; biotin, 20 μg; folic acid, 2 μg; niacin, 400 μg; *p*-aminobenzoic acid, 200 μg; riboflavin, 200 μg; thiamine hydrochloride, 400 μg; H_3BO_3, 500 μg; $CuSO_4{\cdot}5H_2O$, 40 μg; KI, 100 μg; $FeCl_3{\cdot}6H_2O$, 200 μg; $MnSO_4$, 400 μg; $ZnSO_4{\cdot}7H_2O$, 400 μg; $Na_2MoO_4{\cdot}2H_2O$, 200 μg; pH 5.5. All fermentations were conducted at 25 °C in 250-ml Erlenmeyer flasks. Fermentation activities were monitored by weight loss as an estimate of CO_2 production. The end of fermentation was determined by constant weight for three consecutive days.

Microvinification in Merlot must

The selected strains and one commercial wine yeast (F15, Lafford, France, control) were inoculated in the musts of Merlot grape in 2009 (September 24) from Yuma vineyard, Ningxia Province, China. Merlot musts had the following compositions: 244.6 ± 0.7 g/l of sugar, 8.43 ± 0.13 g/l of total acidity as tartaric acid, and a pH of 3.46 ± 0.01. The musts were distributed into equivalent amounts and barrelled in 5 l fermentation vessels (three for each strain). After barrelling, SO_2 was added to a final concentration of 60 ppm in the musts. Yeasts were cultured in YEPD broth (at 25 °C) overnight, washed twice (by centrifugation) with sterile water and inoculated to the must at a rate of 10^6 - cells/ml. Fermentation was undertaken at 25 °C, monitored by taking readings of density and temperature and considered complete when no sugar could be detected. After fermentation, samples were refrigerated for 2 days at 2–4 °C then filtered (for clarification) and stored at −20 °C until composition analysis.

General analysis

After fermentations of "Tripple M" and Merlot must, ethanol production, volatile acid, total acidity and residual sugar were determined according to the National Standard of the People's Republic of China (GB/T 15038-2006, 2006). The production of H_2S in "Tripple M" was determined by Lead acetate column methods according to Linderholm et al. (2008).

GC–MS analysis of volatile compounds

Sixty-seven standards in this study were in HPLC or GC grade and obtained from Aldrich (Milwaukee, WI, USA), Sigma-Aldrich (China, Beijing), Supelco (Bellefonte, PA) and Fluka (Buchs, Switzerland). The purity of standards ranged from 95.0 to 99.9 % (in Supplement Table S1). 4-Methyl-2-pentanol (1.086 g/l in ethanol) was used as the internal standard. Absolute ethanol, tartaric acid and sodium chloride were of analytical grade and purchased from Xi'an chemical factory (Xi'an, China).

Volatile compounds of the Merlot wine samples were extracted by HS-SPME and analyzed using GC–MS according to Zhang et al. (2011). Volatile compounds were identified by comparison of the mass spectra in standard NIST05a.L library, retention times and mass spectra of the reference standards, when available. As for quantification, according to the alcohol degree and total acid in the samples, the synthetic wine with 13.5 % (v/v) ethanol was

Table 1 Distribution of 21 genotypes generated from different grape varieties

Genotype	Code of the strains	Genotype	Code of the strains
XJ1(14)	LFP501, LFP503, LFP506, LFP508, LFP509, LFP512, LFP514-LFP518, LFP525, LFP529, LFN502	XJ8(1)	LFP522
		XJ10(1)	LFN524
		XJ11(3)	LFN510, LFG505, LFG802
XJ2(1)	LFP523	XJ12(4)	LFE1225, LFE1226, LFE1215, LFE1504
XJ3(1)	LFP510	XJ13(1)	LFE1217
XJ4(1)	LFP505	XJ14(1)	LFE1219
XJ5(2)	LFP507, LFP502	XJ15(1)	LFE1809
XJ6(1)	LFP511	XJ16(1)	LFA719
XJ7(1)	LFP513	XJ17(2)	LFA709, LFA711
XJ9(15)	LFN503, LFN504, LFN506, LFN507, LFN508, LFN511, LFN514, LFN517, LFN518, LFN520, LFN521, LFN526, LFN531, LFN532, LFP504	XJ18(1)	LFA414
		XJ19(4)	LFR602, LFR318, LFR323, LFR312
		XJ20(4)	LFG510, LFG511, LFG820, LFG832
		XJ21(1)	LFG521

The isolates from "Mix red" variety were numbered with LFA, Merlot with LFE, 'Mix while' with LFG, Small-berry Thompson Seedless with LFN, Red Globe with LFP, and Big-berry Thompson Seedless with LFR

Table 2 Technological properties of *S. cerevisiae* strains from spontaneous fermentation

Genotype	Strains origin	SO_2 tolerance[a] (mg/l)			Ethanol tolerance (%v/v)			pH tolerance		H_2S production (μg/l)	Residual sugar (g/l)	Ethanol content (%v/v)	Volatile acid (g/l)	Acidity[b] (g/l)
		100	200	250	12	14	16	3.5	2.0					
XJ1	LFP525	+	+	+	+	+	+	+	+	31.08	0.05	10.39	0.21	5.29
	LFP529	+	+	+	+	+	+	+	+	59.46	0.21	11.23	0.24	7.76
	LFN502	+	+	+	+	+	+	+	−	32.97	1.80	11.22	0.21	4.85
XJ2	LFP523	+	+	+	+	+	+	+	+	59.46	1.50	10.86	0.21	6.56
XJ3	LFP510	+	+	+	+	+	+	+	−	50.00	0.81	10.78	0.25	7.07
XJ4	LFP505	+	+	+	+	+	+	+	+	67.03	1.54	10.74	0.17	4.90
XJ5	LFP507	+	+	+	+	+	+	+	−	78.39	0.38	11.67	0.24	5.02
	LFP502	+	+	−	+	−	−	+	−	93.52	0.30	11.14	0.24	7.16
XJ6	LFP511	+	+	+	+	+	+	+	+	40.54	0.45	11.45	0.18	5.29
XJ7	LFP513	+	+	+	+	+	+	+	+	6.48	4.33	10.32	0.21	4.32
XJ8	LFP522	+	+	+	+	+	+	+	+	42.43	0.45	10.95	0.23	5.11
XJ9	LFN506	+	+	+	+	+	+	+	+	10.27	0.85	11.44	0.18	4.85
	LFN517	+	+	+	+	+	+	+	−	36.76	5.27	11.81	0.24	7.76
	LFP504	+	+	+	+	+	+	+	+	51.89	0.75	11.26	0.23	4.90
XJ10	LFN524	+	+	+	+	+	+	+	+	0.81	0.52	11.99	0.16	6.99
XJ11	LFN510	+	+	+	+	+	+	+	+	72.71	1.63	10.77	0.17	4.67
	LFG505	+	+	+	+	+	−	+	+	229.76	0.73	10.77	0.25	7.07
XJ12	LFE1225	+	+	+	+	+	+	+	+	65.95	0.62	10.35	0.23	6.20
	LFE1504	+	+	+	+	+	+	+	+	12.16	0.83	10.48	0.25	7.07
XJ13	LFE1217	+	+	+	+	+	+	+	−	42.43	1.75	11.24	0.24	6.56
XJ14	LFE1219	+	+	+	+	+	+	+	+	67.03	0.55	10.55	0.20	7.33
XJ15	LFE1809	+	+	+	+	+	+	+	+	68.92	0.75	11.05	0.28	6.64
XJ16	LFA719	+	+	+	+	+	+	+	+	114.34	2.81	9.28	0.25	7.07
XJ17	LFA709	+	+	+	+	+	+	+	+	84.06	0.98	10.99	0.23	7.41
	LFA711	+	+	+	+	+	+	+	+	32.97	0.75	11.23	0.23	6.20
XJ18	LFA414	+	+	+	+	+	−	+	−	42.43	1.75	10.81	0.21	6.64
XJ19	LFR318	+	+	+	+	+	−	+	−	290.31	1.66	11.42	0.20	5.11
	LFR602	+	+	+	+	+	+	+	+	57.57	0.82	11.29	0.24	5.38
XJ20	LFG510	+	+	+	+	+	+	+	−	82.17	0.75	9.54	0.18	6.56
	LFG820	+	+	+	+	+	+	+	−	4.59	26.83	9.25	0.20	7.76
XJ21	LFG521	+	+	+	+	+	+	+	+	80.28	5.75	6.39	0.18	6.56

All fermentations were conducted for 10–15 days at 25 °C

[a] Growth within 72 h after inoculation; [b]expressed as tartaric acid. Data show mean value of two duplications

prepared in distilled water, containing 5.5 g/l of tartaric acid and pH was adjusted to 3.7 with 1 mol/l NaOH. Volatile standards were dissolved in synthetic matrixes at concentrations typically found in wine. Five-point calibration curves for each compound were achieved using the method described by Ferreira et al. (2000) and quantitative data were obtained based on the calibration curves of respective standards mentioned.

Odor activity value (OAV) of individual compound is the radio between its concentration and related threshold.

Sensory evaluation

All the wines were evaluated by a panel of 12 judges, including eight graduates and four teachers from College of Enology, Northwest A&F University. Before the arbitrarily presentation to the judges, all the wines were randomly coded. All the judges were asked to score according to wines appearance (clarity/color, 25 points), aroma (typicalness/quality/intensity, 28 points), mouth-feel (typicalness/quality/intensity/duration, 35 points) and harmony (balance, 12 points) (Li 2006).

Table 3 Physicochemical parameters of Merlot wines fermented with eight different wine yeasts (average ± SD)

Strain	Ethanol (%)	Residual sugars (g/l)	Total acidity[A] (g/l)	Volatile acid[B] (g/l)	pH
F15	13.82 ± 0.17a	3.90 ± 0.19a	5.92 ± 0.15a	0.43 ± 0.05a	3.54 ± 0.01a
LFN524	14.02 ± 0.12a	3.84 ± 0.17a	5.80 ± 0.18a	0.48 ± 0.07a	3.49 ± 0.01a
LFP522	13.81 ± 0.15a	3.72 ± 0.26a	6.43 ± 0.22ab	0.33 ± 0.04ab	3.44 ± 0.02a
LFP525	13.52 ± 0.11a	3.66 ± 0.24a	5.87 ± 0.16a	0.27 ± 0.05b	3.46 ± 0.02a
LFE1809	13.72 ± 0.13a	3.65 ± 0.06a	6.64 ± 0.20b	0.31 ± 0.06ab	3.48 ± 0.01a
LFE1225	13.83 ± 0.21a	3.71 ± 0.23a	5.47 ± 0.26ac	0.36 ± 0.07ab	3.54 ± 0.01a
LFE1504	14.11 ± 0.11a	3.89 ± 0.13a	5.78 ± 0.17a	0.34 ± 0.06ab	3.49 ± 0.01a
LFA711	13.62 ± 0.13a	3.87 ± 0.12a	6.69 ± 0.19b	0.36 ± 0.04ab	3.50 ± 0.01a
LFP529	14.12 ± 0.09a	3.72 ± 0.11a	6.02 ± 0.11a	0.39 ± 0.06ab	3.48 ± 0.01a

Different letters in the same column indicate significant differences ($P < 0.05$)

[A] Tartaric acid; [B]acetic acid

Statistical analysis

One-way analysis of variance (ANOVA) was used to evaluate the difference in aromatic composition among the wines studied. Significant difference was calculated at 0.05 levels. Hierarchical cluster analysis was performed to study the relation between phenotypes and genotypes. Principal component analysis (PCA) was applied to sensory data and the volatile compounds. SPSS 17.0 statistical package for Windows (SPSS Inc, Chicago, IL, USA) was used for all statistical analysis.

Results

Diversity of indigenous *S. cerevisiae* isolates

By interdelta sequence analysis, the 59 native *S. cerevisiae* isolates were classified into 21 genotypes, named as XJ1, XJ2-XJ21 in Shanshan County. As can be seen in Table 1, three of these 21 genotypes, XJ1, XJ9 and XJ11 appeared in two fermentations (XJ1, XJ9 in Red Global and Small-berry Thompson Seedless; XJ11 in Small-berry Thompson Seedless and Mixed White). Because Small-berry Thompson Seedless was one variety of Mixed White, XJ11 perhaps existed only in Small-berry Thompson Seedless. It can be seen that the genotypes of indigenous *S. cerevisiae* isolates were restricted to grape varieties.

Yeast pre-selection

Thirty-one isolates were chosen randomly as the representatives of genotypes XJ1-XJ21, and the genotypes with more isolates had one more or two more representatives, such as XJ1, XJ5, and XJ9 in Table 1. To characterize single isolates, the growing abilities in the presence of 10–16 % v/v ethanol and 100–250 mg/l sulfur dioxide, as well as to low medium pH were tested. Furthermore, fermentation potential was also quantified by chemical compositions of wines made from synthetic grape juice in Table 2. For the H_2S production, the values were in the range of 0.81–290.31 μg/l among these isolates. It was remarkable that LFN524 and LFG820 had very low H_2S production, 0.81 and 4.59 μg/l, respectively.

Eight strains LFN524, LFP522, LFP525, LFE1809, LFE1225, LFE1504, LFA711 and LFP529 were selected for the further Merlot fermentation, with F15 as control. These selected yeasts had excellent technological properties, such as high tolerance to SO_2 (250 mg/l), high tolerance to ethanol (16 % v/v), high tolerance to pH (2.0) and high sugar metabolism ability (residual sugar lower than 1 g/l), and also low H_2S production (below 70 μg/l; Table 2).

Fermentation of Merlot must

All strains completed fermentations with residual sugar lower than 4 g/l in Merlot wines and the physicochemical parameters of Merlot wines fermented by them were listed in Table 3. Fifty-two volatile compounds were quantified by GC–MS analysis in Table 4. Quantitatively, alcohols formed the most abundant group in the aromatic components of these nine wines, followed by esters and fatty acids. The total concentrations of aroma compounds ranged from 218.47 to 267.82 mg/l. LFE1225 produced the highest amounts of esters considered favorable for wine flavor. LFN524 produced the highest amount of 2-phenylethyl acetate, which was associated with the flower aroma.

Sensory evaluation

Results of the sensory analysis of the wines maded by different yeast strains showed in Table 5. LEF1225 had the highest score (81.5) of sensory evaluation among all samples made by Merlot, followed by F15 (80.8) and LFN524 (80.7).

Table 4 The threshold values (OTH) and concentrations of volatiles in wines with different yeast strains (μg/l)

Compounds	OTH[A]	Concentration								
		LFE1225	LFP525	LFE1809	F15	LFN524	LFP522	LFE1504	LFP529	LFA711
Esters 13										
Ethyl acetate	7500	28,831.8 ± 660.2ab	17,098.7 ± 297.0e	27,725.4 ± 829.5bc	21,733.5 ± 528.6d	13,616.5 ± 693.0e	8966.4 ± 281.2f	24,215.9 ± 1188.5c	24,740.4 ± 544.9c	32,859.7 ± 1555.5a
Ethyl butyrate	20	nd	16.7 ± 0.4c	18.1 ± 0.2c	16.4 ± 0.3c	20.3 ± 0.3b	5.7 ± 0.8e	26 ± 1.6a	13.9 ± 1.4d	13.2 ± 0.2d
Isoamyl acetate	30	3811.8 ± 171.3a	2989.7 ± 47.5b	2305.7 ± 106.7c	2168.2 ± 54.5 cd	4012.6 ± 100.5a	1911.7 ± 54.5d	1725.1 ± 111.5e	3619.9 ± 36.1a	1974.2 ± 92.6d
Hexyl acetate	670	17 ± 3.8c	32.9 ± 4.3ab	34.3 ± 0.9ab	35.9 ± 2.7ab	38.1 ± 1.7a	34.4 ± 2.7ab	16.5 ± 1.7c	24.3 ± 1.6b	32.3 ± 0.7ab
2-Phenylethyl acetate	250	6 ± 0.6f	40.1 ± 0.4b	2.6 ± 1.1gh	14.1 ± 0.06d	44 ± 0.5a	18 ± 0.3c	4.6 ± 0.4 fg	9.1 ± 1.1e	0.9 ± 0.3 h
Ethyl hexanoate	50	2252.2 ± 199.6b	2416.3 ± 108.3a	1615.5 ± 99.7 cd	1553.3 ± 37.6 cd	2693.7 ± 128.9a	1352.3 ± 37.6d	1517.9 ± 83.2d	1886.1 ± 29.0bc	1546.1 ± 99.7 cd
Ethyl lactate	14,000	10,099.5 ± 726.4 cd	11,345.7 ± 925.6bc	13,351.6 ± 38.9b	5188.9 ± 218.5f	9108.9 ± 655.7de	10,319.2 ± 501.4 cd	23,133.7 ± 655.7a	8423.1 ± 31.8e	10,364.5 ± 17.6d
Ethyl octanoate	20	28,408.4 ± 1528.2b	25,818.1 ± 602.6c	17,897.9 ± 90.0e	18,094.3 ± 107.6e	39,131 ± 1669.6a	18,321.8 ± 531.9e	21,176.5 ± 1457.5d	18,342.7 ± 90.1e	13,886.5 ± 160.7f
Diethyl succinate	500,000	1629.1 ± 113.6a	1874.7 ± 67.0a	1623.1 ± 99.2a	1648.8 ± 45.8a	1716.7 ± 42.9a	1384.7 ± 60.0b	1679.4 ± 42.9a	1191.4 ± 14.4b	1394.3 ± 99.2b
Ethyl decanoate	200	2358.2 ± 149.2 cd	2766.2 ± 49.9b	2074.5 ± 44.0f	2644.2 ± 35.7bc	3297.5 ± 7.7a	2466.1 ± 50.0cde	2340.3 ± 177.4 cd	2262.8 ± 44.1def	2178.5 ± 51.1ef
Ethyl dodecanoate	1500	559.8 ± 24.3b	579 ± 5.3b	468.1 ± 2.9d	577 ± 8.8b	701.1 ± 17.2a	340 ± 4.6e	444.9 ± 3.9d	482.1 ± 1.5 cd	512 ± 3.6c
Methyl octanoate	58	0.5 ± 0.1d	2.2 ± 0.1a	0.2 ± 0.1ef	0.3 ± 0.06de	1.6 ± 0.1b	0.2 ± 0.1ef	0.4 ± 0.1de	1.4 ± 0.03c	nd
Isoamyl hexanoate		nd	nd	nd	nd	5.6 ± 0.1	nd	nd	nd	nd
Subtotal		77,974.3a	64,980.3bc	67,117.0b	53,674.9d	74,387.6a	45,120.5e	76,281.2a	60,997.2c	64,762.2bc
Subtotal (%)		30.04	26.23	29.58	22.83	27.98	20.65	28.48	25.67	26.54
Alcohols 19										
1-Butanol	150,000	382.1 ± 18.2f	665.8 ± 23.1c	343.6 ± 7.8f	678.7 ± 23.1c	811.2 ± 11.1a	542.3 ± 16.0d	750.8 ± 18.2b	707.5 ± 0.7bc	431.8 ± 7.1e
Isobutyl alcohol	40,000	7159.4 ± 143.4f	5819 ± 136.8 g	7251 ± 80.8ef	6066.3 ± 108.6 g	14,364.7 ± 143.4a	12,800.2 ± 73.2c	7789.5 ± 68.7d	7406.7 ± 10.1de	13,280.3 ± 151.5b
Isoamyl alcohol	30,000	143,528.9 ± 2429.9a	143,585.7 ± 2384.9a	125,493.6 ± 774.9c	143,174.7 ± 3092.1a	141,989.7 ± 3844.1a	134,945.5 ± 3092.1b	143,183.6 ± 6672.5a	141,726 ± 845.6a	139,825.3 ± 3603.4ab
1-Pentanol	80,000	18.7 ± 1.2f	10 ± 0.2f	212.6 ± 6.3a	114.9 ± 1.2b	76.3 ± 0.9 cd	70.3 ± 0.4cde	66.8 ± 0.6e	65 ± 5.6de	74.8 ± 5.6c
4-Methyl-1-pentanol	5000	40.2 ± 1d	43.4 ± 0.7c	54.4 ± 0.9a	46 ± 0.08b	53.5 ± 0.9a	25.5 ± 0.6e	42.8 ± 1.7c	40.4 ± 1.1d	40.5 ± 0.9d
2-Heptanol	250	39.6 ± 0.6de	44.2 ± 1.6c	46.9 ± 1.3bc	40.8 ± 1.67d	27.1 ± 0.6 g	36.7 ± 1.7ef	49.9 ± 1.4a	37.1 ± 0.9f	49.4 ± 1.1ab
1-hexanol	4000	316.8 ± 7.1ef	373.9 ± 5.2c	396.9 ± 3.9b	334 ± 1.8e	341.7 ± 6.4d	316.1 ± 6.0f	422.3 ± 5.7a	367.6 ± 3.1c	348 ± 3.1d
(*E*)-3-Hexen-1-ol	1000	54 ± 1.5d	43.5 ± 0.9f	119.8 ± 0.5a	63 ± 1.7c	109.4 ± 2.0b	51.2 ± 1.0d	61.8 ± 0.8c	45.8 ± 0.4e	52.4 ± 0.7d
(*Z*)-3-Hexen-1-ol	400	6.2 ± 0.6 h	31.9 ± 0.2e	25.5 ± 0.7f	57.1 ± 0.5b	31.7 ± 0.8e	53.9 ± 0.3c	58.5 ± 1.0a	36 ± 0.6d	13.8 ± 0.7 g
2-Hexen-1-ol		nd	4.1 ± 0.1e	14 ± 1.8c	7.6 ± 0.1d	13.2 ± 0.3c	42.1 ± 0.2b	52.5 ± 0.2a	3.3 ± 0.08e	1.8 ± 0.5e
1-Heptanol	250	4.2 ± 0.1b	nd	nd	nd	nd	nd	6.9 ± 0.1a	nd	nd
2-Nonanol	58	0.5 ± 0.02c	0.7 ± 0.08b	0.1 ± 0.0d	0.3 ± 0.05c	1.7 ± 0.09a	0.8 ± 0.08b	0.2 ± 0.02c	0.7 ± 0.0b	0.1 ± 0.00d

Table 4 continued

Compounds	OTH[A]	Concentration								
		LFE1225	LFP525	LFE1809	F15	LFN524	LFP522	LFE1504	LFP529	LFA711
2,3-Butanediol	120,000	9.9 ± 0.2 cd	9.9 ± 0.1bc	9 ± 0.3d	9.6 ± 0.2 cd	10 ± 0.01bc	9.2 ± 0.1d	10.2 ± 0.2abc	10.3 ± 0.1a	10.2 ± 0.06ab
Methionol	500	1.3 ± 0.1b	1.2 ± 0.05b	0.5 ± 0.1d	1.7 ± 0.05a	0.8 ± 0.1 cd	0.8 ± 0.1c	1.7 ± 0.1a	0.5 ± 0.1d	1.3 ± 0.09b
1-Dodecanol		2.8 ± 0.3d	14.4 ± 0.6a	2.3 ± 0.9d	8.7 ± 0.4b	5.6 ± 0.2c	3.1 ± 0.4d	0.2 ± 0.02e	0.8 ± 0.2e	0.7 ± 0.9e
1-Octanol	900	6.9 ± 0.3bc	5.4 ± 0.05d	8.2 ± 0.9a	8.3 ± 0.05ab	0.1 ± 0.2e	5.2 ± 0.1d	6.7 ± 0.1c	4.8 ± 0.3d	4.5 ± 0.9d
1-Decanol	400	4.1 ± 0.1b	4.4 ± 0.5b	85.1 ± 5.7a	4.5 ± 0.5b	3.3 ± 0.08b	2.3 ± 0.5b	2.9 ± 0.1b	nd	2.5 ± 0.6b
Benzyl alcohol	200,000	10.3 ± 0.3d	26.6 ± 2.0a	3.3 ± 0.2f	21.9 ± 2.2b	13.2 ± 0.3c	2 ± 0.6f	24.1 ± 1.0b	nd	6.4 ± 0.2e
2-Phenylethanol	10,000	21,804.5 ± 148.5b	23,910.5 ± 964.6b	19,572.4 ± 313.4c	22,907.4 ± 449.5b	23,215.4 ± 360.6b	18,378.6 ± 893.9de	31,272.2 ± 205.0a	18,924.3 ± 242.7 cd	17,904.7 ± 384.1e
Subtotal		173,390.4b	174,594.6b	153,639.2c	173,545.5b	181,068.6a	167,285.8b	183,803.6a	169,376.8b	172,048.5b
Subtotal (%)		66.81	70.47	67.72	73.82	68.09	76.57	68.63	71.29	70.5
Terpenes and norisoprenoids 8										
Myrcene		12.3 ± 0.3d	25.8 ± 0.5b	65 ± 4.4a	6.6 ± 0.5d	22.8 ± 0.2bc	9.6 ± 0.5d	22.9 ± 1.4bc	13.9 ± 0.2c	2.1 ± 0.1de
α-Terpineol	250	0.4 ± 0.08b	0.4 ± 0.01b	3.7 ± 0.6a	0.5 ± 0.01b	0.3 ± 0.08b	0.3 ± 0.01b	0.3 ± 0.08b	0.3 ± 0.2b	0.3 ± 0.03b
Citronellol	100	5.2 ± 0.2d	14.1 ± 0.7a	6.5 ± 1.2 cd	6.2 ± 0.7c	11.9 ± 0.03b	5.6 ± 0.5 cd	3.1 ± 0.1e	6.8 ± 0.6 cd	4.1 ± 0.2e
Nerol	500	8.9 ± 0.1b	9.5 ± 0.1b	17.2 ± 6.3a	9.4 ± 0.1b	9.8 ± 0.1b	8.1 ± 0.1b	8.9 ± 0.07b	8.8 ± 0.1b	8.7 ± 0.2b
β-Damascenone	0.05	1.1 ± 0.2b	1.6 ± 0.04b	4.4 ± 0.7a	1.6 ± 0.04b	1.6 ± 0.2b	1.3 ± 0.04b	2.3 ± 0.3b	1.6 ± 0.08b	1.6 ± 0.8b
Citral		31.3 ± 0.3c	29.2 ± 0.6e	45.5 ± 0.4a	29.2 ± 0.6e	34.9 ± 0.2b	30.7 ± 0.6c	31.1 ± 0.04 cd	29.2 ± 0.2e	30.1 ± 0.4de
Linalool	25	10.1 ± 0.4bc	7.3 ± 0.2bc	33.7 ± 3.5a	12.8 ± 0.1bc	6 ± 0.2c	6.4 ± 0.2bc	9.1 ± 0.2bc	6.3 ± 0.5bc	10.5 ± 0.8b
Limonene	200	40.2 ± 0.6c	30.7 ± 0.4d	21.5 ± 6.0e	22.5 ± 1.0e	43.4 ± 0.3c	41.7 ± 0.7c	61.1 ± 1.4b	8 ± 1.4f	2.5 ± 0.2 g
Subtotal		109.5d	118.6d	197.5a	88.8b	130.7c	103.7d	138.8c	74.9b	59.9e
Subtotal (%)		0.04	0.05	0.09	0.04	0.05	0.05	0.05	0.03	0.02
Acids 6										
Acetic acid	200,000	2821.3 ± 88.8b	1756 ± 32.8 cd	1791.5 ± 19.5c	1627.8 ± 35.0d	1888.5 ± 67.5c	1402.2 ± 32.8e	1328.1 ± 18.1f	1689.5 ± 5.7 cd	3134.9 ± 24.1a
Isobutyric acid	8100	557.7 ± 26.1c	71.3 ± 1.2e	547.9 ± 16.7c	71.3 ± 1.2e	1259.1 ± 19.0a	478.1 ± 4.7d	1110.5 ± 2.1b	71.3 ± 17.0e	71.3 ± 5.4e
Hexanoic acid	420	249.9 ± 4.9 cd	291.3 ± 4.0b	220.3 ± 47.12e	278.4 ± 3.0bc	354 ± 5.6a	210.3 ± 4.0e	235.5 ± 3.4d	256.5 ± 12.4d	262.1 ± 16.8d
Octanoic acid	500	1977.7 ± 18.0bc	2061.5 ± 18.2b	1398.9 ± 6.0e	2038.6 ± 18.2bc	2855.2 ± 20.1a	1223 ± 52.5f	1941.4 ± 10.9c	1562.1 ± 16.7d	1367.4 ± 54.2e
n-Decanoic acid	1000	446.5 ± 7.0 g	610.4 ± 7.6b	438 ± 6.8 g	570.4 ± 8.3c	747.1 ± 4.8a	461.2 ± 6.9 fg	476.3 ± 5.5de	487.2 ± 47.1d	463.8 ± 6.7ef
Isovaleric acid	33	380.7 ± 7.7f	637.5 ± 5.7a	342.3 ± 0.4 g	493.2 ± 9.2c	575.1 ± 4.2b	406.3 ± 3.6e	505.8 ± 4.8c	481.5 ± 4.6d	586.9 ± 0.2b
Subtotal		6433.8a	5428.0c	4738.9b	5079.7d	7679.0f	4181.1e	5597.6d	4548.1 h	5886.4c
Subtotal (%)		2.48	2.19	2.09	2.16	2.89	1.91	2.09	1.91	2.41
Aldehydes and ketone 4										
Nonanal	15	3.6 ± 0.0c	6.7 ± 0.4a	5.5 ± 0.4b	6.9 ± 0.4a	1.3 ± 0.0e	2.2 ± 0.4d	2.7 ± 0.07d	1.8 ± 0.1e	1.6 ± 0.4e
Benzealdehyde	2000	1414.1 ± 6.9c	2218 ± 90.2a	613.2 ± 0.9f	2315.3 ± 19.5a	2131.7 ± 28.1b	1284.5 ± 83.2c	1187.1 ± 35.2d	2129 ± 41.4b	956.2 ± 0.9e
Acetoin	150,000	199.4 ± 11.5f	378.2 ± 12.2 cd	494 ± 19.6b	375.7 ± 5.2d	486.8 ± 4.4b	488.8 ± 9.4b	804.5 ± 4.4a	406 ± 12.5c	335.5 ± 19.6e
Furfural	14,100	nd	nd	65.5 ± 0.8a	nd	nd	nd	nd	nd	nd
Subtotal		1617.1a	2602.9b	1178.2c	2697.9b	2619.8b	1775.5a	1994.3c	2536.8b	1293.3f
Subtotal(%)		0.62	1.05	0.52	1.15	0.99	0.81	0.74	1.07	0.53
Others 2										
p-Cymene		nd	4.4 ± 0.2b	nd	0.3 ± 0.1d	4.8 ± 0.2a	nd	2.6 ± 0.3c	2.7 ± 0.2c	nd
4-Ethylguaiacol	110	nd	14.8 ± 0.3c	nd	9.4 ± 0.4d	16.8 ± 0.1b	nd	3.9 ± 0.2e	38.5 ± 1.4a	3.7 ± 0.1e

Table 4 continued

Compounds	OTH[A]	Concentration									
		LFE1225	LFP525	LFE1809	F15	LFN524	LFP522	LFE1504	LFP529	LFA711
Subtotal	0		19.2c	0	9.7d	21.6b	0	6.5 ± 0.1e	41.2 ± 1.6a	3.7 ± 0.1f
Subtotal (%)	0		0.01	0	<0.01	0.01	0	<0.01	0.02	<0.01
Total		259,525.1b	247,743.7c	226,870.8ef	235,096.5de	265,907.3ab	218,466.6f	267,822a	237,575d	244,054 cd

Different letters in the same line indicate significant differences ($P < 0.05$)

[A] OTHs were not detected in this study, and were referred from the reports by Gil et al. (2006), Jiang and Zhang (2010), Tao and Li (2009), Peinado et al. (2004), Zea et al. (2001) and Guth (1997)

Discussion

The diversity of indigenous *S. cerevisiae* in Shanshan region

In view of the important role of indigenous *S. cerevisiae* strains to introduce local features into winemaking practice (Fleet 2008), genetic diversity and selection of autochthonous *S. cerevisiae* in spontaneous fermentation of different grape varieties have been studied in various regions (Chovanová et al. 2011; Orlić et al. 2010; Ortiz et al. 2013). Recently, Schuller et al. (2012) investigated the biodiversity of local *S. cerevisiae* isolated from 16 vineyards, nine grape varieties in Vinho Verde and Bairrada region (Portugal) by using ten microsatellite markers. They found that the populations of *S. cerevisiae* in vineyards may occur locally due to influences of multi-factors, one of them being the grape variety. In Shanshan County, the indigenous *S. cerevisiae* strains of six grape varieties revealed a low diversity with 21 genotypes by interdelta sequence fingerprinting, and the differences of diversity among varieties were significant (1–9 genotypes per fermentation). The low diversity was attributed to the origin and environment there (Ayoub et al. 2006; Schuller et al. 2012; Settanni et al. 2012).

In this study, representative isolates of the identified genotypes were assessed for their physiological properties. In order to study the relation between phenotypes and genotypes, hierarchical cluster analysis was performed based on the physiological properties of the isolates with the same genotypes. Just as Fig. 1 shown, the relationship between the phenotype and the genotype of *S. cerevisiae* strains was not clear which was in accordance with the previous studies (Agnolucci et al. 2007; Fernández-González and Briones 2013; Ortiz et al. 2013). For example, three isolates of XJ1 did not cluster into the same group; however the isolates from different genotypes such as isolates of XJ11 and XJ19 clustered into the same groups. However, Franco-Duarte et al. (2009) found strains with similar microsatellite allelic pattern were clustered in subgroups by computational techniques with low ethanol resistance, growing at 30 °C and in media containing galactose, raffinose or urea. Moreover, they considered that it still had to be evaluated whether the phenotypic tests with biotechnological relevance can reflect the strains' behavior in larger scales.

Differences of volatile compounds and sensory analysis among different yeast strains

The differences observed in the volatile compositions of Merlot wines obtained from the different yeast strains in

Table 5 Results of the sensory analysis of the wines made by different yeast strains

Attributes		LFE1225	LFP525	LFE1809	F15	LFN524	LFP522	LFE1504	LFP529	LFA711
Visual analysis	Clarify (5)	4.0	4.2	4.0	4.2	4.0	4.3	4.4	4.2	4.2
	Color (10)	8.0	8.3	8.0	8.3	8.0	8.6	8.9	8.3	8.3
Aroma analysis	Aroma purity (6)	4.7	4.5	4.3	4.5	4.4	4.4	4.3	4.3	4.0
	Aroma intensity (8)	6.7	6.5	6.3	6.3	6.4	6.4	6.3	6.5	5.8
	Aroma quality (16)	13.3	12.7	12.0	13.3	12.9	12.3	12.3	12.0	11.7
Taste analysis	Taste purity (6)	4.8	4.0	4.2	4.8	4.9	4.1	4.4	4.3	4.0
	Taste intensity (8)	6.8	5.8	6.3	6.7	6.9	6.1	6.4	6.2	6.0
	Taste Lasting (8)	6.7	6.0	6.0	6.7	6.9	6.1	6.4	6.3	6.2
	Taste quality (22)	17.5	16.0	15.0	16.9	17.3	16.0	16.0	17.0	15.5
Global evaluation	Balance (11)	9.0	8.7	9.0	9.2	9.1	9.0	8.9	8.8	9.0
Total	100	81.5	76.7	75.2	80.8	80.7	77.4	78.3	78.0	74.7

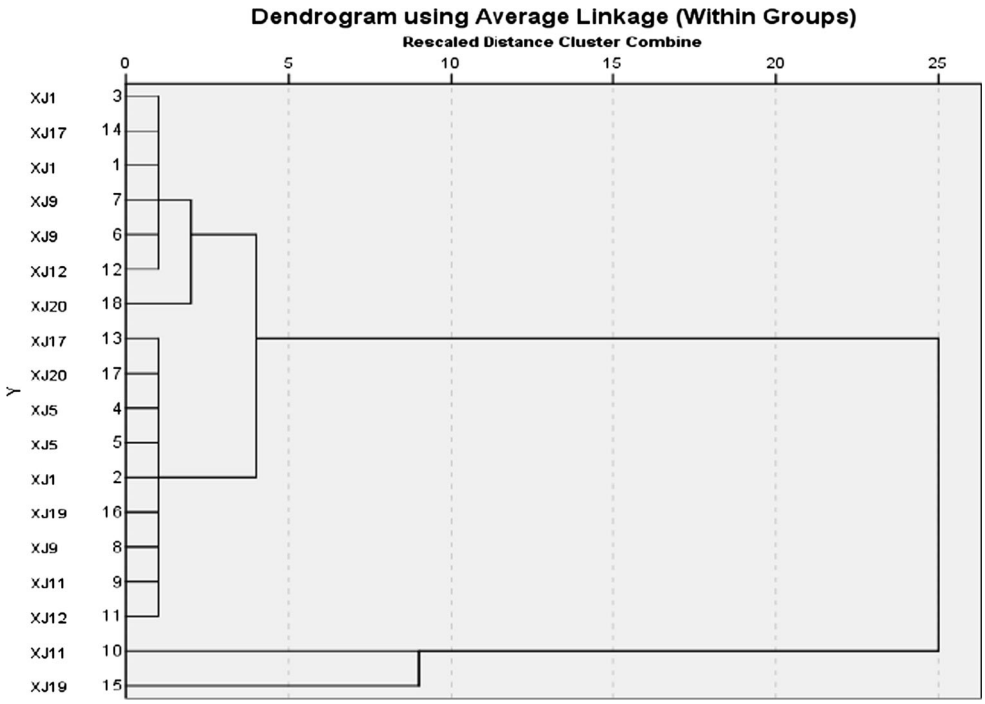

Fig. 1 Hierarchical cluster analysis of representatives of same genotypes

this study appeared to be quantitative rather than qualitative, which were in agreement with the previous studies (Mateo et al. 2001; Romano 2003). In order to assess the influence of the aroma volatiles studied on overall wine aroma, OAV was calculated and the values of 15 compounds were above 1 at least in one wine (shown in Supplement Table S2), contributing individually to the aroma characteristics of wine (Vilanova et al. 2010). However, compounds with OAV lower than 1 could also contribute to the aroma character of wine because of the additive effect of similar compounds with similar structure or odor (Francis and Newton 2005), and compounds with similar OAVs can improve some existing contribution already present through synergy with other compounds (López et al. 2003). In this study aroma compounds with OAVs more than 0.5 at least in one wine (Supplement Table S2) were chosen in the following Principal component analysis (PCA).

PCA was performed to obtain the characters of wines and elucidate differences in aroma profiles and organoleptic properties of nine strains (Fig. 2). After deletion of some volatiles with little importance in loading, 14 volatile components with OAVs above 0.5 were used as variables, as well as sensory parameters in Table 5. PCA explained the 61.86 % of the variability in the first two dimensions. PC1 accounted for 44.03 %, was correlated to the appearance of sensory evaluation (color and clarify) and varietal volatiles (linalool and β-damascenone) on the negative part. The sensory parameters about aroma and

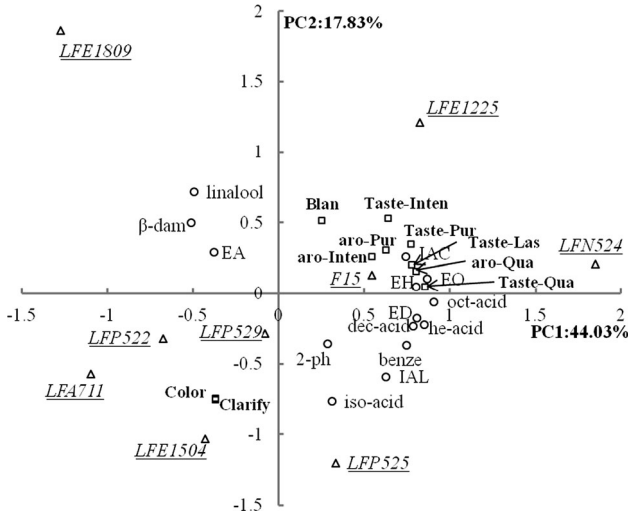

Fig. 2 PCA scores and loadings biplots for the wines fermented with selected strains. *EA* ethyl acetate, *IAC* isoamyl acetate; *EH* ethyl hexanoate, *EO* ethyl octanoate, *ED* ethyl decanoate, *IAL* isoamyl alcohol, *2-ph* 2-phenylethanol, *β-dam* β-damascenone; *he-acid* hexanoic acid, *oct-acid* octanoic acid, *dec-acid n*-decanoic acid, *iso-acid* isovaleric acid, *benze* benzealdehyde

mouth feel, and the most abundant aroma compounds, such as hexanoic acid, isoamyl acetate, were with high loadings on the positive part of PC1. These compounds mentioned above were derived from fermentation and gave wines more pleasant flavor, such as green pepper (hexanoic acid) and banana, pineapple, pear and floral flavor from esters (Guth 1997; Tao et al. 2009). The second principal component (PC2) explained an additional 17.83 % of total variance, closely related to varietal volatiles and the balance of sensory evaluation with higher positive loading, the appearance of sensory evaluation (color and clarify) and isovaleric acid with negative loading.

Figure 2 showed the wines of nine yeast strains were clearly distincted from each other by PCA and yeast strain "signature" was evident. Wine yeast showed a critical role in the volatiles producing during fermentation (Antonelli et al. 1999; Regodón Mateos et al. 2006); and levels of terpene compounds were also highly dependent on yeast strains (Álvarez-Pérez et al. 2012; Loscos et al. 2007). This observation was confirmed by our data, as the wine fermented by LFE1809 presented the maximum of linalool, α-terpineol, β-damascenone and nerol and significantly differed from other strains. The Merlot musts fermented by different yeasts were homogeneous in the study, so the different levels of terpenes and norisoprenoids were the most likely the product of β-glucosidase secreted by yeasts which consequently released the monoterpene alcohol from the bound terpenoid precursor (Hernandez 2003; Pérez et al. 2011; Tosi et al. 2009; Vernocchi et al. 2011). Otherwise, it was reported some terpenoids could also be produced by *S. cerevisiae* via the de novo pathway, including linalool, α-terpineol and β-citronellol (Wu et al. 2015).

Among these wines, LFN524 wine was characterized with higher fermentation aroma (mainly esters) but lower varietal volatiles; others by LFP522, LFP529, LFE1504 and LFA711 laid in the left-down side of the matrix, with lower contents of volatiles and lower sensory scores of aroma and mouth feel; LFP525 wine had higher levels of isovaleric acid and isoamyl alcohol. Compared with strains LFN524, LFP522, LFP525, LFP529 and LFA711, indigenous stains LFE1225 and LFE1809, scored highest in sensory evaluation and characterised with higher content of varietal volatiles separately, were both isolated from Merlot fermentation. According to this result, yeast strains seemed to be "variety-specific". Recently, a hypothesis has also been proposed, the potential of "area-specific" yeast starter cultures could enhance the peculiarity of distinguishing marks of regional productions, which has been confirmed by Tufariello et al. (2014). They studied the dissimilarity of chemical composition of Negroamaro wines produced with two yeast population isolates from two different micro districts in Salento and a natural separation of the wines was achieved based on strains origin area.

In conclusion, indigenous *S. cerevisiae* strains in Shanshan County (Xinjiang, China) showed less genetic diversity, and the potential of "area-specific" yeast starter cultures could enhance the peculiarity of distinguishing marks of regional productions. Because LFE1225 and LFE1809 were isolated from Merlot fermentation, the results of sensory and variety aroma revealed that these yeast strains seemed to be "variety-specific". To confirm this hypothesis, further experiments are now under way by separately using the LFE1225, LFE1809 and LFN524 strains as starter culture for Merlot, Cabernet Sauvignon and Chardonnay fermentations.

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