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ORIGINAL PAPER

Patagonian wines: the selection of an indigenous yeast starter

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Abstract The use of selected yeasts for winemaking has clear advantages over the traditional spontaneous fermentation. The aim of this study was to select an indigenous *Saccharomyces cerevisiae* yeast isolate in order to develop a regional North Patagonian red wine starter culture. A two-step selection protocol developed according to physiological, technological and ecological criteria based on killer interactions was used. Following this methodology, *S. cerevisiae* isolate MMf9 was selected among 32 indigenous yeasts previously characterized as belonging to different strains according to molecular patterns and killer biotype. This isolate showed interesting technological and qualitative features including high fermentative power and low volatile acidity production, low foam and low sulphide production, as well as relevant ecological characteristics such as resistance to all indigenous and commercial *S. cerevisiae* killer strains assayed. Red wines with differential volatile profiles and interesting enological features were obtained at laboratory scale by using this selected indigenous strain.

Keywords Wine flavour · Killer interactions · Microvinifications · Yeast selection · *Saccharomyces cerevisiae*

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Introduction

One of the most significant technological advances in winemaking has been the control of the microbiological process by grape must inoculation using selected yeasts [21]. Today, the use of indigenous wine yeasts selected from each winegrowing area is widespread [20]. These local yeasts are presumed to be more competitive than commercial yeasts because they are better adapted to the ecological and technological features of their own winegrowing area [1, 19]. Therefore, they would be more capable of dominating the fermentation and would become the most important biological agent responsible for the winemaking. Additionally, selection of the appropriate local yeasts would assure the production of quality premium wines maintaining the differential properties of their own area and preserving its natural biodiversity.

The selection of wine yeasts is usually carried out within the species *Saccharomyces cerevisiae*—the most relevant one in winemaking—according to a set of physiological features (criteria) indicative of their potential usefulness for industrial wine production [21]. These criteria include the evaluation of yeast features related to the achievement of an efficient transformation of grape sugars into alcohol and

carbon dioxide at a controlled rate and without off-flavor development. They are evaluated for all isolates by carrying out small-scale fermentations in synthetic or semi-synthetic media [17, 31]. Afterwards, another set of enological properties, generally more related to the kind of wine to be produced, is evaluated for a lower number of preselected isolates usually using sterile grape must [4, 10, 14, 15, 27].

Located at 37°5′ and 40°5′ southern latitude, Argentinean North Patagonia is the southernmost winegrowing region of the world. This region has optimal agro-ecological conditions for high quality viticulture and a long winemaking tradition [6]. Both spontaneous and conducted grape juice fermentations are carried out and young dry red (85%) and white wines (12%) from neutral *Vitis vinifera* varieties are mostly produced. However, indigenous yeast strains for enological use had not been selected until this study. In this work, 32 indigenous North Patagonian *S. cerevisiae* isolates of enological origin, previously characterized as belonging to different strains by means of molecular patterns and killer biotype [7, 8], were extensively evaluated for their technological and qualitative traits using a two-step selection protocol. Because killer character is widely distributed among native wine yeasts in North Patagonia [24, 26], additional ecological criteria based in killer interactions were included in the protocol and an indigenous yeast isolate with potential application in the elaboration of more aromatic young red wines was selected.

Materials and methods

Yeasts and growth conditions

Thirty-two indigenous *S. cerevisiae* isolates obtained from North Patagonian natural Malbec wine fermentations were used in this work. These isolates have been previously characterized by mtDNA-RFLP, PCR amplified inter δ sequence DNA polymorphism analysis and killer biotype as belonging to 32 different molecular patterns [7, 8] (Table 1). Two commercial *S. cerevisiae* starter strains commonly used in North Patagonian red winemaking were used as reference standards.

Killer and sensitive strains

Forty-seven killer yeasts and the sensitive *S. cerevisiae* P351 strain (PROIMI yeast collection) were used in killer assays. Killer yeasts were the K2-type killer reference *S. cerevisiae* NCYC 738 strain, 10 *S. cerevisiae* killer commercial starters widely used in North Patagonian winemaking and 35 indigenous killer yeasts including 10 *S. cerevisiae* and 25 non-*Saccharomyces* isolates (*Debaryomyces hansenii*, 1 isolate; *Hanseniaspora uvarum*, 4 isolates; *Metschnikowia pulcherrima*, 7 isolates; *Pichia anomala*, 4 isolates; *Pichia kluyveri*, 4 isolates; *Torulaspora delbrueckii*, 2 isolates; *Rhodotorula glutinis*, 2 isolates; and *Zygosaccharomyces rouxii*, 1 isolate). All indigenous killer isolates were obtained from Malbec and Merlot grapes and musts as well as cellar surfaces in North Patagonia.

For all assays, the yeast cells were previously growth overnight in GPY Agar medium (0.5% w/v yeast extract, 0.5% w/v peptone, 4% w/v glucose, and 2% w/v agar, pH 4.5).

Killer assays

Killer activity and sensitivity were determined using the seeded-agar-plate technique on YEPD-MB medium (1% w/v yeast extract, 2% w/v glucose, 2% w/v peptone, 2% w/v agar, 0.0003% w/v methylene blue) buffered at pH 4.6 with 0.5 M phosphate–citrate as described by Sangorrín et al. [25].

Fermentations

Small-scale fermentations were carried out in 250 ml Erlenmeyer flasks containing 200 ml of semi-synthetic medium (200 g l^{-1} of sucrose, 10 g l^{-1} of yeast extract, 1 g l^{-1} of ammonium sulphate, 1 g l^{-1} of potassium phosphate, 1 g l^{-1} of magnesium sulphate). After sterilization, citric acid (10 g l^{-1}) was added to the fermentation flask in order to decrease the medium pH to 3.5, a pH similar to the wine [13]. After this, they were inoculated with 17 ml of each indigenous *S. cerevisiae* young cultures containing 10^7 cells ml^{-1}. Flasks were plugged with glass fermentation traps containing sulphuric acid to allow only CO_2 to evolve from the system and they were kept at 27 °C without shaking [31]. The fermentation evolution was daily followed by weight loss until constant weight during two consecutive measures. At this time, the fermented media were racked and centrifuged at 4,000 g for 5 min and the clear fermented media were assayed for ethanol production and total and volatile acidity as described below. The fermentations were carried out in triplicate in separate trials.

Microvinifications

Microvinifications were carried out in 5 l flasks containing 3.5 l of pasteurized (90 °C, 3 min) Malbec must (240 g l^{-1} of sugars, pH 3.4) supplemented with sodium metabisulphite at a final concentration of 150 mg l^{-1}. *S. cerevisiae* indigenous isolates were inoculated at a cellular density of 10^7 cells ml^{-1}. The winemaking was conducted at 25 °C and its kinetics was followed by measuring the Baumé degrees (1°Baumé = 17 g l^{-1} of fermentable sugars). When alcoholic fermentations were completed (<1°Baumé)

Table 1 Origin, identity and some technological and qualitative properties of North Patagonian indigenous *S. cerevisiae* isolates

No.	Strain[a]	N[b]	Isolate[c]	Origin	KP[d]	SH_2[e]	Foam	Flo[f]
1	I	3	MIf10	Final stage	K−R−	++	lower	(−)
2	II	5	MMf17	Final stage	K−R−	++	lower	(+)
3	III_b	2	MIf4	Final stage	K−R−	++	middle	(−)
4	III_a	11	MMf8	Final stage	K−R−	+	lower	(−)
5	IV	2	MMf1	Final stage	K−R−	++	higher	(−)
6	V	4	MIf8	Final stage	K−R−	++	lower	(−)
7	VI_a	2	MIf9	Final stage	K−R−	++	lower	(−)
8	VI_b	1	MIi20	Initial stage	K−R−	+	lower	(−)
9	VII	3	MIi8	Initial stage	K−R−	+++	lower	(+)
10	VIII	3	MIm19	Middle stage	K−R−	++	lower	(+)
11	IX_a	1	MMf4	Final stage	K−R−	++	higher	(+)
12	IX_b	1	MIm17	Middle stage	K−R−	+	lower	(−)
13	X	1	MIf6	Final stage	K−R−	++	lower	(+)
14	XI	1	MIf7	Final stage	K−R−	++	lower	(−)
15	XII	1	MIm16	Middle stage	K−R−	+++	lower	(−)
16	XIII	1	MIm4	Middle stage	K−R−	++	lower	(−)
17	XIV	1	MIm6	Middle stage	K−R−	+++	lower	(+)
18	XV	1	MIm8	Middle stage	K−R−	+	lower	(−)
19	XVI	1	MIi3	Initial stage	K−R−	++	lower	(−)
20	XVII	1	MIi4	Initial stage	K−R−	+	lower	(+)
21	XVIII	1	MIi10	Initial stage	K−R−	++	lower	(−)
22	XIX	1	MIi13	Initial stage	K−R+	+	lower	(+)
23	XX	1	MMf3	Final stage	K−R−	+	middle	(+)
24	XXI	1	MMf7	Final stage	K−R−	+	higher	(−)
25	XXII	1	MMf9	Final stage	K+R+	+	lower	(−)
26	XXIII	1	MMf11	Final stage	K−R−	+	lower	(+)
27	XXIV	1	MMf12	Final stage	K−R−	+	higher	(−)
28	XXV	1	MMf16	Final stage	K−R−	++	middle	(−)
29	XXVI	1	MIi9	Initial stage	K−R−	++	lower	(+)
30	XXVII	1	MIf16	Final stage	K−R−	+	lower	(−)
31	XXVIII	1	MIi6	Initial stage	K−R−	++	middle	(−)
32	XXIX	1	MIf12	Final stage	K−R−	++	middle	(−)
33	BC	1	BC starter	Commercial	K−R−	+	lower	(−)
34	F10	1	F10 starter	Commercial	K−R−	+++	middle	(−)

[a] Defined as a particular combined molecular (mtDNA-RFLP and PCR amplified inter δ sequence DNA polymorphism analysis) and killer sensitivity pattern [7, 8]

[b] Number of isolates exhibiting the same molecular and killer pattern

[c] Isolate selected among those belonging to the same strain

[d] Killer sensitivity against K2-type killer producer strain *S. cerevisiae* NCYC 738 and killer activity against *S. cerevisiae* P351 sensitive strain

[e] High (+++), medium (++) and low (+) sulphide producers

[f] Flocculation

young wines were racked, centrifuged (4,000 g, 5 min) and their enological parameters determined.

Enological parameters

All enological parameters were determined according to the methods proposed by Ribereau-Gayon et al. [22]. Therefore, ethanol concentration was determined by steam distillation and expressed as fermentative power or Gay Lussac degrees (FP or °GL, % v/v) and specific productivity [SP, ethanol (g) biomass (dry weight g^{-1}) hour (h^{-1})]. The biomass values were obtained by subtracting the initial yeast culture dry weight from the final one. For dry weight determination 5 ml culture samples taken at initial and end of each fermentation were centrifuged at 10,000 g 5 min, washed twice and resuspended in cold sterile distilled water; these cell suspensions were placed on preweighed dishes and dried at 105 °C to constant weight. Volatile acidity (VA) was determined by steam distillation followed by titration with NaOH 0.1 N and was expressed as acetic acid ($g\ l^{-1}$). Total acidity was determined at 20 °C by direct titration using NaOH 0.1 N and expressed as tartaric acid ($g\ l^{-1}$). Free sulphur dioxide was determined by direct titration with iodine and total sulphur dioxide was determined by sample treatment with KOH (1 N) followed by titration with iodine. Malic acid and glycerol were enzymatically determined using Boehringer Mannheim commercial kits.

Higher alcohols and esters determinations

Aliquots of the cell-free wine samples were analysed by headspace solid-phase-microextraction sampling (SPME) using poly (dimethylsiloxane) (PDMS) fibres (Supelco, Sigma-Aldrich, Barcelona, Spain) and GC according to Rojas et al. [23]. Aliquots of 1.5 ml of the samples were placed in 15 ml vials and 0.3 g of NaCl and 15 ul of 0.1% (v/v) 2-octanol in ethanol (Fluka) were added as internal standard. The vials were closed with screwed caps and 3-mm thick teflon septa. Solutions were stirred for 2 h at 25 °C to get the required headspace–liquid equilibrium. PDMS fibers were injected through the vial septum and exposed to the headspace for 7 min and then desorbed during 4 min in an HP 5890 series II gas chromatograph equipped with an HP Innowax column (Hewlett-Packard) (length, 15 m; inside diameter, 0.25 mm; film thickness, 0.25 um). The injection block and detector (FID) temperatures were kept constant at 220 and 300 °C, respectively. The oven temperature was programmed as follows: 40 (10 min) to 200 °C at 4 °C/min, and 200 to 260 °C at 20 °C/min and kept 2 min at 260 °C.

The following standards were purchased from Fluka: isobutilic alcohol, isoamylic alcohol, 1-hexanol, bencylic alcohol, 2-phenyl ethanol, ethyl acetate, isobutyl acetate, ethyl lactate, isoamyl acetate, hexyl acetate, diethyl succinate, bencyl acetate, ethyl caprylate, ethyl 3-hydroxibutanoate, 2-penylethyl acetate. All standards were of greater than 99% purity.

Ester and higher alcohols concentrations calculated using standard solutions were the average of three independent assays.

Hydrogen sulfide production

Test tubes (16 mm × 16 mm) containing 15 ml of sterile red grape juice, supplemented with sodium metabisulphite at a final concentration of 150 mg l^{-1}, were inoculated with young yeast cultures. At this time, paper saturated with Lead acetate (PbAcO) was suspended from the tube top using its screwed cap. Assays were carried out at 26 °C during 15 days. The qualitative hydrogen sulfide production was detected by the blackening of the PbAcO paper [10] and the isolates were classified as high (+++), medium (++) and low (+) sulphide producers.

Foam production

Foam height was measured every day throughout the fermentation assays described above. Yeasts were classified into three categories based on the maximum foam height reached: lower (foaming lower than 2 mm), middle (foaming between 2 and 4 mm) and higher (foaming greater than 4 mm) according to Martínez-Rodríguez et al. [10].

Ethanol tolerance

Ethanol tolerance was determined using fermentation assays proposed by Kurtzman and Fell [5]. Five, 10 and 15% v/v ethanol were added into the fermentation media before the yeast inocula. Fermentation tubes without ethanol were used as controls.

Statistical analysis

ANOVA and Tukey honest significant difference tests (HSD) with $\alpha = 0.05$ were performed by mean comparison. The data normality and variance homogeneity in the residuals were verified by Lilliefors and Bartlet tests respectively.

Results and Discussion

Yeast preselection

Technological and qualitative properties

In this first step, local yeasts were evaluated in their key technological and qualitative properties using both small-scale fermentations carried out on semi-synthetic media (fermentative power, FP; volatile acidity, VA; and foam production) and specific assays (SH_2 production, ethanol tolerance, killer behaviour). For comparative purposes, two commercial *S. cerevisiae* strains, (BC and F10), were also evaluated.

The results obtained from small-scale fermentations showed little foam production as well as similar FP and VA values in most of the isolates (Table 1, Fig. 1). Statistical analysis of FP data evidenced that: (a) there was not significant difference between commercial starters, (b) the isolates MIi4, MIf4 and MMf11 showed significantly lower values than BC (black symbols in Fig. 1) and (c) the isolates MIi8, MIi20, MIm17, MMf1, MMf8, MMf9, MIf16 and MMf17 showed values significantly higher than F10 ($p < 0.01$) as well as than other indigenous isolates (grey symbols in Fig. 1). When ethanol production was standardized by biomass and time (specific productivity, SP) significant differences were only detected among some indigenous isolates. Isolates MMf9 and MIm16 showed the highest SP values (data not shown). With regards to VA production, the commercial F10 strain as well as 16 indigenous isolates—indicated as triangles in Fig. 1—showed significantly lower VA values than BC starter ($p < 0.01$).

On the other hand, the results obtained from the specific assays evidenced that all the yeasts including commercial starters have capability for sulphide production, however, most of them (51.7%) showed an intermediate production level (Table 1). On the other hand, all the isolates were

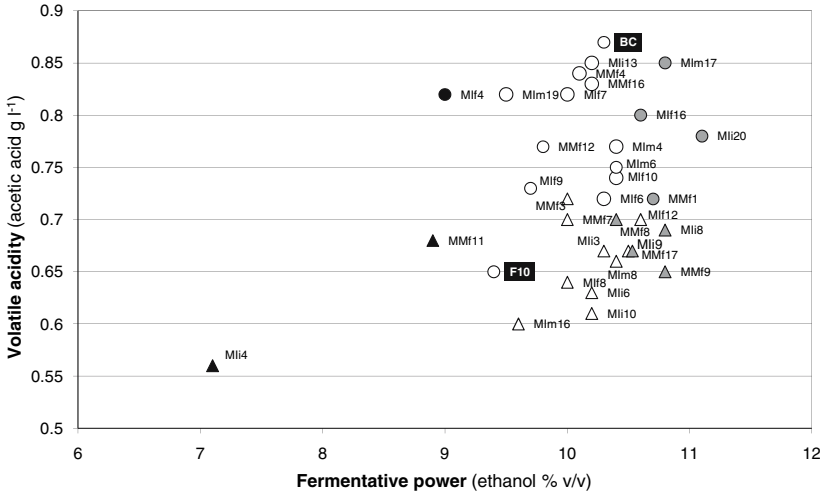

Fig. 1 Correlation between fermentative power (*FP*) and volatile acidity (*VA*) values obtained with all studied indigenous isolates and both BC and F10 commercial starters. *Black shadowed symbols* FP values significantly lower than BC ones. *Grey shadowed symbols* FP significantly higher than F10 ones. *Triangles* VA values significantly lower than BC ones ($p < 0.01$, $n = 3$)

capable to ferment sugars in the presence of 5 and 10% of ethanol in the media. However, only MIf8, MMf3 and MMf9 isolates were capable to ferment with 15% of ethanol.

With regards to the local yeasts killer properties, the technological aspects of this phenomenon were firstly evaluated. Several killer toxin types has been described in *S. cerevisiae*, however, K2-type toxin has proved to be the most important one in winemaking because it is active under the wine pH conditions [30]. Evaluation of the killer phenotype using sensitive and K2-type killer reference *S. cerevisiae* strains showed that all indigenous isolates but two, MMf9 (killer isolate, K^+R^+) and MIi13 (neutral isolate, K^-R^+), exhibited the sensitive (K^-R^-) phenotype (Table 1).

Additional ecological criteria

The evaluation of killer phenotype using K2-type killer and sensitive reference strains is a frequently used technological criterion in yeast selection programmes [16, 29]. However, ecological aspects of this phenomenon, such as killer interactions of the studied isolates against other indigenous non-*Saccharomyces* and *Saccharomyces* yeasts from the same winemaking area, become relevant in areas where killer yeasts are widespread such as the North Patagonian region [24, 26]. There is evidence that in guided fermentations the dominance of the starter can be subordinated to the specific winemaking conditions settle down during the initial stages [18]. Indigenous and/or commercial killer yeasts present at the beginning of fermentation, originated from grapes or winery surfaces, could hinder the implantation of *S. cerevisiae* starters inducing stacking fermentations [16, 30]. In this context, killer sensitivity of the studied isolates was tested against 10 *S. cerevisiae* killer commercial starters, and 10 *S. cerevisiae* and 25 non-*Saccharomyces* killer indigenous isolates.

The isolates response against indigenous *S. cerevisiae* killer yeasts was the same than that observed against the K2-type killer reference strain showed in Table 1. However, the 50% of the indigenous isolates was resistant to, at least, one commercial killer starter. Under assays conditions, MMf9 and MIi13 isolates showed resistance phenotype against all commercial killer strains whereas the isolates MMf17 and MIf6 as well as MIi8, MIi20, MMf4, MIm17, MMf7 and MIi6 showed resistance to 50 and 20% the commercial strains, respectively. These results evidence the relevance of studying additional regionally significant yeast killer interactions, different from the classical technological evaluation using K2-type reference strains.

On the other hand, all analyzed isolates including those showing resistant phenotype (MMf9 and MIi13 *S. cerevisiae* isolates) were sensitive to four *P. anomala* and two *P. kluyveri* indigenous killer isolates (data not shown) evidencing a different target molecule in *S. cerevisiae* cell wall. The relevance of these killer interactions in winemaking is currently been evaluated in our laboratory.

Among all evaluated properties, FP values and VA production as well as ecological criteria were prioritized in the preselection stage. The indigenous isolates MIi20 and MMf9 showing appropriate combinations of FP and VA features were selected to continue the study. Moreover, MMf9 isolate showed killer character against sensitive collection strains and resistant phenotype against K2 killer collection strain, all the indigenous and commercial *S. cerevisiae* killer yeasts (Table 1) whereas MIi20 showed resistant phenotype against some of the later. Both isolates additionally showed other remarkable qualitative traits such as low SH_2 production—malodorous compound in wines [12], little foam production (Table 1) and capability to ferment sugars in medium containing 10–15% of ethanol, an important feature for ensure the dryness of must.

Finally, it is interesting to note that the results obtained from semi-synthetic medium assays shows a good correlation

Table 2 Comparison among some strain properties evaluated in semisynthetic medium and grape must

Isolate	Properties and medium					
	Semi-synthetic			Grape must		
	FP[a]	SP[b]	VA[c]	FP[a]	SP[b]	VA[c]
BC	10.3 ± 0.1	0.29 ± 0.06	0.87 ± 0.06	14.8 ± 0.1	0.14 ± 0.02	0.78 ± 0.06
F10	9.4 ± 0.2	0.24 ± 0.05	0.65 ± 0.04	12.8 ± 0.1	0.11 ± 0.03	0.59 ± 0.06
MMf9	10.8 ± 0.2	0.36 ± 0.02	0.65 ± 0.03	14.8 ± 0.1	0.18 ± 0.02	0.55 ± 0.06
MIi20	11.1 ± 0.3	0.31 ± 0.04	0.78 ± 0.02	14.8 ± 0.2	0.16 ± 0.02	0.66 ± 0.02

[a] Fermentative power: ml of ethanol 100 ml^{-1} of wine

[b] Specific productivity: ethanol (g) biomass (g^{-1}) hour (h^{-1})

[c] Volatile acidity: expressed as acetic acid (g l^{-1}) ($n = 3$)

with those obtained from sterile must (Table 2). Comparison between PF and VA values obtained from both media (Table 2) shows that even when absolute values were significantly modified, differences among isolates were kept (ANOVA with no significant interaction, p: 0.5321).

Selection

In young wines obtained from neutral *V. vinifera* varieties the final aromatic quality is strongly influenced by the fermentative flavour originated from yeast metabolism. In the selection stage, small-scale red grape musts fermentations using the preselected indigenous isolates (MIi20 and MMf9) were carried out in order to evaluate both the aromatic profiles (Higher alcohols, HA; and Esters) and the physicochemical characteristics of the obtained wines. Additionally, an isolate (MMf8) representing the most frequent molecular pattern (pattern III_a in Lopes et al. [7]) was also included in this selection stage. Fermentations carried out with both BC and F10 commercial strains were used as controls.

As a whole, the results indicate that all Malbec grape must fermentations took place properly and that the all wines had a composition considered normal for this wine-making scale. However, significant differences were observed among some physicochemical characteristics as well as the volatile compound concentrations of the wines produced by different yeast strains (Tables 3, 4).

It is interesting to note that MMf8 and MMf9 indigenous isolates were the lowest acetic acid and total higher alcohol producers. Elevated concentrations of both acetic acid (more than 0.8 g l^{-1}) and higher alcohols (more than 300 mg l^{-1}) are related to defective wines [28]. Additionally, isoamyl alcohol and 2-phenyl ethanol, the major constituents

Table 3 Enological features of Malbec wine from 5 l fermentations with two commercial strains and three selected indigenous isolates

Enological features	Yeasts used in microvinifications					p value
	Commercial starters		Indigenous preselected			
	BC	F10	MIi20	MMf8	MMf9	
Ethanol (°GL)[a]	14.4 ± 0.3[b]	13.4 ± 0.6[a]	14.6 ± 0.1[b]	13.2 ± 0.4[a]	14.4 ± 0.1[b]	0.0298
Volatile acidity (g l^{-1})[b]	0.50 ± 0.03[b,c]	0.56 ± 0.03[c]	0.51 ± 0.04[b,c]	0.44 ± 0.02[a,b]	0.39 ± 0.03[a]	0.0115
Total acidity (g l^{-1})[c]	5.98 ± 0.35	6.06 ± 0.34	6.29 ± 0.50	5.9 ± 0.30	5.75 ± 0.42	0.9459
pH	2.99 ± 0.03	3.00 ± 0.21	2.96 ± 0.06	2.93 ± 0.03	2.97 ± 0.04	0.9574
Free SO_2 (mg l^{-1})	11 ± 2	12 ± 1	12 ± 1	13 ± 2	12 ± 2	0.9333
Total SO_2 (mg l^{-1})	30 ± 4	39 ± 6	30 ± 3	38 ± 6	30 ± 3	0.2094
L-Malic acid (g l^{-1})	1.74 ± 0.31	1.35 ± 0.07	1.35 ± 0.14	1.41 ± 0.15	1.80 ± 0.14	0.1322
Glycerol (g l^{-1})	4.47 ± 0.04	4.57 ± 0.01	5.02 ± 0.05	5.02 ± 0.03	4.83 ± 0.02	0.2183
TRS (g l^{-1})[d]	5.00 ± 0.71[a]	10.80 ± 0.56[b]	6.60 ± 0.71[a]	11.00 ± 1.41[b]	5.20 ± 1.13[a]	0.0001

Values not shearing the same superscript letter within the horizontal line are significantly different (ANOVA and Tukey HSD test, $n = 2$)

[a] GL: Gay Lussac degrees (ml of ethanol 100 ml^{-1} of wine)

[b] Expressed as acetic acid

[c] Expressed as tartaric acid

[d] Total reducing sugars ($n = 2$)

Table 4 Volatile compounds (mg l^{-1}) produced in Malbec wine fermentations (5 l) using two commercial strains and three selected indigenous isolates

Volatile compounds	Yeasts used in microvinifications					*p* value
	Commercial starters		Indigenous preselected			
	BC	F10	MIi20	MMf8	MMf9	
Higher alcohols						
Isobutyl alcohol	29.80 ± 1.13^{d}	35.72 ± 1.27^{c}	26.28 ± 1.10^{b}	24.77 ± 0.32^{a}	21.64 ± 0.81^{a}	0.00021
Isoamyl alcohol	$130.08 \pm 1.68^{b,c}$	$102.54 \pm 4.63^{a,b}$	138.36 ± 11.94^{c}	96.21 ± 4.86^{a}	87.33 ± 0.73^{a}	0.0016
1-Hexanol	0.60 ± 0.01^{b}	0.53 ± 0.05^{b}	0.84 ± 0.03^{c}	0.40 ± 0.01^{a}	0.39 ± 0.01^{a}	0.0001
Benzyl alcohol	0.52 ± 0.04^{b}	0.51 ± 0.02^{b}	0.55 ± 0.01^{b}	0.13 ± 0.01^{a}	0.15 ± 0.01^{a}	0.0000
2-Phenyl ethanol	58.83 ± 2.07^{b}	55.94 ± 4.22^{b}	53.76 ± 5.13^{b}	35.17 ± 2.47^{a}	48.54 ± 0.23^{b}	0.0047
Total higher alcohols (A)	219.83 ± 0.71^{c}	195.24 ± 10.16^{b}	219.79 ± 5.74^{c}	156.66 ± 2.74^{a}	158.04 ± 0.17^{a}	0.0001
Esters						
Ethyl acetate (B)	29.11 ± 1.48^{a}	30.08 ± 0.61^{a}	28.60 ± 2.05^{a}	26.99 ± 2.77^{a}	16.42 ± 0.86^{b}	0.0026
Isobutyl acetate (μg l^{-1})	$19.25 \pm 0.21^{a,b}$	27.15 ± 4.03^{b}	16.30 ± 1.56^{a}	18.45 ± 1.77^{a}	16.35 ± 0.50^{a}	0.0164
Ethyl lactate	2.64 ± 0.08^{c}	1.82 ± 0.03^{b}	1.15 ± 0.06^{a}	$1.40 \pm 0.12^{a,b}$	3.79 ± 0.15^{d}	0.0000
Isoamyl acetate	0.53 ± 0.02^{b}	$0.45 \pm 0.05^{a,b}$	0.55 ± 0.01^{b}	0.42 ± 0.02^{a}	0.38 ± 0.02^{a}	0.0059
Hexyl acetate (μg l^{-1})	0.55 ± 0.07^{c}	0.15 ± 0.03^{a}	0.11 ± 0.00^{a}	0.41 ± 0.00^{b}	$0.35 \pm 0.01^{a,b}$	0.0002
di-Ethyl succinate (μg l^{-1})	44.60 ± 0.71^{b}	93.30 ± 7.99^{c}	49.50 ± 5.79^{b}	16.20 ± 1.41^{a}	17.80 ± 0.28^{a}	0.0001
Bencyl acetate	$1.25 \pm 0.06^{b,c}$	1.42 ± 0.12^{c}	$1.19 \pm 0.02^{b,c}$	0.77 ± 0.01^{a}	1.02 ± 0.02^{b}	0.0009
Ethyl caprilate (μg l^{-1})	$77.05 \pm 0.64^{a,b}$	144.60 ± 15.98^{d}	53.15 ± 5.02^{a}	$95.10 \pm 8.77^{b,c}$	$125.15 \pm 2.48^{c,d}$	0.0007
Ethyl 3-hidroxybutirate	0.38 ± 0.01^{c}	0.31 ± 0.02^{b}	0.38 ± 0.01^{c}	0.24 ± 0.01^{a}	0.41 ± 0.03^{c}	0.0005
2-Phenyl ethyl acetate	2.44 ± 0.20^{b}	1.56 ± 0.09^{a}	$1.73 \pm 0.10^{a,b}$	$1.73 \pm 0.11^{a,b}$	1.41 ± 0.06^{a}	0.0024
Total esters (C)	36.46 ± 1.82^{b}	35.89 ± 0.61^{b}	33.73 ± 1.97^{b}	31.69 ± 2.77^{b}	23.59 ± 0.79^{a}	0.0041
C−B/A + B relation	2.95 ± 0.13^{b}	$2.59 \pm 0.12^{a,b}$	2.07 ± 0.09^{a}	2.28 ± 0.31^{a}	4.11 ± 0.06^{c}	0.00036

Values not sharing the same superscript letter within the horizontal line are significantly different (ANOVA and Tukey HSD test, $n = 2$)

of the higher alcohols fraction; have been related to caustic notes and floral flavour notes in wines, respectively. MMf9 wines showed the lowest levels of isoamyl alcohol as well as the highest proportion of 2-phenyl ethanol with regard to the total higher alcohols values (30.7% of the total HA) (Table 4).

The fresh, fruity aroma of young wines is largely derived from a mixture of esters produced during fermentation [3]. However, high levels of ethyl acetate (EA), the main ester in wine, can spoil the wine bouquet with an unpleasant aroma [28] whereas long chain esters (esters different to ethyl acetate) contribute positively to wine complexity and quality [2, 28]. In this context, some authors have proposed that a relationship as [long chain esters]/[HA]+[EA] can be related to the olfactory quality of wine [11]. This relation (R) was regarded as good selection criterion in this stage; wines obtained with MMf9 isolate showed the highest values for this relationship (Table 4). Similar behaviour was observed using the same strain in both Malbec and Merlot type musts (data not shown).

This poly-phasic selection study allowed us to have a complete and real picture of the indigenous yeast strain physiology and ecology. As a result, one indigenous *S. cerevisiae* strain (MMf9) was selected in order to be developed as a regional North Patagonian wine starter culture. Results obtained from implantation studies carried out with this indigenous strain at industrial level (10,000 l fermentations) in two regional cellars [9] enabled us confirmed its aptitude for enological use evidencing that the methodology proposed for the yeast selection was adequate.

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