J Ind Microbiol Biotechnol (2007) 34:139–149
DOI 10.1007/s10295-006-0178-0

ORIGINAL PAPER

Patagonian wines: implantation of an indigenous strain of *Saccharomyces cerevisiae* in fermentations conducted in traditional and modern cellars

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Received: 24 May 2006 / Accepted: 2 September 2006 / Published online: 29 September 2006

Abstract In this work we evaluate the implantation capacity of the selected *S. cerevisiae* indigenous strain MMf9 and the quality of the produced wines in a traditional (T) and a modern (M) cellar with different ecological and technological characteristics in North Patagonia (Argentina). Red musts were fermented in 10,000 l vats using the indigenous strain MMf9 as well as the respective controls: a fermentation conducted with a foreign starter culture (BC strain) in M cellar and a natural fermentation in T cellar. Since commercial *S. cerevisiae* starters are always used for winemaking in M cellar and in order to compare the results, natural fermentations and fermentations conducted by the indigenous strain MMf9 were performed at pilot (200 l) scale in this cellar, concomitantly. Thirty indigenous yeasts were isolated at three stages of fermentation: initial, middle and end. The identification of the yeast biota associated to vinifications was carried out using ITS1-5.8S-ITS2 PCR-RFLP. The intra-specific variability of the *S. cerevisiae* populations was evaluated using mtDNA-RFLP analysis. Wines obtained from all fermentations were evaluated for their chemical and volatile composition and for their sensory characteristics. A higher capacity of implantation of the indigenous MMf9 strain was evidenced in the fermentation carried out in M cellar (80% at end stage) than the one carried out in T cellar (40%). This behaviour could indicate that each cellar differs in the diversity of *S. cerevisiae* strains associated to wine fermentations. Moreover a higher capacity of implantation of the native starter MMf9 with regard to the foreign (BC) one was also found in M cellar. The selected indigenous strain MMf9 was able to compete with the yeast biota naturally present in the must. Additionally, a higher rate of sugar consumption and a lower fermentation temperature were observed in vinifications conducted by MMf9 strain with regard to control fermentations, producing wines with favourable characteristics. Even when its implantation in T fermentation was lower than that observed in M one, we can conclude that the wine features from MMf9 fermentations were better than those from their respective controls. Therefore, MMf9 selected indigenous strain could be an interesting yeast starter culture in North Patagonian wines.

Keywords *Saccharomyces* diversity · Wine yeast selection · Wine flavour

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Introduction

It is firmly established that differences in the geographical characteristics between wine producing areas and different oenological practices condition the diversity of the indigenous yeasts communities associated to fermentation musts or cellar surfaces [4, 7, 18, 27] and that in conducted fermentations the dominance of the inoculated strain can be subordinated to the specific conditions of the vinification, mainly of those that settle down in the initial stage [20].

North Patagonia is the southernmost wine-producing region of Argentina and one of the most Southern regions in the world. Both, traditional cellars in which their production is essentially based on the natural fermentation of the grape musts, and modern cellars using foreign commercial yeast starters in conducted fermentations are established in this region. In order to overcome the wine-quality variation in traditional cellars and the loss of wines' regional identity in modern ones, an indigenous strain of S. *cerevisiae* has been selected from this wine producing region in our laboratory [13]. Due to the great extension of this region and different winemaking practices adopted by the different cellars inside, two of these cellars with particular ecological and technological characteristics were selected to evaluate the fermentative behaviour of the selected indigenous *S. cerevisiae* strain at industrial scale.

The traditional (T) cellar used in this work is a small traditional family cellar located in the Upper Valley of the Colorado River. In this cellar, wine yeast starter cultures were never used and an appropriate control of the temperature during vinification is not carried out due to their technological backwardness. The modern (M) cellar is the main wine producer of the North Patagonian region and is located in the Upper Valley of the Negro River. The use of yeast commercial starters is a common practice in this cellar, which has the necessary equipment for the correct temperature control during the fermentative process.

The aim of this work was to evaluate the capacity of implantation of the selected *S. cerevisiae* indigenous MMf9 strain, and the quality of the produced wines under real conditions of vinification (10,000 l) in a traditional and a modern cellar from North Patagonia (Argentina).

Materials and methods

Yeasts and musts

Saccharomyces cerevisiae selected indigenous strain MMf9 and commercial strain BC (Danstar Ferment) were used for inoculated fermentations.

Oenologically high quality Cabernet Sauvignon (total reducing sugars 210 g/l, free SO_2 47 mg/l, pH 3.72) and Merlot (total reducing sugars 270 g/l, free SO_2 45 mg/l, pH 3.82) red musts were used for vinification assays in the modern (M) and the traditional (T) cellar, respectively.

Fermentations

Two red wine fermentations in 10,000-l-vats were performed in each cellar: an experimental fermentation (inoculated with the indigenous strain) and a control one.

A natural fermentation was used as control fermentation in T cellar, where conducted fermentations had never been carried out. This cellar was chosen for natural fermentation assays because it is far from those cellars using commercial starters that could contaminate natural fermentations.

Because commercial yeast starters are always used for vinifications in M cellar, a fermentation inoculated with commercial yeast (BC) was utilized as control. With comparative purposes the same must was additionally fermented in new 200-l-vats in the same cellar, but natural and MMf9 inoculated fermentations were carried out in them.

Eight hundred liter pre-cultures using the same musts were used to inoculate each 10,000 l inoculated fermentations. Fermentation processes were followed by temperature and sugar (°Baumé) content. Must samples were taken at different fermentations stages.

Isolation and identification of yeast

Aliquots of appropriate dilutions (0.1 ml of each one) were spread onto YEPD agar (composition in g/l: yeast extract 10, glucose 20, peptone 20, and agar 20; pH 4.5) supplemented with 100 ppm of ampicilline (Sigma, Steinheim, Germany). After incubation at 28°C for 2–6 days, those plates containing between 30 and 300 yeast colonies were examined and 20–30 colonies from each fermentation stage were isolated according to their macroscopic features and frequencies.

Yeast identification was performed by PCR-RFLP analysis of the ITS1-5.8S-ITS2 region from the nuclear rDNA gene complex [http://www.yeast-id.com, 8]. Additionally, yeast isolates belonging to *S. cerevisiae* species were characterized by using mtDNA-RFLP analysis according to Lopes et al. [14].

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 Gay Lussac degrees (GL, ml of alcohol/100 ml of wine). Volatile acidity was determined by steam distillation followed by titration and it was expressed as acetic acid (g/l). Total acidity was determined by direct titration and expressed as tartaric acid (g/l). Free SO_2 was determined by direct titration with iodine and total SO_2 by treatment with KOH 1N followed by titration with iodine.

Higher alcohols and esters

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. [24]. Aliquots of 1.5 ml of the samples were placed in 15-ml vials and 0.3 g of NaCl and 15 µl 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 µm). 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 200°C at 4°C/min, and 200–260°C at 20°C/min and kept for 2 min at 260°C.

The following standards were purchased from Fluka: isobutyl alcohol, isoamyl alcohol, 1-hexanol, benzyl 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.

Sensory evaluation

A panel of seven judges carried out sensory evaluation of wine by applying the triangle test as well as the Kramer method. Wines were bench-tasted at INTA, Lujan de Cuyo, Mendoza, Argentina. All sensory attributes were rated by the judges on 5-cm line scales [9].

Statistical analysis

Principal components analysis (PCA) on the centred and standardized quantitative variables evaluated in wines (oenological parameters, higher alcohols and esters) was performed using the NTSYS programme (Numerical Taxonomic System version 2.11) [23]. ANOVA and Tukey honest significant difference (HSD) test 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

Fermentation kinetics

Figure 1 shows the kinetic of the fermentations carried out with the indigenous strain MMf9 and its respective controls. As is depicted in Fig. 1a (see day-0 in both natural fermentations), the initial density of indigenous yeasts was higher in traditional (T) than in modern (M) cellar fermentations. For this reason the proportion between inoculated and natural yeasts showed significant differences among fermentations (80–20% in T and 99–1% in M fermentations respectively), even when the same number of starter cells was used for inoculation in all cases. Nevertheless, differences are not significant towards fermentations middle stage. A lower yeast density was observed in T cellar fermentations at the final stage in which the alcohol concentrations reached 15% v/v.

In all cases the monitoring of the fermentations was carried out by registration of the sugar content (measured as Baumé degrees) and the temperature, routine measurements in the cellars (Fig. 1b). No significant differences in the sugar consumption and the total time required to complete the fermentation processes were found between the industrial vinifications conducted by both indigenous (MMf9) and commercial (BC) yeasts in M cellar. Conversely, a higher rate of sugar consumption and a lower temperature were observed in both M pilot scale and T fermentations conducted by MMf9 strain with regard to their respective natural fermentations. However, no differences were detected in the total time required for fermentation and the residual sugars content in resulting wines (Fig. 1b). It is interesting to note that toward the third day of fermentation (stage of tumultuous fermentation) in T cellar the natural process reached a temperature of 32°C, while the conducted one never surpassed 30°C.

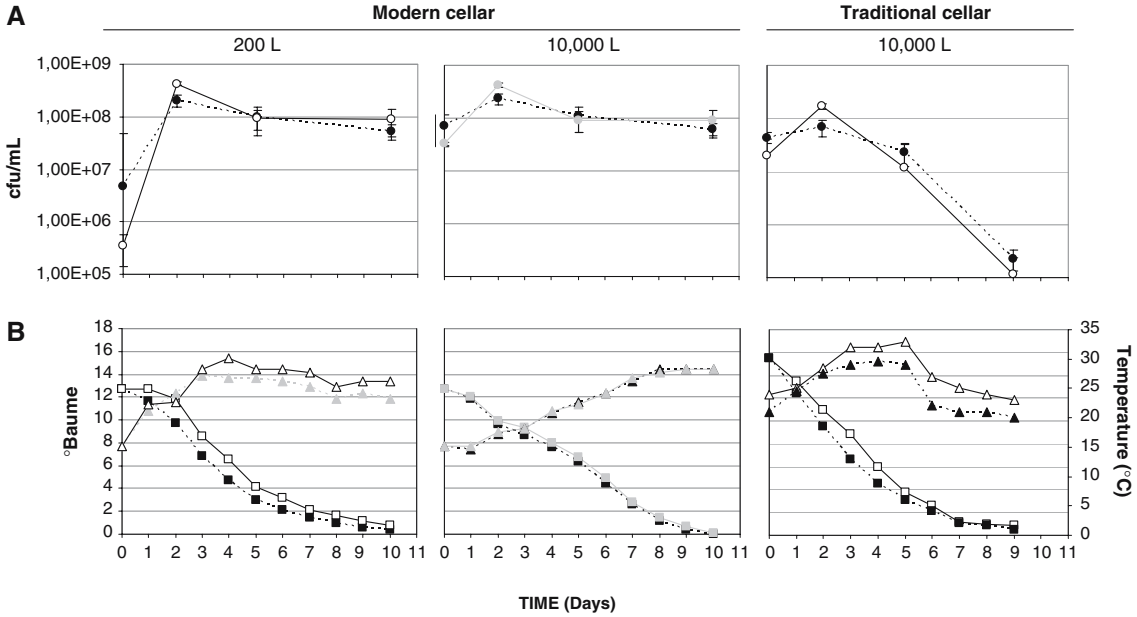

Fig. 1 Yeast growth (**a**) and time course of main parameters (**b**) in natural fermentations (*white*) and fermentations inoculated with BC strain (*grey*) and MMf9 indigenous strain (*black*) yeast starters. *circles* (cfu/ml), *triangles* (temperature, °C), and *squares* (°*Baumé*)

Yeast diversity

Three different ITS1-5.8S-ITS2 PCR-RFLP patterns were detected among all the yeast isolates associated to vinifications and they corresponded to *Hanseniaspora uvarum, Pichia guilliermondii* and *Saccharomyces cerevisiae* species. Their frequencies during the fermentative processes are shown in Fig. 2. In M cellar fermentations the whole biota identified before the inoculation (must) corresponded to the species *H. uvarum*, while in T cellar a high proportion of *S. cerevisiae* isolates was detected (60%). Additionally, isolates belonging to *P. guilliermondii* (11%) were also detected in must from T fermentations (Fig. 2).

The addition of the starter produced a dilution of the non-*Saccharomyces* biota (day 0), and from the second day of fermentation 100% of the characterized biota corresponded to *S. cerevisiae.* The same *S. cerevisiae* dominance (100%) from the second day of fermentation was observed in the natural fermentation

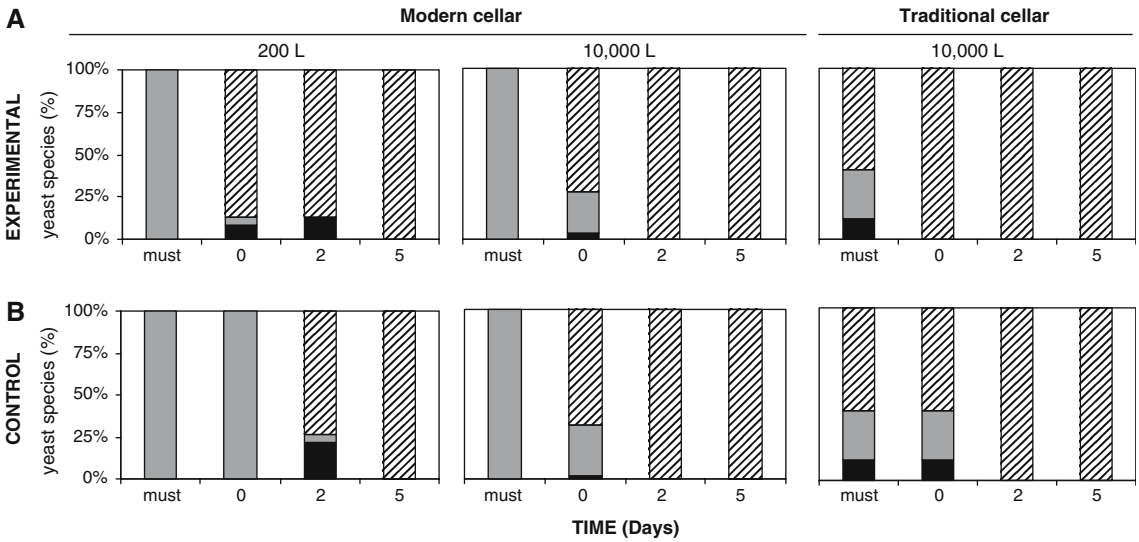

Fig. 2 Succession of yeast species during fermentations. *Hanseniaspora uvarum* (*light shaded square*), *Pichia guilliermondii* (*dark shaded square*) and *Saccharomyces cerevisiae* (*slashed square*)

(Fig. 2b). In pilot scale fermentations the non-*Saccharomyces* biota was observed until second day of fermentation in both natural and inoculated vinification.

Dynamics of the S. *cerevisiae* populations

In order to evaluate the capacity of the selected native strain to dominate a fermentation, the dynamics of the *S. cerevisiae* populations were determined by means of mtDNA-RFLP analysis. As a result of this analysis, indigenous MMf9 strain dominance evidenced in M cellar fermentations (80 and 100% in the end stage of 10,000 and 200 l, respectively) was higher than that observed in the fermentation carried out in T cellar (40%)(Tables 1, 2). Moreover a higher implantation capacity of the MMf9 indigenous starter with regard to the foreign (BC) one was also found (80 against 30%, respectively) in M fermentations. No indigenous isolates were detected when MMf9 was used as starter in 200 l fermentations.

A total of 17 mtDNA-RFLP patterns were found in M cellar natural fermentation (200 l) (Table 1). Two of which (patterns XXX, XXXVI) were also detected in both BC and MMf9 conducted fermentations carried out at 10,000 l in the same cellar (Table 1 and Fig. 3).

On the other hand, 23 different mtDNA-RFLP patterns were detected in T cellar, five of which (named XLVII, LIV, LV, LIX, and LX) were common to both conducted and natural fermentations, and five (patterns LXV, LXVI, LXVII, LXVIII and LXIX) were only present in MMf9 conducted one (Table 2). As was also observed in natural fermentation in M cellar, a sequential substitution of mtDNA-RFLP patterns was observed during the different fermentations days.

The MMf9 mtDNA-RFLP pattern was not detected either in the natural fermentations or in the BC inoculated one (Fig. 3).

Chemical composition of wine

Wines obtained from different fermentations showed significant differences in their compositions. In particular, both wines obtained by fermentation with MMf9 indigenous strain showed significantly lower values of volatile acidity and a higher relation of free SO_2/total SO_2 than the values from their respective controls (Table 3). Additionally, the yield of the alcoholic fermentation (expressed as ethanol produced/ethanol estimated, %) was higher in M cellar (99%) than in T cellar (91%).

The analysis of the volatile composition evidenced that MMF9 strain produced significantly lower levels of ethyl acetate and higher levels of other esters (fundamentally ethyl lactate) than the levels reached in the control fermentations (Table 4).

Table 1 Frequencies of the *S. cerevisiae* mtDNA-RFLP patterns associated with experimental (MMf9 conducted) and control (natural and BC conducted) fermentations carried out in the modern cellar

mtDNA pattern	No. (%) of isolates corresponding to mtDNA patterns											
	200 l						10,000 l					
	Natural			MMf9			BC			MMf9		
	Day 0	Day-5	Day-10	Day-0	Day-5	Day-10	Day-0	Day-5	Day-10	Day-0	Day-5	Day-10
XXX	10(50)	4(20)	4(20)	–	–	–	2(10)	5(25)	5(25)	–	–	1(5)
XXXI	1(5)	–	–	–	–	–	–	–	–	–	–	–
XXXII	3(15)	–	–	–	–	–	–	–	–	–	–	–
XXXIII	2(10)	–	–	–	–	–	–	–	–	–	–	–
XXXIV	2(10)	–	–	–	–	–	–	–	–	–	–	–
XXXV	2(10)	–	–	–	–	–	–	–	–	–	–	–
XXXVI	–	3(15)	5(25)	–	–	–	–	3(15)	4(20)	–	2(10)	3(15)
XXXVII	–	4(20)	–	–	–	–	–	–	–	–	–	–
XXXVIII	–	4(20)	–	–	–	–	–	–	–	–	–	–
XXXIX	–	1(5)	2(10)	–	–	–	–	–	1(5)	–	–	–
XL	–	2(10)	–	–	–	–	–	–	–	–	–	–
XLI	–	2(10)	–	–	–	–	–	–	2(10)	–	–	–
XLII	–	–	2(10)	–	–	–	–	–	2(10)	–	–	–
XLIII	–	–	1(5)	–	–	–	–	–	–	–	–	–
XLIV	–	–	3(15)	–	–	–	–	–	–	–	–	–
XLV	–	-	1(5)	-	-	-	-	-	-	-	-	-
XLVI	–	–	2(10)	–	–	–	–	–	–	–	–	–
MMf9 (XXII)	–	–	–	20(100)	20(100)	20(100)	–	–	–	20(100)	18(90)	16(80)
BC	–	–	–	–	–	–	18(90)	12(60)	6(30)	–	–	–
TOTAL	20 (100)	20 (100)	20 (100)	20(100)	20(100)	20(100)	20(100)	20(100)	20(100)	20(100)	20(100)	20(100)

Table 2 Frequencies of the *S. cerevisiae* mtDNA patterns associated with experimental (MMf9 conducted) and control (natural) fermentations carried out in the traditional cellar

mtDNA pattern	No. (%) of isolates corresponding to mtDNA patterns					
	Natural			MMf9		
	Day-0	Day-5	Day-10	Day-0	Day-5	Day-10
XLVII	2 (12.5)	–	–	–	2 (10)	–
XLVIII	2 (12.5)	–	–	–	–	–
XLIX	4 (25)	–	–	–	–	–
XLX	3 (18.8)	–	–	–	–	–
LI	1 (6.25)	–	–	–	–	–
LII	2 (12.5)	–	–	–	–	–
LIII	2 (12.5)	–	–	–	–	–
LIV	–	2 (12.5)	–	–	–	2 (10)
LV	–	6 (37.5)	2 (10)	–	4 (20)	–
LVI	–	4 (25)	4 (20)	–	–	–
LVII	–	2 (12.5)	–	–	–	–
LVIII	–	2 (12.5)	2 (10)	–	–	–
LIX	–	–	2 (10)	–	–	1 (5)
LX	–	–	3 (15)	–	2 (10)	3 (15)
LXI	–	–	1 (5)	–	–	–
LXII	–	–	2 (10)	–	–	–
LXIII	–	–	2 (10)	–	–	–
LXIV	–	–	2 (10)	–	–	–
LXV	–	–	–	–	–	2 (10)
LXVI	–	–	–	–	–	2 (10)
LXVII	–	–	–	–	–	2 (10)
LXVIII	–	–	–	–	2 (10)	–
LXIX	–	–	–	2 (10)	–	–
MMf9 (XXII)	–	–	–	18 (90)	10 (50)	8 (40)
Total	16 (100)	16 (100)	20 (100)	20 (100)	20 (100)	20 (100)

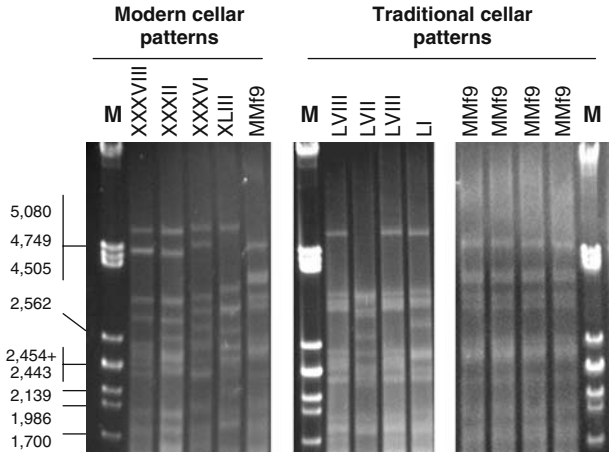

Fig. 3 mtDNA-RFLP patterns of some indigenous *S. cerevisiae* isolates from natural and inoculated fermentations. The strains identities are indicated by *Roman numbers* at the top of gels. MMf9: selected indigenous strain used as control (*left gel*, *sixth lane*) and as starter (*right gel*). *M* Weight marker-lambda DNA digested with *Hin*dIII; *arabic numbers* indicate the sizes (bp) of the different restriction fragments

Total higher alcohols, total esters and ethyl acetate do not show significant differences between both 200 l conducted and natural fermentations, however, the levels of isobutyl and benzyl alcohols in particular were significantly higher in the MMf9 conducted fermentation (Table 4).

Finally, only significant differences among minor compounds were observed when wines obtained at industrial scale using the indigenous MMf9 isolate in both T and M fermentations were compared.

Sensory evaluation of wine

In order to compare the MMf9 isolate behaviour over wines sensory properties, we only evaluate those processes representing real conditions of fermentation in cellars (10,000 l). According to the test of difference (triangular test), the analysed wines were different from their respective controls for a $P = 0.05$ (Fig. 4). Wines obtained with MMF9 strain showed values of the descriptors violet hue and eucalyptus (Merlot) or pepper and cassis (Cabernet Sauvignon) that were higher than those in the control wines. Moreover, the wine obtained with MMf9 strain in T cellar exhibited lower values in the phenol aroma descriptor than the naturally fermented wine.

At last, the wines obtained by fermentation with the selected indigenous yeast were the favourite for the seven judges in front of their respective controls according to the Kramer test of preference.

Table 3 Chemical characteristics of wines obtained in modern and traditional cellars

Characteristics	Cellar, scale of fermentation and inoculum							
	Modern cellar					Traditional cellar		
	200 l			10,000 l		10,000 l		
	MMf9	Natural	*P* value*	MMf9	BC	MMf9	Natural	*P* value**
Alcohol (°GL)[a]	12,9 ± 0.2	12,9 ± 0.2	*0.8998*	13,5 ± 0.2[a]	13,5 ± 0.2[a]	15.0 ± 0.2[b]	15.0 ± 0.2[b]	*0.0134*
Volatile acidity (g/l)[b]	0,15 ± 0.02	0,32 ± 0.03	*0.0234*	0,20 ± 0.02[a]	0,78 ± 0.02[c]	0.34 ± 0.03[a]	0.46 ± 0.03[b]	*0.0097*
Total acidity (g/l)[c]	7,65 ± 0.23	7,12 ± 0,31	*0.2353*	4,72 ± 0.25	5,47 ± 0.25	5.23 ± 0.30	6.20 ± 0.22	*0.9476*
TRS (g/l)[d]	1,20 ± 0.12	1,19 ± 0.21	*0.3547*	2,02 ± 0.4	2,25 ± 0.5	4.6 ± 0.5	3.6 ± 0.4	*0.0556*
Free SO_2 (g/l)	11 ± 2	8 ± 2	*0.0676*	20 ± 1[ab]	27 ± 1[b]	17 ± 2[a]	32 ± 1[c]	*0.0219*
Total SO_2 (g/l)	13 ± 1	14 ± 1	*0.0788*	55 ± 4[ab]	63 ± 4[b]	31 ± 3[a]	78 ± 3[b]	*0.0279*
pH	3,42 ± 0.02	3,52 ± 0.04	*0.2113*	3,87 ± 0.03	3,82 ± 0.03	4.24 ± 0.02	4.08 ± 0.03	*0.0654*

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

[a] Gay Lussac degrees (ml of alcohol/100 ml of wine)

[b] Expressed as acetic acid

[c] Expressed as tartaric acid

[d] Total Reducing Sugars

*Significant differences $P < 0.05$ between 200 l scale

**Significant differences $P < 0.05$ between 10,000 l-scale

Discussion

The capacity of a selected isolate to take over industrial fermentations and its influence over wine quality are the last features to be evaluated in all wine yeast selection programmes. Several works have evidenced that the dominance of the starter is not always guaranteed [5, 10, 12, 16] and that the growth of indigenous yeasts can significantly affect the effectiveness of the dominance process [2, 3, 28]. Several factors as those related to the cellar operations, must type, geographical characteristics, among others, can affect the diversity of the indigenous biota also influencing the implantation capacity of a wine yeast starter.

Our work evidences that the dominance of the indigenous MMf9 strain in traditional (M) cellar fermentation was lower than the one in modern (M) cellar (Tables 1, 2). This different behaviour could be related to significant differences in both extension and diversity of the indigenous biota at the beginning of the fermentations among cellars. While the must fermented in M cellar showed an initially low density of cells (10^5 cells/ml), the initial biota in T cellar fermentations was significantly higher (10^7 cells/ml). The inoculation with the same volume of a 10^8 cells/ml pre-culture determines a particular relationship between the inoculated strain and the indigenous biota at fermentations initial stages. Subsequently, in T cellar where the implantation capacity of MMf9 strain was lower (40%), the relationship starter/ indigenous biota was also lower. Additionally, the initial biota in M fermentations belonged to *H. uvarum* species (100%), and the one from T fermentations showed a great proportion of *S. cerevisiae* isolates (60%). In a previous work in which the killer sensitivity patterns of the same natural fermentations used in this work were evaluated [15], we demonstrated that the indigenous *S. cerevisiae* biota from T cellar is mostly resistant to the *S. cerevisiae* K2 type killer toxin, while the *S. cerevisiae* biota related to M cellar is particularly sensitive to that toxin. The same behaviour is observed among *S. cerevisiae* isolates from surfaces of traditional and modern cellars and could be due to selective pressure exerted by years of inoculation with K2 factor bearing commercial starters in these cellars [27]. According to these results the isolates coming from T cellar could compete more efficiently with the inoculated native strain (K2 type killer strain) than those from M cellar.

On the other hand, the industrial vinifications carried out in M cellar evidenced a higher dominance of the MMf9 indigenous strain (80%) than BC commercial one (30%). In this case, the relationship starter/ indigenous biota was the same for both fermentations (99/1). This result indicates a higher competition capacity of indigenous MMf9 isolate with regard to commercial BC strain. This experimental evidence supports the hypothesis proposing that selected indigenous strains are better adapted to the particular characteristic of a wine producing region than foreign starters [25, 31].

Regarding the influence of inoculation over intraspecific *S. cerevisiae* diversity, contrary to reports for

Table 4 Concentration of volatile compounds (mg/l) in wines obtained from industrial and pilot fermentations in modern and traditional cellars

Compounds	Cellar, scale of fermentation and inoculum							
	Modern cellar					Traditional cellar		
	200 l			10,000 l		10,000 l		
	MMf9	Natural	*P* value*	MMf9	BC	MMf9	Natural	*P* value**
Higher alcohols								
Isobutyl alcohol	25.92 ± 0.02	20.08 ± 0.81	0.0095	21.41 ± 1.70^{a}	25.04 ± 0.16^{ab}	23.18 ± 1.56^{ab}	27.27 ± 1.14^{b}	0.0393
Isoamylic alcohol	113.80 ± 10.93	118.33 ± 9.56	0.7023	54.05 ± 2.43	59.21 ± 3.54	59.01 ± 1.56	54.45 ± 2.83	0.3172
1-Hexanol	1.98 ± 0.06	2.08 ± 0.05	0.2017	1.40 ± 0.04^{b}	1.50 ± 0.10^{b}	0.66 ± 0.02^{a}	0.47 ± 0.03^{a}	0.0001
Benzyl alcohol	1.12 ± 0.03	0.69 ± 0.05	0.0102	1.61 ± 0.11^{c}	0.80 ± 0.03^{a}	0.17 ± 0.02^{b}	0.17 ± 0.01^{b}	<0.0001
2-Phenyl ethanol	108.51 ± 11.63	166.92 ± 17.84	0.0605	45.98 ± 3.15^{ab}	51.60 ± 3.17^{b}	38.67 ± 2.77^{b}	22.16 ± 2.40^{a}	0.0020
Total alcohols (A)	235.56 ± 22.17	308.11 ± 28.31	0.1040	124.44 ± 2.56^{ab}	138.16 ± 0.61^{b}	121.64 ± 13.65^{a}	104.52 ± 0.72^{a}	0.0370
Esters								
Ethyl acetate (B)	11.89 ± 1.74	8.92 ± 2.25	0.2779	20.34 ± 0.16^{a}	37.85 ± 1.36^{b}	22.23 ± 2.08^{a}	28.99 ± 1.59^{b}	0.0096
Isobutyl acetate (μg/l)	11.92 ± 0.98	6.44 ± 1.15	0.0360	16.46 ± 0.11^{a}	15.90 ± 0.14^{a}	17.87 ± 0.01^{b}	21.89 ± 0.26^{c}	<0.0001
Ethyl lactate	6.64 ± 0.15	3.92 ± 0.72	0.0349	14.99 ± 1.22^{c}	7.02 ± 0.34^{c}	9.47 ± 0.59^{b}	1.98 ± 0.14^{a}	0.0002
Isoamyl acetate (μg/l)	250 ± 0.00	170 ± 0.00	0.0389	140.89 ± 4.06^{a}	138.73 ± 26.16^{a}	195.81 ± 1.86^{b}	87.77 ± 0.71^{a}	0.0060
Hexyl acetate (μg/l)	0.10 ± 0.00	0.32 ± 0.02	0.0049	0.15 ± 0.00^{a}	0.23 ± 0.03^{a}	0.20 ± 0.00^{a}	0.40 ± 0.01^{b}	0.0119
Diethyl succinate (μg/l)	64.44 ± 1.44	48.21 ± 2.67	0.0171	37.95 ± 3.46^{c}	25.65 ± 2.33^{b}	12.80 ± 1.13^{a}	14.50 ± 0.42^{a}	0.0010
Bencyl acetate	1.26 ± 0.01	1.18 ± 0.08	0.2823	3.56 ± 0.66^{bc}	4.83 ± 0.3317^{c}	2.22 ± 0.21^{a}	0.46 ± 0.03^{a}	0.0014
Ethyl caprylate (μg/l)	0.108 ± 0.01	0.092 ± 0.01	0.0716	0.12 ± 0.01^{a}	0.13 ± 0.00^{a}	0.33 ± 0.02^{b}	0.31 ± 0.02^{b}	0.0002
Ethyl 3-Hydroxibutanoate	0.14 ± 0.01	0.15 ± 0.01	0.1282	0.29 ± 0.01	0.38 ± 0.04	0.49 ± 0.07	0.36 ± 0.05	0.0580
2-Penylethyl acetate	1.42 ± 0.04	1.57 ± 0.33	0.5983	0.78 ± 0.04^{a}	1.69 ± 0.24^{a}	4.27 ± 0.33^{b}	5.41 ± 0.24^{c}	0.0001
Total esters (C)	21.78 ± 1.61	16.05 ± 3.41	0.1643	40.02 ± 1.75^{a}	51.74 ± 1.10^{b}	38.79 ± 1.62^{a}	37.30 ± 1.52^{a}	0.0021
Relation C-B/A+B	4.15 ± 0.01	2.38 ± 0.02	0.0313	13.58 ± 1.10^{b}	7.89 ± 0.18^{a}	11.60 ± 1.58^{b}	6.23 ± 0.17^{a}	0.0051

*Significant differences $P < 0.05$ between 200 l scale

**Significant differences $P < 0.05$ between 10,000 l scale

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

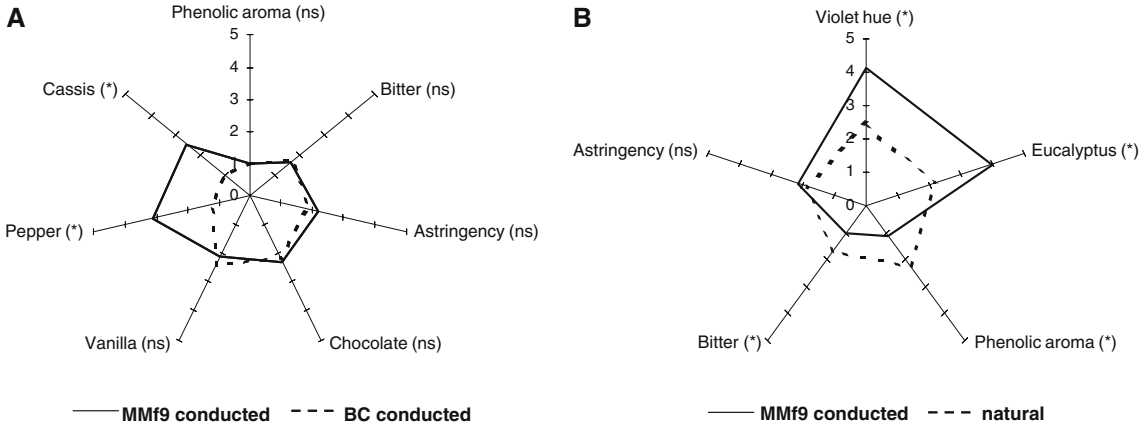

Fig. 4 Sensory profile plot of mean intensity rating for colour, aroma and flavour descriptors of wines obtained in traditional (**a**) and modern (**b**) cellars (0 minimum, 5 maximum) from four different fermentations. *Asterisk* significant difference, *NS* not significant difference

cellars in Valencia (Spain) [21], our results evidence a higher diversity in natural fermentations than in conducted ones (Tables 1, 2). Moreover, five different patterns were common to both inoculated and natural T cellar fermentations, and other five patterns were only detected in the former. This fact evidences that inoculation does not only modify the populations by starter numerical dominance but it also modifies the supplementary *S. cerevisiae* biota. Inoculation allows the development of indigenous isolates (detected by mtDNA-RFLP analysis in this work) that can not develop in natural fermentation. Additionally, no *S. cerevisiae* mtDNA-RFLP patterns common to both cellars were found. This fact, added to the above mentioned different dominance percentages of the indigenous MMf9 strain and the differential killer phenotype of indigenous isolates [15] in both cellars, could indicate that each cellar has a particular *S. cerevisiae* diversity associated to its wine fermentations. However, new investigations in this respect should be carried out in order to characterize either cellars or regions thoroughly and to use this knowledge in the development of appropriate yeasts starters.

Although different relationships starter/ indigenous biota and differences in the MMf9 strain dominance percentages were observed, the indigenous starter was responsible for driving all three fermentations inoculated with it. MMf9 strain was also capable of depleting sugars even when the levels of ethanol overcome 15% v/v (T fermentation) and of evidencing a high rate of substrate consumption without temperature increase (Fig. 1b), two interesting features from an industrial point of view.

Finally, wines obtained from MMf9 fermentations show some differential characteristics, detected in the chemical as well as in the sensory analyses (Tables 3, 4; Fig. 4). Both MMf9 and BC conducted fermentations rendered wines whose values of the free SO_2/total SO_2 relationship were significantly higher than the ones in natural fermentations (Table 3). Because only the SO_2 free portion is responsible for impeding microbial contaminations and considering the health risks associated with elevated SO_2 concentrations, the selection of *S. cerevisiae* strains responsible for a high free SO_2/ total SO_2 relation in wine became particularly interesting. The use of these selected strains would allow to improve the efficiency in the use of the SO_2 diminishing the necessary quantity of this compound to be added to the wine [6, 26]. Another relevant difference is that levels of volatile acidity in wines obtained with the MMf9 were always lower than the ones in control wines (Table 3).

As a whole, the concentrations of individual higher alcohols and esters differed depending on the yeast strain responsible for fermentation (Table 2), which is in accordance with results obtained by Romano et al. [25] and Plata et al. [19]. However, the volatile composition analysis of wines evidenced higher values of the relation C-B/A+B (directly related to wine complexity) for MMf9 conducted fermentations (Table 4). Similar results were observed for the MMf9 strain in 5-l-laboratory scale fermentations using Malbec and Merlot type musts [13].

A direct relationship could not be settled down among volatile compounds and sensory features of wines. Nevertheless, because cassis, pepper and eucalyptus tones are related to the primary aroma of these wines [11, 29], it can be concluded that in the wines obtained by inoculation with the indigenous MMf9 strain there was a significant improvement of this type

of flavours, resulting in more aromatic young wines compared to control wines. Similar results have been reported in Sauvignon Blanc wines obtained from fermentations driven with a selected strain of *S. cerevisiae* [30]. It is interesting to note that some off flavours (phenolic aroma) detected in wine obtained by natural fermentation in T cellar, were not detected when the same must was fermented with MMf9 strain (Fig. 4). These phenolic aromas could be related to the higher numbers of *P. guilliermondii* in the naturally fermented wines than in the conducted one. Recent studies have related the production of phenolic compounds to strains of this species in laboratory assays [17]. Moreover, a reduction in *P. guilliermondii* phenolic compounds production has also been associated with the use of *S. cerevisiae* starters [1].

In conclusion, MMf9 selected indigenous strain is able to compete with the yeasts biota naturally present in the must and to support the stress conditions, particularly the high concentrations of ethanol (more than 15% v/v). This strain produces young wines with chemical and sensory differential characteristics in both kind of musts used in this work.

Acknowledgments This work was supported by Universidad Nacional del Comahue project I-117 and CONICET post-doc fellowship of C. Lopes. The authors wish to thank S. Genovés for help in IATA and C. Catania (INTA, Lujan de Cuyo, Mendoza, Argentina) for sensory analysis as well as to Valencia University and CSIC for permission to consult www.yeast-id.com database.

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