A regional starter for high quality wines: an Argentinean Patagonia experience

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ABSTRACT

The demands of an increasingly competitive international market suffering from oversupply, and consumer demand for new styles of wine, impose challenges to winemakers that require technological innovation. Within the productive chain, appropriate management of the yeast biota responsible for conducting the alcoholic fermentation is strategic to differentiate the character of wine. The objective of this study was to characterize the ability of a Patagonian *Saccharomyces cerevisiae* strain to drive red vinifications and to improve the quality of the regional wines.

Pilot scale vinifications (200 L) were carried out in Pinot noir, Merlot and Malbec varieties, during three consecutive vintages (2012 to 2014). Sulfited musts industrially obtained were inoculated with *S. cerevisiae* ÑIF8-LVI, an autochthonous strain, (initial cellular density of 10^6 cel/mL). Alcoholic fermentation (AF) was followed by ºBaumé and total reducing sugars, and implantation capacity was studied by viable counts in GPY agar and restriction profile analysis from mitochondrial DNA with *Hinf* I endonuclease. In all cases, assays with a *S. cerevisiae* F15 (Laffort), a commonly used commercial starter, were carried out as a comparison. When alcoholic fermentations were finished, wines were devatted and transferred to the 50 L tanks where spontaneous malolactic fermentation (MLF) developed at 20±2ºC. They were stabilized, clarificated, filtered and bottled. Quality was evaluated through physicochemical parameters (INV and enzymatic methods), sensorial analysis and consumer preference polls.

Both indigenous and commercial starters were able to satisfactorily implant, yielding normal processes and products. However, *S. cerevisiae* ÑIF8 vinifications showed significantly faster kinetics than F15 controls and the oenological characteristics of these wines were significantly improved, allowing to obtain a higher score than controls in the sensorial evaluation and to be preferred for the consumers (paired-preference test $p<0.05$). These results reassure the oenological potential of *S. cerevisiae* ÑIF8 strain for the formulation of a regional starter culture already confirmed at laboratory scale.

1. INTRODUCTION

Patagonian Region, located at 37°5` and 40°5` southern latitude, is the southernmost wine-producing region of Argentina and one of the most Southern regions dedicated to this activity in the world. This area presents advantageous agro-ecological conditions for high quality viticulture (LLORENTE et al. 2005) and its production is mainly guided towards young dry wines from red *Vitis vinifera* varieties, such as Merlot and Pinot noir, which have met in this region the optimal conditions for their full oenological expression (CATANIA et al. 2010). However, the demands of an increasingly competitive international market suffering from oversupply and consumer demands for new styles of wines impose challenges to Patagonian winemakers that require technological innovation.

Within the productive chain, appropriate management of the yeast biota responsible for conducting the alcoholic fermentation by grape must inoculation using selected yeasts is strategic to differentiate the character of wine, a practice that it is nowadays widespread in each winegrowing area (RAINIERI. et al. 2000). Local yeasts are presumed to be more competitive than commercial ones because they are better adapted to the ecological and technological features of their own area (QUEROL et al. 1996). Therefore, they would be capable to dominate the fermentation and to become the most important biological agent responsible for winemaking (DEGRE. 1993). Hence, selection of appropriate local yeasts would ensure the production of quality premium wines, maintaining the differential properties of their own area and preserving its natural biodiversity (FLEET. 2008).

Commercial starters for alcoholic fermentation found actually in the market are composed by yeast strains isolated from the most important winegrowing areas in the world, except Argentina. Microbiological studies carried out during several years in the Patagonian region allowed to characterize the biota associated to cellars (SANGORRIN et al. 2004) and red vinification environments (LOPES et al. 2007, CABALLERO et al 2008, DEL MONACO et al. 2014) and to constitute an important collection of local *Saccharomyces cerevisiae* and non-saccharomyces strains relevant for oenological application and wine starter elaboration.

In this context, the aim of this study was to characterize the ability of a Patagonian *S. cerevisiae* strain to drive red vinifications and to improve the quality of regional wines.

2. MATERIALS AND METHODS

2.1 Yeasts

Two *S. cerevisiae* strains were used in this work: ÑIF8 (F8) isolated from the Patagonian region and characterized as indigenous by molecular methods (DEL MONACO et al. 2014) and F15, a commercial starter (Laffort).

2.2 Vinifications

Patagonian Pinot noir, Merlot and Malbec musts obtained at industrial scale were vinificated at pilot scale (200 L) from 2012 to 2013 vintages. Alcoholic fermentations were carried out by grape must inoculation with indigenous ÑIF8 strain or with F15 commercial strain (Laffort) to reach initial cellular densities of 10^6 cel/mL in the tanks and they were maintained at 25±2°C until grape must dryness was achieved (total reducing sugars ≅ 2 g/L). At this time, wines were devatted and transferred to 50 L tanks to develop malolactic fermentation at 20±2ºC. In all cases, malolactic fermentation was carried out spontaneously. At the end of this process, wines were racked, stabilized and finally their free SO_2 contents were adjusted to 40 mg/L and bottled. Alcoholic and malolactic fermentations kinetics were followed by total reducing sugars (TRS) and malic acid evolution, respectively. Must samples were taken in duplicates during AF at the initial (14ºBmé) and final (≅2 g/L TRS) stages.

2.3 Isolation and identification of yeasts

Aliquots (0.1 ml) of appropriate wine dilutions were spread onto YEPD agar (composition in g/L: 10 yeast extract, 20 glucose, 20 peptone, and 20 agar; pH 4.5) supplemented with 100 ppm of ampicillin (Sigma, Steinheim, Germany). Plates were incubated for three days under aerobic conditions at 25ºC and viable cell were counted (CFU/mL). Yeast colonies were isolated from each fermentation stage according to their macroscopic features and frequencies. Isolates were re-isolated onto YEPD-ampicillin and pure cultures were maintained at -80ºC in YEPD with glycerol (20% v/v).

Yeast identification was performed by PCR-RFLP analysis of the ITS1-5.8S-ITS2 region from the nuclear rDNA gene complex (ESTEVE-ZARZOSO. 1999). Additionally, *S. cerevisiae* strains were discriminated by using mtDNA-RFLP analysis with *Hinf I* restriction enzyme according to LOPES et al. (2007).

2.4 Wine physicochemical analysis

Oenological parameters were determined according to the methods proposed by the National Viticulture Institute (Instituto Nacional de Vitivinicultura, INV). Therefore, ethanol concentration was determined by distillation and expressed as Gay Lussac degrees (GL, mL of alcohol/100 mL of wine); volatile acidity was quantified by steam distillation followed by titration and titratable acidity was determined by direct titration with NaHO 0.1N and total reducing sugars (TRS) by Fehling-Causse-Bonnans method. D (+) glucose, D (-) fructose, L (-) malic acid and L (+) lactic acid were determined enzymatically using commercial detection kits (Megazyme, International Ireland Ltd.).

2.5 Wine sensorial analysis

Sensorial quality of wine was qualitatively and quantitatively evaluated. Qualitative analysis was performed by a panel of nine experts (INTI, Segundas Jornadas Patagónicas de Elaboradores de Vino Casero y Artesanal, 2012) or seventeen experts (Cátedra de Enología de la Facultad de Ciencias Agrarias de la Universidad Nacional de

Cuyo, 2013). In these cases, descriptive tests with scaling techniques were used, which involved words (attributes valuation related to visual, smell and mouth feel aspects) and numerical scales (global quality valuation). Additionally, a quantitative paired-preference test (two-tailed) with consumers was performed and its significance determined using Roessler expanded tables (ROESSLER et al. 1978).

3. RESULTS AND DISCUSSION

Vinification studies were carried out using Pinot noir (figure 1), Merlot and Malbec grape musts as substrates and alcoholic fermentations were guided by inoculation with the two *S. cerevisiae* strains: indigenous F8 and commercial F15.

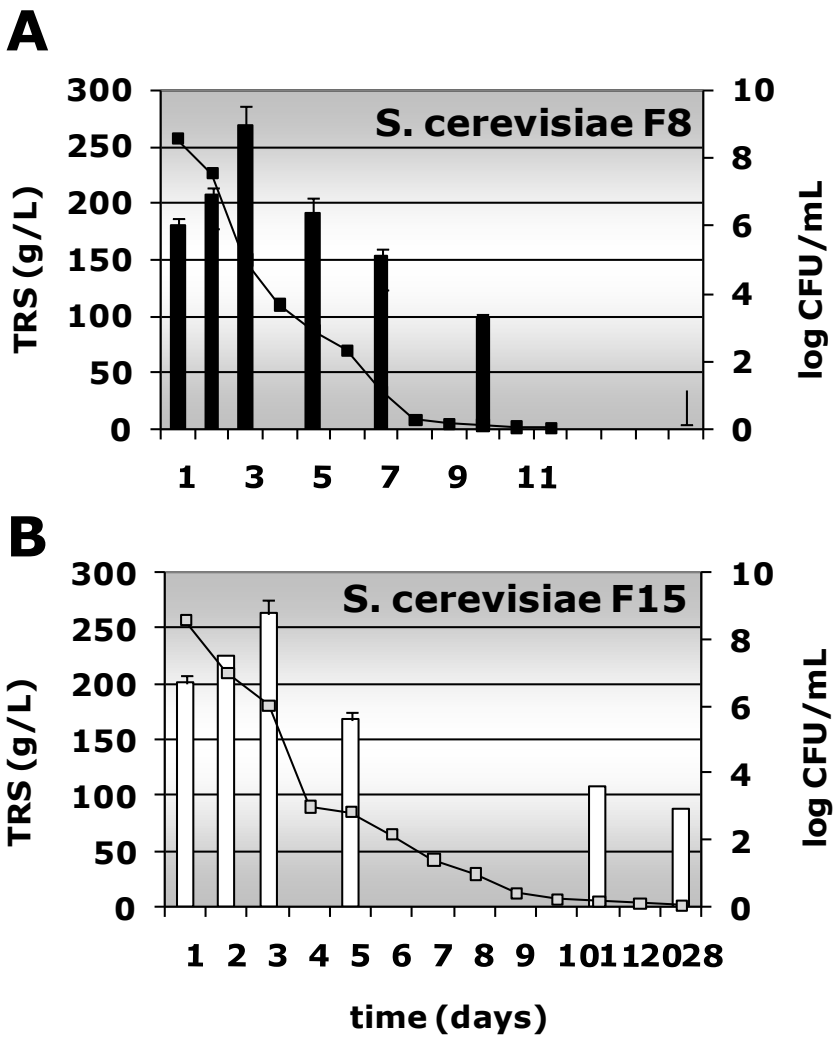

Figure 1: Yeast growth (bars) and total reducing sugars evolution (squares) during pilot scale Pinot noir vinifications guided by indigenous F8 (upper panel, black symbols) and commercial F15 (bottom panel, white symbols) *S. cerevisiae* strains.

The initial cellular densities and biomass evolution were similar in Pinot noir fermentations (figure 1) as well as in Merlot and Malbec fermentations (data no shown). Additionally, fermentations were mostly completed to dryness (TRS ≤ 2 g/L, figure 1 and table 1) but the sugar consumption rates during dryness stages, such as it is displayed in figure 1 for Pinot noir, were ever higher in F8 guided fermentations than in F15 fermentations. As a consequence, fermentative processes guided by the indigenous starter were faster than those guided by the commercial starter.

In order to evaluate the capacity of the indigenous starter to dominate the fermentations, the dynamics of the *S. cerevisiae* populations were determined by means of mtDNA-RFLP analysis. The results obtained from these studies, and partially showed in figure 2, evidence that indigenous F8 and commercial F15 *S. cerevisiae* were the strains mostly found at the initial and final stages of their respective fermentations proving their very good and similar implantation capabilities.

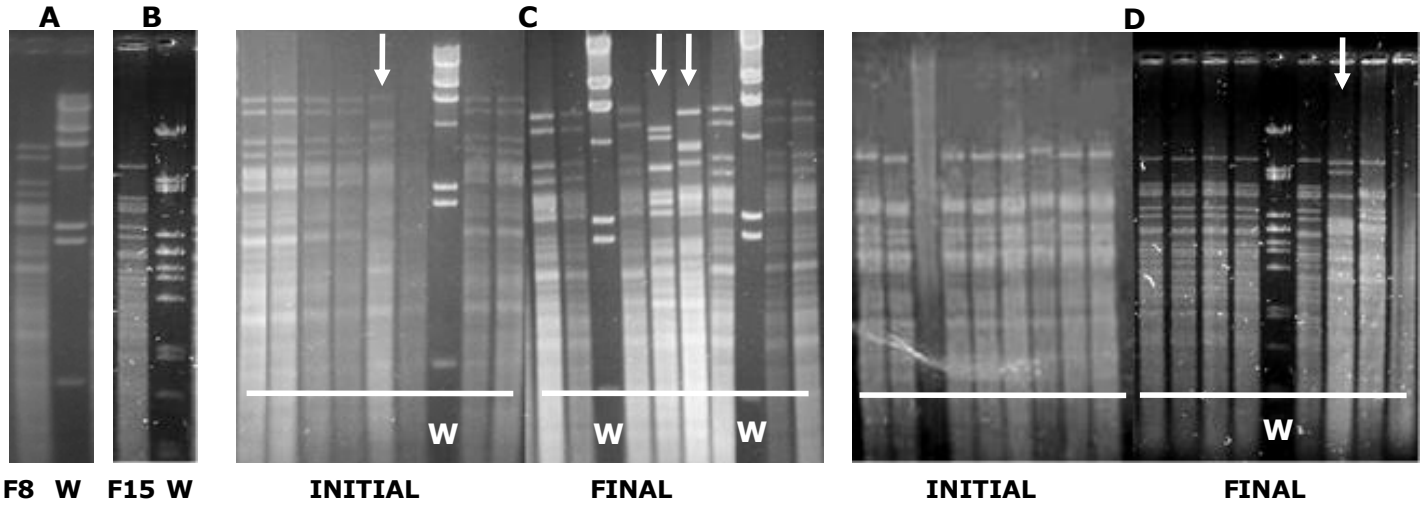

Figure 2: mtDNA-RFLP patterns of indigenous (A) and commercial (B) starters and of S. cerevisiae isolates obtained from F8 (C) and F15 (D) guided Pinot noir fermentations (2013 Vintage) at the initial and final stages. Arrows indicate isolates with mtDNA-RFLP patterns different from the inoculated starters. W = molecular weight marker.

On the other hand, physicochemical analysis of the wines obtained during different years from Pinot noir, Merlot and Malbec varieties are shown in table 1. Wine properties were highly similar between both inoculated strains, where every product was considered acceptable for local young wines. Nonetheless, sensorial analysis carried out by experts and consumers using qualitative and quantitative tests, respectively, showed significant differences between F8 and F15 wines.

Table 1: Enological features of Patagonian wines obtained from pilot scale fermentations with indigenous *S. cerevisiae* ÑNF8 (F8) and commercial *S. cerevisiae* F15 (F15) strains during 2012 to 2014 vintages.

Parameter	Malbec 2014 (n=1)		Merlot 2013 (n=2)		Pinot noir 2012 (n=2)	
	F8	F15	F8	F15	F8	F15
TRS (g/L)	0,90	2,50	1,25±0,50	2,30 ±1,10	6,40 ±3,81	8,94±2,30
Glucose (g/L)	0,10	1,23	Nd	0,57 ±0,80	0,55±0,71	Nd
Fructose (g/L)	1,03	1,3	1,55±0,35	1,77 ±0,25	6,85±2,69	8,77±2,02
pH	3,78	3,75	3,78±0,01	3,78 ±0,03	3,72±0,08	3,66±0,28
TA*	8,79	8,57	5,70±0,00	5,75 ±0,35	6,35±0,08	6,75±0,57
VA#	0,56	0,62	0,45±0,10	0,40 ±0,00	0,63±0,18	0,66±0,14
Ethanol (% v/v)	12,75	12,25	15,20±0,00	15,12±0,00	14,25±0,50	14,00±0,14
Glicerol (g/L)	8,47	6,68	8,65±0,15	10,10±0,24	11,35±2,76	11,30±1,70
L-Malic acid (g/L)	1,48	1,71	2,28±0,10	2,51±0,21	3,52±0,60	3,91±0,30
L-Lactic acid (g/L)	0,76	0,56	2,76±0,07	2,60±0,28	1,65±0,36	0,80±0,20

*Titratable acidity expressed as tartaricacid (g/L); #Volatile acidity expressed as acetic acid (g/L). Nd = not detected.

Qualitative analysis was performed by a panel of experts using descriptive tests. As a whole, the global quality scores obtained in this analysis by F8 Pinot noir (68 = good) and Merlot (6.6 = pleasant) wines were higher than those obtained by F15 Pinot noir (52 = correct) and Merlot (5.7 = slightly pleasant) wines. Particular descriptions evidenced that Pinot noir F8 wines had good color intensities (showing a red color typical for the variety) and aromas of red fruits (cherries) with notes of sherry. In mouth they were described as middling fruity, slightly rusty, sweet y alcoholic. Meanwhile, Pinot noir F15 wines showed limpid and bright aspect and an intense reduced aroma that did not disappear with agitation. In mouth, they were perceived as slightly fruity and bitter, astringent and tannic. On the other hand, both F8 and F15 Merlot wines showed a limpid and bright aspect and an intense brick red color but the F8 aroma was more intense than the F15 aroma, being both aromas of medium quality. Pepper, red fruits, butter, leather, spice and vanilla were the aromatic descriptors highlighted in the former and green pepper, cooked red fruits, spices and pepper were described for the later. In the mouth, both wines showed good acidity and body, and they were persistent. However, F8 wines were described as round and equilibrated while F15 wines showed a tart taste.

At last, F8 Pinot noir wines were the favorite for the consumers with 72 favorable responses out of 119 questioned ($p<0,05$), F8 Merlot wines were selected by 13 out of 17 consumers questioned and F8 Malbec wines were the favorite for 74 consumers out of 122 questioned ($p<0,05$). Polls were performed in the years 2013 and 2014, and compared in each case with their F15 controls according to the paired-preference test.

4. CONCLUSION

Results presented in this work show that S. cerevisiae ÑIF8-LVI strain drives red vinifications improving the quality of the local fermented products. This fact reassures

the oenological potential of the strain to formulate a regional starter culture for the production of well-balanced and physicochemical stable Patagonian young red wines.

5. REFERENCES

CABALLERO A. et al. **Viñedos y Bodegas del Neuquén, nuevas fuente de levaduras para el desarrollo de cultivos iniciadores regionales**. Informe final PFIP: Proyectos Federales de Innovación productiva. Agencia Promoción Científica y Técnica. MECyT Resol Nº 1028/04. 2008. Universidad Nacional del Comahue.

CATANIA C., AVAGNINA S. **La interpretación sensorial del vino**. INTA y Editora Andina Sur. 2010, Cap. III. El terruño argentine, p. 111-121.

DEGRÉ R. **Wine microbiology and biotechnology.** Fleet G ed., Harwood Academic Publishers, Switzerland, 1993, p. 421–447.

DEL MÓNACO S., BARDA N., RUBIO N., CABALLERO A. **Selection and characterization of a Patagonian *Pichia kudriavzevii* for wine deacidification**. J Appl Microbiol., 2014, vol. 117, p. 451-464.

ESTEVE-ZARZOSO B., BELLOCH C., URUBURU F., QUEROL A. **Identification of yeasts by RFLP analysis of the 5.8S rRNA gene and the two ribosomal internal transcribed spacers.** Int J Syst Bacteriol., 1999, vol. 49, p. 329–337.

FLEET, G. H. **Wine yeasts for the future.** FEMS. Yeast Res., 2008, vol. 8, p. 979-995.

LLORENTE, A. CASAZZA. M. El vino y su industria, 2005, vol.40, p. 7-13.

LOPES CA, VAN BROOCK M, QUEROL A, CABALLERO AC (2002) **Saccharomyces cerevisiae wine yeast populations in a cold region in Argentinean Patagonia**. A study at diverent fermentation scales. J Appl Microbiol 93:608–615

LOPES CA., RODRÍGUEZ M. E., SANGORRÍN M., QUEROL A., CABALLERO A. **Patagonian wines: implantation of an indigenous strain of Saccharomyces cerevisiae in fermentations conducted in traditional and modern cellars**. J Ind Microbiol Biotechnol 34: 139–149 (2007)

QUEROL A., RAMÓN D. **The application of molecular techniques in wine microbiology.** Trend Food Sci Technol., 1996, vol. 7, p. 73–78.

RAINIERI S., PRETORIUS I.S. **Selection and improvement of wine yeasts**. Ann. Microbiol., 2000, vol. 50, p. 15–30.

ROESSLER E. B., PANGBORN R. M., SIDEL J. L., STONE H., J. **Expanded statistical tables** for **estimating significance in paired-preference, paired-difference, duo-trio and triangle tests.** Food Sci., 1978, vol. 43, p. 940-947.

SANGORRÍN M., RODRIGUEZ, M.E., CABALLERO A. **Diversidad de levaduras nativas de la Región Vitivinícola Sur.** El vino y su industria, 2004, vol. 19, p. 56-68.