Evolution of Yeast Populations during Different Biodynamic Winemaking Processes

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This work was performed to evaluate the evolution of indigenous yeasts during wine productions carried out following the principles of biodynamic agriculture. Five trials were designed with different technological interventions consisting of the addition of nitrogen (in the form of ammonium salt), thiamine salt, oxygen, and pied de cuvée at varying concentrations. Yeasts were estimated by haemocytometer chamber and plate counts and identified by sequencing of the D1/D2 domain of the 26S rRNA gene. The isolates identified as *Saccharomyces cerevisiae* were found to dominate must fermentations and were genetically differentiated by interdelta sequence analysis (ISA). Several non-*Saccharomyces* species, in particular *Hanseniaspora* spp. and *Candida* spp., were found at subdominant levels during must fermentation. The trial added with both nitrogen and thiamine (NTV) showed the highest fermentation rate and microbial richness. The internal surfaces of the cellar equipment were characterised by a certain yeast biodiversity and hosted the species found during winemaking; the wooden surfaces represented the primary source of inoculation of a strain of *S. cerevisiae* found dominant in all winemaking trials.

INTRODUCTION

In recent years, the interest in wines with definite "terroir" has led to a rediscovery of fermentation performed by indigenous yeasts occurring on the grapes and/or in the winery (Le Jeune *et al*., 2006; Renouf *et al*., 2006; Francesca *et al*., 2010). Several authors have investigated the evolution of native yeasts during the alcoholic fermentation (AF), finding out that the first stage of spontaneous fermentation is characterised by a large microbial biodiversity derived from the vineyards and the cellar (Ciani *et al*., 2004; Le Jeune *et al*., 2006), but that *S. cerevisiae* is the dominant species at increasing ethanol concentrations (Zott *et al*., 2008). However, several factors, such as climate conditions, age of vineyards and oenological practices, may affect the composition of species and strains, influencing the quality of the resulting wines (Santamaria *et al*., 2005; Zott *et al*., 2008).

Biodynamic agriculture is a particular kind of organic farming that emphasises the interrelationship between soil, plants and animals as a self-nourishing system without external inputs (Lorand, 1996). Regarding grape cultivation and wine production, it excludes the use of chemicals agent and microbial starter cultures, in order to let the spontaneous microbiota drive the fermentations (Council Regulation EC, 2007).

The present work was aimed at evaluating the effect of some technological variables on the native yeasts during AF under the biodynamic regime. The dominant yeast strains were investigated to find their origin in the winery ecosystem.

MATERIALS AND METHODS

Wine production

Winemaking was carried out at the Azienda Agricola "Emidio Pepe" winery (Torano Nuovo, Abruzzo, Italy). Wines were made with grapes of the Trebbiano *cv.* cultivated according to the principles of biodynamic agriculture. Additions were limited to diammonium phosphate and diammonium sulphate (1:1) salts (AS) to integrate the nitrogen availability of the must.

Soft crushing was performed in a wooden crusher, after which the must was transferred to cement vats. Five different trials (Fig. 1) were carried out at 28 ± 2°C. Oxygenation of the trial NOV (nitrogen-oxygen vinification) was carried out by daily racking of 30% of the must from the second to the fourth day of fermentation. At the end of AF the wines were racked and transferred to stainless steel vats for spontaneous malolactic fermentation.

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Chemical and microbiological analysis

The chemical parameters of the must and wines were measured by FT-IR using a FOOS WineScan (FOSS, DK). The concentration of promptly assimilable nitrogen (PAN) was determined as described by Nicolini *et al*. (2004). Analysis of variance (ANOVA), elaborated with the program SAS 2004, version 9.1.2 (Statistical Analysis System Institute Inc., Cary, NC, USA), was used to evaluate differences among the vinifications. The significance level was set at $P < 0.05$. The advancement of alcoholic fermentation was monitored by measuring the decrease in must density with a hydrometer (HYDROMETER GmbH, Germany).

Fungal attack of the grapes was determined as described by Chellemi and Marois (1992). Five berries were collected from each bunch, forming a total of 1 000 berries (200 bunches) analysed per vineyard.

Microbiological analysis of the grapes (one week before harvesting), the bulk must (just after grape crushing), and the musts during the alcoholic fermentation (after 5, 50, and 90% sugar consumption) were performed as described by the OIV (2010). The cellar equipment was analysed as listed in the ISO (2004). Wallerstein Laboratory (WL) agar medium (Oxoid, Basingstoke, UK) and lysine agar (LA) (Oxoid), incubated at 25°C for four days, were employed in the enumeration of total and non-*Saccharomyces* yeasts respectively. Lactic acid bacteria (LAB) were enumerated onto tomato juice agar (TJA) (Fluka, D), while acetic acid bacteria (AAB) were enumerated onto Kneifel agar medium

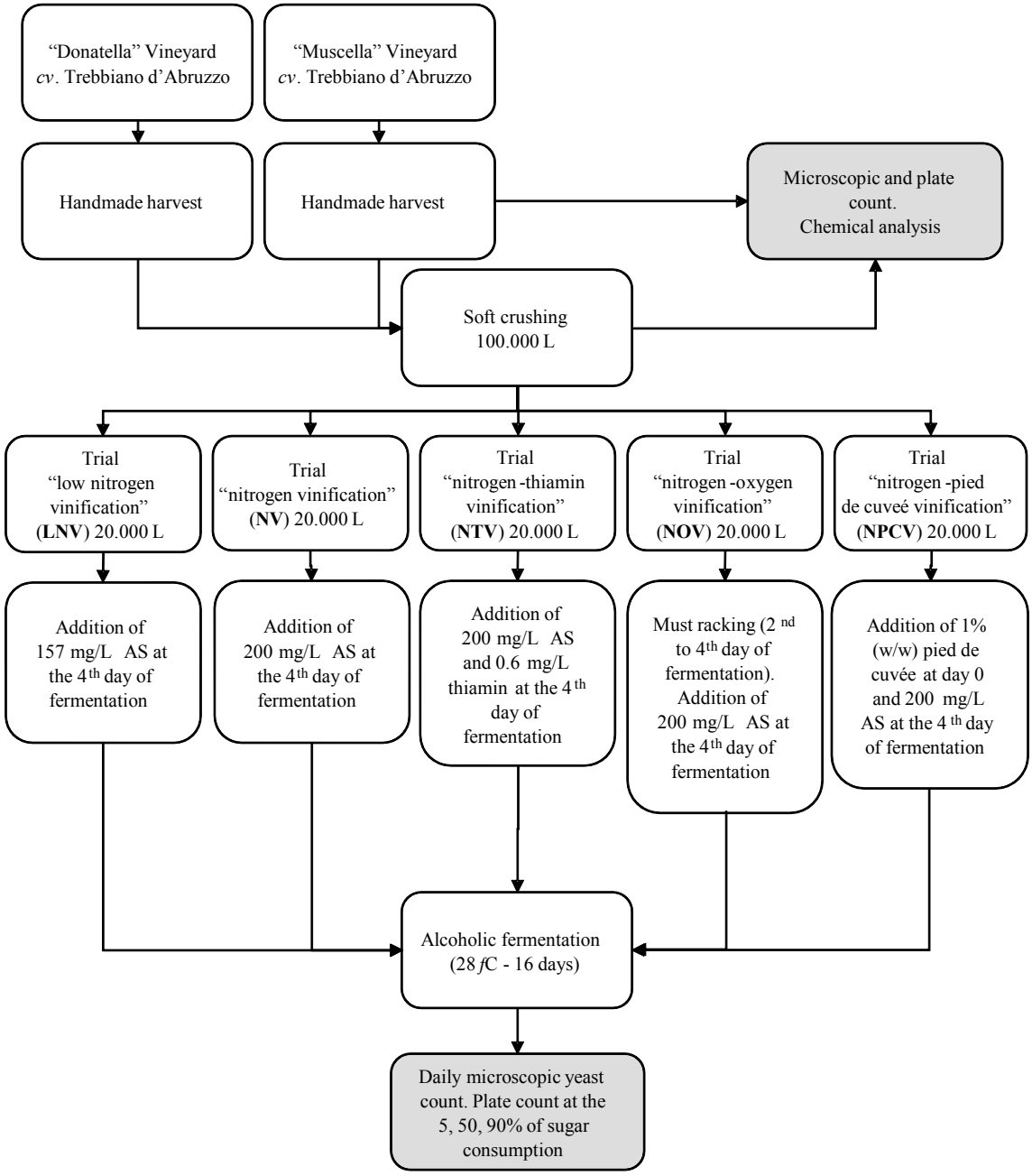

FIGURE 1
Flow diagram of winemaking processes.

(CAAR) (OIV, 2010), both incubated at 25°C for 10 days – the LAB in anaerobic conditions and the AAB in aerobic conditions. Analyses were carried out in duplicate.

Yeast evolution during AF was also monitored by microscopic counts using a Bürker chamber (OIV, 2010).

Identification of yeasts and genotypic differentiation of *Saccharomyces cerevisiae*

After growth and counting, at least three colonies with identical morphology were randomly chosen and purified to homogeneity. Total genomic DNA was extracted and purified as described by Querol *et al*. (1992). Sequencing of the D1/D2 domain of the large subunit 26S rRNA was carried out following the methodology of Kurtzman and Robnett (1998). The sequences (600-pb), obtained by MWG Biotech AG (Ebersberg, Germany), were compared with those available in the GenBank DNA database (http://www.ncbi.nlm.nih.gov/).

Typing of *S. cerevisiae* was performed by interdelta sequence analysis (ISA), as described by Legras and Karst (2003). Conversion, normalisation and analysis of the amplification profiles were carried out using the Fingerprinting II Informatix Software package (Bio-Rad, USA). Similarities between bands were assessed using the Pearson coefficient, and correlation coefficients were calculated by the unweighted pair group method with arithmetic averages (UPGMA).

RESULTS AND DISCUSSION

Chemical parameters of must and fermentation processes

The must was characterised by the following chemical composition: 19.0°Bx (corresponding approximately to 218 g/L of sugars); 6.65 ± 0.25 g/L TTA; 3.57 ± 0.18 g/L malic acid; < 0.20 g/L lactic acid; 4.24 ± 0.20 g/L tartaric acid; 21.5 ± 9.8 mg/L PAN; 2.81 ± 1.10 mg/L; pH was 3.25 ± 0.2. The only limiting factor for yeast growth (Bisson & Butzke, 2000) was PAN; the other parameters were not essential for development.

The advancement of AF is reported graphically in Fig. 2. No differences were observed among the five trials in the early stages of AF. From the fourth day of the AF trials LNV, NV and NTV, the decreases in density slowed down, suggesting the risk of a stuck fermentation due to the scarce amount of PAN in the musts. Trials NOV and NPCV showed more regular decreases in must density; however, according to the experimental plan, their PAN concentration also increased to 200 mg/L. After the additions (Fig. 1), all the other trials, with the exception of LNV, restarted sugar consumption, displaying similar fermentative behaviour. Regarding trial LNV, the addition of AS to 157 mg/L, which is considered to be the minimum concentration warranting a fermentation (Renouf *et al*., 2006), was not enough to speed up the process. After seven days, this trial was supplemented with 3% (v/v) of a vigorously fermenting must to avoid the loss of the entire LNV production.

Microbiological analysis

Vineyard A did not show significant fungal attacks, while vineyard B was characterised by some damaged berries, and thus grapes partially contaminated by *Botrytis cinerea* (grey mould) and *Erysiphe necator* (powdery mildew), even though a low incidence and negligible severity of the diseases were found.

The microbial population on the grape surface (Table 1) was in the range of concentration reported by other authors (Fleet, 1993; Fugelsang, 1997; Francesca *et al*., 2010, 2011).

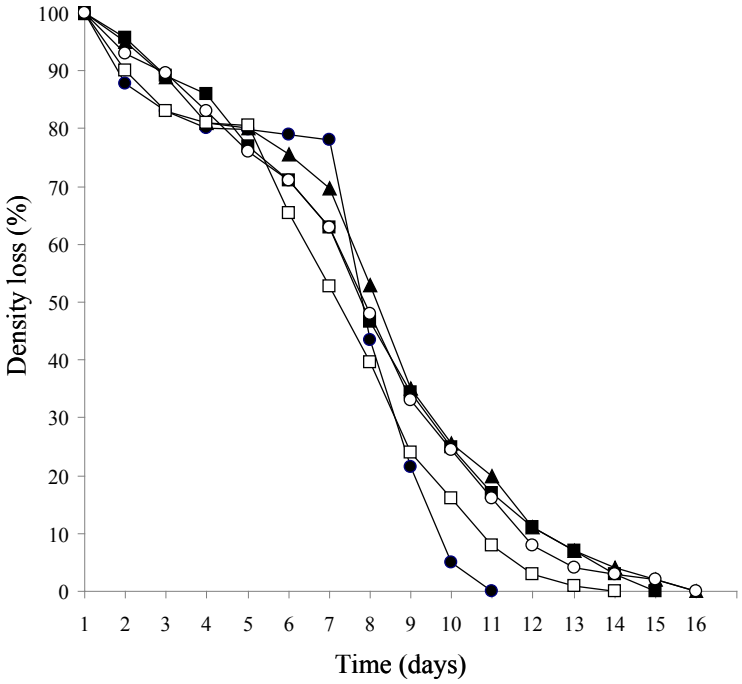

FIGURE 2

Advancement of alcoholic fermentation. Symbols: ●, low nitrogen vinification (LNV); ▲, nitrogen vinification (NV); □, nitrogen-thiamine vinification (NTV); ■, nitrogen-oxygen vinification (NOV); ○, nitrogen-pied de cuvée vinification (NPCV).

A higher number of total yeasts were observed on the surface of grapes from vineyard B, probably because of their worse sanitary state, which determined a higher sugar availability for microbial proliferation than in the grapes from vineyard A. No significant differences were found between yeasts counted on WL and LA, showing that the majority of yeasts did not belong to the *Saccharomyces* genus. Bacterial populations, in the same range of concentration as those reported by other authors (Fleet, 1993; Fugelsang, 1997; Francesca *et al*., 2010, 2011), constituted a limited portion of the microbial community. LAB were counted at one order of magnitude lower than yeasts in vineyard A, and AAB were not detectable.

The daily microscopic counts provided careful monitoring of yeast populations during AF (Fig. 3). In trial LNV, yeasts remained constant up to the sixth day; a sudden increase in the cell count was observed only after the addition of vigorously fermenting must (day 7). In trial NV, the addition of AS to 200 mg/L of PAN stimulated yeast growth up to 4×10^7 cell/mL, which concentration was reached on the eighth day. Trial NTV showed the fastest increase in yeasts, reaching a concentration of approximately 10^7 cell/mL in about two days. The cell concentration remained high during the whole fermentation process; only after 90% of the sugar had been consumed did the decrease become consistent. Trial NOV showed initial behaviour similar to trial NV, but the decrease in live cells started on the twelfth day. Trial NPCV proceeded similarly to trial NOV. Nitrogen supplementation in winemaking has been thoroughly investigated in species *S. cerevisiae*, since it is performed mainly to stimulate the development of this species. However, nitrogen cannot be added in big amounts because it allows the vigorous development of non-*Saccharomyces*, often unwanted, species (Bell & Henschke, 2005).

The plate counts (Table 1) confirmed the data acquired from microscopic counts. Regarding the composition of the yeast population, at 5% sugar consumption, non-*Saccharomyces* yeasts were at least 1 $\log_{10}$ unit lower than yeasts counted on WL, reaching three to four (or higher) orders of magnitude of difference at the end of fermentation for the majority of trials.

Among the two classical approaches used to reveal yeast concentration, the microscopic count showed the best results. It offered the possibility to retrieve, in real time, the order of magnitude of live yeasts, thus preventing the risk of stuck fermentation by being able to adopt the most appropriate solution quickly.

LAB and AAB were found at the end of AF. The trial showing the highest bacterial concentrations (both for LAB and AAB) was NTV. However, the bacteria did not exert any negative interaction on the yeasts, since no stuck fermentation was registered.

As shown in Table 1, all cellar equipment surfaces hosted yeasts in the order of 10^2 cfu/cm^2, with the highest

TABLE 1
Microbial concentrations[a] of grape, must and equipment surface samples[b]

Samples	Total yeasts	Non-*Saccharomyces* yeasts	LAB	AAB
Grapes A	$1.1 (\pm 0.3) \times 10^3$	$1.1 (\pm 0.2) \times 10^3$	$1.4 (\pm 0.3) \times 10^2$	$<5 \times 10$
Grapes B	$2.8 (\pm 0.3) \times 10^3$	$2.3 (\pm 0.4) \times 10^3$	$<5 \times 10$	$<5 \times 10$
Bulk must	$7.5 (\pm 0.7) \times 10^4$	$1.7 (\pm 0.5) \times 10^4$	n.d.	n.d.
Must LNV_5	$5.1 (\pm 0.3) \times 10^7$	$1.4 (\pm 0.4) \times 10^5$	n.d.	n.d.
Must NV_5	$1.6 (\pm 0.2) \times 10^7$	$1.4 (\pm 0.3) \times 10^5$	n.d.	n.d.
Must NTV_5	$2.1 (\pm 0.3) \times 10^7$	$4.6 (\pm 0.3) \times 10^5$	n.d.	n.d.
Must NOV_5	$1.6 (\pm 0.4) \times 10^7$	$1.7 (\pm 0.2) \times 10^6$	n.d.	n.d.
Must $NPCV_5$	$7.2 (\pm 0.4) \times 10^7$	$5.5 (\pm 0.5) \times 10^5$	n.d.	n.d.
Must LNV_{50}	$2.1 (\pm 0.4) \times 10^7$	$4.6 (\pm 0.5) \times 10^5$	n.d.	n.d.
Must NV_{50}	$4.2 (\pm 0.3) \times 10^7$	$1.1 (\pm 0.3) \times 10^4$	n.d.	n.d.
Must NTV_{50}	$9.6 (\pm 0.3) \times 10^7$	$1.8 (\pm 0.4) \times 10^4$	n.d.	n.d.
Must NOV_{50}	$8.0 (\pm 0.4) \times 10^7$	$1.1 (\pm 0.3) \times 10^5$	n.d.	n.d.
Must $NPCV_{50}$	$3.9 (\pm 0.3) \times 10^7$	$5.9 (\pm 0.3) \times 10^4$	n.d.	n.d.
Must LNV_{90}	$2.3 (\pm 0.4) \times 10^7$	$1.8 (\pm 0.2) \times 10^5$	$2.3 (\pm 0.3) \times 10^3$	$2.0 (\pm 0.2) \times 10^2$
Must NV_{90}	$2.7 (\pm 0.3) \times 10^7$	$<5 \times 10^3$	$2.8 (\pm 0.3) \times 10^3$	$1.8 (\pm 0.3) \times 10^2$
Must NTV_{90}	$6.8 (\pm 0.1) \times 10^7$	$<5 \times 10^3$	$1.6 (\pm 0.5) \times 10^5$	$1.5 (\pm 0.4) \times 10^3$
Must NOV_{90}	$7.6 (\pm 0.3) \times 10^7$	$1.8 (\pm 0.2) \times 10^4$	$6.0 (\pm 0.4) \times 10^4$	$5.0 (\pm 0.2) \times 10^3$
Must $NPCV_{90}$	$3.8 (\pm 0.2) \times 10^7$	$5.0 (\pm 0.1) \times 10^3$	$1.8 (\pm 0.3) \times 10^3$	$1.0 (\pm 0.2) \times 10^2$
Wooden crusher	$6.8 (\pm 0.2) \times 10^2$	n.d.	n.d.	n.d.
Wooden hand press	$1.1 (\pm 0.2) \times 10^2$	n.d.	n.d.	n.d.
Cement vat	$1.6 (\pm 0.4) \times 10^2$	n.d.	n.d.	n.d.

[a] $\log_{10}$ CFU/g for grapes, $\log_{10}$ CFU/mL for musts; $\log_{10}$ CFU/cm^2 for internal surfaces of cellar equipment.
[b] subscript numbers (5, 50 and 90) refer to the percentage of total carbohydrate consumption (5, 50 and 90% respectively).
LNV, low nitrogen vinification; NV, nitrogen vinification; NTV, nitrogen-thiamine vinification; NOV, nitrogen-oxygen vinification; NPCV, nitrogen pied de cuvée vinification; n.d., not determined.

counts (6.8×10^2 cfu/cm^2) registered inside the wooden vat used for grape crushing.

Chemical analysis of wines

The wine obtained with LNV was characterised by the highest concentration of total and free SO_2 (Table 2). This observation could be due to the highest concentration of non-*Saccharomyces* yeasts (Table 1) counted at 90% of sugar consumption. The high level of SO_2 could partially have inhibited the activity of bacteria; this vinification showed low concentrations of LAB and AAB. Volatile acidity was registered at the highest level for trial NOV, which showed the highest concentration of AAB. On the contrary, the lowest volatile acidity was found for trial NPCV, characterised by the lowest concentration of AAB. Lactic acid was more concentrated in trials NTV and NOV, which showed the highest levels of LAB.

SO_2 production by yeasts is variable and may reach high levels, which negatively influence malolactic fermentation, stopping it completely in the case of low pH values (Arnik & Henick-Kling, 2005). Generally, commercial starter yeasts produce low levels of SO_2, but this production by indigenous strains is unpredictable, thus representing one of the major risks of spontaneous wine fermentations.

Yeast identification

A total of 1 800 yeast colonies were collected from WL and 1 200 from LA, and all were identified. Two representative isolates of each group were subjected to genotypic identification. The results of analysis are reported in Table 3.

S. cerevisiae was present on the grape surface at low concentrations, confirming that this specie is not dominant in the vineyard (Clavijo *et al*., 2010; Shuang-Shi *et al*., 2010). In total, eight different species, belonging to seven genera, were recognised. The biodiversity of bulk must was lower than that of the grape surface: only *H. uvarum*, *P. guillermondii* and *S. cerevisiae* were identified. *S. cerevisiae* dominated the whole process of fermentation. According to previous works (Sipiczki, 2003; Tofalo *et al*., 2009), the non-*Saccharomyces* genera found most frequently during AF are *Candida* and *Hanseniaspora*. It is noteworthy that the species *C. jaroonii* (Imanishi *et al*., 2008) was found in wine AF for the first time. At the end of AF, another species, *Cr. victoriae*, which commonly is isolated from environments different to vineyards and cellars (Branda *et al*., 2010; Brandao *et al*., 2011), was found. Several species isolated in must were also found to be hosted on the surface of the fermentation vats and crusher. Among these, *C. boidinii, P. guilliermondii, H. uvarum* and *R. mucilagionsa* were the most frequent species. However, *S. cerevisiae* was clearly recognised. Regarding yeast populations associated with biodynamic wine production, very little has been published in the literature, since the studies on this topic have focussed mainly on the chemical characteristics of soil and grapes. However, an investigation conducted recently on the indigenous yeasts associated with organic vineyards in Spain (Cordero-Bueso *et al*., 2011) provided evidence for, besides *S. cerevisiae*, the presence of most of the species found in our study.

S. cerevisiae isolates were analysed by ISA, obtaining five main patterns (corresponding to five diverse strains) (Fig. 4). As reported in Table 3, ISA3 was the main profile found during the whole winemaking process in all five trials. Furthermore, since the same band pattern was also showed by the isolates present on the internal surfaces of the winery equipment, it can be stated that the strain characterised by the ISA3 profile colonised the wine production environment.

CONCLUSIONS

Different conclusions may be drafted: a moderate addition of nitrogen is of paramount importance to ensure the fermentation of must under a biodynamic regime; oxygen provided encouraging results; the addition of pied de cuvée, together with nitrogen, provided the most interesting data, since the fermenting must is already adapted to the process conditions and the corresponding trial showed the lowest level of AAB. Overall, the main finding of this work is

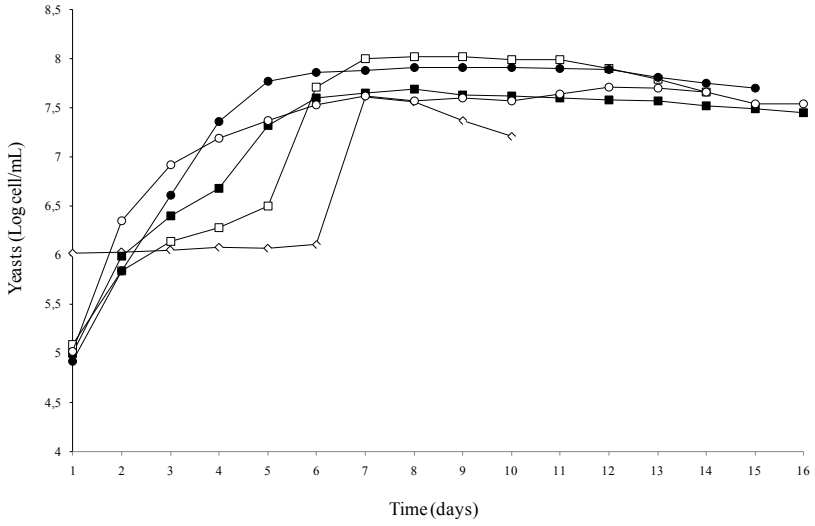

FIGURE 3

Yeast cell concentration determined by haemocytometer chamber. Symbols: ◊, low nitrogen vinification (LNV); ■, nitrogen vinification (NV); □, nitrogen-thiamin viinification (NTV); ●, nitrogen-oxygen vinification (NOV); ○, nitrogen-pied de cuvée vinification (NPCV).

TABLE 2
Chemical parameters[a] of wines

Parameters:	LNV	NV	NTV	NOV	NPCV
pH	3.40 ± 0.01	3.41 ± 0.01	3.58 ± 0.01	3.52 ± 0.01	3.43 ± 0.01
TTA (g/L tartaric acid)	5.49 ± 0.02	5.65 ± 0.03	5.94 ± 0.01	5.88 ± 0.02	5.71 ± 0.02
Tartaric acid (g/L)	2.14 ± 0.01	2.27 ± 0.01	2.39 ± 0.03	2.31 ± 0.03	2.33 ± 0.04
VA (g/L acetic acid)	0.68 ± 0.01	0.64 ± 0.01	0.71 ± 0.02	0.79 ± 0.01	0.61 ± 0.02
Alcohol (% v/v)	13.1 ± 0.1	13.0 ± 0.1	12.9 ± 0.1	13.0 ± 0. 1	12.9 ± 0.1
Reducing sugars (g/L)	<1.0	<1.0	<1.0	<1.0	<1.0
Total SO_2 (mg/L)	74.41 ± 0.24	47.98 ± 0.15	38.70 ± 0.12	49.64 ± 0.16	55.14 ± 0.35
Free SO_2 (mg/L)	23.50 ± 0.06	8.03 ± 0.03	6.14 ± 0.06	8.31 ± 0.03	11.25 ± 0.06
Malic acid (g/L)	1.20 ± 0.01	0.71 ± 0.01	0.38 ± 0.02	0.45 ± 0.02	0.84 ± 0.01
Lactic acid (g/L)	0.88 ± 0.01	1.15 ± 0.02	2.10 ± 0.01	1.90 ± 0.01	1.38 ± 0.02

[a] results indicate mean value ± S.D. of three independent measurements.
LNV, low nitrogen vinification; NV, nitrogen vinification; NTV, nitrogen-thiamine vinification; NOV, nitrogen-oxygen vinification; NPCV, nitrogen pied de cuvée vinification; nd, not detected (value < detection limit of method).

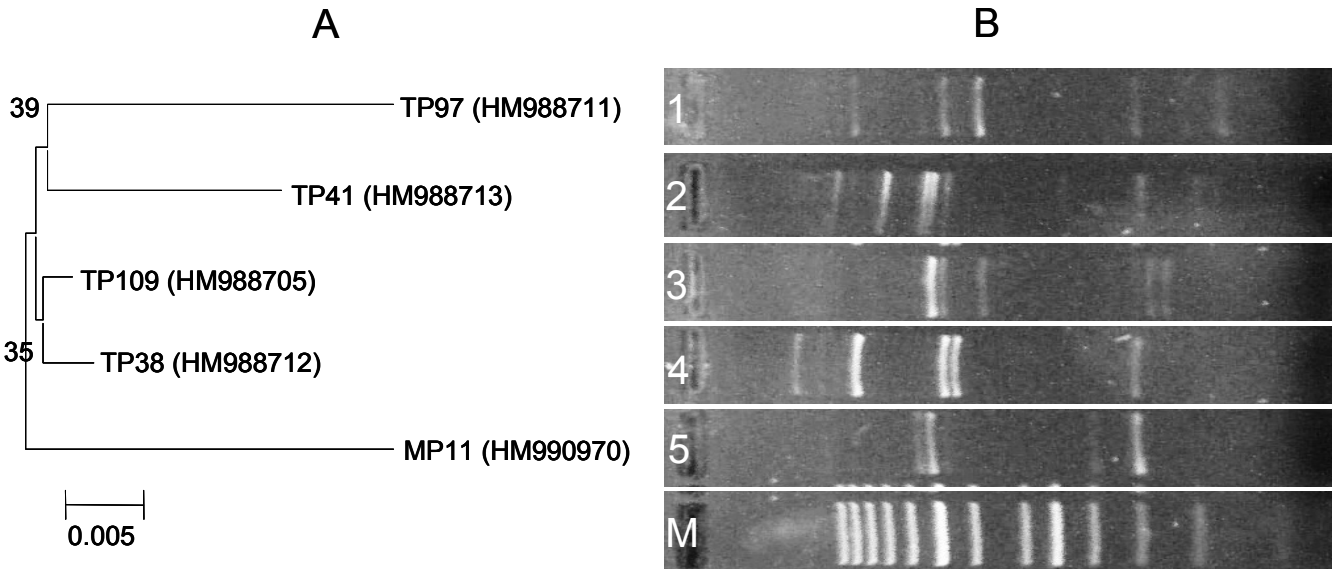

FIGURE 4
Genotypic characterisation of *S. cerevisiae* strains. **A**, phylogram based on the partial 26S rRNA gene sequences. Sequence alignment was performed with the CLUSTALX program (Thompson *et al*., 1997). Sequence and alignment manipulations were performed with GeneDoc program version 2.5.000 (K.B. Nicholas and H.B. Nicholas, unpublished data). Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 3.1 (Kumar *et al*., 2004). Bar, 0.005 nucleotide substitution per site. **B**, ISA patterns; M, molecular marker.

TABLE 3
Yeast species identified from Trebbiano grapes and musts during biodynamic wine productions

Species	Source of isolation	GenBank Acc. No.	ISA profile
C. boidinii	Cement vat	HM988725	–
C. diversa	Grapes B	HM988691	–
C. friedrichii	Must LNV_{90}	HM988724	–
C. ishiwadae	Bulk must	HM988700	–
	Must $NPCV_{50}$	HM988722	–
C. jaroonii	Must NV_{90}	HM988719	–

TABLE 3 (CONTINUED)

Species	Source of isolation	GenBank Acc. No.	ISA profile
C. zemplinina	Must NV_5	HM988714	–
	Must NTV_5	HM988707	–
	Must NV_{50}	HM988717	–
	Must NTV_{50}	HM988718	–
Cr. victoriae	Must NOV_{90}	HM988710	–
D. hansenii	Grapes A	HM988685	–
	Grapes B	HM988696	–
	Must NOV_{50}	HM988708	–
H. uvarum	Grapes A	HM854034	–
	Grapes B	HM988692	–
	Bulk must	HM988697	–
	Must LNV_5	HM988683	–
	Must NV_5	HM988687	–
	Must NOV_5	HM988693	–
	Must $NPCV_5$	HM988968	–
	Must LNV_{50}	HM988701	–
	Must NV_{50}	HM988703	–
	Must NTV_{50}	HM988704	–
	Must NOV_{50}	HM988716	–
	Wooden hand press	HM988721	–
	Cement vat	HM988723	–
I. occidentalis	Grapes A	HM988684	–
I. terricola	Grapes A	HM988682	–
M. pulcherrima	Must NTV_5	HM988706	–
P. guillermondii	Grapes B	HM988686	–
	Bulk must	HM988699	–
	Wooden vat	HM988720	–
P. membranifaciens	Must $NPCV_{90}$	HM988709	–
R. mucilaginosa	Grapes A	HM988688	–
	Grapes B	HM988694	–
	Wooden vat	HM988695	–
	Wooden hand press	HM988689	–
	Cement vat	HM988690	–
S. cerevisiae	Must NOV_5	–	1
	Must NTV_{90}	–	1
	Must NOV_{90}	HM988711	1
	Must $NPCV_{90}$	–	1
	Must LNV_{90}	–	2
	Must NV_{90}	HM988713	2
	Must NTV_{90}	–	2
	Bulk must	HM988705	3
	Must LNV_5	–	3
	Must NV_5	–	3
	Must NTV_5	–	3
	Must NOV_5	–	3
	Must $NPCV_5$	–	3
	Must LNV_{50}	–	3
	Must NV_{50}	–	3
	Must NTV_{50}	–	3
	Must NOV_{50}	–	3
	Must $NPCV_{50}$	–	3
	Must LNV_{90}	–	3
	Must NV_{90}	–	3
	Must NOV_{90}	–	3
	Wooden vat	–	3

TABLE 3 (CONTINUED)

Species	Source of isolation	GenBank Acc. No.	ISA profile
S. cerevisiae	Wooden hand press	–	3
	Cement vat	–	3
	Grapes B	–	4
	Must LNV_{90}	HM988712	4
	Must NTV_{90}	–	4
	Must $NPCV_{90}$	–	4
	Grapes A	HM990970	5
	Must LNV_{5}	–	5
	Must NV_{50}	–	5
	Must NTV_{50}	–	5
	Must NOV_{50}	–	5
T. delbrueckii	Bulk must	HM988702	–
Z. meyerae	Must $NPCV_{5}$	HM988715	–

LNV, low nitrogen vinification; NV, nitrogen vinification; NTV, nitrogen-thiamine vinification; NOV, nitrogen-oxygen vinification; NPCV, nitrogen pied de cuvée vinification.

that the cellar equipment is responsible for the inoculation of a *S. cerevisiae* strain that becomes dominant during the fermentation process, independently of the technological intervention applied. This strain is highly adapted to the cellar conditions of the winery investigated and it easily develops in the bulk must, thus it may be considered an *ad hoc* starter for that winery.

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