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ORIGINAL PAPER

Characterization of natural *Oenococcus oeni* strains for Montepulciano d'Abruzzo organic wine production

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Abstract

Montepulciano d'Abruzzo is a red wine grape variety of *Vitis vinifera* L., grown in Central Italy. It is mainly identified with Abruzzo region, where it currently accounts for around 50% of the regional vineyard. Malolactic fermentation (MLF) has a crucial role in red wines giving microbial stabilization, biological deacidification through the decarboxylation of L-malic acid to L(+)-lactic acid and carbon dioxide, and increasing complexity of wine aroma. Studies are focusing on the selection of yeast starter cultures for this wine, while few studies are available on malolactic bacteria. Therefore, a technological (ability to grow up at different pH, concentration of SO_2, ethanol, presence of *hdc*, *tdc* and *odc* genes, conversion of malic acid into lactic acid) and genetic characterization of autochthonous *Oenococcus oeni* strains was performed. Moreover, *O. oeni* strain with the best traits was selected and produced by a local starter industry and used in cellar to produce Montepulciano d'Abruzzo organic wine without added SO_2. Obtained wines not only maintained the typical traits of Montepulciano d'Abruzzo wines but also showed healthy characteristics since wines were histamine free. Selected starter is actually produced and dispensed on demand and in a frozen concentrate culture for wineries.

Keywords *Oenococcus oeni* · Organic wine · Biogenic amines · Montepulcianod'Abruzzo

Introduction

Abruzzo is one of the most important Italian regions for wine production and Montepulciano d'Abruzzo is one of the highest quality wines produced. It is obtained from a red wine grape variety of *Vitis vinifera* L. and it has been cultivated for over two centuries. Nowadays, its production extends for around 50% of the regional vineyard (18.500 ha) (Regione Abruzzo, https://www.regione.abruzzo.it/). In 2003, Colline Teramane Montepulciano d'Abruzzo wine gained its DOCG (Designation of Controlled and Guaranteed Origin) recognition. The importance of Montepulciano d'Abruzzo wine is recognized worldwide, but the knowledge of its oenological characteristics needs further investigation. Studies on *Saccharomyces* and non-*Saccharomyces* yeasts underlined the importance of autochthonous strains to shape Montepulciano d'Abruzzo wine aroma profile [1–4]. Yeasts are the principal actors of alcoholic fermentation (AF) which is crucial in vinification, and lactic acid bacteria (LAB) are responsible for malolactic fermentation (MLF).

MLF has a huge role, especially in red wines giving microbial stabilization, biological deacidification by decarboxylation of L-malic acid to L(+)-lactic acid and carbon dioxide, and increasing complexity of wine aroma and organoleptic properties [5, 6]. Aside from impact on acidity, LAB can also metabolize other precursors present in wine during fermentation and affect the final product [7]. MLF is carried out by LAB, mainly belonging to *Lactobacillus*, *Pediococcus*, *Leuconostoc*, and *Oenococcus* genera. During spontaneous MLF, *Oenococcus oeni* is the major bacterial species found in wines, due to its ability to grow in harsh wine conditions such as low pH, high ethanol and SO_2 concentrations, low nutrients, and low temperatures [6]. The ability of the cells to survive and grow under wine

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environment depends on the physicochemical intrinsic properties of wine and winemaking practices [8].

In specific conditions, spontaneous MLF is often long (many months after alcoholic fermentation), unpredictable and hazardous [6]. In fact, natural LAB could have undesirable effects on wine quality due to the increasing of volatile acidity, the formation of metabolites that can affect human health such as biogenic amines (BAs), and the production of off-flavours [9].

Nowadays, use of malolactic starter cultures has become a common winemaking practice to promote a reliable and rapid MLF in wines produced from different grape varieties [9]. The use of the same commercial bacterial starters worldwide could affect the different properties that characterize typical regional wines [9]. For this reason, the application of a starter culture well adapted to the conditions of a specific wine-producing area has been proposed [10]. Several studies have been performed on *O. oeni* biodiversity with the aim of selecting autochthonous starter cultures within a specific region or grape variety from different countries (Australia, China, France, Germany, Greece, Italy, Portugal, Spain) [11–13]. In this context, it is essential to consider the compatibility *O. oeni*/yeast as some yeast strains can have inhibitory, neutral and stimulatory effects on LAB growth. Moreover, strain compatibility is different between yeast–LAB co-inoculation and sequential inoculation (LAB inoculated after AF) procedures [14, 15].

Recently, many studies focused on *Saccharomyces* and non-*Saccharomyces* populations associated with spontaneous grape must fermentation, to be used as starters to produce Montepulciano d'Abruzzo wines with peculiar/typical flavour [2].

Conversely, an ad hoc starter for malolactic Montepulciano d'Abruzzo fermentation is still lacking. For this reason, the aim of this research was to select tailored *O. oeni* strains to improve Montepulciano d'Abruzzo wine production.

Materials and methods

Strain isolation and growth conditions

Oenococcus oeni strains were isolated from 12 Montepulciano d'Abruzzo organic wines obtained with grapes from two different vineyards in a cellar located in Orsogna (Chieti, Abruzzo, Central Italy) where wine is produced based on spontaneous fermentation and without any commercial preparations. Strains were isolated using MRS medium supplemented with fructose (5 g/L), malic acid (6 g/L) and cysteine (0.5 g/L) at pH 4.8 [16]. Higher dilutions were used to isolate colonies to increase the probability to pick up strains belonging to the dominant species [1, 17, 18]. Plates were incubated at 28 °C under anaerobic conditions for 7 days. Isolates were identified as putative *O. oeni* by positive Gram staining and negative catalase assay. Gram-positive cocci and catalase-negative bacteria were purified and stored in MRS supplemented with glycerol (20% v/v) (Sigma-Aldrich Srl, Milan, Italy) at − 80 °C. Strains belong to the Culture Collection of the Faculty of BioScience and Technology for Food, Agriculture and Environment (University of Teramo).

Identification of malolactic bacteria and 16S rRNA gene amplification

Genomic DNA was extracted with InstaGene matrix (Bio-Rad, Milan, Italy) according to the manufacturer's instructions. Isolates were identified according to Zapparoli et al. [19] using the following primer pairs: On1 (5′-TAATGTGGTTCTTGAGGAGAAAAT-3′) and On2 (5′-ATCATCGTCAAACAAGAGGCCTT-3′); On3 (5′-AATATTCAATACGAATCACG-3′) and On4 (5′-GATTCCAGTTCCTTGAATA-3′). Bacterial assignment species was also assessed by 16S rRNA gene sequence analysis using Lac16S-for (5′-AATGAGAGTTTGATCCTGGCT-3′) and Lac16S-rev (5′-GAGGTGATCCAGCCGCAGGTT-3′) primer set [20]. Amplified fragments were purified using QIAquick Purification Kit (Qiagen), according to the manufacturer's instructions and delivered to BMR Genomics (Padua University, Padua, Italy) for sequencing. The sequences obtained in FASTA format were compared with those deposited in GenBank DNA database (https://www.ncbi.nlm.nih.gov/) using the basic BLAST search tools [21].

RAPD-PCR

Strain fingerprinting was carried out by RAPD-PCR using M13 primer (5′-GAGGGTGGCGGTTCT-3′) as previously described [22]. Amplification was performed on a Perkin-Elmer GeneAmpPCR System 2400 with an initial denaturation at 94 °C for 4 min followed by 35 cycles consisting of 30 s at 94 °C, 20 s at 45 °C, 2 min at 72 °C and a final extension of 7 min at 72 °C. The repeatability of RAPD-PCR fingerprints was determined by triplicate loading of independent triplicate reaction mixtures prepared with the same strain and the repeatability of the assay was 95%. Conversion, normalization, and further analysis of the RAPD-PCR patterns were carried out with Fingerprinting II Informatix™ software program (Bio-Rad). Similarities among profiles were calculated by clustering the Pearson's *r* correlation matrix using the Unweighted Pair-Group Method with Average (UPGMA) algorithm.

Evaluation of L-malic acid consumption

Laboratory-scale fermentations were conducted in triplicate under static conditions at 25 °C in sterile Erlenmeyer 2-L flasks. Each flask contained 1 L of pasteurized organic Montepulciano d'Abruzzo must (sugar 235 g/L, malic acid 2.09 g/L, lactic acid 0.02 g/L, 7.91 titratable acidity (TTA) and pH 3.38) and were inoculated with a commercial yeast strain AV0 (Dalton Biotecnologie, Srl) according to the manufacturer's instructions. After 24 h, malolactic bacteria were inoculated at a final concentration of 10^7 CFU/mL [23]. Fermentations kinetics were monitored by gravimetric determinations, evaluating the loss of weight due to the production of CO_2. When samples reached constant weight, they were stored at − 20 °C for chemical analysis. When CO_2 evolution stopped (i.e. at constant weight) fermentation were considered ended. The dynamics of the MLF fermentation was monitored using an HPLC 200 series (Perkin Elmer, Monza, Italy) connected to a UV–Vis detector at 210 nm. ROA Organic Acid H^+ column (Phenomenex, Bologna, Italy) was used for the analyses. All determinations were performed isocratically with a flow rate of 0.4 mL/min at 25 °C using H_2SO_4 solution 0.009 N as the mobile phase [24].

pH, ethanol and SO_2 tolerance determination

Strains (10^6 CFU/mL) were inoculated in a synthetic wine (4 g/L yeast extract, 2 g/L glycerol, 6 g/L malic acid, ethanol 10%, pH adjusted to 4) as previously described [25]. The synthetic wine was modified to test different conditions: pH (3 and 3.6), ethanol (10 and 15% v/v) and SO_2 (10 and 50 ppm) [26–29]. Microbial growth was evaluated by a spectrophotometer (Lambda Bio 20, Perkin-Elmer, Waltham, MA, USA) at 600 nm for 7 days.

Evaluation of strains' decarboxylation activity potential

The presence of tyrosine decarboxylase (*tdc*), histidine decarboxylase (*hdc*) and ornithine decarboxylase (*odc*) was performed according to Torriani et al. [30], Coton and Coton [31], Bonnin-Jusserand et al. [32], respectively. Amplification was performed on a Perkin-Elmer GeneAmpPCR System 2400. PCR conditions and primer sets are reported in Table 1.

Cellar vinifications

MALOBACT-T1 biomass production was carried out at 26 °C, at constant pH (pH 5) by Dalton Biotecnologie Srl according to the industrial protocol. The above conditions were constantly monitored and regulated. The biomasses produced were aseptically taken from the fermenter and kept at 4 °C. Biomass viability was determined at weekly intervals, by taking a sample under sterile conditions and count by plating on MRS agar medium. Maximum reached cell concentration in the culture was 10^9 CFU/mL. Biomass was harvested by centrifugation and cross flow microfiltration reaching a final concentration of 10^{10} CFU/mL. Harvested cells were stabilized by cryogenic pelletizing in liquid nitrogen, a specific cryoprotectant was added to enhance cells resistance to freezing, measured survival rate 1 day after freezing was 68%, after 3 months, at −40 °C, cell survival loss was lower than 10%. Vinifications were carried out in a local winery in Chieti province. Montepulciano d'Abruzzo must (256 g/L fermentable sugars, malic acid 2.11 g/L, 7.81 titratable acidity (TTA) and pH 3.47) was separated in tanks of 50 L. Vinifications were carried out in triplicate according to Montepulciano d'Abruzzo winemaking procedures at room temperature (maximum temperature variation from 8 to 18 °C). *Saccharomyces cerevisiae* strain AV0 was inoculated at a final concentration of 10^6 CFU/mL and after 24 h, the malolactic starter was added at different concentrations: 40 g/hL and 80 g/hL; one batch was not inoculated and used as control (CTR). The dynamics of the MLF fermentation was monitored evaluating the sugar consumption and the transformation of malic acid in lactic acid by Fourier transform infrared spectroscopy (FTIR), employing the WineScan Flex (FOSS Analytical, DK). Biogenic amines were determined according to Tofalo et al. [2], using an HPLC system (Waters, Milford, MA, USA), equipped with a Waters 2695 separation module connected to a Waters 2996 photodiode array detector (PDA), set at

Table 1 PCR amplification conditions and primer sets used in this study

Primer	Sequence (5′–3′)	Conditions
Tyr3	CGTACACATTCAGTTGCATGGCAT	94 °C for 5 min, 35 cycles at 94 °C for 20 s, 58 °C for 30 s, 72 °C for 45 s
Tyr4	ATGTCCTACTTCTTCTTCCATTTG	
Hdc3	GATGGTATTGTTTCKTATGA	95 °C for 5 min, 32 cycles at 95 °C for 45 s, 52 °C for 45 s, 72 °C for 1 min
Hdc4	CAAACACCAGCATCTTC	
ODCV1	AATAAGAGTTTAC ATTGGGGAA	95 °C for 5 min, 35 cycles at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 2 min
ODCV3	TGAGTTTCTGCAGGTGTCATT	

254 nm. A Supelcosil LC-18 column (5 µm particle size, 250 × 4.6 mm i.d.) from Sigma was used. The system was governed by Waters Empower personal computer software. All analyses were performed in triplicate.

Results and discussion

The selection of efficient malolactic starter cultures is one of the main challenges for oenological research [33–35]. In particular—as for yeasts—the use of autochthonous malolactic starter culture, well adapted to the conditions of a specific wine-producing area, has been suggested since it can maintain regional typicity of wines [36]. The development of autochthonous starter cultures for wine fermentation implies the study and the characterization of distinctive features focusing on traits for their commercialization. It is essential to identify oenological and genetic differences. For malolactic bacteria there are some strain-dependent characteristics which should be evaluated such as L-malic acid consumption rate, the ability to survive in harsh conditions and the presence of enzymatic activities involved in aroma compounds release [11, 26, 28, 29, 37].

Identification and typing

To study *O. oeni* in Montepulciano d'Abruzzo wine environment, bacterial population from organic wines undergoing spontaneous MLF was isolated by plating wine onto a modified MRS medium. Dominant bacterial populations were isolated from the highest dilution plates. Cell counts ranged from 10^5 to 10^7 CFU/mL in agreement with previous studies [38]. Ten putative *O. oeni* strains (Gram positive, catalase negative and cocci shaped) were identified using species-specific primers designed on a conserved region of the malolactic enzyme [19]. For nine out of ten isolates, a PCR fragment of 653 bp was obtained indicating that they belonged to *O. oeni* species. Only ISO345 strain gave a negative result and was subjected to the sequence analysis of the 16S rRNA gene and was identified as *Pediococcus parvulus* with a similarity level of 100%. The results of a general fingerprinting of strains is shown in Fig. 1. M13 RAPD-PCR was applied since it has already been proven useful to typing indigenous bacterial strains [11, 39, 40]. Clusters were arbitrarily identified at a similarity level of 90%. Four different clusters were obtained. Cluster I was made up of three strains (ISO349, ISO351, ISO350), cluster II contained only a strain (MALOBACT-T1), and clusters III and IV were formed by 3 (ISO359, ISO360, ISO358), and 2 (ISO344, ISO352) strains, respectively.

Technological characterization

The success of MLF depends on the ability of *O. oeni* to face the stressful environment of wines (low pH, high ethanol and SO_2 content, presence of inhibitory compounds, etc.). *O. oeni* adaptability is related to its genome plasticity which is associated with its fitness [41, 42]. The ability of natural tested strains to tolerate oenological stresses in terms of ethanol (10 and 15% v/v), SO_2 (10 and 50 ppm) and pH (3 and 3.6) was evaluated. All strains showed an acceptable survival at pH 3.6. MALOBACT-T1, ISO358, ISO350, and ISO359 strains were able to survive at pH 3.0. The presence of SO_2 was selective for the majority of strains: none of them was able to survive at 50 ppm and only two of them (ISO351 and MALOBACT-T1) survived in the presence of concentrations of 10 ppm. To guarantee a correct outcome of MLF, strains should be ethanol resistant, since this compound negatively impacts *O. oeni* membrane causing a delay of MLF [43, 44]. As

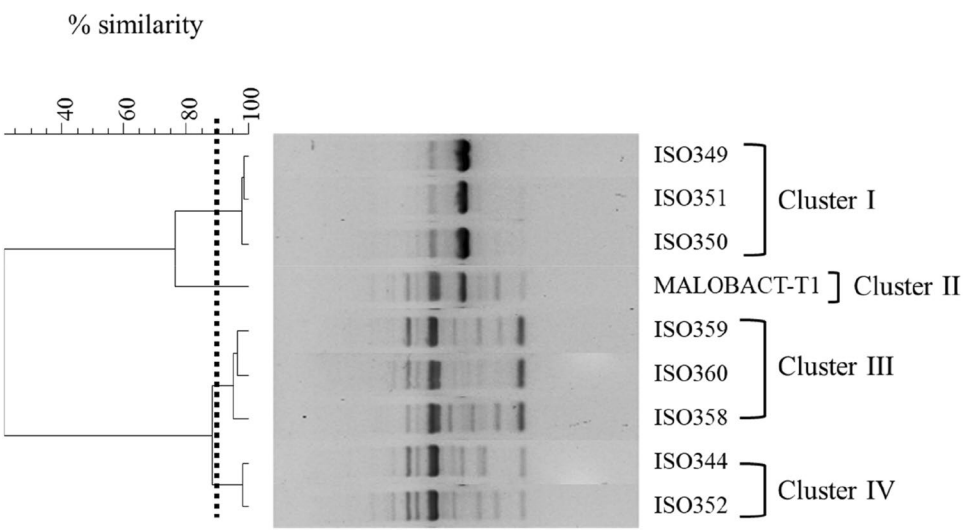

Fig. 1 Dendrogram showing the similarity among RAPD-PCR patterns. Similarities were calculated using UPGMA

expected, all the strains grew at a concentration of 10% (v/v) ethanol, with the only exception of ISO349. Five of them were able to resist also at 15% (v/v) ethanol (MALOBACT-T1, ISO351, ISO350, ISO359, ISO360). Regarding the other strains, even though they were isolated from an environment with a concentration of ethanol > 13% v/v, they were unable to tolerate high ethanol concentration. It is probably due to the lack of phenolic compounds and specific nutrients in the synthetic wine used in this study in agreement with previous observations [29, 33]. Also, pH is essential to determine the success of MLF. In general, it occurs without problems in wines with a pH of about 3.3, in fact, malolactic activity is higher at pH 3.5–4.0 [45]. Sulphur dioxide is generally added at the beginning of fermentation process to control the development of non-*Saccharomyces* yeasts and bacteria [45, 46] which showed that *O. oeni* strains are generally characterized by a tolerance to sulphite of 30 mg/L. An increased sulphite tolerance is observed when cells are adapted to low pH and a sub-lethal concentration of sulphite (15 mg/L) is added during the adaptation step in acidic medium (pH 3.5) [46].

To evaluate the malolactic ability of *O. oeni* strains, microvinification was performed in Montepulciano d'Abruzzo musts and monitored for 20 days. MALOBACT-T1, ISO358 and ISO360 consumed all the malic acid releasing the highest concentrations of lactic acid (1.3 g/L for ISO358 and ISO360 and 1.45 g/L for MALOBACT-T1). A lower consumption was observed for the other strains with residual concentrations ranging from 0.12 g/L (ISO352) to 0.55 g/L (ISO351) at the end of fermentation (Fig. 2). The decrease of malic acid and the subsequent increase of lactic acid positively impact wine flavour through the reduction of titratable acidity and making a smoother mouthfeel [47]. Malate metabolism is a strain-dependent trait and could be beneficial for bacterial survival in wine [6]. Obtained data agree with this observation, in fact, MALOBACT-T1 not only showed a faster malic acid degradation rate but also the best tolerance to the stressing conditions tested. From a technological point of view, this strain showed the best performances. Its different behaviour is in line with the genetic results; in fact, it was well differentiated from the other strains. Similar results were

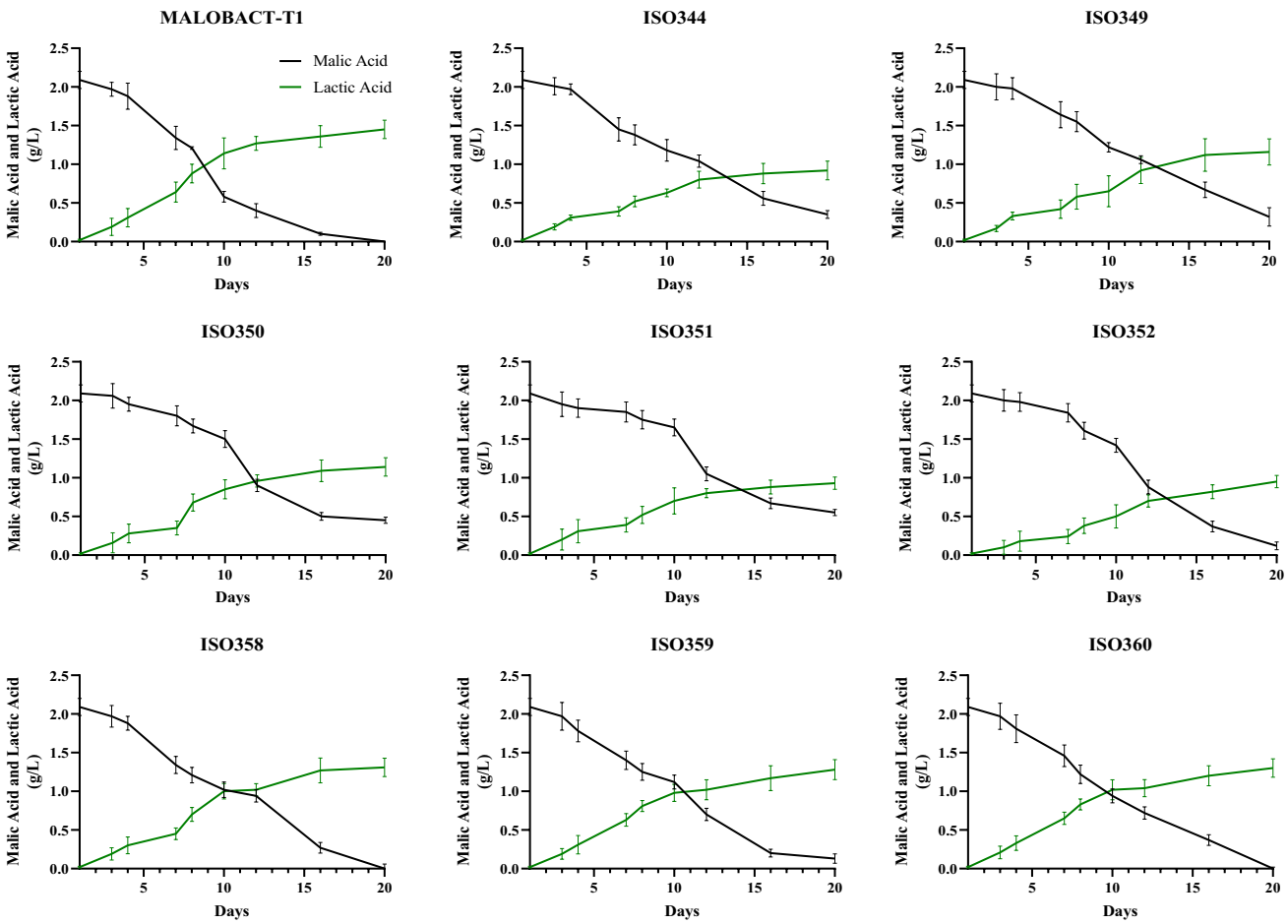

Fig. 2 Malic acid degradation and lactic acid production for all nine *O. oeni* strains

observed by Guerrini et al. [48] and Delaherche et al. [49] who found a correlation between phenotypic and genotypic traits of *O. oeni* strains of different origin.

Biogenic amine gene detection

Strains were characterized for their ability to produce histamine and tyramine at genetic level. All strains were unable to synthetize these two BAs since *hcd* and *tdc* genes were absent. The occurrence of *O. oeni* histamine-non-producing strains has been reported [50]. However, other studies showed the presence of producing strains [51] highlighting the strain dependence of this character. These contradictory results are probably due to the presence of an instable 100 kb plasmid in some strains [52] but this hypothesis is not confirmed yet. Concerning tyramine, the majority of studies reported that *O. oeni*-producing strains are rare [51]. This feature is of great interest for oenological industries in terms of human health and should be included as a selective criterion for starter cultures [53].

Cellar vinifications

MLF is a crucial step in wine production; therefore, the development of new starter cultures is an attention-grabbing challenge in oenology. In particular, the exploitation of autochthonous malolactic starter is more and more required because of their adaptation to a specific wine-producing area [54]. One of the key factors for a successful MLF is the establishment of a proper inoculum size. In this study, two different concentrations of MALOBACT-T1 strain were tested: 40 g/hL and 80 g/hL. Obtained data highlighted that the degradation of L-malic acid was successfully completed in wines inoculated with 80 g/hL of inoculum after 10 days. On the other hand, the inoculum size of 40 g/hL was not able to metabolize all malic acid even after 20 days of fermentation. In the control sample, the indigenous malolactic population was not able to perform MLF at least during the first 20 days (Fig. 3). Montepulciano d'Abruzzo wine characteristics were evaluated to monitor the fermentation performance. In addition to the complete malic acid degradation, the physical–chemical parameters of wines are presented in Table 2. Fermentation with MALOBACT-T1 and *S. cerevisiae* AV0 strain allowed to obtain a final product with characteristics typical of Montepulciano d'Abruzzo wine [2]. The volatile acidity was below 1.2 g/L of acetic acid which is the legal limit [55] since higher values can confer to wine undesired acidic flavour. Ethanol, tartaric acid and glycerol had concentrations in line with this red wine [2].

A proper inoculum of selected bacteria at the beginning of wine-making process did not influence the fermentation outcome. In agreement with other authors, a gradual acclimation of bacterial starter is essential during the first steps

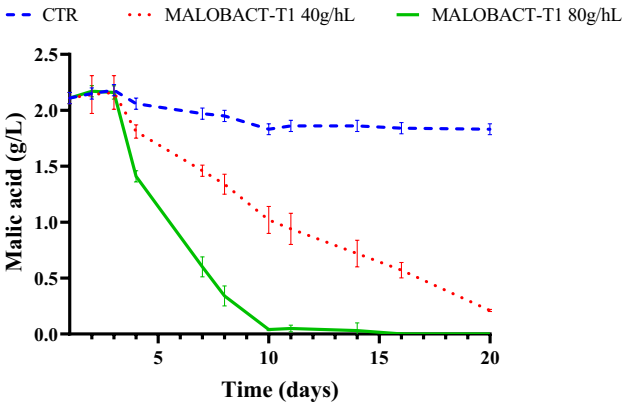

Fig. 3 Malic acid degradation in cellar vinification with MALOBACT-T1 at different inoculum size (40 g/hL and 80 g/hL). CTR: *S. cerevisiae* AV0 without the addition of MALOBACT-T1

Table 2 Characteristics of Montepulciano d'Abruzzo wine obtained with *S. cerevisiae* AV0 strain alone (CTR) and with MALOBACT-T1 40 g/hL and MALOBACT-T1 80 g/hL

Parameters	CTR	MALOBACT-T1 40 g/hL	MALOBACT-T1 80 g/hL
Alcohol (% v/v)	14.21 ± 0.41	14.19 ± 0.69	14.26 ± 0.74
Sugars (g/L)	0.98 ± 0.11	1.12 ± 0.15	1.03 ± 0.23
TA (g/L)	7.1 ± 0.29	6.98 ± 0.31	7.06 ± 0.36
VA (g/L)	0.4 ± 0.06	0.3 ± 0.07	0.37 ± 0.04
pH	3.38 ± 0.26	3.38 ± 0.17	3.39 ± 0.14
Malic acid (g/L)	1.83 ± 0.16	0.21 ± 0.05	0.03 ± 0.01
Lactic acid (g/L)	0.28 ± 0.01	1.82 ± 0.14	1.98 ± 0.13
Tartaric acid (g/L)	3.16 ± 0.35	3.45 ± 0.67	3.23 ± 0.25
Glycerol (g/L)	10.07 ± 0.71	10.11 ± 0.21	10.12 ± 0.37

Values are expressed in g/L. Ethanol concentration is expressed in g/100 mL. Data are expressed as average ± SD

TA total acidity (expressed as tartaric acid), *VA* volatile acidity (expressed as acetic acid)

of fermentation when ethanol content starts to increase [23]. Generally, MLF is strongly influenced by several factors and the compatibility of the LAB starter culture with the wine environment and the yeast starter (*Saccharomyces* and non-*Saccharomyces*) is the main one [15, 56].

In final wine, BAs were also monitored since MLF is a critical step for the production of these toxic compounds (Table 3). Ethanolamine was the main BA detected with values of 21 ± 0.32 mg/L. Its presence is usually related to *S. cerevisiae* and *Candida stellata* metabolism [4] and it is usually associated with grapes [57]. It is also a precursor for the formation of phosphatidylcholine, the most important membrane lipid of eukaryotic cells, and it could be released externally due to phospholipid regulation pathways [57]. The

Table 3 BA content in final wine inoculated with selected *O. oeni* strain (MALOBACT-1)

BAs (mg/L)	Wine
Putrescine	11.35 ± 0.33
Histamine	nd
Ethanolamine	21 ± 0.32
Phenylamine	2.5 ± 0.11
Tyramine	nd
Spermine	nd
Spermidine	nd
Isoamylamine	4.2 ± 0.51
Ethylamine	7 ± 0.34
Methylamine	2 ± 0.19
Cadaverine	1.2 ± 0.09

values of putrescine were 11.35 ± 0.33 mg/L and it was the second BA detected, and it is usually found in red wines. Putrescine can originate from grapes and red wine vinifications are conducted with grape lees and pulp and this BA could be released and found in the final product. Moreover, it could originate from agmatine by LAB [58].

Isoamylamine and ethylamine had values of 4.2 ± 0.51 and 7 ± 0.34 mg/L, respectively. It is interesting to point out that 36%–54% of wines usually have 5–10 mg/L of this BA [59].

Tyramine, spermine, spermidine and histamine were not detected in final wines. Histamine is frequently found in wines since its production is related to AF and MLF [60, 61] and it is considered the most important cause of wine intolerance. Histamine production is strictly strain dependent and is associated with the presence of a histidine decarboxylase.

Obtained data suggested that MALOBACT-T1 has an excellent potential that would make it a suitable commercial starter culture in line with more recent studies which underline the importance of autochthonous starter strain to preserve wine-specific traits.

Conclusions

This is the first report that focuses on natural *O. oeni* strains of Montepulciano d'Abruzzo must. Data allowed to identify a potential autochthonous starter to be applied in Montepulciano d'Abruzzo wine production. These starter cultures could represent a valid solution to improve the attributes of typical regional wines. This investigation also illustrates the preparation and validation of an *O. oeni* starter formulation that could be successfully adopted for the industrial production of Montepulciano d'Abruzzo wines. These findings could be applied to better investigate the use of autochthonous strains as industrial starters to enhance the organoleptic complexity of wines both in co-culture with *S. cerevisiae* and applying a sequential inoculation strategy. In conclusion, the proposed malolactic bacteria could represent the ideal solution to enhance the specific features of typical regional wines and could be produced on demand and distributed to the wineries as frozen concentrate culture. Further studies are needed to better understand the metabolic interactions between S. *cerevisiae*/non-*Saccharomyces* (e.g. *St. bacillaris*, *H. uvarum*)/*O. oeni* autochthonous strains to preserve the typical character of Montepulciano d'Abruzzo wine.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Compliance with ethics requirements The authors declare that this research did not involve human participants or animals.

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