Vitis **47** (2), 123–129 (2008)

Performance of malolactic fermentation by inoculation of selected *Lactobacillus plantarum* and *Oenococcus oeni* strains isolated from Rioja red wines

I. López[1], R. López[2], P. Santamaría[2], C. Torres[1], and F. Ruiz-Larrea[1]

[1] Department of Food and Agriculture, University of La Rioja, Logroño, Spain
[2] Servicio de Investigación y Desarrollo Tecnológico de La Rioja (CIDA), Logroño, Spain

Summary

Malolactic fermentations (MLF) of wines inoculated with selected lactic acid bacteria strains of the species *Oenococcus oeni* and *Lactobacillus plantarum* were studied and compared with spontaneous MLF. Bacterial populations were monitored along the whole process of MLF and bacteria identifications were carried out at species and strain level. Macrorestriction analysis with SfiI endonuclease and subsequent PFGE was carried out in order to identify *O. oeni* individual strains. *L. plantarum* active lyophila did not survive competing with the indigenous microbiota in a wine with 15.3 % (vol/vol) alcohol, whereas the selected *O. oeni* strains carried out wine MLF. The highest production of histamine took place during MLF in those wines that underwent spontaneous MLF with a mixed population of indigenous strains. The lowest levels of histamine were obtained with the selected commercial *O. oeni* strain that succeeded 100 % over the indigenous microbiota. Results indicate that development of MLF leaded by selected *O. oeni* active lyophila provides negligible histamine levels in red wines of quality that can be submitted to subsequent ageing in wood.

K e y w o r d s : *Lactobacillus plantarum*, *Oenococcus oeni*, malolactic fermentation, wine, PFGE.

Introduction

Malolactic fermentation (MLF) is generally considered a desirable transformation in winemaking processes. Lactic acid bacteria (LAB) are the microbiological agents that perform MLF and convert the L-malic acid into L-lactic acid and CO_2, and this leads to a decrease in wine acidity. The metabolism of LAB involves a significant number of compounds, many of them with relevant effects on wine taste and flavour (De Revel *et al.* 1999, Liu 2002, Ugliano *et al.* 2003). Spontaneous MLF implies several risks, such as a considerable increase in volatile acidity, consumption of residual sugars and formation of undesirable metabolites, such as biogenic amines, that can affect human health and lead to low quality wines (Mira de Orduña *et al.* 2000, Liu 2002, Pripis-Nicolau *et al.* 2004, Marcobal *et al.* 2006). In recent years, wine industries have moved towards using pure starter cultures of selected LAB to promote a reliable and rapid malic acid bioconversion, and thus ensuring better control and predictability of the reaction (Fuster and Krieger 2002, Zhang and Lovitt 2006, Gindreau and Charlotte 2007). Commercial strains directly inoculated into wine improved significantly the control of MLF (Nielsen *et al.* 1996). The use of MLF starter cultures of LAB strains selected from the wine indigenous microbiota of each region takes advantage of the natural adaptation of strains to wine characteristics, and may simultaneously maintain regional peculiarities (Zapparoli *et al.* 2003, Izquierdo *et al.* 2004). Improvements in the quality and the speed of MLF are frequently attributed to the utilised starter cultures (Pilatte *et al.* 1997, Carbó *et al.* 1998, Nielsen *et al.* 1999, Delaquis *et al.* 2000), although few times an adequate identification of strains is carried out to check which really are the responsible strains of this MLF. Molecular methods are used to identify individual strains. One of the most reproducible techniques, which provides profiles quite easy to analyze, is macrorestriction analysis of DNA by Pulsed Field Gel Electrophoresis (PFGE) (Izquierdo *et al.* 2004, Rodas *et al.* 2005, López *et al.* 2007).

Previous studies had shown that *Lactobacillus plantarum* strains can grow in wines (Navarro *et al.* 2000, Sehovic *et al.* 2003, Du Plessis *et al.* 2004, Rojo-Bezares *et al.* 2007 b) and possess resistance mechanisms to tolerate ethanol and low pHs of the growth medium (G-Alegría *et al.* 2004, Rojo-Bezares *et al.* 2007). Moreover, it was demonstrated that one *L. plantarum* strain isolated from wine (Navarro *et al.* 2000) and another *L. plantarum* strain isolated from grape must (Rojo-Bezares *et al.* 2007 b) possess interesting antimicrobial activities that inhibit cell growth of other oenological LAB strains. These characteristics leaded us to investigate the viability of inoculating selected oenological *L. plantarum* and *O, oeni* strains in wine as MLF starters. This study presents the results obtained when inoculating red wine with one commercial *O. oeni* strain, two *O. oeni* and one *L. plantarum* indigenous strains selected from a collection of LAB isolates from Spanish red wines of Appellation of Origin Rioja. Control of implantation along the MLF process was carried out by PFGE.

Material and Methods

S e l e c t e d L A B s t r a i n s : *L. plantarum* strain J51 isolated from Rioja red wine under spontaneous MLF, which had been shown to possess antimicrobial activity

Correspondence to: Dr. F. Ruiz-Larrea, Department of Food and Agriculture, University of La Rioja, Av. Madre de Dios 51, 26006 Logroño, Spain. Fax: +34-941-299-721. E-mail: fernanda.ruiz@unirioja.es

(Navarro et al. 2000), and *O. oeni* strains IS18 and IS159, isolated as well from Rioja red wines under spontaneous MLF, were selected for inoculation. These strains were grown, submitted to a pre-treatment and lyophilised for direct inoculation in wine at the labs of Lallemand Inc. The commercial *O. oeni* strain Uvaferm®Alpha (Lallemand Inc., St. Simon, France) was as well inoculated.

Wine elaboration and lyophila inoculation: Inoculations of the LAB strains were performed at the experimental winery of C.I.D.A. Research Centre, and at one commercial winery of the Spanish northern region of Rioja. Alcoholic fermentation was conducted following traditional procedures, i.e. wines were elaborated from 'Tempranillo' local grapes in the presence of grape skins, seeds and stalks, with the indigenous *Saccharomyces cerevisiae* yeast strains, and after SO_2 addition. At the end point of alcoholic fermentation, wine was drawn off from the lees and placed homogeneously into fermentation vats for MLF (racking), this wine will be referred to as “initial wine”. Temperature was maintained around 22 ºC.

The chemical composition of the wine elaborated at the experimental winery was as follows: alcohol 15.3 % v/v; pH 3.65; volatile acidity 0.43 $g·l^{-1}$; total acidity 7.81 $g·l^{-1}$(as tartaric acid); malic acid 2.88 $g·l^{-1}$. MLF was carried out in stainless steal vats (25 l) and experiments were carried out in triplicates. Twelve vats underwent MLF: three control samples were not inoculated and performed MLF with the indigenous microbiota, three were inoculated with *L. plantarum* J51, three were inoculated with *O. oeni* IS-18, and three were inoculated with the commercial *O. oeni* strain Uvaferm®Alpha. The chemical composition of the wine elaborated at the commercial winery was: alcohol 13.5 % v/v; pH 3.70; volatile acidity 0.36 $g·l^{-1}$; total acidity 5.80 $g·l^{-1}$ (as tartaric acid); malic acid 2.97 $g·l^{-1}$. MLF was carried out in eight oak barrels (250 l): three barrels were inoculated with *O. oeni* IS-159, two with *O. oeni* IS-18, and three control wine barrels underwent spontaneous MLF without any inoculation.

In all cases lyophila were rehydrated in mineral water at 20-30 ºC for 15 min and added to wines for MLF, obtaining around 10^6 $cfu·ml^{-1}$. MLF was followed by measuring wine L-malic acid content using the L-malic acid Enzymatic BioAnalysis (Boehringer-Mannheim/R-Biopharm, Darmstadt. Germany). Wine samples were taken from fermentation tanks at different moments: before inoculation, 48 h after inoculation, consumption of 10 % (initial MLF), 30 % (intermediate MLF) and 60 % (full MLF) of the initial malic acid. Samples were transported to the laboratory for microbiological analysis and kept under refrigeration (4ºC). When MLF had finished (L-malic acid concentration < 0.5 $g·l^{-1}$, final MLF), samples were taken for chemical and final microbiological analyses.

Bacterial isolation and growth conditions: Ten ml wine samples were processed as described before (López et al. 2007). Samples were spun at 100 x g for 3 minutes at 4 °C (Sorwall RC-5 B Refrigerated Superspeed Centrifuge). Pellets containing fermentation debris were discarded and supernatants were spun at 1,000 x g for 10 min. Pellets were collected and after appropriate dilutions in sterile saline solution (0.9 % NaCl), they were seeded in duplicates onto MRS agar (Scharlau Chemie S.A., Barcelona. Spain) plates with 200 µg of nystatin per ml (Acofarma, S. Coop., Terrassa. Spain). Samples were incubated at 30 ºC under strict anaerobic conditions (GasPak. Oxoid Ltd., Basingstoke. England) for at least five d. Ten colonies of the initial wine sample, and six colonies of each of the subsequent inoculated wine samples were taken for reisolation. They were grown onto MRS agar plates, at 30 ºC under 98 % humidity and 10 % CO_2 atmosphere. Strains were stored in 20 % sterile skim milk (Difco, Madrid. Spain) at -20 ºC.

Species identification: Species identification was carried out by previously recommended methods (Holt et al. 1994) and by the API 50 CHL kit and APILAB Plus software using the API 50 CHL version 4.0 database (BioMérieux S.A., Marcy l’Etoile. France). *L. plantarum* and *O. oeni* species were confirmed by the species-specific PCR method described by Quere et al. (1997) and Zapparoli et al. (1998).

Typification of strains: PFGE was carried out according to the method described by Birren and Lai (1993), with some modifications (López et al. 2007) for agarose block preparation. *L. plantarum* cells from an overnight culture on MLO plates (Scharlau Chemie S.A.) with 3 % of bacto agar (Difco), containing 40 mM of glycine (Sigma Chemical Co., St. Louis. MO) at 30 °C under 10 % CO_2 atmosphere, were suspended in 3 ml of saline solution to a turbidity equivalent of McFarland standard N° 1.5 (BioMérieux S.A.). Twenty five µl samples of cell suspensions were inoculated in tubes containing 3 ml of MLO broth (Scharlau Chemie S.A.) with 40 mM glycine to facilitate cellular lysis in following steps, and were grown at 18 ºC during 23 hours with agitation. *O. oeni* cells from a fresh overnight culture on MLO agar, were suspended in 3 ml of saline solution to a turbidity equivalent of McFarland standard N° 1.

L. plantarum and *O. oeni* cells were harvested, washed with 3 ml of 50 mM EDTA (pH 8) and submitted to macrorestriction analysis with *Sfi*I endonuclease by PFGE following the method reported by Lopez et al. (2007).

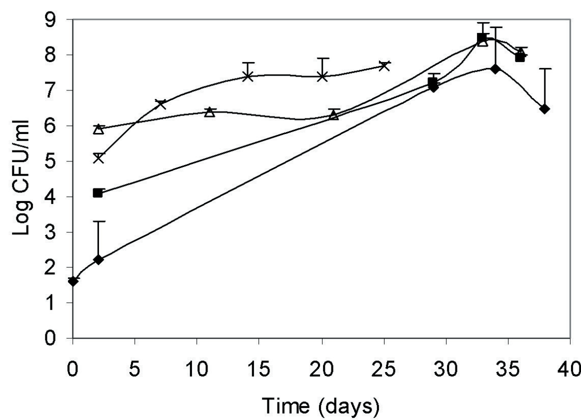
Analysis of biogenic amines: The method used to determine biogenic amines in wines was the method reported by Crespo and Lasa (1994). A Perkin Elmer 410 chromatographic system, with a LS-4 fluorometric detector and a PE LCI-100 integrator, was used. Chromatographic separations were carried out in a Spherisorb ODS 2 column (15 x 0.46 cm, 3 µm particle size). O-phthaldialdehyde was used in the derivatization reaction. Mobile phase composition was as follows: methanol and sodium acetate buffer (pH 6, 0.05 M) and tetrahydrofurane (99:1). The excitation and emission wavelengths were 340 nm and 420 nm, respectively. The amount of sample injected was 20 µl and a constant temperature of 45 ºC was maintained.

Results

Development of malolactic fermentation at the experimental winery:

MLF of the inoculated wines lasted for 25 to 36 d and the spontaneous MLF lasted for 38 d. The development of the MLF was related to the viable population of LAB and there was a correlation between bacterial population and the decrease in malic acid. Fig. 1 shows the viable bacterial counts (A) and the unconsumed malic acid percentage (B) during MLF. 48 h after inoculation, bacterial populations were around 10^5 CFU·ml^{-1} for both the commercial and IS-18 strains, 10^4 CFU·ml^{-1} for strain J-51, and 10^2 CFU·ml^{-1} for wines with spontaneous MLF (control wines). The maximum of 10^8 CFU·ml^{-1} was reached after 25 d for the commercial strain, 33 for both IS-18 and J-51, and 34 d for controls. One of the three control wines underwent a delayed MLF, as reflected by the high standard deviation (Fig. 1 A). The behaviour of MLF was more homogenous for the inoculated wines. The commercial strain was the most efficient and the duration of MLF was shortened 12 days when compared with control wines with spontaneous MLF.

A



B

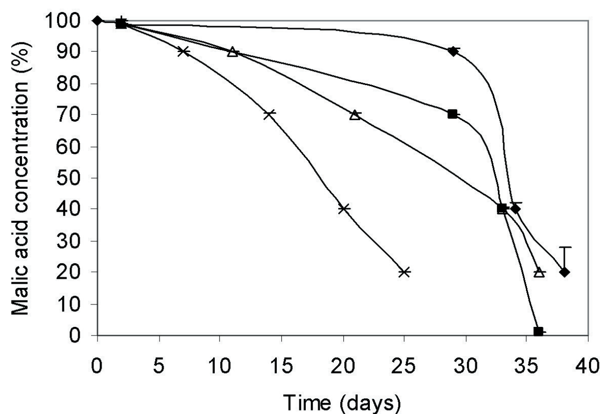


Fig. 1: MLF of wines at the experimental winery. A, Viable LAB counts [mean ± SD (n=3)] during MLF in wine: ♦ Spontaneous MLF; ■ Inoculated with *L. plantarum* J-51; Δ Inoculated with *O. oeni* IS-18; X Inoculated with the commercial *O. oeni* strain Uvaferm®Alpha. B, Percentage of non-consumed L-malic acid [mean ± SD (n=3)]: ♦ Spontaneous MLF, ■ Inoculated with *L. plantarum* J-51; Δ Inoculated with *O. oeni* IS-18; X Inoculated with with the commercial *O. oeni* strain Uvaferm®Alpha.

The Table shows species identification of bacterial isolates and the percentage of implantation during MLF in those inoculated wines. Out of the 72 total LAB isolates obtained from the spontaneous MLF at the experimental winery, three of them were *Pediococcus spp*. and appeared 48 h after wine racking, all the other isolates were identified as *O. oeni*. *L. plantarum* species was only found 48 h after inoculation of *L. plantarum* selected strain J51, and all the isolates (n=54) obtained thereafter from these inoculated wines were identified as *O. oeni*.

Typification analysis showed that the isolates from the initial wine rendered two PFGE patterns corresponding to two clearly distinct strains. As shown in Fig. 2, one of the patterns (A) was the most abundant (70 % of the total isolates, n=10) for isolates from the initial wine. Peculiarly, pattern B became clearly dominant and was the only one shown by isolates from control vats when the malic acid consumption was 10 % (Fig. 2) and 60 % (data not shown). The inoculation with the commercial strain was successful and all the PFGE patterns were identical to that of the inoculated strain, as shown in Fig. 3, and the percentage of implantation was 100 % (Table).

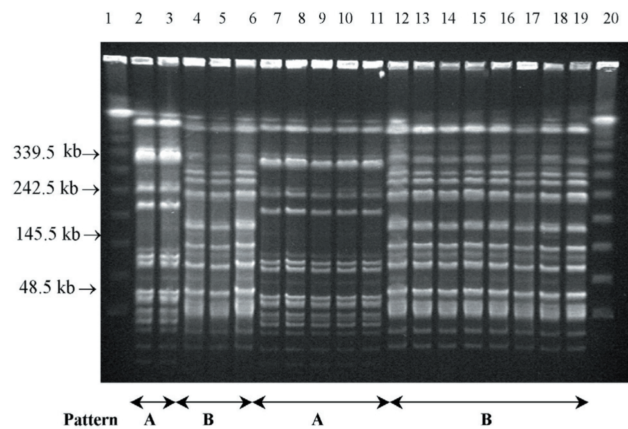


Fig. 2: PFGE patterns of SfiI digests of genomic DNA from *O. oeni* isolates obtained from wines elaborated at the experimental winery. Lanes 1 and 20: Lambda ladder DNA. Lanes 2-11: isolates from initial wine. Lanes 12-19: isolates from spontaneous fermentation at the initial MLF.

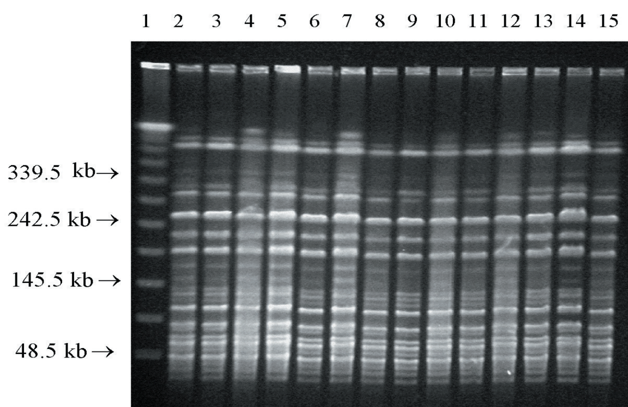


Fig. 3: PFGE patterns of SfiI digests of genomic DNA from *O. oeni* isolates. Lane 1: Lambda ladder DNA. Lanes 2-13: isolates from wine inoculated with commercial *O. oeni* at the intermediate MLF. Lanes 14 and 15: control pattern of commercial *O. oeni*.

Table

Species identfication and percentage of implantation during MLF

	Fermentation tank	Moment	Number of *O.oeni* isolates	Number of *Pediococcus spp.* isolates	Number of *L. plantarum* isolates	% Implantation*
Experimental winery	Initial wine	Before inoculation	10			
	Spontaneous MLF	48 h after racking	15	3		
		Initial MLF	18			
		Full MLF	18			
		Final MLF	18			
	L. plantarum J-51	48 h after inoculation			18	100
		Intermediate MLF	18			0
		Full MLF	18			0
		Final MLF	18			0
	Commercial *O. oeni* strain	48 h after inoculation	18			100
		Initial MLF	18			100
		Intermediate MLF	18			100
		Full MLF	18			100
		Final MLF	18			100
	O.oeni IS-18	48 h after inoculation	18			100
		Initial MLF	18			100
		Intermediate MLF	18			67
		Full MLF	18			50
		Final MLF	18			50
Commercial winery	Initial wine	Before inoculation	10			
	Spontaneous MLF	Initial MLF	18			
		Intermediate MLF	18			
		Full MLF	18			
		Final MLF	18			
	O.oeni IS-18	48 h after inoculation	12			100
		Full MLF	12			100
		Final MLF	12			100
	O.oeni IS-159	48 h after inoculation	18			100
		Full MLF	18			100
		Final MLF	18			100

*% Implantation: % PFGE patterns undistinguishable from the inoculated strain pattern.

Isolates obtained when malic acid consumption was 10 %, from the wines inoculated with *O. oeni* IS-18, showed the same pattern as that of the inoculated strain. Thereafter and until MLF completion both pattern B and IS-18 pattern appeared together, and when 30 % of the initial malic acid was consumed pattern B appeared in 33 % of the isolates (Table). This percentage continued increasing up to 50 % and coexisted with the inoculated IS-18 strain (Fig. 4). Therefore, it can be concluded that both strains carried out together MLF.

Development of malolactic fermentation at the commercial winery: MLF was performed in oak barrels and it lasted 20-21 d for the inoculated wines, and 36 days for the spontaneous MLF (Fig. 5). As in the case of wines from the experimental winery, the behaviour of the spontaneous MLF was also more irregular than the inoculated MLFs, and standard deviations were higher. Fig. 5 shows the viable bacterial counts and the unconsumed malic acid percentage during MLF. 48 h after inoculation, bacterial population was around 10^5 CFU

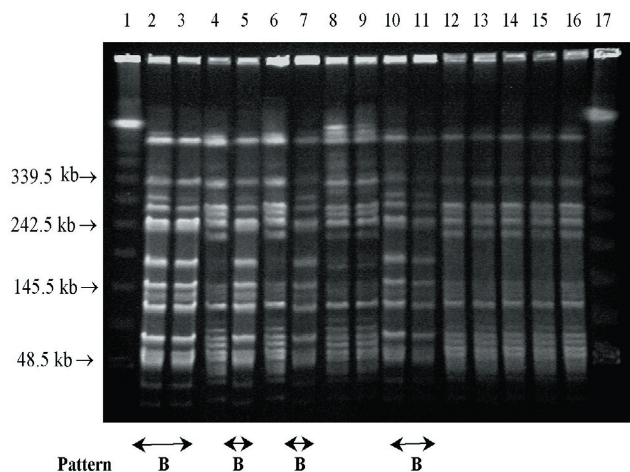
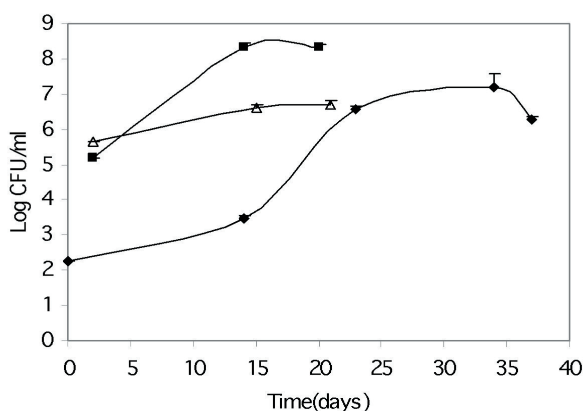


Fig. 4: PFGE patterns of SfiI digests of genomic DNA from *O. oeni* isolates. Lanes 1 and 17: Lambda ladder DNA. Lanes 2-13: isolates wine inoculated with *O. oeni* IS-18 at the full MLF. Lanes 14 to 16: control pattern of *O. oeni* IS-18.

A



B

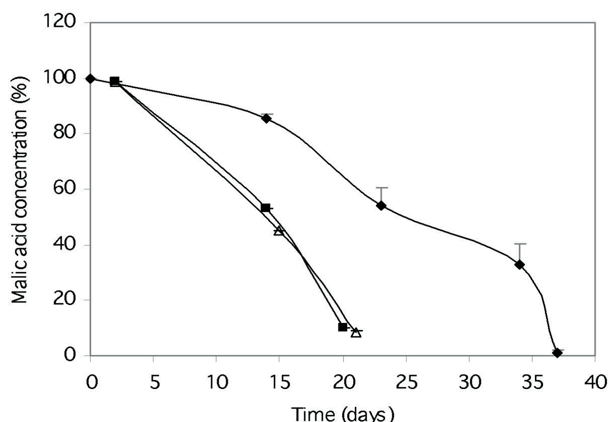


Fig. 5: MLF of wines at the commercial winery. A, Viable LAB counts [mean ± SD] during MLF in wine: ♦ Spontaneous MLF (n=3); ■ Inoculated with *O. oeni* IS-18 (n=2); Δ *O. oeni* IS-159 (n=3). B, Percentage of non-consumed L-malic acid [mean ± SD] in wine: ♦ Spontaneous MLF (n=3); ■ Inoculated with *O. oeni* IS-18 (n=2); Δ Inoculated with *O. oeni* IS-159 (n=3).

ml^{-1} for the inoculated IS-159 and IS-18 strains, whereas control wines showed an indigenous bacteria population of 10^2 CFU·ml^{-1}. Maximal bacteria populations (10^6-10^8 CFU ml^{-1}) were reached after 14 d for IS-18 strain, 15 d for IS-159, and after 34 days for control wines (Fig. 5). Therefore, again it was demonstrated that the inoculation stimulated the development of MLF, diminished considerably the period of latency and shortened the average duration of the fermentation process in 16 days respect to control wines with spontaneous MLF.

The identification results revealed that all the isolates were *O. oeni* in all the studied moments (Table). Typification analysis showed that the inoculation was successful in both cases and that 100 % of the PFGE patterns were indistinguishable from the inoculated strain pattern (Table).

Histamine concentrations: Regarding histamine concentrations in wines elaborated at the experimental winery, results are shown in Fig. 6. The initial wine, before MLF, showed a histamine content of 0.13 ± 0.02 mg·l^{-1}. Histamine levels were higher in wines that underwent spontaneous MLF (3.85 ± 1.10 mg·l^{-1}) with the wild indigenous *O. oeni* microbiota (lane 2 of Fig. 6), followed by histamine levels of wines that were inoculated with *L. plantarum* (1.93 ± 0.38 mg·l^{-1}) but underwent MLF by the indigenous *O. oeni* strains (lane 5 of Fig. 6, and the Table). The lowest values were obtained in wines inoculated with the commercial *O. oeni* strain (0.10 ± 0.05 mg·l^{-1}), which imposed 100 % over the indigenous microbiota (lane 3 of Fig. 6, and the Table). The selected strain IS-18 leaded MLF of the inoculated wines; nevertheless, at the end of MLF 50 % of the isolates were wine indigenous strains. Histamine levels of these wines were low (0.70 ± 0.05 mg·l^{-1}) (lane 4 of Fig. 6), but slightly higher than those of the wines inoculated with the commercial *O. oeni* strain.

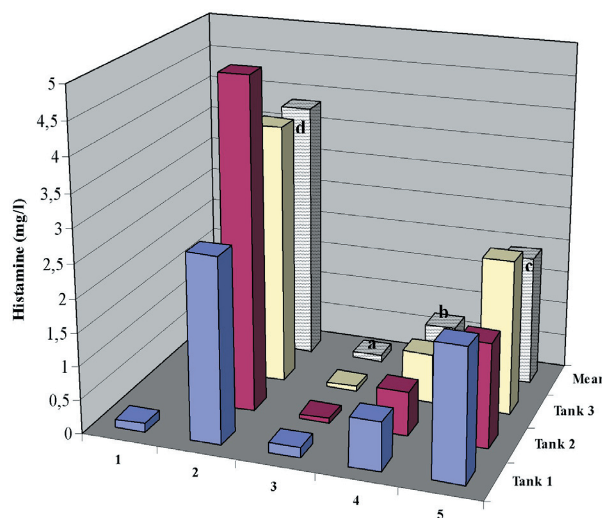


Fig. 6: Histamine content (mg·l^{-1}) in wines before and after MLF at the experimental winery. Lanes: (1) initial wine; (2) spontaneous MLF; (3) commercial *O. oeni* strain; (4) *O. oeni* IS-18; (5) *Lb. plantarum* J-51. Different letters indicate significance at the $p < 0.05$ level.

Discussion

The inoculation with malolactic cultures has always generated doubts about the real intervention of the added LAB. The effectiveness of these commercial starters has been justified by many winemakers by the fact that the MLF began several days or weeks before in the inoculated tanks than in the control ones (without inoculation), or that the disappearance of malic acid was faster. Nevertheless, these observations are not enough to conclude that the inoculated LAB have succeeded *i.e.* being the one growing in the wine and leading MLF. Genetic typification analysis is necessary to identify a certain strain throughout the MLF, and to evaluate the incidence of starters in the wine at analytical and sensorial level. In this study, the implantation of *O. oeni* strains was confirmed and an advantage with respect to the non-inoculated tanks was observed. MLF of wines inoculated with *O. oeni* selected strains lasted 25 d in the stainless steal vats, and 20-21 d in the barrels, whereas spontaneous MLFs lasted 38 and 36 d, respectively. In our study a good correlation between MLF duration and the percentage of implantation of the inoculated strain was observed in all cases. Thus, it can be concluded that MLF duration depended on the degree of LAB implantation. In those wines whose MLF lasted for 20 to 25 d, the inoculated strains appeared in 100 % of the studied isolates, and viable counts 24 h after inoculation were next to 10^6 $CFU \cdot ml^{-1}$. In control wines with spontaneous MLF, in which a competition between different strains of *O. oeni* was observed, the MLF average duration was prolonged up to 36 or 38 d. The concentration of histamine seemed to correlate with the level of implantation of the inoculated strain, and thus the lowest value was obtained for the wines inoculated with the commercial strain (100 % implantation). The initial wine showed a low concentration of histamine (0.13 $mg \cdot l^{-1}$), and after spontaneous MLF wines displayed the highest concentration of this biogenic amine (3.85 $mg \cdot l^{-1}$), whereas histamine concentrations in the inoculated wines were intermediate (Fig. 6). These results seem to indicate that effective inoculation with selected bacteria diminishes amino acid metabolism, and consequently the risk of biogenic amine formation. This effect could be due to the massive bacteria sowing that prevents multiplications and reduces amino acid metabolic reactions.

The indigenous *O. oeni* strain that prevailed in control wines carried out MLF, and appeared together with IS-18 in the wines inoculated with IS-18, was able to compete with the inoculated IS-18 strain and could grow in a wine of high alcoholic graduation (15.3 % vol/vol). Carbó *et al.* (1995) reported that the development of MLF with a single strain could not be guaranteed in any type of wine and the interest of mixed inoculations with selected strains based on different resistance parameters. The selection of the best-adapted strains to each type of wine, that maintain the characteristics of the wines and provide the best quality, has to follow basic selection criteria (Suárez and Leal 2004, Zambonelli *et al.* 2004, Zapparoli *et al.* 2004) and *O. oeni* strains IS-18 and IS-159 isolated from Rioja wines in spontaneous MLF (López *et al*. 2007) revealed as excellent candidates for selection as they succeeded 100 % in a red wine with 13.5 % (vol/vol) ethanol that was ready to undergo a subsequent process of ageing in wooden barrels. The results of this study showed as well that the molecular typification method of PFGE enabled us to precisely follow the level of implantation of *O. oeni* strains during the whole MLF. On the other hand, our results confirmed that our *L. plantarum* strain was not resistant enough to survive competing with indigenous microbiota in a red wine with 15.3 % (vol/vol) ethanol, whereas the commercial *O. oeni* strain did, and fully conducted MLF with no significant production of histamine, preventing indigenous strains from growing and producing the undesirable metabolites.

Acknowledgements

This work was supported by the I.N.I.A. grant VIN03-043-C3-2 and FEDER of the European Community (2FD97-1475) and by the Autonomous Community of La Rioja grant ACPI/2004. I. López was supported by a doctorate grant of the Spanish Ministry of Education and Science (AP99 16580683). We acknowledge the support of Lallemand Inc. that provided active lyophila of bacterial strains for direct inoculation in wine.

References

Birren, B.; Lai, E., 1993: Preparation of DNA for pulsed field analysis. In: Hartcourt Brace Jovanich (Ed.): Pulsed field gel electrophoresis. A practical guide, 25-74. Academic Press, San Diego.

Carbó, R.; Conde, M.; Gordún, E.; 1995: Aislamiento y selección de bacterias lácticas en vino. Riv. Vitic. Enol. **4**, 29-38.

Carbó, R.; Soler, B.; Gordón, E.; De Castro, J.; Sancho, J.; 1998: Adaptación de las bacterias lácticas para la fermentación maloláctica del vino. Riv. Vitic. Enol. **4**, 39-45.

Crespo, M. I.; Lasa, B. V.; 1994: Determination of biogenic-amines and other amines in wine by an optimized HPLC method with polarity gradient elution. Am. J. Enol. Vitic. **45**, 460-463.

De Revel, G.; Martín, N.; Pripis-Nicolau, L.; Lonvaud-Funel, A.; Bertrand, A.; 1999: Contribution to the knowledge of malolactic fermentation influence on wine aroma. J. Agr. Food Chem. **47**, 4003-4008.

Delaquis, P.; Cliff, M.; King, M.; Girard, B.; Hall, J.; Reynolds, A.; 2000: Effect of two commercial malolactic cultures on the chemical and sensory properties of chancellor wines vinified with different yeasts and fermentation temperatures. Am. J. Enol. Vitic. **51**, 42-48.

Du Plessis, H. W.; Dicks, L. M. T.; Pretorius, I. S.; Lambrechts, M. G.; Du Toit, M.; 2004: Identification of lactic acid bacteria isolated from South African brandy base wines. Int. J. Food Microbiol. **91**, 19-29.

Fuster, A.; Krieger, S.; 2002: Contrôle de la fermentation malolactique: Une approche practique. Rev. Francaise Oenol. **194**, 14-17.

G-Alegría, E.; López, I.; Ruiz, J.; Saenz, J.; Fernandez, E.; Zarazaga, M.; Dizy, M.; Torres, C.; Ruiz-Larrea, F.; 2004: High tolerance of wild *Lactobacillus plantarum* and *Oenococcus oeni* strains to lyophilisation and stress environmental conditions of acid pH and ethanol. FEMS Microbiol. Lett. **230**, 53-61.

Gindreau, E.; Charlotte, A.; 2007: Nouveautés dans les levains malolactiques. Rev. Oenol. **122**, 13-15.

Holt, J. G.; Krieg, N.; Sneath, P.; Staley, J.; Williams, S.; 1994: Bergey's Manual of Determinative Bacteriology. Lippincott, Williams and Wilkins, Baltimore.

Izquierdo, P.; García, E.; Martínez, J.; Chacón, J.; 2004: Selection of lactic bacteria to induce malolactic fermentation in red wine of cv. Cencibel. Vitis **43**, 149-153.

Liu, S.; 2002: A review. Malolactic fermentation in wine beyond deacidification. J. Appl. Microbiol. **92**, 589-601.

López, I.; Tenorio, C.; Zarazaga, M.; Dizy, M.; Torres, C.; Ruiz-Larrea, F.; 2007: Evidence of mixed wild populations of *Oenococcus oeni* strains during wine spontaneous malolactic fermentations. Eur. Food Res. Technol. (In press DOI 10.1007/s00217-006-0529-0).

Marcobal, A. ; Martin-Alvarez, P.; Polo, M. C.; Muñoz, R.; Moreno-Arribas, R.; 2006: Formation of biogenic amines throughout the industrial manufacture of red wine. J. Food Protect. **69**, 397-404.

Mira de Orduña, R.; Liu, S.; Ratchett, M.; Pilone, G.; 2000: Ethyl carbamate precursor citrulline formation from arginine degradation by malolactic wine lactic acid bacteria. FEMS Microbiol. Lett. **183**, 31-35.

Navarro, L.; Zarazaga, M.; Sáenz J.; Ruiz-Larrea, F.; Torres, C.; 2000: Bacteriocin production by lactic acid bacteria of Rioja red wines. J. Appl. Microbiol. **88**, 44-51.

Nielsen, J.; Prahl, C.; Lonvaud-Funel, A.; 1996: Malolactic fermentation in wine by direct inoculation with freeze-dried *Leuconostoc oenos* cultures. Am. J. Enol. Vitic. **47**, 42-48.

Nielsen, J.; Richelieu, M.; 1999: Control of flavor development in wine during and after malolactic fermentation by *Oenococcus oeni*. Appl. Environ. Microbiol. **65**, 740-745.

Pilatte, E.; Prahl, C.; 1997: Biological deacidification of acid grape varieties by inoculation on must with a freeze-dried cultura of *Lactobacillus plantarum*. Abstr. 48th Annual Meeting of the ASEV (San Diego).

Pripis-Nicolau, L.; De Revel, G.; Bertrand, A.; Lonvaud-Funel, A.; 2004: Methionine catabolism and production of volatile sulphur compounds by *Oenococcus oeni*. J. Appl. Microbiol. **96**, 1176-1184.

Quere, F.; Deschamps, A.; Urdaci, M.; 1997: DNA probe and PCR-specific reaction for *Lactobacillus plantarum*. J. Appl. Microbiol. **82**, 783-790.

Rodas, A.; Ferrer, S.; Pardo, I.; 2005: Polyphasic study of wine *Lactobacillus* strains: taxonomic implications. Int. J. Syst. Evol. Micr. **55**, 197-207.

Rojo-Bezares, B.; Sáenz, Y.; Navarro, L.; Zarazaga, M.; Ruiz-Larrea, F.; Torres, C.; 2007 b: Coculture-inducible bacteriocin activity of *Lactobacillus plantarum* strain J23 isolated from grape must. Food Microbiol. **24**, 482-91.

Rojo-Bezares, B.; Sáenz, Y.; Zarazaga, M.; Torres, C.; Ruiz-Larrea, F.; 2007 a: Antimicrobial activity of nisin against *Oenococcus oeni* and other wine bacteria. Int. J. Food Microbiol. (in press).

Sehovic, D.; Bozanic, R.; Runjic-Peric, V.; Maric, V.; 2003: Comparison of malolactic fermentation in wine by two bacterial strains. Periodicum Biologorum **105**, 263-267.

Suárez Lepe, J. A.; Leal, B. I.; 2004: Microbiología Enológica. Fundamentos de Vinificación. 3rd edn. Ed. Mundi-Prensa, Madrid.

Ugliano, M.; Genovese, A.; Moio, L.; 2003: Hydrolysis of wine aroma precursors during malolactic fermentation with four commercial starter cultures of *Oenococcus oeni*. J. Agr. Food Chem. **51**, 5073-5078.

Zambonelli, C.; Tini, V.; Coloretti, F.; Benevelli, M.; 2004: I batteri della fermentazione malolattica. Vigne Vini **9**, 66-69.

Zapparoli, G.; Spinelli, P.; Torriani, S.; Dellaglio, F.; 2003: The spontaneous malolactic fermentation in wines of the Valtellina area: A study of the kinetic parameters and of the dominant *Oenococcus oeni* strains. Riv. Vitic. Enol. **2**, 47-56.

Zapparili, G.; Moser, M.; Dellaglio, F.; Tourdot-Marechal, R. Guzzo, J.; 2004: Typical metabolic traits of two *Oenococcus oeni* strains isolated from Valpolicella wines. Lett. Appl. Microbiol. **39**, 48-54.

Zapparoli, G.; Torriani, S.; Pesente, P.; Dellaglio, F.; 1998: Design and evaluation of malolactic enzyme gene targeted primers for rapid identification and detection of *Oenococcus oeni* in wine. Lett. Appl. Microbiol. **27**, 243-246.

Zhang, D.; Lovitt, R.; 2006: Performance assessment of malolactic fermenting bacteria *Oenococcus oeni* and *Lactobacillus brevis* in continuous culture. Appl. Microbiol. Biot. 69, 658-664.

Received June 27, 2007