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Occurrence of Lactic Acid Bacteria During the Different Stages of Vinification and Conservation of Wines

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We showed that the growth of lactic acid bacteria during alcoholic fermentation depends on the composition of the must. We illustrated how the addition of sulfur dioxide to the must before fermentation and the temperature of storage both affect the growth of these bacteria in the wine. Whereas species of *Lactobacillus* and *Leuconostoc mesenteroides* were isolated from grapes and must, *Leuconostoc oenos* was the only species isolated after alcoholic fermentation. This organism was responsible for the malolactic fermentation. Isolates of this species varied in their ability to ferment pentoses and hexoses. The survival of *Leuconostoc oenos* in wines after malolactic fermentation depended on wine pH, alcohol concentration, SO_2 concentration, and temperature of storage.

Numerous studies have been conducted on the lactic acid bacteria that occur on grapes, in grape musts, and in wines (1, 5, 6, 12, 13; P. Bidan, Document de Travail Office Internationale de la Vigne et du Vin no. 14, 1967). However, little information exists on the development of these bacteria during the vinification process. Some recent studies suggest that a succession of species actually occurs (3, 4, 9, 10). With the development of rapid methods for the identification of wine lactic acid bacteria, it is now practical to quantitatively examine the species that evolve during the different stages of vinification (7, 9).

It is common practice in wine making to add various levels of sulfur dioxide (SO_2) to grape musts, before fermentation, to control the development of unwanted yeast species and bacteria (12; S. Domercq, Dr. Ingénieur thesis, University of Bordeaux—Talence, France, 1956). However, little is known of what effects such additions have on the levels of lactic acid bacteria in the musts or on the ability of these bacteria to grow in the wine later and to conduct a desirable malolactic fermentation.

It is known that the capacity of lactic acid bacteria to grow in wines after the primary fermentation is determined by wine pH, alcohol concentration, and sulfur dioxide concentration (8, 12). Such information is largely qualitative, and little attempt has been made to examine these influences on a quantitative basis. In wineries, it is widely known that the temperature of wine conservation after the alcoholic fermentation will determine the time at which the malolactic fermentation commences. Higher temperatures favor a rapid onset of this fermentation, and lower temperatures delay it. This empirical knowledge, however, has not been well documented, microbiologically.

This study examines the levels and species of lactic acid bacteria that develop in wines during the different stages of vinification, from the grape must until several months after the completion of the malolactic fermentation. The effects of SO_2 addition to the must and the temperature of wine storage on both the growth of lactic acid bacteria in the wine and the commencement of the malolactic fermentation are reported. The effects of pH, alcohol concentration, SO_2 addition, and temperature on the survival of lactic acid bacteria in wines after completion of the malolactic fermentation are also presented.

MATERIALS AND METHODS

Wine production. Three types of wine were considered in this study: a red wine produced in Bordeaux, France, a white wine produced at Bordeaux, and a white wine base produced at Cognac for brandy distillation.

With the vinification of red wines, the alcoholic fermentation was conducted in the presence of grape skins, seeds, and stalks after the addition of SO_2. The temperature was between 25 and 30°C to favor the extraction of color. The duration of this step depended on the quality of the grape and the type of wine desired. After sufficient color extraction and fermentation, the wine was run off into metal or cement tanks or oak barrels, where the alcoholic fermentation went to completion and the malolactic fermentation commenced.

For the production of dry white wines, the must of white grapes was used. The must was sulfured and

partially clarified by sedimentation before fermentation, which was conducted at temperatures below 20°C to favor the production and preservation of yeast aromatic substances. After the alcoholic fermentation was completed, the wine was clarified by filtration or centrifugation, eventually after the addition of SO_2.

For the brandy base wine, the must was extracted from white grapes and fermented at around 25°C without prior clarification or addition of SO_2. The wine was kept on the lees after the alcoholic fermentation was completed and until distillation. In this case, most often the malolactic fermentation developed immediately after cessation of the alcoholic fermentation.

Grape and wine samples. Grapes were aseptically harvested from vines, transferred to sterile plastic bags, and transported to the laboratory for microbiological analysis. Samples (100 g) were aseptically homogenized in a Sorvall blender, and 1.0-ml aliquots were used to inoculate isolation media.

Must and wine samples from large tanks and barrels were obtained by using large, sterilized glass pipettes. Generally, sample volumes of 500 ml were taken, transferred to a sterile glass container, and transported to the laboratory under refrigeration (4°C).

Enumeration and isolation of lactic acid bacteria. Nutrient medium for the enumeration and isolation of lactic acid bacteria had the following components (per liter): glucose, 40 g; Casamino Acids (Difco), 5 g; yeast extract, 4 g; DL-malic acid, 20 g; KH_2PO_4, 0.6 g; KCl, 0.45 g; $CaCl_2 \cdot 2H_2O$, 0.13 g; $MgSO_4 \cdot 7H_2O$, 0.13 g; $MnSO_4 \cdot H_2O$, 0.003 g; tomato juice, 10 ml. The pH was adjusted to 4.8, and the medium was sterilized at 10 lb (ca. 4.5 kg) of pressure for 20 min.

At the moment of use, 5.0 ml of the medium was dispensed into sterilized petri dishes, along with 0.1 ml of 0.5% pimaricin solution to inhibit the growth of yeasts and molds. The medium was inoculated with 1.0 ml of sample, and 5 ml of molten (40°C) 3% agar was added and then carefully mixed. After solidification, the plates were incubated at 27°C under anaerobic conditions (GasPak) for 8 to 10 days. Anaerobic conditions were used to prevent the growth of acetic acid bacteria. After incubation, colonies were counted, and 5 to 10 were selected for restreaking and identification.

Identification of isolates. After purification, isolates were identified according to the tests given by Buchanan and Gibbons (3) and Ribéreau-Gayon et al. (12). Criteria examined included Gram reaction, cell morphology, homo- and heterofermentation of glucose, isomeric form of lactic acid produced from glucose metabolism (2), and resistance to quaternary ammonium compounds. In addition, isolates were tested for their reactions in API 50CHL batteries. Arginine dihydrolase and Vosges-Proskauer reactions were tested by using API batteries. Details of these operations have been published elsewhere (7). L-Malic acid was measured enzymatically (2).

RESULTS

Development of lactic acid bacteria during the vinification of red wine. In 1979, the Cabernet grapes used for the wine in this study gave a must of pH 3.4, containing 204 g of sugar and 3.4 g of malic acid per liter. Samples of 600 liters each were sulfured with either 50 or 100 mg of SO_2 per liter and transferred to tanks for fermentation. One sample was left unsulfured. Fermentation commenced naturally and occurred at 18 to 30°C. After the alcoholic fermentation, which lasted 10 days, the wines (alcohol, 12% by volume; pH 3.4) were transferred to oak barrels without further addition of SO_2. However, one barrel for each condition was further sulfured at this stage by the addition of 50 mg of SO_2 per liter. Barrels were stored at 14°C. One liter from each barrel was maintained in a bottle for observations on storage at 19°C. These bottles were not given the additional 50 mg of SO_2 per liter as described above.

Figure 1 shows the development of lactic acid bacteria in the various barrels maintained at 14°C, from the must during alcoholic fermentation through conservation. The initial population of lactic bacteria in the must was of the order of 10^4 cells per ml and was not altered by the addition of 50 mg of SO_2 per liter. However, the addition of 100 mg of SO_2 per liter reduced this population to approximately 10^3 cells per ml. During the alcoholic fermentation, the population of lactic acid bacteria in all samples decreased to about 200 cells per ml. Separation of the wine from the maceration and transfer to barrels were accompanied by an enrichment in the population, and at the commencement of

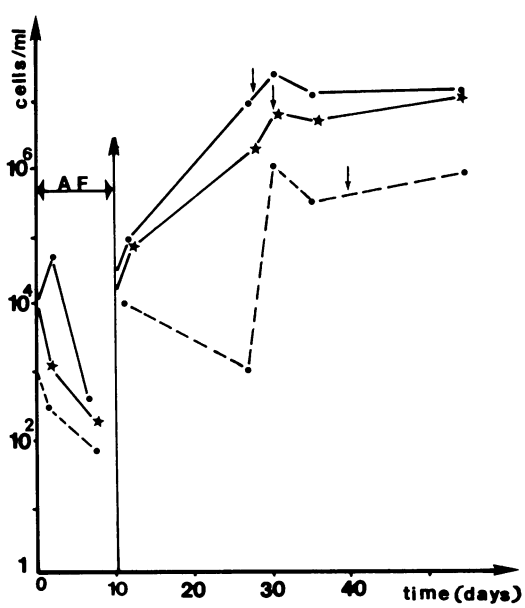

FIG. 1. Evolution of lactic acid bacteria in red wine during production. Temperature of conservation, 14°C. Symbols: (●—●) must without SO_2; (★) must with 50 mg of SO_2 per liter; (●--●) must with 100 mg of SO_2 per liter. AF, Alcoholic fermentation. ↓, Malolactic fermentation.

TABLE 1. Effects of SO_2 addition and temperature of storage on the development of lactic bacteria populations and the time for completion of malolactic fermentation

Vintage	SO_2 to must (mg/liter)	SO_2 to wine (mg/liter)	Temp of storage (°C)	Maximum population (cells per ml)	Time to completion of malolactic fermentation (days)
1979	0	0	14	3×10^7	16
1979	50	0	14	1×10^7	21
1979	100	0	14	1×10^6	31
1979	0	50	14	1×10^4	—[a]
1979	50	50	14	5×10^4	—
1979	100	50	14	1×10^4	—
1979	0	0	19	1×10^8	10
1979	50	0	19	9×10^7	17
1979	100	0	19	4×10^7	19
1980	0	0	18	1×10^8	16
1980	50	0	18	8×10^7	17
1980	100	0	18	5×10^7	24

[a] —, No fermentation after 200 days.

barrel storage, the levels of lactic acid bacteria were again around 10^4 cells per ml.

Lactic acid bacteria quickly developed in the wines originating from the nonsulfured and sulfured (50 mg/liter) musts (Fig. 1), reaching a population of 10^7 cells per ml by 25 to 30 days; malic acid degradation was complete at this time. For the wine from the 100-mg/liter-sulfured must, the development of lactic acid bacteria was retarded, as shown by slight initial decreases. Growth recommenced after 28 days, however, reaching a final level of about 10^6 cells per ml by 32 days. Malic acid degradation was not completed until after 40 days.

For the corresponding wines held in bottles at 19°C, the maximum populations of lactic acid bacteria obtained were slightly elevated: 10^8 cells per ml for the unsulfured and 50-mg/liter-sulfured musts and 4×10^7 cells per ml for the 100-mg/liter-sulfured must. For these wines degradation of malic acid was more rapid and was completed after 10, 17, and 19 days, respectively. In all cases, at both 14 and 19°C, the population of lactic acid bacteria remained at levels of 10^6 to 10^7 cells per ml for several weeks after the fermentation of malic acid (Table 1).

For the wines given an additional 50 mg of SO_2 per liter at the time of barreling, a different development was observed. The populations of lactic acid bacteria in these samples decreased considerably, and subsequent growth did not exceed maximum levels of 10^4 to 10^5 cells per ml. Malic acid fermentation in these wines was very slow; after 50 days, only 100 mg/liter had been degraded. Similar results were obtained at both 14 and 19°C storage.

The above studies were repeated during the 1980 vintage, using a Cabernet-Sauvignon wine of similar analytical characteristics. Levels of bacteria in unsulfured grape musts were only about 10 cells per ml (Table 1). Addition of 100 mg of SO_2 per liter reduced the bacteria to undetectable levels. At the end of the alcoholic fermentation, levels of lactic acid bacteria were around 100 cells per ml for both the sulfured and nonsulfured musts.

After separation of the wine and transfer to barrels, the levels of bacteria in wines from the nonsulfured and 50-mg/liter- and 100-mg/liter-sulfured musts were 2.5×10^4, 2.5×10^3, and 5×10^2 cells per ml, respectively. These developed to maximum levels of 10^7 to 10^8 cells per ml, and the malolactic fermentation was completed after 16, 17, and 30 days, respectively. For this vintage, the species of lactic acid bacteria occurring during the different stages of vinification were isolated and identified (Table 2). *Lactobacillus hilgardii* and *Lactobacillus plantarum* occurred on the grapes, but in all samples of wines taken during the alcoholic fermentation or subsequent barrel conservation *Leuconostoc oenos* was the only species found.

Development of lactic acid bacteria during the vinification of white wines. (i) Vinification at Bordeaux. Must extracted from a mixture of Colombard and Ugni Blanc grapes gave a sugar level of 196 g/liter and a pH of 3.2. The must was sulfured with 50 mg of SO_2 per liter and fermented naturally at 19°C in 90-liter stainless-steel vats. Lactic acid bacteria were present in the must at only 10 cells per ml, although three species, *Leuconostoc oenos*, *Leuconostoc mesenteroides*, and *Lactobacillus plantarum* were isolated. Alcoholic fermentation lasted 15 days, during which the lactic acid bacteria decreased until none could be recovered. Only *Leuconostoc oenos*, at levels around 5 cells per ml, could be isolated from the fermenting must at 5, 8, and 11 days.

At the end of the alcoholic fermentation, the wine (alcohol, 11.5% by volume; pH 3.2) was separated after sedimentation and returned to the same vats without the addition of SO_2. After these operations, lactic acid bacteria were again detected at levels around 10^2 to 10^3 cells per ml. This level decreased to 10 cells per ml during the next 20 days of conservation at 18°C, after which multiplication commenced. *Leuconostoc oenos* was the only species isolated from fermenting must and the wine during conservation. Between 20 and 50 days, the population of this species gradually increased to 10^4 cells per ml, but malic acid was not degraded during this time and remained at the initial level (4.2 g/liter). The experiment was terminated after 80 days with no malolactic fermentation.

TABLE 2. Enumeration and identification of lactic acid bacteria during the vinification of 1980 red Bordeaux wine[a]

Sample	Time of sample (day)	Cells per ml	Species isolated (no. of strains identified)
Grape	1 mo before harvest	3	*Lactobacillus hilgardii* (2)
	Harvest	2	*Lactobacillus plantarum* (2)
Must (no SO_2)	1	7	*Leuconostoc oenos* (7)
	9	6×10^2	*L. oenos* (10)
	15	2.5×10^4	*L. oenos* (8)
	28	4.5×10^7	*L. oenos* (8)
Must (50 mg of SO_2 per liter)	1	5	*L. oenos* (2)
	9	5×10^2	*L. oenos* (8)
	15	2.5×10^3	*L. oenos* (8)
	35	7×10^7	*L. oenos* (3)
Must (100 mg of SO_2 per liter)	1	0	
	9	2×10^2	*L. oenos* (9)
	15	4.5×10^2	*L. oenos* (8)
	35	2.5×10^6	*L. oenos* (8)

[a] Draining of wine from fermentation tank occurs at day 14.

(ii) Vinification at Cognac of a wine base for distillation. Must extracted from Ugni Blanc grapes had the following properties: sugar, 166 g/liter; malic acid, 7.6 g/liter; pH 3.2. It was not sulfured and was naturally fermented in a 500-liter cement tank at 18 to 28°C. The alcoholic fermentation quickly commenced and lasted 4 days before the exhaustion of fermentable sugars. During this time the initial population of lactic acid bacteria of 10^4 cells per ml increased about 10-fold, and this was accompanied by a small release of 270 mg of D-(−)-lactic acid per liter. Malic acid was reduced to 5.6 g/liter by the end of the alcoholic fermentation. The wine (alcohol, 9.7% by volume; pH 3.2) was retained in the tank on the lees for subsequent storage. The growth of lactic acid bacteria continued quickly during storage, reaching a maximum level of 10^8 cells per ml during the next 6 days, by which time malic acid had been totally degraded. After this time the population decreased slightly to around 10^6 to 10^7 cells per ml and remained at this level until 120 days, at which time the wine was distilled. Wine temperature decreased to 10°C during this period.

Table 3 shows the numbers and species of lactic acid bacteria isolated from the wine during vinification. The grapes, 15 days before harvesting, showed the presence of two species, *Lactobacillus hilgardii* and *Lactobacillus casei*, at very low levels. However, these or other species were not isolated at the time of harvest. Nevertheless, *Leuconostoc mesenteroides* and *Leuconostoc oenos* were isolated from freshly produced must and, in addition, *Lactobacillus casei* and *Lactobacillus brevis* were found in the pressings along with *Leuconostoc oenos*. *Leuconostoc mesenteroides* predominated in samples taken after day 1 of fermentation, but *Leuconostoc oenos* and *Lactobacillus plantarum* were also isolated. *Leuconostoc mesenteroides* continued to predominate into day 2 of fermentation, but by day 3 *Leuconostoc oenos* had commenced its growth, and this was the only species recovered thereafter. It continued its growth during conservation and was responsible for the malolactic fermentation.

Development of lactic acid bacteria in wines during their conservation. The assays were conducted in the laboratory with a red Cabernet-Sauvignon wine produced under commercial conditions and taken immediately after the completion of the malolactic fermentation. The wine studied had the following basic properties: alcohol, 11.25%; pH 3.6; free SO_2, 0 mg/liter; total SO_2, 200 mg/liter. Samples of the wine were taken, and one property was adjusted while the other base properties were kept constant. In this way, three pH values (3.3, 3.6, and 3.9), three alcohol concentrations (10, 11.25, and 12.50% by volume), and three SO_2 concentrations (0, 20, and 40 mg/liter) were examined. The pH was adjusted by the addition of NaOH or HCl, and the alcohol concentration was adjusted by dilution with sterile distilled water or the addition of pure ethanol. The various samples were then kept in the laboratory at 19°C for 202 days, during which time examinations were made for the levels of lactic acid bacteria. One set of unadjusted samples was kept at either 4, 12, 18, or 26°C to study the effect of temperature on bacterial development. The results are presented in Fig. 2.

At pH 3.9 and 3.6 (Fig. 2), lactic acid bacteria

TABLE 3. Enumeration and identification of lactic acid bacteria during the vinification of a white wine base for Cognac distillation (1981)

Sample	Time of sample	Cells per ml	Species isolated (no. of strains identified)
Grape	1 mo before harvest	7	*Lactobacillus hilgardii* (1) *L. casei* (6)
	Harvest	0	
Must before fermentation	First juice	800	*Leuconostoc mesenteroides* (2) *L. oenos* (1)
	Pressed juice	1,300	*Lactobacillus casei* (1) *L. brevis* (1) *Leuconostoc oenos* (1)
Wine during fermentation	Day 1	10^4	*L. mesenteroides* (6) *L. oenos* (2) *Lactobacillus plantarum* (2)
	Day 2	2.7×10^4	*Leuconostoc mesenteroides* (7)
	Day 3	2.7×10^4	*L. mesenteroides* (4) *L. oenos* (8)
	Day 4	5.6×10^4	*L. oenos* (5)
Wine during conservation	Day 7	3.2×10^5	*L. oenos* (12)
	Day 11	4.8×10^7	*L. oenos* (11)
	Day 16	2×10^8	*L. oenos* (12)
	Day 18	8.2×10^7	*L. oenos* (12)
	Day 119	2×10^5	*L. oenos* (5)

remained at their initial level of 10^6 to 10^7 cells per ml for around 50 days. After this time slight decreases in cell numbers were recorded. At pH 3.3 there was a notable and progressive decrease in cell numbers, and by day 177 the bacteria were not detectable.

For the wines of 10 and 11.25% (by volume) alcohol, cell populations remained initially constant around 10^6 to 10^7 cells per ml but had gradually reduced to 10^4 to 10^5 cells per ml by 200 days. Bacteria in the 12.5% alcohol wine exhibited progressive reduction in viability and were not detectable after 117 days (Fig. 2B).

Cell populations remained virtually constant during storage at 4 and 12°C, decreased slightly (to 10^4 to 10^5 cells per ml) at 18°C, and decreased rapidly at 26°C. Lactic acid bacteria were not detected in the wine after 80 days at 26°C (Fig. 2C).

Addition of either 20 or 40 mg of SO_2 per liter to the wines resulted in rapid loss of cell viability (Fig. 2D) compared to the control. Curiously, however, lactic acid bacteria recommenced growth in these wines after 100 days, and by 200 days they reached levels of 10^6 cells per ml. No growth was observed in the control unsulfured wine. The renewed growth was due to *Leuconostoc oenos*, which was the only species encountered in all of these storage samples.

At the end of the storage period, samples of all the wines were examined for their content of D-(−)-lactic acid and acetic acid (Table 4). Note the elevated levels of acetic acid after storage of wines at pH 3.9 and at the alcohol concentration of 10%, where the final values were 0.64 and 0.62 g/liter, respectively. This was accompanied by an increase in the levels of D-(−)-lactic acid. Acetic acid levels were also increased by storage at 18°C as compared to 4 and 12°C. However, no augmentation occurred at 26°C, when the population was rapidly killed.

DISCUSSION

In both red and white musts with high concentrations of sugar, there was a notable and consistent decrease in the population of lactic acid bacteria during the alcoholic fermentation. In some cases it was not possible to detect any lactic acid bacteria in the newly fermented wine. These observations are consistent with previous reports (12; C. S. Pan, G. H. Fleet, G. J. Morrison, P. J. Costello, and T. H. Lee, Annu. Meet. Am. Soc. Vitic. Enol., 1980). The reasons for this decrease are not known but may be related to the fact that the strains of lactic acid bacteria found on grapes and in musts, namely, *Lactobacillus plantarum, Lactobacillus hilgardii*, and *Leuconostoc mesenteroides*, may not be tolerant to the alcohol generated during fermentation.

The addition of sulfur dioxide, up to 50 mg/liter, to musts did not reduce the initial

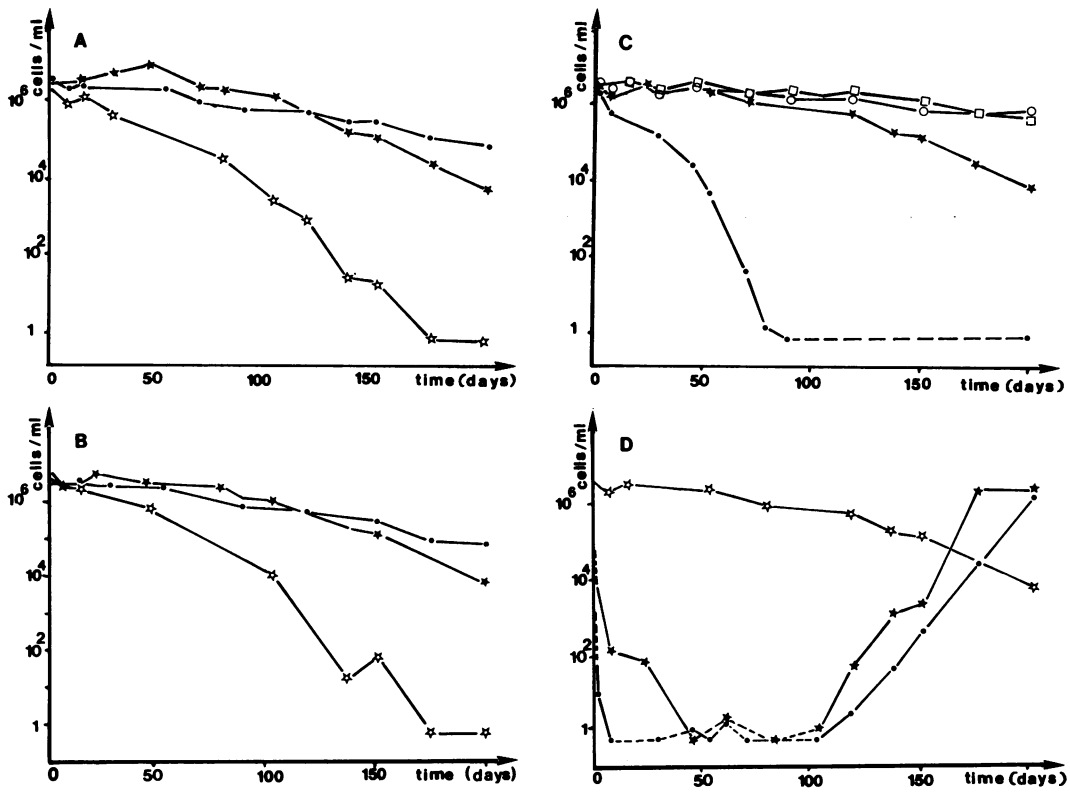

FIG. 2. Survival of lactic acid bacteria in wines after the malolactic fermentation. (A) Effect of (●) pH 3.9, (★) pH 3.6, or (☆) pH 3.3. (B) Effect of alcoholic concentrations of (●) 10%, (★) 11.25%, or (☆) 12.5% by volume. (C) Effect of storage at (□) 4°C, (○) 12°C, (★) 18°C, or (●) 26°C. (D) Effect of addition of (☆) 0, (★) 20, or (●) 40 mg of SO_2 per liter.

population of lactic acid bacteria, although 100 mg/liter did reduce it by around 10-fold.

The depressed population, however, was reinstated after the various manipulations for subsequent conservation at the end of the alcoholic fermentation. Immediately after these operations, levels of lactic acid bacteria were generally around 10^2 to 10^4 cells per ml. It would seem that these bacteria, largely *Leuconostoc oenos*, for the most part originate from winery equipment and materials. They serve as "natural" inoculum for the subsequent malolactic fermentation in the case of the red wines.

This study illustrated how the degree of sulfur dioxide addition and the temperature of storage may affect the subsequent development of *Leuconostoc oenos*, the species responsible for the malolactic fermentation (Table 1).

In the case of the white wine base for distillation, lactic acid bacteria did not decrease during the alcoholic fermentation, but actually increased. Furthermore, the commencement of their growth after alcoholic fermentation was

TABLE 4. Effects of various factors on levels of lactic and acetic acids in wine during conservation after malolactic fermentation

Factor	Acid concn (g/liter)	
	D-(−)-Lactic acid	Acetic acid
pH		
3.3	0.31	0.39
3.6	0.41	0.56
3.9	0.46	0.64
Temp		
4°C	0.31	0.35
12°C	0.32	0.36
18°C	0.41	0.56
26°C	0.32	0.38
Alcohol		
10%[a]	0.41	0.62
11.25%	0.41	0.56
12.5%	0.34	0.54
SO_2		
0 mg/liter	0.41	0.56
20 mg/liter	0.34	0.55
40 mg/liter	0.31	0.45

[a] By volume.

rapid and strong. This different behavior might be associated with the lower concentration of sugars in this unsulfured and unclarified must.

This study has also provided some insight into the factors that affect the growth or survival of lactic acid bacteria in wines during conservation after completion of the malolactic fermentation. Under standard conditions, lactic acid bacteria (*Leuconostoc oenos*) remained viable in the wine during storage, exhibiting no tendency for further growth and showing only a slow progressive decline in viability over the 200-day storage period. Temperature, however, exerts a very important influence on this survival. At the higher temperatures, and especially above 20°C, a rapid decline in viability was noted. At 26°C no lactic acid bacteria were detected after a storage period of 80 days. High temperature, low pH, and wine alcohol presumably combine to exert a lethal effect, possibly acting at the site of the cell membrane. Decrease in cell viability during conservation was accelerated by lowering the wine pH, increasing alcohol concentration, and adding SO_2. Although SO_2 addition to the wines resulted in rapid loss of cell viability, growth recommenced at a later stage. The basis of this regrowth is not understood and is being investigated further.

In some aspects of this study we have attempted to relate cell numbers to the species present. Although *Lactobacillus plantarum, Lactobacillus hilgardii, Leuconostoc mesenteroides*, and *Leuconostoc oenos* may be found on grapes in musts, only *Leuconostoc oenos* survives the alcoholic fermentation; even this species occurs in very low numbers at the end of this fermentation. In all cases, *Leuconostoc oenos* was the only species found in wines after the alcoholic fermentation, and it was the species responsible for the malolactic fermentation. This specific involvement of *Leuconostoc oenos* appears to be unique to the Bordeaux region, as during and after the malolactic fermentation (9–11; C. S. Pan, G. H. Fleet, G. J. Morrison, P. J. Costello, and T. H. Lee, Annu. Meet. Am. Soc. Vitic. Enol., 1980).

During the course of this study, 166 strains of *Leuconostoc oenos* were isolated and identified by the API system. Among these isolates, 71% fermented arabinose, 71% fermented ribose, and 2% fermented xylose; 47% of the isolates were not able to metabolize glucose or fructose within a 48-h incubation period, but for some strains this ability was noted after 15 days of incubation at 25°C under an atmosphere of CO_2. Only 11% of the strains degraded both glucose and fructose. Of those able to metabolize one or the other of the hexoses, 83% fermented fructose and 27% fermented glucose.

API batteries provide a rapid means for identifying wine lactic acid bacteria (7, 9). In addition, as revealed in this study, they showed a certain heterogeneity among the strains of *Leuconostoc oenos* that were isolated. In particular, this relates to the ability to ferment the pentoses (arabinose and xylose) and also to the affinity towards the hexoses (glucose and fructose). Such strain heterogeneity has been observed before (12), but its enological significance is not clear. Finally, it was noted that all *Leuconostoc oenos* isolates gave a strong reaction for esculin hydrolysis. This could mean that such strains may have the ability to hydrolyze phenolic heterosides in the wine.

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