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Review

Lactobacillus plantarum, a New Biological Tool to Control Malolactic Fermentation: A Review and an Outlook

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Received: 26 February 2020; Accepted: 1 April 2020; Published: 7 April 2020

Abstract: Malolactic fermentation (MLF) in wine is an important step in the vinification of most red and some white wines, as stands for the biological conversion of L-malic acid into L-lactic acid and carbon dioxide, resulting in a decrease in wine acidity. MLF not only results in a biological deacidification, it can exert a significant impact on the organoleptic qualities of wine. This paper reviews the biodiversity of lactic acid bacteria (LAB) in wine, their origin, and the limiting conditions encountered in wine, which allow only the most adapted species and strains to survive and induce malolactic fermentation. Of all the species of wine LAB, *Oenococcus oeni* is probably the best adapted to overcome the harsh environmental wine conditions and therefore represents the majority of commercial MLF starter cultures. Wine pH is most challenging, but, as a result of global warming, *Lactobacillus* sp. is more often reported to predominate and be responsible for spontaneous malolactic fermentation. Some *Lactobacillus plantarum* strains can tolerate the high alcohol and SO_2 levels normally encountered in wine. This paper shows the potential within this species for the application as a starter culture for induction of MLF in juice or wine. Due to its complex metabolism, a range of compositional changes can be induced, which may positively affect the quality of the final product. An example of a recent isolate has shown most interesting results, not only for its capacity to induce MLF after direct inoculation, but also for its positive contribution to the wine quality. Degrading hexose sugars by the homo-fermentative pathway, which poses no risk of acetic acid production from the sugars, is an interesting alternative to control MLF in high pH wines. Within this species, we can expect more strains with interesting enological properties.

Keywords: malolactic fermentation; *Lactobacillus plantarum*; *Oenococcus oeni*; facultative hetero-fermentative; starter cultures

1. Introduction

Malolactic fermentation (MLF), the process of biological de-acidification in winemaking, is based on the L-malic acid decarboxylation to L-lactic acid and CO_2. It can occur during or after alcoholic fermentation as a result of the metabolic activity of lactic acid bacteria (LAB), which are present in wine at all stages of winemaking. Four genera were identified as the principal organisms involved in MLF: *Lactobacillus*, *Leuconostoc*, *Oenococcus*, and *Pediococcus* [1]. Wine pH is most selective, and, at a pH below 3.5, generally only strains of *Oenococcus oeni* can survive and express malolactic activity. *O. oeni* is probably the best adapted to overcome the harsh environmental wine conditions and therefore most of the commercial MLF starter cultures consist of strains from this species. Traditionally, when selected wine bacteria are used, inoculation is performed at the completion of alcoholic fermentation (AF). Since 1980, researchers have explored the possibility of inoculating wine LAB into the grape must together

with the yeast or shortly after the yeast at the beginning of the alcoholic fermentation. Today, we have identified two different timings throughout the winemaking process for inoculating wine LAB into the wine: co-inoculation with yeast (selected wine bacteria added 24 to 72 h after yeast addition) or sequential inoculation, when selected wine lactic acid bacteria are added at the end of, or just after the completion of, AF.

Wine pH has been increasing gradually for the last several years. Red wines with pHs over 3.5–3.6 are more and more frequent. At these pH levels, we can observe very fast growth of various indigenous microorganisms, some of which are spoilage bacteria that can cause loss of wine quality. Among these species, *Lactobacillus plantarum* strains have shown most interesting results for their capacity to induce MLF under high pH conditions, their facultative hetero-fermentative properties that avoid acetic acid production from hexose sugars and their more complex enzymatic profile and different metabolism compared to *O. oeni*, which could play an important role in the modification of wine aromas.

Besides pH, the ethanol produced by the yeast during alcoholic fermentation is another limiting factor for bacterial growth and survival in wine. Radler [2], Peynaud and Domercq [3], and Henick-Kling [4] reported an increasing inhibition above 5% (*v/v*). The degree of ethanol tolerance is, however, strain dependent. Specific details of alcohol sensitivity for the various species of wine LAB are contradicting. Davis et al. [5] reported strains of *Lactobacillus* and *Pediococcus* being in general more tolerant to high ethanol concentrations than *O. oeni*. In contrast to this, Henick-Kling [6] reported *O. oeni* being only partially inhibited by ethanol concentrations above 5% (*v/v*) and able to tolerate up to 14% (*v/v*) alcohol, while the growth of *L. plantarum* stops at ethanol concentrations of 5–6% (*v/v*). The first *L. plantarum* starter culture was introduced in the late 1980s in the United States and later also in Europe. Prahl et al. [7,8] proposed to inoculate the grape juice before alcoholic fermentation using a facultative hetero-fermentative *L. plantarum* starter culture. In EP0398957B1 [7], they disclosed a method of introducing an important freeze-dried biomass of *L. plantarum* directly into must or fruit juice to induce MLF without significant consumption of sugars present in the must or fruit juice and substantially without any production of volatile acidity. This malolactic bacteria strain had little alcohol tolerance and had been unable to survive in the fermented wine. The application of this culture had only been recommended for partial malic acid degradation in low pH white wines.

In 2004, Bou and Krieger [9] filed a patent on "Alcohol-tolerant malolactic strains for the maturation of wines with average or high pH" under the international application number PCT/FR2004/001421. The patent relates to alcohol tolerant LAB strains of the genera *Lactobacillus* and *Pediococcus* capable of initiating and carrying out a complete MLF upon direct inoculation in dried, frozen, or lyophilized state into a wine with an alcohol content of 10% (*v/v*) or more and an average high pH level. Dating back to 2005, a new selection of *L. plantarum* at Università Cattolica del Sacro Cuore in Italy resulted in a very effective *L. plantarum* culture V22, adapted to high pH wines, and showing good alcohol tolerance [10].

In 2012, Soerensen et al. [11] filed a patent application on "*Lactobacillus plantarum* cells with improved resistance to high concentrations of ethanol" (WO 2012/17200). The invention relates to cycloserine resistant mutants of lactic acid bacteria having improved resistance towards ethanol. The cycloserine resistant mutants of lactic acid bacteria had been proposed for use to induce malolactic fermentation in wine having high alcohol levels, but, as outlined above, alcohol tolerant *L. plantarum* strains can also be isolated from nature.

In 2016, a new highly concentrated *Lactobacillus plantarum* starter culture was introduced to the markets [12]. The new starter culture, called ML Prime™, is issued from an optimized process that promotes very high malolactic activity as soon as it is added to must. Despite the good alcohol tolerance of this pure *Lactobacillus plantarum* culture with the homo-fermentative metabolism of hexose sugars, its most interesting application is in co-inoculation (inoculation 24 h after the wine yeast) without any risk of volatile acidity production during MLF even under high pH conditions. Due to the very high malolactic enzymatic activity and early inoculation shortly after the selected wine yeast into the fermenting must, MLF is therefore completed in record time (3–7 days) during alcoholic fermentation.

This way, wines can be stabilized early and protected from further contamination and thus retain their sensory integrity. More recently, this *L. plantarum* starter culture ML Prime™ had been also proposed for a specific application in white wine to achieve a partial malolactic fermentation under lower pH conditions. In the white wine application, co- and sequential inoculation can be applied.

The majority of the selected wine lactic acid bacteria starter cultures are the pure single strain cultures, but, in 2008, the Institute for Wine Biotechnology at Stellenbosch University [13] launched a project to study on the possible application of mixed MLF starter cultures consisting of one selected *L. plantarum* and one selected *O. oeni* strain deriving from the Stellenbosch strain collection. In 2010, the first mixed LAB species culture was as Co-Inoculant NT202 and proposed for simultaneous inoculation together with a specific yeast strain NT202. The authors claim the importance of the *L. plantarum* strain in the mix for its sensory contribution and of the *O. oeni* strain for its malolactic enzyme activity. Certain strains within the *L. plantarum* species have been found to possess even more diverse enzymatic activities, which could contribute to the wine aroma profile than *O. oeni*.

2. Biodiversity of Lactic Acid Bacteria in Wine

Winemaking is a microbiological process involving a very complex system and it involves numerous microbial transformations comprising a complex succession of various yeast and bacterial species. Malolactic fermentation (MLF) can occur during or after alcoholic fermentation and is carried out by one or more species of lactic acid bacteria (Figure 1).

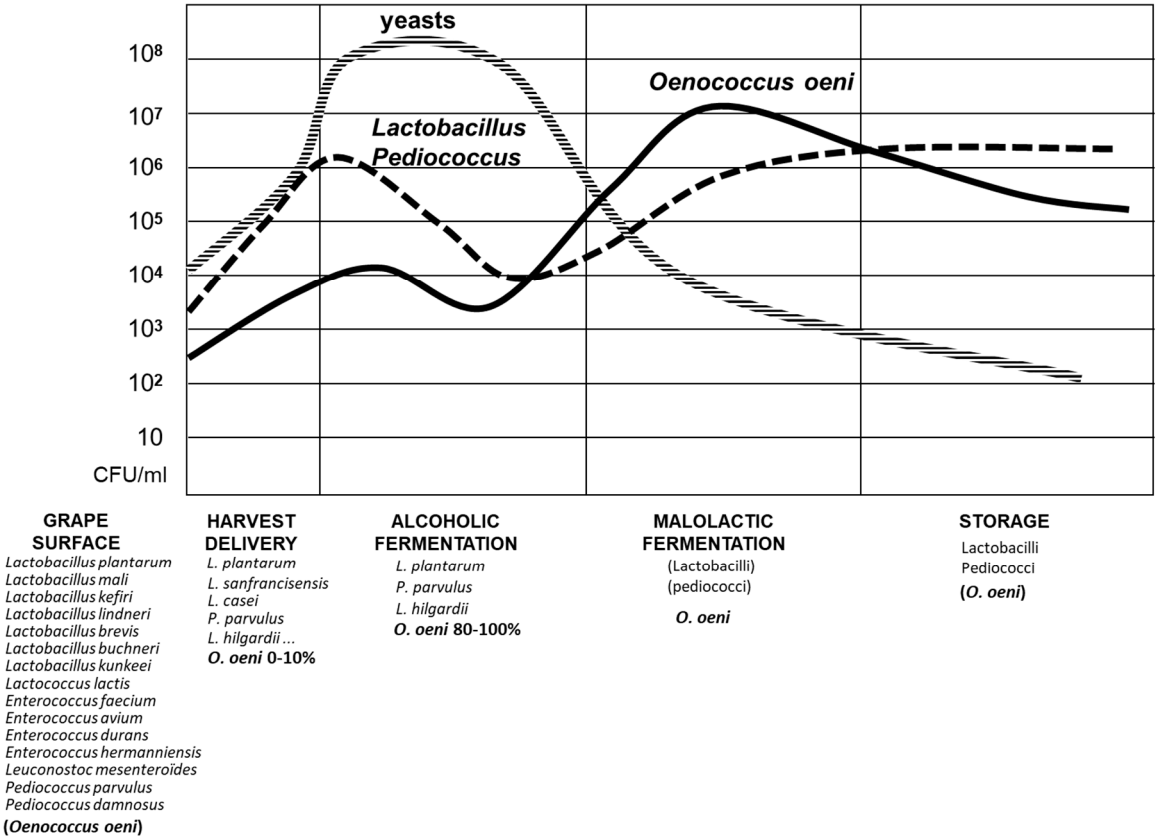

Figure 1. Population kinetics of wine lactic acid bacteria from the vineyard to the wine (Modified from Patrick Lucas, 2016, International ML School Lallemand Toulouse).

Different LAB enter into grape juice/wine from the surface of grape berries, stems, leaves, and soil and winery equipment. In the vineyard, LAB species diversity associated with grape surfaces is rather limited, mainly due to their nutritional requirements [14,15]. The population density of LAB is very limited, especially in comparison to the indigenous yeast population found on grapes [16].

Pediococcus, *Leuconostoc*, and *Lactobacillus* species occur on grapes more frequently than *O. oeni* [17]. In addition to grape surfaces, bacterial strains can also be isolated from the cellar environment, such as fermentation tanks and barrels and poorly sanitized winery equipment, such as pipes and valves [17,18]. Shortly after crushing and the start of AF, the LAB population in the grape must generally range from 10^3 to 10^4 cfu/mL (colony forming units per milliliter), and the LAB species largely belonging to the species of *Lactobacillus* and *Pediococcus* disappear progressively during the AF [19]. The decrease could be attributed to increased ethanol concentrations, high SO_2 concentrations, initial low pH, low temperatures, the nutrient depletion, and/or competitive interactions with the yeast culture [16,20]. During spontaneous MLF, *O. oeni* is the major bacterial species found, however, several species can be occasionally detected, mainly *Lactobacillus*, *Pediococcus,* and *Leuconostoc* [1,19]. In some of the warmer wine growing regions, *L. plantarum* is more frequently isolated from spontaneous malolactic fermentations [13,21–23]. Lerm et al. reported three *O. oeni* and three *L. plantarum strains* from South Africa wine isolates for use as MLF starter cultures. Bergeral et al. [21] had studied the properties of *Lactobacillus plantarum* strains isolated from grape must fermentation Apulian wines in order to select suitable starter for MLF, and Valdés La Hens et al. [22] reported the Prevalence of *L. plantarum* and *O. oeni* during spontaneous fermentation in Patagonian red wines. More recently, Lópes-Seijas et al. [23] evaluated malolactic bacteria associated with wine from the Albariño variety. Different to what has been described from other wine growing regions, the predominant species in the region of Val do Salnés in Spain were *L. hilgardii*, *L.* paracasei, and *L.* plantarum. Nevertheless *O. oeni* is most frequently the predominant species at the later stages of vinification (Figure 1), since it is best adapted to the limiting conditions encountered in wine. Over centuries of selective pressure, *O. oeni* has acquired and perfected various adaptive strategies that enable it to outcompete with other wine lactic acid bacteria during the later stages of vinification and thus to dominate in wine [15]. It proliferates in wine and cider during or after the yeast-driven alcoholic fermentation and reaches population levels above 10^6 cells/mL, thus becoming sometimes the only detectable bacterial species [24,25].

Wine pH is most selective, and, at a pH below 3.5, generally only strains of *O. oeni* can survive and express malolactic activity, while, in wines with a pH above 3.5, some *Lactobacillus* species have also shown a good ability to conduct MLF. Generally, the most frequent lactobacilli isolated from wine belongs to *Lb. plantarum*, *Lb. brevis*, *Lb. buchneri*, *Lb. hilgardii,* and *Lb. fructivorans*, although their occurrence and that of other species (i.e., *Lb. fermentum*, *Lb. kunkeei*, *Lb. mali*, *Lb. vini*) can be found depending on the grape varieties and typologies of wines [1]. Among them, *Lb. plantarum* is certainly the most important in wine because it is found frequently on grapes and in wine and is often involved in spontaneous MLF under high pH conditions. This versatile bacterium tolerates ethanol up to 14% (*v/v*) and can have similar SO_2 tolerance like *O. oeni*. Moreover, *Lb. plantarum* has a more diverse array of enzymes and can potentially exert positive effects on organoleptic properties of wine [1]. Some selected *L. plantarum* strains have shown interesting results for their capacity to induce MLF under high pH conditions, and, unlike *Oenococcus oeni*, *L. plantarum* has a facultative hetero-fermentative metabolism that prevents acetic acid production from hexose sugars. Due to these characteristics, selected strains of *Lb. plantarum* are currently being commercialized to induce MLF in wine [1,26].

Pediococcus damnosus is the other species well represented in the wine environment. It is often found after alcoholic fermentation in wines with rather high pH, along with *Lactobacillus* sp. and *O. oeni*. As it has been identified in most ropy wines, its presence is considered undesirable. In reality, only certain strains of *P. damnosus* are responsible for this spoilage and they are easily identified through polymerase chain reaction (PCR). Little research has been published on the possibility of using these organisms as wine LAB starter cultures. A study in which indigenous strains of *P. damnosus* dominate a starter culture of *O. oeni* and conduct the MLF shows that it is very capable of surviving in wine [27].

3. Selected Wine Lactic Acid Bacteria Starter Cultures and Wine Challenging Factors

Grape juice and (especially) grape wine contain a challenging matrix, with sugar, ethanol, organic acids, amino acids, fatty acids, other metabolites deriving from the yeast metabolism during alcoholic

fermentation, phenol contents, pH, and SO_2 determining the growth of wine microorganisms. Various papers reported many factors that influence the occurrence of LAB and MLF in wines. Henick-Kling [28] and Wibowo et al. [29] listed, besides oxygen and CO_2, carbohydrates, amino acids, vitamins and minerals, organic acid content, the alcohol level, pH, and SO_2 level.. The interrelationships between LAB and wine yeast [30] or other wine microorganisms and the method of vinification have been reported to be the most influential factors to affect LAB growth. The wine pH is one of the most important factors that limits LAB growth and MLF in wine [2,29,31] and determines the type of LAB which will be present. Ideally, for table wines, the pH should be between 3.1 and 3.6 [32], but due to global warming wine pH has increased in recent years in almost all wine regions.

3.1. Well-Known Factors that Affect Malolactic Fermentation and Bacteria Vitality

The best understood factors that govern successful MLF are SO_2, pH, alcohol, and temperature.

3.1.1. pH

The pH of the media has a drastic influence on the MLF itself as low pH inhibits the growth of the wine LAB. Most LAB are neutrophilic [33], and the optimum pH for the growth of lactic acid bacteria is close to neutrality. Some bacteria strains of the genera of *Lactobacillus* and *Oenococcus* show more acidophilic behavior. At pH values less than 3.0, bacterial growth is very difficult or impossible [34]. *Oenococcus oeni*, which is the organism of choice to conduct MLF under acidic conditions, will generally dominate, and in wine of pH above 3.5, strains of the genera of *Lactobacillus* or *Pediococcus* can be more present. The ability of the bacteria to obtain energy from the metabolism of glucose is inhibited at the low pH of wine [28]. *L. plantarum* shows a preference for malate as an energy source at low pH [35], even in the presence of glucose, which suggests this species as a starter for malate decarboxylation in fermenting musts [36]. Prahl [8] and Bou and Krieger [9]) proposed *Lactobacillus plantarum* as most promising for use as a starter culture in higher pH wines.

3.1.2. Ethanol

Ethanol is known for its bactericidal properties and it is the main yeast metabolite produced during alcoholic fermentation. It can play an integral role in the ability of wine LAB to survive in wine and induce the malolactic fermentation. It is difficult to specify the concentrations which will completely prevent LAB development. Radler [2], Peynaud and Domercq [3], and Henick-Kling [4] reported an increasing inhibition above 5% (*v/v*). Wibowo et al. [29] stated in their review that the ability of LAB to survive and grow in wine decreases as the alcohol concentration increases above 10% (*v/v*). Henick-Kling [6] indicated a strong impact of temperature on the toxicity of ethanol. A temperature of 25 °C and above, combined with alcohol levels above 14.5% (*v/v*) can inhibit bacterial growth and the malolactic fermentation. However, the ethanol tolerance is very strain dependent. Information in the literature is contradictory regarding the alcohol sensitivity for the various species of wine LAB. Davis et al. [5] reported strains of *Lactobacillus* and *Pediococcus* being in general more tolerant to high ethanol concentrations than *O. oeni*. From the observation of Wibowo et al. [29], most *Lactobacillus spp*. can tolerate about 15% (*v/v*). Britz and Tracey [37] acknowledged that all *O. oeni* strains are able to survive and grow at 10% (*v/v*) ethanol at pH 4.7. Studying the combined effects of pH, temperature, ethanol, and malate concentrations on *L. plantarum* and *O. oeni*. Guerzoni et al. [36] suggest *L. plantarum* being more competitive in early steps of alcoholic fermentation. However, more severe conditions, e.g., ethanol concentrations higher than 6% (*v/v*), favor *O. oeni*. Most robust commercial *O. oeni* strains, which are produced with a specific process for pre-adaptation (MBR™ process) to different wine conditions, show good survival and good malolactic activity up to 16% (*v/v*) alcohol, depending on other environmental factors even higher. With regard to commercial *L. plantarum* starter culture preparations for the induction of MLF in wine, there are two different approaches: Pre-inoculation: Prahl et al. [7] proposed inoculating must before alcoholic fermentation using a direct inoculum of a freeze-dried facultative hetero-fermentative *L. plantarum* starter culture. Inoculation before the

wine yeast addition was recommended due to the sensitivity of the described *L. plantarum* strain towards alcohol. Contrastingly, a patent filed in 2004 [9] on "Alcohol-tolerant malolactic strains for the maturation of wines with average or high pH" relates to LAB strains of the genera *Lactobacillus* and *Pediococcus* displaying a good alcohol tolerance and the capability to induce a complete MLF upon direct inoculation into a wine with an alcohol content of 10% (*v/v*) or more and an average high pH level. More recently, more alcohol resistant *Lactobacillus* starter culture had been released, which can tolerate up to 15% (*v/v*) [10].

3.1.3. Temperature

In wine, the optimum temperature of growth is different from what is obtained in laboratory culture. The optimum range is dependent on other physical and chemical parameters of the wine, notably the ethanol content. A higher ethanol content will lead to a decrease in the optimum growth temperature. In general, MLF usually occurs at sub-optimal LAB temperatures (below or around 18 °C). At 15 °C or lower, the chance of bacterial growth is slight [38]. Guerzoni et al. [36] studied the effects of several chemico-physical factors (pH, SO_2, ethanol concentration and temperature) on *L. plantarum* and *O. oeni*. A temperature increase only positively affected the lag phase of *O. oeni*, but not of *L. plantarum*. A temperature increase exhibited a negative and positive influence on *O. oeni* and *L. plantarum*, respectively. The combination of high temperatures and high alcohol increase the toxicity of ethanol as outlined above. Low temperatures are not lethal but decrease the enzymatic activity.

3.1.4. Sulphur Doxide

Sulphur dioxide (SO_2) is another compound well known for its bactericidal action and plays an essential role in the growth of LAB and development of MLF [32]. This component is found in wine with variable concentrations according to the winemaking conditions and the yeast strain responsible for alcoholic fermentation. SO_2 is purposely added to wines to inhibit the growth of undesirable microorganisms and for its antioxidant effect. Sulphur dioxide in its free form, as well as in its bound form with aldehydes and ketones, is a potent inhibitor of many microbes, including LAB. Three liberated forms of SO_2 are present in wine: molecular SO_2, bisulphite (HSO_3^-), and sulphite (SO_3^{2-}). Molecular SO_2 is effective as a bacterial preservative [39], and a well-known synergistic effect is the impact of pH on the level of molecular SO_2. The lethal level of molecular SO_2 for most wine LAB is low (0.3 mg/L), but it is possible that certain selected wine LAB strains could have a better resistance to molecular SO_2. Depending on the pH of the juice/wine, the amount of molecular SO_2 is between 1% and 7% of the free SO_2 content. The molecular SO_2 increases with a decrease in pH and an increase in temperature and/or alcohol.

For MLF to be successful, the values of these chemical parameters described above must correspond to those that allow the bacterial cultures to function successfully. A favorable level of any of these components may compensate for an unfavorable level of one or several of the others. It is important to remember these factors function synergistically, i.e., their actions together have a greater total effect than the sum of their individual actions.

3.2. Lesser-Known Factors that Affect Malolactic Fermentation

A number of lesser known, but equally important, factors can influence the course of MLF and are outlined below.

3.2.1. Yeast Strain Selection

It has been known for some time that certain yeasts selected to conduct the alcoholic fermentation (AF) interact better with certain wine LAB for the successful achievement of MLF. Under specific conditions, certain yeast strains may produce high concentrations of SO_2, which has a negative influence on the growth and survival of the wine LAB. Similarly, yeast strains that exhibit an inordinate need for nutrients could exhaust the medium to such an extent that no reserve nutrients are available

for the bacteria. Implementing a specific nutrition strategy for a particular yeast in the early stages of AF can largely surmount this [40–43] and avoid the production of certain unwanted metabolites or toxins derived from yeast stress. More recently, other bacterial growth inhibiters derived from yeast metabolism have been reported, such as medium-chain fatty acids [44] and yeast peptides (between 5 and 10 kDa) [45,46]. More recently, Liu et al. [30] reported certain peptides being stimulating for *O. oeni*. These effects depend on the nature and the level of fatty acids in the wine or the size of the yeast peptides, and can be exacerbated by low pH. On the other hand, the contact with the yeast lees has a very stimulating effect on MLF. The autolysis process releases amino acids and vitamins, and the must become richer in nutrients for the LAB. There may also be a detoxifying effect by yeast polysaccharides, as they may adsorb inhibitory compounds or complex them. Fumi et al. [10] reported yeast strains compatible with *O. oeni* starter cultures being also compatible with a *Lb. plantarum* starter culture strain.

3.2.2. Organic Acids

From practical experience, wines with L-malic acid levels below 1 g/L are not as conducive to MLF by *O. oeni*, as are wines with L-malic acid concentrations between 2 and 4 g/L. Wines with levels of L-malic acid above 5 g/L often start L-malic acid degradation, but do not go to completion. The cause is thought to be the result of the inhibition of the bacteria by increasing concentrations of L-lactic acid derived from the MLF itself. Since acidification with the organic acids lactic acid, L(−) or DL malic acid, L(+) tartaric acid and citric acid is authorized in many wine regions, and Vincent Gerbaux from the Institut Français de la Vigne et du Vin (IFV) in France has studied the influence of organic acid additions on the development of MLF (data not published). In this study, six selected wine LAB strains were inoculated into a Chardonnay wine, and five selected *O. oeni* wine LAB strains were inoculated into a Pinot noir wine, both of which were adjusted to a pH of 3.25. Increasing the amounts of L-malic acid, 0.75 to 5.2 g/L for Chardonnay and 3.0 to 5.7 g/L for Pinot noir, increased the time required to complete MLF, but the speed of L-malic acid degradation increased with increasing content of L-malic acid. The differences between selected wine LAB strains were observed. The addition of D-malic acid had no noticeable effect on MLF.

The presence of L-lactic acid in the wine inhibits the implantation and growth of the inoculated *O. oeni* strains, resulting in an inhibition of MLF. An initial content of L-lactic acid in the range of 1.5 g/L slows MLF, but a content of 3.0 g/L inhibits MLF by most of the tested *O. oeni* strains. Problems inducing MLF by inoculation with selected wine LAB cultures may be encountered when L-lactic acid was added to must or wine or in wines with a partial MLF. The impact of DL-lactic acid and D-lactic acid has yet to be investigated.

3.2.3. Tannins

Some red grape cultivars, such as Merlot, Tannat, and Zinfandel, may experience great difficulty undergoing successful MLF [47,48]. This may be related to certain grape tannins exerting a negative influence on the growth and survival of wine LAB, and consequently on the MLF. Research has been conducted exploring the impact of polyphenols on the growth and viability of wine LAB and their ability to degrade L-malic acid, often with inconsistent results. Polyphenols can have either stimulatory or inhibitory effects on the growth of wine LAB, depending on their type and concentration, and on the selected wine LAB strain in question. Figueiredo et al. [49], Chasseriaud et al. [50], and Stivala et al. [51] showed that tannin compositions containing a high percentage of condensed tannins can strongly affect the viability of *O. oeni* cells, whereas tannin blends consisting of anthocyanins and condensed tannins or catechin and epicatechin monomers and dimers, respectively, only slowed down the growth of bacteria when they were used at the highest concentration. These results are also in agreement with previous studies that showed no effect or a stimulatory effect of these compounds [49,52,53]. The successive activity of a 3-O-galloyl esterase and gallate decarboxylase, as it has been found in *L. plantarum* [54], may explain the stimulation by the addition of grape tannins.

3.2.4. Nutrient Deficiencies

In order to successfully complete MLF, proper nutrition for the wine LAB is of the utmost importance, because wine LAB are characterized as having complex nutritional requirements. Contrary to the fermentation yeast *Saccharomyces cerevisiae*, the bacteria *O. oeni* and other wine LAB cannot utilize inorganic nitrogen sources. Instead, sufficient amounts of organic nitrogen in the form of amino acids and peptides must be supplied. The vitamins pantothenic acid, thiamine, and biotin, as well as the trace elements Mg, Mn, and K, must also be provided to ensure bacterial growth and malolactic activity. Terrade and Orduña [55] investigated the essential growth requirements of four strains of wine LAB from the genera *Oenococcus* and *Lactobacillus*. The two *Oenococcus oeni* strains revealed a larger number of auxotrophies (18 and 23), the two *Lactobacillus* strains only had 11 and 14 auxotrophies. Despite the complex nutritional needs of wine LAB, the amounts they require are, in fact, quite small. Normally, the amount of nutrients contained in the wine matrix is sufficient to meet the needs of LAB, but certain vinification practices can result in nutrient deficiency.

3.2.5. Residual Lysozyme Activity and Chitosan Formulation

If lysozyme is used to control indigenous LAB during the production of wine, it is possible that residual levels of this enzyme may impact the duration of the subsequent MLF [56]. In most cases, racking the wine off the gross lees is recommended. Strains of *O. oeni* are more sensitive to the effects of lysozyme than strains of *Lactobacillus* or *Pediococcus* are.

Certain forms of chitosan, a natural polymer derived from chitin, exhibit antimicrobial properties. Chitosan is well known for its antimicrobial properties against yeast, bacteria, and fungi [57,58], and a preparation extracted from a fungal source has been used to neutralize contamination by *Brettanomyces bruxellensis* [59]. More recently, Chitosanglucan formulation has been released to inhibit wine lactic acid bacteria and delay or inhibit malolactic fermentation. To ensure timely MLF, as well as wine protection, the application of chitosan is recommended at the completion of MLF.

3.2.6. Others

Other inhibitory compounds include certain fungicides and pesticides, especially the former, which may have a detrimental effect on wine LAB. The residues of systemic fungicides are the inhibitoriest and they are often used in the later stage of grape maturation to control the fungus *Botrytis cinerea* [60]. More recently, mixes of medium-chain fatty acid or the addition of fumaric acid had been proposed to inhibit the growth of wine lactic acid bacteria and the resulting malolactic fermentation.

4. Selected Wine Lactic Acid Bacteria Starter Cultures and the Timing of Inoculation

Even when desirable malolactic acid bacteria are established in a winery, the onset of the MLF may take several months and may occur in some barrels and tanks but not in others. There are several options available to control MLF: firstly, the selection of conditions to encourage the growth of the indigenous malolactic flora; secondly, the induction of MLF in wines by inoculating with wines already undergoing MLF; or, thirdly, the induction of MLF by inoculation with either laboratory-prepared or commercial strains of LAB. Increased recognition of the influence of MLF on wine quality has led winemakers in recent years to seek better control over the occurrence and outcome of MLF. For this reason, the induction of the MLF by the use of selected LAB starter cultures is fast becoming the preferred option. Due to a massive inoculum with bacteria at 10^6 cfu/mL, less time is required for the bacteria to grow up to a cell density high enough to rapidly degrade the malate present in wine. Undesirable bacteria can be suppressed, which prevents wine alterations [1].

In the 1980s, commercially available strains of wine LAB from around the world became available to the wine industry. These LAB strains were selected from good spontaneous MLF and screened for good MLF kinetics, reliable performance under wine conditions, and desired sensory properties. They will tolerate the difficult growth and survival conditions found in wine and will produce compounds

that impart positive sensory impacts to the wine. In the early 1990s, direct inoculation MLB starter cultures became available, and the most effective are pre-acclimatized during the production process. This step allows them to survive being added directly to wine, with no decrease in viable cell numbers, and no loss of malolactic activity. These wine LAB preparations can be added directly to wine or rehydrated in water for a short time prior to their addition.

Traditionally, when selected cultures of known wine LAB are used, inoculation is performed at the completion of AF. That said, already in 1985, Beelman and Kunkee [61] explored the possibility of inoculating wine LAB into juice along with the yeast used to conduct AF. Current thinking identifies the following times during wine production when selected wine LAB can be added (Figure 2).

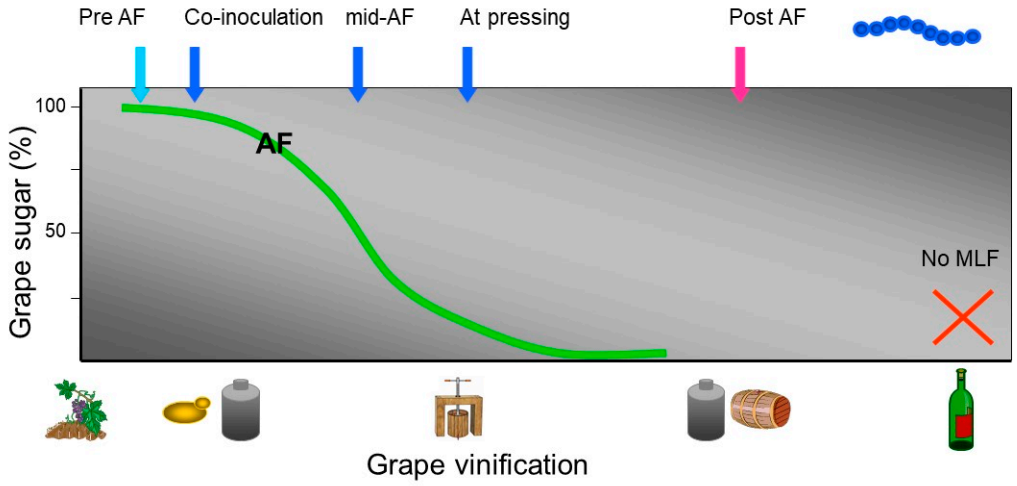

Figure 2. Inoculation regimes for selected wine lactic acid bacteria Adapted from Bartowsky, Australian Wine Research Institute (AWRI), Entretiens Scientifiques Lallemand Dubrovnik 2011.

4.1. Co-inoculation with Selected Yeast and Selected Wine Lactic Acid Bacteria

Before 2003, researchers at the Université de Bordeaux recommended making the wine LAB addition only after the completion of AF. They felt this timing would avoid the production of acetic acid and D-lactic acid, compounds derived from the hetero-fermentative carbohydrate metabolism of LAB [62]. They proposed that wine LAB added at earlier points during AF may result in slow or stuck yeast fermentation, or result in MLF inhibition due to yeast antagonism. To date, none of these concerns have been observed when both AF and MLF have been properly managed.

But other researchers proposed the inoculation of selected wine LAB into juice along with yeast because it was felt nutrient availability would be enhanced, and the absence of alcohol would allow wine LAB to better acclimatize to environmental conditions and grow more vigorously. Beelman and Kunkee [61] showed that MLF in the presence of fermentable sugars does not necessarily lead to the production of excessive amounts of acetic acid, as long as yeast fermentation starts promptly and goes to completion [63,64]. For a successful co-inoculation, some parameters are crucial for its success—choosing the right wine yeast, correctly rehydrated, good temperature management, and the proper yeast nutrition strategy are key points to integrate for any fermentation. Well-fed and healthy wine yeast and bacteria leads to complete and regular alcoholic and malolactic fermentations.

Since 2003, co-inoculation has gained increasing interest across all winemaking regions. Today, co-inoculation is understood as the practice of inoculating selected wine lactic acid bacteria at the beginning of the winemaking process shortly after yeast inoculation, usually 24 to 48 h after yeast inoculation. This technique is advantageous because not only will it secure the malolactic fermentation under most difficult conditions, but also because there are definite advantages that are recognized by winemakers and professionals: First bullet

- MLF can be completed in between 3 days and 2 weeks depending on the type of musts and the bacteria used.

- Co-inoculation to produce fresh wine styles with low diacetyl content: Co-inoculation always result in more fruit-driven wine styles and very low diacetyl content in wines. Early results also show that in the case of co-inoculation the high content of sugars could repress the metabolism of the diacetyl, as opposed to post-alcoholic fermentation inoculation. Moreover, under the reductive conditions generated by the active yeast, diacetyl produced will be immediately reduced to the less active metabolites, acetoin and butanediol.
- Co-inoculation to limit the development of *Brettanomyces* and off-flavors: The increase in sugar levels, pH, and sometimes lower SO_2 addition can influence the development of spoilage microorganisms, especially *Brettanomyces*, which can produce phenolic off-odors in wines. It is well known that the period from the end of AF to the start of MLF is particularly conducive to the development of *Brettanomyces*. Early inoculation with wine bacteria, either right after AF or in co-inoculation (24 h after inoculation with yeast), has proven to be a simple and effective method for preventing the development of *Brettanomyces* and the production of ethyl phenols off-flavors. Recent studies with IFV in Burgundy (Gerbaux) show co-inoculation with selected *O. oeni* strains can inhibit the growth of *Brettanomyces* (below 10 cell/mL) as opposed to the spontaneous control that is still contaminated with 500 cell/mL of *Brettanomyces* while the MLF is not completed and the wine is not stabilized [65].
- As a bio-control agent for low acidity/high pH wines, *Lactobacillus plantarum* with its facultative hetero-fermentative sugar metabolism is ideal as it completes MLF in 3–5 days during the alcoholic fermentation with no risk of increased volatile acidity due to its specific metabolism. It enables early stabilization of wines, as soon as the AF is finished.
- Co-inoculation as a tool for sustainability. In the frame of National Spanish R&D Project (VINySOST) involving six wineries, two companies of auxiliary industry, and several research centers (New strategies vine and winemaking for sustainable management in the production in great surfaces and increase in competitiveness of wineries in the international market—CDTI (strategic program CIEN, call 2014)), one of the studies were focused on the carbon footprint and analysis of life cycle from different axes involving wine producers. Within the study of carbon footprint and energy cost related to malolactic fermentation, co-inoculation with selected wine LAB had been compared to spontaneous MLF. Co-inoculated wine finished MLF with four after termination of the alcoholic fermentation whereas the spontaneous MLF took more than one and a half months to finish MLF. An electrical network analyzer was used after the energy consumption. Co-inoculation reduces the electricity consumption by more than 60%, as there was no need to heat the tanks to achieve a malolactic fermentation.

4.2. Sequential Inoculation with Selected Wine Lactic Acid Bacteria Post-alcoholic Fermentation

The traditional inoculation at the end of AF does not pose the risk of the bacterial decomposition of sugars and the resultant increase in VA, nor does the production of excessive amounts of lactic acid, known as "piqûre lactique," occur. Inoculation at this point avoids much of the toxicity attributed to some carboxylic acids, such as fumaric acid, as their concentration declines after AF [38]. The merit of inoculation at the end of AF can also be related to the availability of the bacterial nutrients, nitrogen-containing bases, peptides, amino acids, and vitamins that have arisen from yeast death and subsequent autolysis [66]. Another advantage may be simply from a logistical point of view. When using sequential inoculation, the wines that should undergo MLF can be separated from the wines where acidity is to be conserved. The vinification process can be conducted so that only one type of fermentation at a time is monitored. Often, this is perceived as less risk for cross-contamination.

However, exposure to high levels of ethanol that are present may result in delayed MLF, especially in wines produced in hot climates. If wine conditions are not limiting, selected wine LAB added after the AF are able to achieve cell concentrations comparable to those inoculated into must. In cases of nutrient limitation or adverse wine chemical parameters, the addition of a bacterial nutrient will support MLF. In instances where alcohol levels exceed 14.5% (*v/v*), selected wine LAB strains tolerant

to alcohol must be used or they must be acclimatized before inoculating into wine. The additive, inhibiting effect of ethanol, pH, and SO_2 must be considered, and the strain best adapted to the conditions must be chosen.

5. Advantages of *Lactobacillus plantarum* Starter Cultures

Lactobacillus plantarum strains have shown most interesting results for their capacity to induce MLF under high pH conditions (Table 1), their facultative hetero-fermentative properties that avoid acetic acid production (Figure 3), and their more complex enzymatic profile compared to *O. oeni*, which could play an important role in the modification of wine aroma.

Table 1. Characteristics of Lactobacillus plantarum vs. Oenococcus oeni.

Species	*Lactobacillus plantarum*	*Oenococcus oeni*
Fermentation of sugars (hexoses)	Homo-fermentative = 2 × lactate	Hetero-fermentative = Lactate + acetate + CO_2
Wine parameter for best performance	pH > 3.5 Alcohol < 15.5% (*v/v*) Total SO_2 < 50 ppm Temperature 20–26 °C	pH > 3.1 Alcohol < 15.5% (*v/v*) Total SO_2 < 50 ppm Temperature > 17 °C
Genetic preposition for enzyme activities	MOST strains: Malolactic enzyme/ Glycosidase/Protease/Esterase/Lipase/Citrate lyase	Only very FEW strains: Malolactic enzyme Esterase/Protease/ Citratelyase/Methionine synthase c
Genetic preposition for bacteriocins production	Good potential	Only a FEW strains

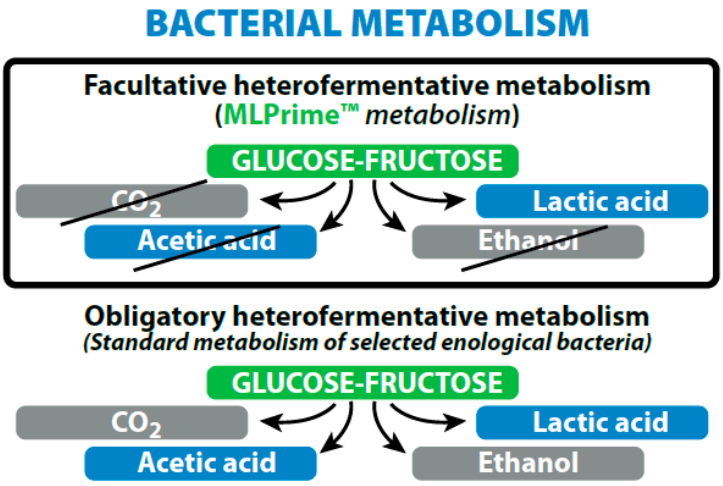

Figure 3. Sugar metabolism of wine lactic acid bacteria.

5.1. *Lactobacillus plantarum* Starter Cultures for the Induction of Malolactic Fermentation in Must and Wine

In 1988, the first malolactic starter culture was introduced to the wine industry. Prahl et al. [7] proposed to inoculate must before alcoholic fermentation using a facultative hetero-fermentative *L. plantarum* starter culture making use of non-proliferating cells. In EP 0398957B1, they disclosed a method of introducing a freeze-dried biomass of *L. plantarum* directly into must or fruit juice to induce MLF without significant consumption of sugars present in the must or fruit juice and substantially without any production of volatile acidity. The malolactic bacteria had been unable to survive in the fermented wine. The application under practical conditions asked for an inoculation 24 to 48 h prior to the addition of yeast with 10 g/hl of the freeze-dried preparation, corresponding to an inoculation level of 5×10^7 cells/mL wine [8]. Malic acid degradation had initiated rapidly; it slowed down and stopped when alcohol levels reached about 5–8% (*v/v*). At this stage, the *Lactobacillus* cultures died off. Depending on the wine parameters, mainly pH, temperature, and the speed of yeast fermentation,

more or less malic acid is degraded. The advantage would have been partial malic acid degradation in low pH white wines. The disadvantages had been that microbial stability could not be achieved, since a part of the malic acid stayed in the wine as a source for the growth of other microorganisms. The amount of malic acid degraded is varying and not predictable. Furthermore, the application of this culture had only been recommended for low pH white wines, since above pH 3.5 the *L. plantarum* could grow and metabolize glucose producing L/D-lactic acid. This risk is quite high since lactic acid production would be significant and even yeast growth could be triggered by the excessive growth of this microorganism under high pH conditions. The use of this starter culture was limited, as the degree of malic acid conversion was variable and rarely complete, and due to the limited application in low pH wines only, along with the risk to leave the wines for two days without SO_2 and yeast addition.

In 2004, Bou and Krieger [9] filed a patent on an "Alcohol-tolerant malolactic strains for the maturation of wines with average or high pH". The patent had been published in 2004 under the international application number PCT/FR2004/001421. The patent relates to LAB strains of the genera *Lactobacillus* and *Pediococcus* capable of initiating and carrying out a complete MLF upon direct inoculation in dried, frozen, or lyophilized state, without a previous acclimatization step at a concentration of between 10^6–5×10^7 cfu/mL, into a wine with an alcohol content of 10% (*v/v*) or more and an average high pH level. The resistance to alcohol is apparent with an excellent survival rate on inoculation and a rapid start of malic acid degradation. Claims had been supported by examples of the successful induction of MLF under various wine conditions with *Lactobacillus* strains DSM-9916 and CNCM I-2924. These strains had been chosen from a pool of LAB strains selected for their good tolerance to various limiting conditions and specifically for high alcohol tolerance.

A more recent Italian selection led to *L. plantarum* strain V22 [10]. As part of a European project, where chemical adjuvant, wine yeast, and LAB were screened for their ability to degrade Ochratoxin A (OTA) in must and wine, three *L. plantarum* strains were selected at the University Catolica Sacro Cuore in Piacenza (UCSC) [67]. Ochratoxin A is a mycotoxin suspected of being nephrotoxic, teratogenic, hepatotoxic, and carcinogenic. *Lactobacillus plantarum* V22 showed the highest degradation of OTA under the experimental conditions. The three strains were tested in freeze-dried MBR® form for the induction of MLF in wine. Dried MLF starter cultures in MBR® form will allow direct inoculation into wine without significant loss of MLF activity. The *L. plantarum* strain V22 was the most robust under the tested conditions. This strain had been tested during three vintages under various high pH (>pH 3.5) and high alcohol conditions (≥14% (*v/v*)), proving to be as fast as *O. oeni* starter cultures when inoculated after alcoholic fermentation. Due to its facultative hetero-fermentative properties, *L. plantarum* is most interesting for co-inoculation without the risk of volatile acid formation when inoculated in the presence of sugars. Again, it proved to have a faster fermentation rate compared to *O. oeni*, if wine pH was higher than pH 3.5.

5.2. Specific Feature of Lactobacillus plantarum of Oenological Interest

Iorizzo et al. [68] selected 11 *L. plantarum* isolates from spontaneous MLF in wines from Southern Italy and characterized them according to their oenological characteristics and for their potential use as starter cultures for MLF in wine. None of the 11 strains produced biogenic amines which is an important criteria for its potential use as MLF starter culture. Cappozzi et al. [69] studied the biogenic amine degradation by *L plantarum* and found two strains able to degrade putrescine and tyramine under wine-like conditions.

Knoll et al. [70] studied LAB isolated from South African red wines during alcoholic and MLFs and 9 commercial malolactic bacteria starter strains including *L. plantarum* V22 for antimicrobial activity. Of the entire screened isolates, 26 strains, belonging to *L. plantarum*, *L. paracasei*, *L. hilgardii,* and *O. oeni*, showed activity towards various wine-related and non-wine-related indicator strains on a synthetic medium. A PCR-based screening revealed the presence of the plantaricin encoding genes plnA, plnEF, plnJ, and plnK in five selected *L. plantarum* strains, including V22. These strains have also been screened for bacteriocin activity by plate assays, on normal MRS media, MRS pH 3.5 and MRS 10% (*v/v*) ethanol

(unpublished data). All 20 strains were tested against nine different sensitive organisms. Seven strains, including *L. plantarum* V22, showed bacteriocin inhibitory activity against all of the sensitive strains tested under those pH and ethanol conditions, but under real wine conditions bacteriocin producing activity was not expressed. Iorizzo et al. [68] could not detect a bacteriocin-producing activity within their selection of 11 *L. plantarum* strains from South Italian wines.

5.3. Mixed Oenococcus oeni and Lactobacillus plantarum Starter Cultures for the Induction of MLF

Lerm et al. [71] studied various *Oenococcus oeni* and *Lactobacillus plantarum* strains isolated from the South African wine environment for their potential use as malolactic starter cultures. These strains were characterized with regards to their properties of oenological interest, including the genetic screening for enzyme-encoding genes (enzymes implicated in wine aroma modification, as well as the absence of enzyme negatively impacting on of the final wine quality or integrity such as biogenic amine formation or production of ethylcarbamate), the ability to survive in wine, their fermentation capabilities, as well as their volatile acidity production. A total of three *O. oeni* and three *L. plantarum* strains were selected at the completion of this study. These strains showed the most potential during the characterization and were able to successfully complete MLF in Pinotage wine. It was again found that *L. plantarum* strains displayed a more diverse enzyme profile than *O. oeni* strains, particularly with regards to the presence of the aroma-modifying enzymes β-glucosidase and phenolic acid decarboxylase (PAD), which implies the future use of this species in the modification of the wine aroma profile and use as commercial starter culture. It was concluded that *Lactobacillus plantarum* strains might have an added beneficial influence in that it has the genetic potential to influence the wine aroma profile to a larger extent than *O. oeni*, due to its cache of enzymes. Based on outcome of this study a mixed starter culture consisting of an *Oenococcus oeni* and a *Lactobacillus plantarum* strain has been introduced 2011 as "Co-Inoculant" for simultaneous inoculation together with the yeast for induction of malolactic fermentation. This was the first commercially available blend of its kind in the world recommended for co-inoculation in high pH grape musts (>pH 3.4) only. Nowadays, a second blend *O. oeni/L. plantarum* is on the market, which can work at lower pH (>pH 3.2).

5.4. A New Concept of Lactobacillus plantarum Starter Cultures for High pH Red Wines.

Although co-inoculation (inoculation of selected wine lactic acid bacteria 24 to 48 h after the inoculation with selected wine yeast) is getting very popular and is more and more applied because of its various benefits outlined in chapter 4.1, some winemakers still consider co-inoculation with *O. oeni* as risky because of their obligatory hetero-fermentative properties. They wrongly fear co-inoculation, although this practice has more than proven itself to be a secure choice for high pH red wines (above pH 3.5) in which the native flora is even more critical. The biggest fear is to get a stuck alcoholic fermentation due to antagonism with the wine LAB, and having the bacteria taking over, resulting in an important increase in volatile acidity due to the hetero-fermentative metabolism of the residual sugars. More recently, a new starter culture called ML Prime™ (a pure *Lactobacillus plantarum* ferment) was released. Due to its specific optimized production process, this *Lactobacillus plantarum* starter culture expresses extremely high malolactic enzymatic activity as soon as it is added to must. MLF is therefore completed in record time (3–7 days in average) during alcoholic fermentation (Figures 4 and 5), and, unlike classical inoculum with *O. oeni*, no further growth is needed, which explains the very rapid onset of MLF upon inoculation into the must without any impact on yeast vitality and alcoholic fermentation. As explained before, *L. plantarum* degrades hexose sugars only via the homo-fermentative pathway, so there is no risk of acetic acid production from residual sugars that may be present in high pH wines, or still present, when MLF has finished before the end of the alcoholic fermentation.

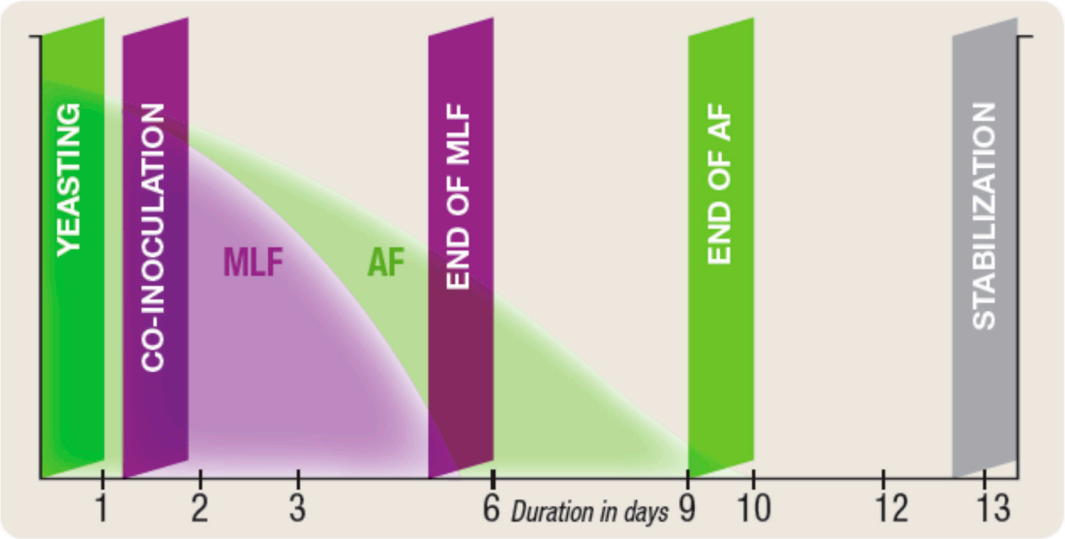

Figure 4. Vinification with a new generation starter culture of *Lactobacillus plantarum.*

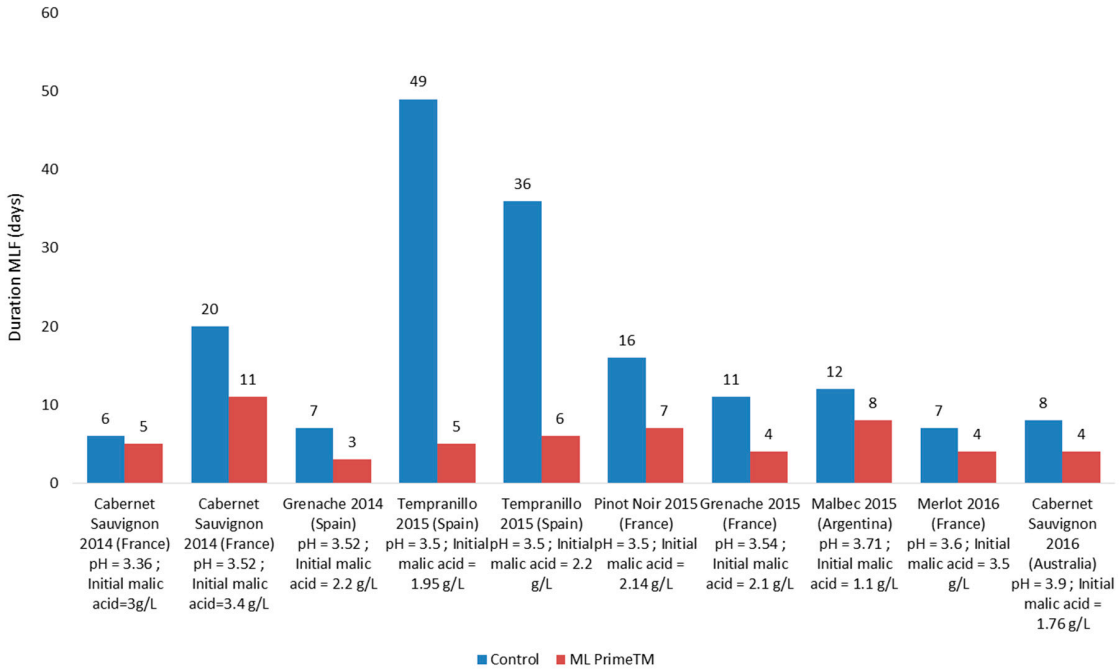

Figure 5. Duration of Malolactic fermentation (MLF) in various trials (days) inoculated with *L. plantarum* ML-PRIME™ (internal data).

5.4.1. Control of Microbial Contamination

As a result of the global warming, wines with a pH of over 3.5–3.7 are more and more frequent. At those pH levels, we can observe very fast growth of various indigenous microorganisms, some of which are spoilage bacteria that can cause a loss of wine quality or present health concerns. Co-inoculation is advantageous because it allows for the early dominance of a selected wine LAB strain and the faster onset and completion of MLF and early wine stabilization [42].

A more recent OIV regulation (OIV-Oeno-264-2014) on good vinicultural practices for controlling *Brettanomyces* proposed the co-inoculation of selected yeast and selected wine bacteria to shorten the lag phase between the end of alcoholic fermentation and the start of malolactic fermentation and consequently limit the implantation and the growth of *Brettanomyces*, another wine spoilage microorganism with a detrimental impact on wine quality. It also states that the use of malolactic starters is a good way to limit the development of *Brettanomyces* and the production of the undesired compounds

4-ethylphenol, 4-ethylguaïacol, and 4-ethylcatechol. These volatile phenols are characterized by animal-like off-flavors described as horse and barnyard, and/or pharmaceutical odors characterized as medicinal. Because of the high initial vitality of *L. plantarum* ML Prime™, an immediate onset of malolactic fermentation can be observed, as evidenced in Figure 4 where malic acid was degraded during alcoholic fermentation. However, it is important to respect the windows of application summarized in Table 2, which is narrower than for an *O. oeni* starter culture.

Table 2. Optimum conditions for the use of *Lactobacillus plantarum* ML Prime™.

Types of Wines	**Reds–Traditional Vinification (Short or Medium Maceration–Thermovinification (Liquid Phase) Initial Sugar/Potential Alcohol up to 260 g/L/15,5% (*v*/*v*))**
Timing of Bacteria Inoculation	Only co-inoculation Addition of ML Prime™, 24 h after adding yeast
SO2 Addition on Grapes/Must	≤5 g/hL
pH **Acid Malic Content**	≥ 3.4 maximum 3 g/L
Temperature During AF	20° to 26 °C

5.4.2. Malolactic Fermentation and Red Wine (Pinot Noir) Color

In Pinot Noir, MLF is often delayed because the resulting wines have anecdotally been reported to have superior color. Delayed MLF in a Pinot noir wine, for up to 4 months, showed improved wine color intensity [72]. Pinot noir wine color presents its own unique challenges, particularly because of its low tannin and anthocyanin content, with a bias towards the less stable acetylated form. The formation of wine color is a complex reaction with many different factors having and integral role. It is known that microbial metabolites, acetaldehyde, and pyruvic acid play a role in the formation of polymeric pigments [73]; however, the degradation of these compounds by *O. oeni* and the impact it could have on red wine color is not been well understood. A study in Pinot noir wine showed that there was no significant impact on color loss (A520) when MLF was delayed by up to six months; however, there was an impact on the formation of polymeric pigments [74] This study demonstrated the role of acetaldehyde and/or pyruvic acid degradation by *O. oeni* during MLF as a cause for reduced polymeric pigment formation independent of the pH change.

The results from a research collaboration with the Oregon State University and the team of James Osborn [75] showed different LAB species and strains can metabolize acetaldehyde at different rates (Table 3), which then in turn will affect red wine color post MLF (Figure 6). *Lactobacillus plantarum* (ML-Prime™) metabolizes acetaldehyde at a slower rate to *O. oeni* strains (*O. oeni* OM and *O. oeni* AL).

Table 3. Acetaldehyde concentration (mg/L) in a Pinot noir wine pre- and post-MLF and a control wine without MLF extracted from Bartowsky and Krieger-Weber [75].

	Pre-MLF	**Post-MLF**
Control	8.37	9.2
***L. plantarum* ML-Prime™**	8.37	7.67
***O. oeni* OM**	8.37	2.33
***O. oeni* AL**	8.37	1.7

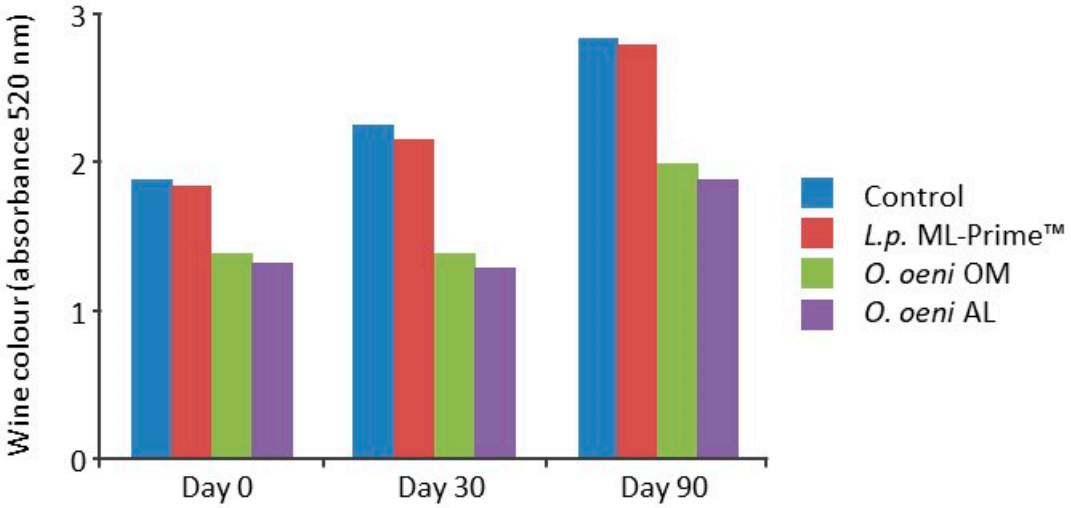

Figure 6. Wine color (520 nm) of Pinot noir wine that did not undergo MLF (Control) or MLF with different malolactic bacteria strains; the wines were stored at 13 °C and extracted from Bartowsky and Krieger-Weber [75].

When color and polymeric pigment values were measured in the wines post-MLF (Day 0) and after 30 and 90 days storage at cellar temperatures (Figure 6), a reduction in color was observed in wines that underwent MLF with *O. oeni* AL or *O. oeni* OM, whereas less loss of color was noted in wines that underwent MLF with the *L. plantarum* (ML-Prime™). After 30 or 90 days of aging, no loss of polymeric pigment was noted in wines that underwent MLF with ML-Prime™.

The overall color of Pinot noir wine can be better managed by selecting a specific wine lactic acid bacteria with consideration of the acetaldehyde metabolism and timing of MLF inoculation. Delaying MLF can actually also promote the combination of tannins and anthocyanins, resulting in a lesser impact of SO_2 on color. However, this approach to use a delayed MLF for more and stable color must be carefully weighed up against potential microbial spoilage, including Brettanomyces and biogenic amine formation (indigenous LAB).

5.5. A New Concept of Lactobacillus plantarum Starter Cultures for Low pH White Wines

Although it was out of the comfort zone for *L. plantarum*, ML-Prime (pH ≥ 3.4 and malic acid maximum 3 g/L) was also tested in white wines, and the results had been surprisingly good. Due to the optimized production process resulting in a very high de-acidification activity, it showed an excellent performance when added into the must (24 h after the yeast) or wine with initial low pH and high malic acid content. Even at a pH as low as 3.05 it allows a partial degradation of the malic acid in the white wine vinification process. The percentage of malic acid degradation depends on the specific must or wine conditions (pH, acid malic content, the total acidity, temperature, and the SO_2 content) and the grapes varietals, and can vary between 20% and 90%. Figure 7 shows the kinetics of malic acid degradation with *L. plantarum* (ML-Prime™) in a 2017 Chardonnay from South of France with an initial malic acid concentration of 3.6 g/L adjusted to pH 3.1, pH 3.2, pH 3.3, pH 3.4, or pH 3.5, respectively. Chardonnay is known for its difficulties to undergo MLF. *L. plantarum* was able to degrade about 30% of the malic acid (between 1.1 and 1.5 g/L malic acid had been degraded) when the wine pH ranged from pH 3.1 to pH 3.5, whereas, at pH 3.5, a complete malic acid degradation was achieved.

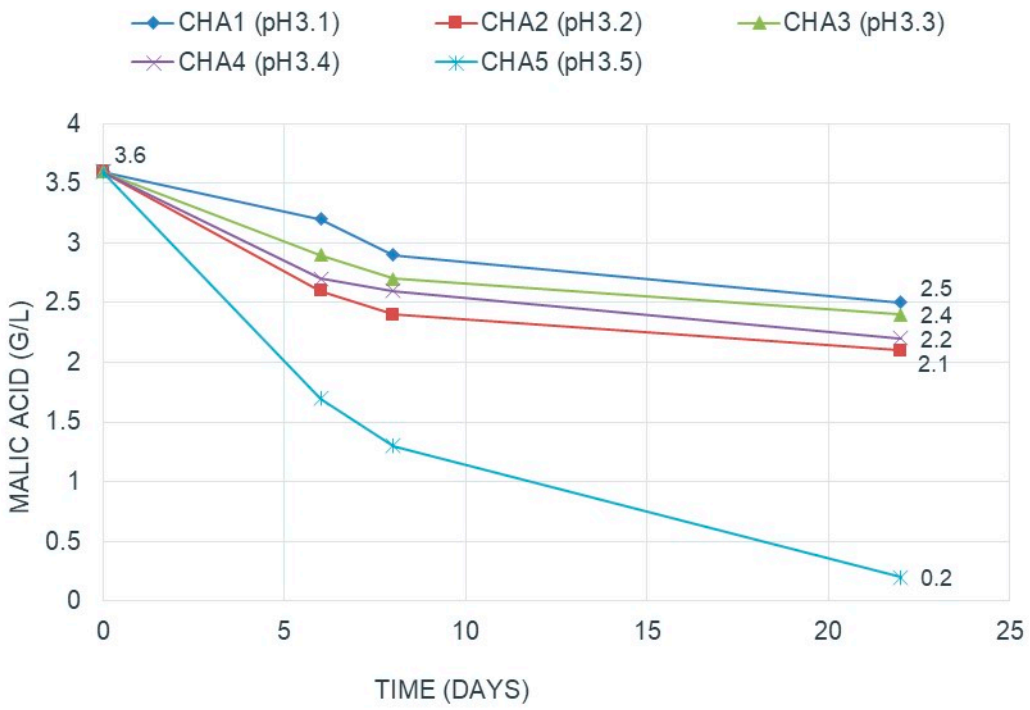

Figure 7. Kinetics of malic acid degradation in a 2017 Chardonnay (South-France) after co-inoculation with *L. plantarum* (ML-Prime™) depending on the pH. Initial must analyses: malic acid concentration 3.6 g/L, total sugars 189 g/L, potential alcohol 11.2% (*v/v*), 7.56 g/L total acidity (in tartaric acid), nitrogen 193 mg/L (Internal laboratory trials Lallemand SAS).

As the wine matrix is very versatile, a precise prediction of how much malic acid will remain in the wine is not possible. Figure 8 shows the malic acid degradation in sequential inoculation in a 2019 Chardonnay from Germany. Again, the matrix was a difficult Chardonnay with a pH of 3.21, 13.2% (*v/v*) alcohol, and 55 mg/L total SO_2, the temperature was at 17 °C. Under these challenging conditions, most of the *O. oeni* strains failed in sequential inoculation. Only one *O. oeni* strain OM started malolactic fermentation, but only after re-inoculation and with a very slow degradation. The *L. plantarum* culture (ML-Prime™) finished MLF when inoculated with the normal inoculation ratio after 25 days, and, when doubling the inoculation dosage MLF, it was finished within 10 days. In this experiment, the *L. plantarum* cultures maintained a high cell viability throughout the malolactic fermentation. Contrastingly, this Chardonnay wine must have contained a toxic compound, which had trigged *O. oeni*, as upon inoculation a sharp die-off of the *O. oeni* population could be observed. Only *O. oeni* Oo OM showed better survival and could finally implant, regrow, and induce MLF in this wine.

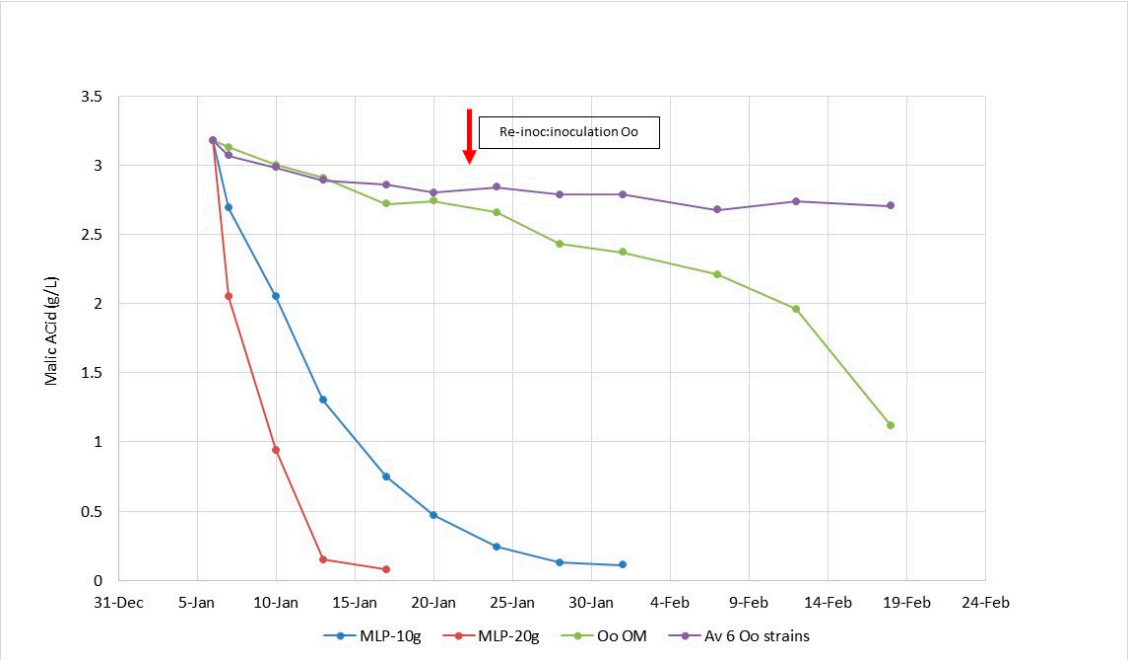

Figure 8. Kinetics of malic acid degradation in a 2019 Chardonnay (Germany) after sequential inoculation with *L. plantarum* (ML-Prime™), and different *O. oeni* strains. The kinetics show malic acid degradation with the *L. plantarum* culture inoculated with a single and double dosage and *O. oeni* strain Oo OM, and the average of 6 other *O. oeni* strains inoculated at a single dosage and re-inoculated at a double dosage. Wine analyses before inoculation: malic acid concentration 3.18 g/L, pH 3.21, 13.2% (*v/v*), 55 mg/L total SO_2 (Internal data).

Knowing the specifications above, it can be a tool for winemakers who want to achieve only a partial MLF in case of white wines vinification or a biological de-acidification instead of chemical de-acidification. The window of application for *L. plantarum* ML-Prime™ is outlined below:

- pH: ≥3.05
- Malic acid content: ≤8 g/L
- Temperature range: from 17 °C to 22 °C
- Total SO_2 tolerance in must up to 5 g/hL
- Free SO_2 tolerance in wines: less than 10 mg/L

5.6. Interesting Sensory Properties of Lactobacillus plantarum in Wine Application

As outlined by Lerm et al. [71], *Lactobacillus plantarum* strains might have an added beneficial influence, as it has the genetic potential to influence the wine aroma profile to a larger extent than *O. oeni*, due to its cache of enzymes. Mtshali et al. [76] conducted a genetic screening for wine-related enzymes within *Lactobacillus* species isolated from South African wines. They found a range of genes encoding for b-glucosidase, protease, esterase, citrate lyase (a-, b- and c-subunits), and phenolic acid decarboxylase. These findings indicated a possible use of *L. plantarum* not only for conducting MLF but also as the potential source of enzymes to impact positively on wine aroma, but expression under wine conditions needs further investigation. The commercial starter strain *L. plantarum* strain V22 had been included in a genetic screening of winemaking LAB starter strains mainly belonging to the species *O. oeni* for wine-relevant enzymatic activities [11]. The enzymes of interest that were screened for included β- glucosidase, esterase, protease, and phenolic acid decarboxylase (PAD). The V22 strain was found to possess more diverse enzymatic profiles related to aroma than *O. oeni*. The biggest differences were observed for the presence of esterase, protease, and PAD. The findings of Iorizzo et al. [68] reported the release of free volatiles from odorless glyosidic aroma precursors by all 11 *L. plantarum*

strains in their study in a synthetic wine medium. Interestingly, the *L. plantarum* strain M10 was not only a major producer of 1-octanol, but also released a considerable amount of other odorant compounds with low odor thresholds. Still, these findings need to be validated in a real wine matrix. Spano et al. [77] reported that the expression of β-glucosidase gene in *L. plantarum* is regulated by abiotic stresses such as ethanol, temperature, and pH.

Still, the application of this species in grape must and wine is rather new since only recently commercial starter cultures had been made available, which can survive also at higher alcohol levels and can induce a reliable malolactic fermentation in wine. Further research is needed to elaborate the sensory contribution of these species to the wine aroma profile.

5.7. Other Applications of Lactobacillus plantarum Apart from the Induction of Malolactic Fermentation

However, the application of *L. plantarum* in vinification should not be restricted to de-acidification through malolactic fermentation only, Lucio et al. [78,79] have most recently proposed *L. plantarum* for the biological acidification of wines. Within the project CENIT CDTI 2008, they selected *Lactobacillus* strains, which show a high potential as biological acidification starters for winemaking when inoculated prior to the alcoholic fermentation into high pH grape must. WO 2015/110484A2 patent application [80] proposes reverse inoculation (inoculation prior to the inoculation with selected wine yeast) or the co-inoculation (inoculation together with the wine yeast) of selected homo-fermentative or facultative hetero-fermentative lactic acid bacteria strains to produce fermented fruit beverages, such as wine or cider, with a reduced alcohol level. Moreover, the international patent application WO 2015/110484 A2 [81] relates to the use of lactic acid bacteria as bio-protective agents against unwanted microorganisms, such as mold and gram-negative bacteria, such as acetic acid bacteria. The inventors propose a specific *L. plantarum* strain as an antimicrobial agent in the process of winemaking.

6. Conclusions

Lactobacillus is one of the most diverse group of microorganisms associated with the wine environment. Some *Lactobacillus* species have also displayed the ability to survive the harsh wine conditions, and, within this group, the species *Lactobacillus plantarum* has shown the most potential as a starter culture for the induction of malolactic fermentation. Global warming and the trend towards harvesting higher maturity grapes have resulted in the processing of higher pH musts. Microbial stability as a result of lactic acid bacteria activity will play a more important role in the warmer climates. Under these high pH conditions, *Lactobacillus plantarum* bacteria have shown especially interesting results, not only for their capacity to induce malolactic fermentation when inoculated either shortly after the yeast (co-inoculation) into the must or in sequential inoculation after alcoholic fermentation, but also for their homo-fermentative properties for the metabolism of hexose sugars, which minimizes the risk of acetic acid production. *L. plantarum* was found to dispose over a more complex enzymatic system, which could play role in the modification of wine aroma. More research is certainly needed to study the expression of these enzyme activities in wine.

Applying a strong *Lactobacillus plantarum* inoculum with high malolactic activity assures the early onset of predictable and complete MLF in a short period of time (during AF) and allows an early stabilization of the wine. Even under limiting white wine conditions, a complete or partial malolactic fermentation can be induced. Since this species is very versatile, other application for bio-protection and acidification mat play a more important role in the use of this starter culture.

Funding: This research received no external funding.

Acknowledgments: The authors thank research partner James Osborne (Oregon State University USA) for the research collaboration on impact of malolactic fermentation on Pinot Noir color, and our colleague Magali Déléris-Bou from our research Laboratory at Lallemand SAS in Toulouse, for conducting the internal experiments.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Du Toit, M.; Engelbrecht, L.; Lerm, E.; Krieger-Weber, S. *Lactobacillus*: The next generation of malolactic fermentation starter cultures-an overview. *Food Bioprocess Technol.* **2011**, *4*, 876–906. [CrossRef]
2. Radler, F. Die mikrobiologischen Grundlagen des Säureabbaus in Wein. *Zentralbl Bakteriol Parasitenkd* **1966**, *120*, 237–287.
3. Peynaud, E.; Domercq, S. Étude de quatre cents souches de coques hétérolactiques isolées de vins. *Ann. Inst. Pasteur* **1968**, *19*, 159–170.
4. Henick-Kling, T. Growth and Metabolism of *Leuconostoc oenos* and *Lactobacillus plantarum* in Wine. Ph.D. Thesis, University of Adelaide, Adelaide, Australia, 1986.
5. Davis, C.R.; Wibowo, D.; Fleet, G.H.; Lee, T.H. Properties of wine lactic acid bacteria: Their potential enological significance. *Am. J. Enol. Vitic.* **1988**, *39*, 137–142.
6. Henick-Kling, T. Malolactic Fermentation. In *Wine Microbiology and Biotechnology*, 1st ed.; Fleet, G.H., Ed.; Harwood Academic Publishers: Chur, Switzerland, 1993; pp. 289–326.
7. Prahl, C. Method of Inducing the Decarboxylation of Malic Acid in Must or Fruit Juice. European Patent International Application Number PCT/DK89/00009, 24 January 1989.
8. Prahl, C. La décarboxylation de l'acide L-malique dans le moût par l'ensemencement de lactobacilles homofermentaires. *Revue des Œnologues* **1989**, *54*, 13–17.
9. Bou, M.; Krieger, S. Alcohol-Tolerant Malolactic Strains for the Maturation of Wines with Average or High pH. U.S. Patent 7,625,745, 9 June 2004.
10. Fumi, M.D.; Krieger-Weber, S.; Déléris-Bou, M.; Silva, A.; du Toit, M. A New Generation of Malolactic Starter Cultures for High pH Wines. WB3 Microorganisms—Malolactic-Fermentation. In Proceedings of the International IVIF Congress, Düsseldorf, Germany, 21–23 March 2010.
11. Soerensen, K.; Kibenich, A.; Johansen, E. Lactobacillis planaturm Ceels with Improved Resistance to High Concentrations of Ethanol. International Patent Application Number PCT/EP2012/061296, 14 June 2012.
12. Krieger-Weber, S.; Silvano, A.; Déléris-Bou, M.; Vidal, F.; Dumont, A. Neues Konzept für die Mikroflora. *Das Deutsche Weinmagazin* **2017**, *19*, 30–32.
13. Lerm, E. The Selection and Characterization of Lactic Acid Bacteria to be Used as a Mixed Starter Culture for Malolactic Fermentation. Master's Thesis, Agricultural Science, Stellenbosch University, Stellenbosch, South Africa, 2010.
14. Bae, S.; Fleet, G.H.; Heards, G.M. Lactic acid bacteria associated with wine grapes from several Australian vineyards. *J. Appl. Microbiol.* **2006**, *100*, 712–727. [CrossRef]
15. Ribéreau-Gayon, P.; Dubourdieu, D.; Donèche, B.; Lonvaud, A. Handbook of Enology, Volume 1. In *The Microbiology of Wine and Vinifications*; Ribéreau-Gayon, P., Ed.; Wiley: Chichester, UK, 2006; pp. 149–151.
16. Fugelsang, K.C.; Edwards, C.G. *Wine Microbiology: Practical Applications and Procedures*; Fugelsang, K.C., Edwards, C.G., Eds.; Springer: New York, NY, USA, 1997.
17. Jackson, R.S. Origin and Growth of Lactic Acid Bacteria. In *Wine Science: Principles and Applications*; Jackson, R.S., Ed.; Academic Press: Cambridge, MA, USA, 2008; p. 394.
18. Boulton, R.B.; Singleton, V.L.; Bisson, L.F.; Kunkee, R.E. *Principles and Practices of Winemaking*; Boulton, R.B., Ed.; Chapman and Hall Publishers: New York, NY, USA, 1996.
19. Dicks, L.M.T.; Endo, A. Taxonomic status of lactic acid bacteria in wine and key characteristics to differentiate species. *S. Afr. J. Enol. Vitic.* **2009**, *30*, 72–90. [CrossRef]
20. Volschenk, H.; van Vuuren, H.J.J.; Viljoen-Bloom, M. Malic acid in wine: Origin, function and metabolism during vinification. *S. Afr. J. Enol. Vitic.* **2006**, *27*, 123–136. [CrossRef]
21. Berbegal, C.; Peña, N.; Russo, P.; Grieco, F.; Pardo, I.; Ferrer, S.; Spano, G.; Capozzi, V. Technological properties of *Lactobacillus plantarum* isolated from grape must fermentations. *Food Microbiol.* **2016**, *57*, 187–194. [CrossRef]
22. Valdés La Hens, D.; Bravo-Ferrada, B.M.; Delfederico, L.; Caballero, A.C.; Semorile, L.C. Prevalence of *Lactobacillus plantarum* and *Oenococcus oeni* during spontaneous malolactic fermentation in Patagonian wines revealed by polymerase chain reaction-denaturing gradient gel electrophoresis with two targeted genes. *Aust. J. Grape Wine Res.* **2015**, *21*, 49–56. [CrossRef]

23. López-Seijas, J.; García-Fraga, B.; Da Silva, A.F.; Zas-García, X.; Lois, L.C.; Gago-Martínes, A.; Leão-Martins, J.M.; Sieiro, C. Evaluation of malolactic bacteria associated with wines from Albariño variety as potential; starter cultures for quality and safety. *Foods* **2020**, *9*, 99. [CrossRef]
24. Fleet, G.H.; Lafon-Lafourcade, S.; Ribereau-Gayon, P. Evolution of yeasts and lactic acid bacteria during fermentation and storage of Bordeaux wines. *Appl. Environ. Microbiol.* **1984**, *48*, 1034–1038. [CrossRef] [PubMed]
25. Lonvaud-Funel, A. Lactic acid bacteria in the quality improvement and depreciation of wine. *Antonie Van Leeuwenhoek* **1999**, *76*, 317–331. [CrossRef] [PubMed]
26. Bartowky, E.J.; Costello, P.J.; Chambers, P.J. Emerging trends in the application of malolactic fermentation. *Aust. J. Grape Wine Res.* **2015**, *21*, 663–669. [CrossRef]
27. Juega, M.; Costantini, A.; Bonello, F.; Cravero, M.C.; Martinez-Rodriguez, A.J.; Carrascosa, A.V.; Garcia-Moruno, E. Effect of malolactic fermentation by *Pediococcus damnosus* on the composition and sensory profile of Albariño and Caiño white wines. *J. Appl. Microbiol.* **2013**, *116*, 586–595. [CrossRef]
28. Henick-Kling, T. *Yeast and Bacteria Control in Winemaking, Modern Methods of Plant Analysis*; Linskens, R.S., Jackson, J.F., Eds.; Springer: Berlin/Heidelberg, Germany, 1988; Volume 6, pp. 276–315.
29. Wibowo, D.; Eschenbruch, R.; Davis, D.R.; Fleet, G.H.; Lee, T.H. Occurrence and growth of lactic acid bacteria in wine: A review. *Am. J. Enol. Vitic.* **1985**, *36*, 302–313.
30. Liu, Y.; Forcisi, S.; Harir, M.; Deleris-Bou, M.; Krieger-Weber, S.; Lucio, M.; Longin, C.; Degueurce, C.; Gougeon, R.D.; Schmitt-Kopplin, P.; et al. New molecular evidence of wine yeast-bacteria interaction unraveled by non-targeted exometabolomic profiling. *Metabolomics* **2016**, *12*, 1–16. [CrossRef]
31. Dittrich, H.H.; Grossmann, M. Der Mirobielle Säureabbau. In *Mikrobiologie des Weines, Handbuch der Getränketechnologie*, 3rd ed.; Dittrich, H.H., Grossmann, M., Eds.; Ulmer Verlag: Stuttgart, Germany, 2005; pp. 151–169.
32. Amerine, M.A.; Berg, H.W.; Kunkee, R.E.; Ough, C.S.; Singleton, V.L.; Webb, A.D. The Composition of Grapes and Wines. In *The Technology of Wine Making*, 4th ed.; Amerine, M.A., Berg, H.W., Eds.; Avi Publishing Company: Westport, CT, USA, 1980.
33. Nicolas, D. Caractérisation Physiologique et Moléculaire de Préparations Malolactiques de *Oenococcus oeni* Destinées à L'ensemencement Direct des Vins. Ph.D. Thesis, University of Dijon, Dijon, France, 2005.
34. Lonvaud-Funel, A. Microbiology of the malolactic fermentation: Molecular aspects. *FEMS Microbiol. Lett.* **1995**, *126*, 209–214. [CrossRef]
35. Cox, D.J.; Henick-Kling, T. Chemiosmotic energy from malolactic fermentation. *J. Bacteriol.* **1989**, *171*, 5750–5752. [CrossRef]
36. Guerzoni, M.E.; Sinigaglia, M.; Gardini, F.; Ferruzzi, M.; Torriani, S. Effects of pH, temperature, ethanol, and malate concentration on *Lactobacillus plantarum* and *Leuconostoc oenos*: Modelling of the malolactic activity. *Am. J. Enol. Vitic.* **1995**, *3*, 368–374.
37. Britz, T.J.; Tracey, R.P. The combination effect of pH, SO_2, ethanol and temperature on the growth of *Leuconostoc oenos*. *J Appl. Bacteriol.* **1990**, *68*, 23–31. [CrossRef]
38. Lafon-Lafourcade, S. Chapter III: Lactic Acid Bacteria and the Malolactic Fermentation. In *Wine and Brandy in Biotechnology*; Rehm, H.J., Redd, G., Eds.; Verlag Chemie: Weinheim, Germany, 1983; pp. 81–163.
39. Chang, I.S.; Kim, B.H.; Shin, P.K. Use of sulfite and hydrogen peroxide to control bacterial contamination in ethanol fermentation. *Appl. Environ. Microbiol.* **1997**, *63*, 1–6. [CrossRef] [PubMed]
40. Massera, A.; Soria, A.; Catania, A.; Krieger, S.; Combina, M. Simultaneous Inoculation of Malbec (*Vitis vinifera*) Musts with Yeast and Bacteria: Effects on Fermentation Performance, Sensory and Sanitary Attributes of Wines. *Food Technol. Biotechnol.* **2009**, *47*, 192–201.
41. Abrahamse, C.; Bartowsky, E. Inoculation for MLF reduces overall vinification time. *Aust. N. Z. Grapegrow. Winemak.* **2012**, *577*, 41–46.
42. Azzolini, M.; Tosi, E.; Vagnoli, P.; Krieger, S.; Zapparoli, G. Evaluation of technological effects of yeast-bacterial co-inoculation in red table wine production. *Ital. J. Food Sci.* **2010**, *3*, 257–263.
43. Jussier, D.; Dubé Morneau, A.; Mira de Orduña, R. Effect of simultaneous inoculation with yeast and bacteria on fermentation kinetics and key wine parameters of cool-climate Chardonnay. *Appl. Environ. Microbiol.* **2006**, *72*, 221–227. [CrossRef]
44. Renouf, V.; La Guerche, S.; Moine, V.; Murat, M.L.; Bowyer, P.K. Malolactic fermentability of wines: The role of octanoic and decanoic acid. *Aust. N. Z. Grapegrow. Winemak.* **2010**, *560*, 93–98.

45. Osborne, J.P.; Charles, G.E. Inhibition of malolactic fermentation by a peptide produced by *Saccharomyces cerevisiae* during alcoholic fermentation. *Int. J. Food Microbiol.* **2007**, *118*, 27–34. [CrossRef]
46. Nehme, N.; Mathieu, F.; Taillandier, P. Impact of the co-culture of *Saccharomyces cerevisiae—Oenococcus oeni* on malolactic fermentation and partial characterization of a yeast-derived inhibitory peptidic fraction. *Food Microbiol.* **2010**, *27*, 150–157. [CrossRef]
47. Lonvaud-Funel, A. Interactions between Lactic Acid Bacteria of Wine and Phenolic Compounds. In *Nutritional Aspects II, Synergy between Yeast and Bacteria*; Lallemand Technical Meeting: Perugia, Italy, 2001; pp. 27–32.
48. Vivas, N.; Augustin, M.; Lonvaud-Funel, A. Influence of oak wood and grape tannins on the lactic acid bacterium *Oenococcus oeni* (*Leuconostoc oenos*). *J. Sci. Food Agric.* **2000**, *80*, 1675–1678. [CrossRef]
49. Figueiredo, A.R.; Campos, F.; de Freitas, V.; Hogg, T.; Couto, J.A. Effect of phenolic aldehydes and flavonoids on growth and inactivation of *Oenococcus oeni* and *Lactobacillus hilgardii*. *Food Microbiol.* **2008**, *25*, 105–112. [CrossRef]
50. Chasseriaud, L.; Krieger-Weber, S.; Déléris-Bou, M.; Sieczkowski, N.; Jourdes, M.; Teissedre, P.L.; Claisse, O.; Lonvaud-Funel, A. Hypotheses on the effects of enological tannins and total red wine phenolic compounds on *Oenococcus oeni*. *Food Microbiol.* **2015**, *52*, 131–137. [CrossRef]
51. Stivala, M.G.; Villecco, M.B.; Enriz, D.; Fernandez, P.A. Effect of phenolic compounds on viability of 2 wine spoilage lactic acid bacteria. A structure-activity relationship study. *Am. J. Enol. Vitic.* **2017**, 1–12. [CrossRef]
52. Reguant, C.; Bordons, A.; Arola, L.; Rozes, N. Influence of phenolic compounds on the physiology of *Oenococcus oeni* from wine. *J. Appl. Microbiol.* **2000**, *88*, 1065–1071. [CrossRef] [PubMed]
53. Garcia-Ruiz, A.; Moreno-Arribas, M.V.; Martin-Alvarez, P.J.; Bartolome, B. Comparative study of the inhibitory effects of wine polyphenols on the growth of enological lactic acid bacteria. *Int. J. Food Microbiol.* **2011**, *145*, 426–431. [CrossRef] [PubMed]
54. Jimeñez, N.; Esteban-Torres, M.; Mancheño, J.M.; De Las Rivas, B.; Muñoz, R. Tannin degradation by a novel tannase enzyme present in some *Lactobacillus plantarum* strains. *Appl. Environ. Microbiol.* **2014**, *80*, 2991–2997. [CrossRef] [PubMed]
55. Terrade, N.; Mira de Orduña, R. Determination of the essential nutrient requirements of wine-related bacteria from the genera *Oenococcus* and *Lactobacillus*. *Int. J. Food Microbiol.* **2009**, *133*, 8–13. [CrossRef]
56. Liburdi, K.; Benucci, L.; Esti, M. Lysozyme in wine: An overview of current and future applications. *Compr. Rev. Food Sci. Food Saf.* **2014**, *13*, 1062–1073. [CrossRef]
57. Kong, M.; Chen, X.G.; Xing, K.; Park, H.J. Antimicrobial properties of chitosan and mode of action: A state of the art review. *Int. J. Food Microbiol.* **2010**, *144*, 51–63. [CrossRef]
58. Bağder Elmacı, S.; Gülör, G.; Tokath, M.; Erten, H.; İşci, A.; Özcelik, F. Effectiveness of chitosan against wine-related microorganisms. *Antonie Van Leeuwenhoek* **2014**. [CrossRef] [PubMed]
59. Nardi, T.; Vagnoli, P.; Minacci, A.; Gautier, S.; Sieczkowski, N. Evaluating the impact of fungal-origin chitosan preparation on *Brettanomyces bruxellensis* in context of wine aging. *Wine Stud.* **2014**, *3*, 13–15. [CrossRef]
60. Schildberger, B. Influence of Specific Botryticides on Malolactic Fermentation. In *Mitteilungen Klosterneuburg, Rebe und Wein, Obstbau und Früchteverwertung*; Wissensbericht; Lehr- und Forschungszentrum für Wein und Ostbau: Klosterneuburg, Austria, 2011; pp. 168–172.
61. Beelman, R.B.; Kunkee, R.E. Inducing co-inoculation malolactic-alcoholic fermentation in red table wines. In *Proceedings of the Australian Society of Viticulture and Oenology Seminar on Malolactic Fermentation*; Australian Wine Research Institute: Urrbrae, South Australia, 1985; pp. 97–112.
62. Ribéreau-Gayon, J.; Peynaud, E.; Ribéreau-Gayon, P.; Sudraud, P. *Sciences et Techniques du Vin*; Dunod: Paris, France, 1975; Volume 2.
63. Krieger, S.; Zapparoli, G.; Veneri, G.; Tosi, E.; Vagnoli, P. Simultaneous and sequential alcoholic and malolactic fermentations: A comparison for Amarone-style wines. *Aust. N. Z. Grapegrow. Winemak.* **2007**, *517*, 71–77.
64. Sieczkowski, N. Maîtrise et intérêt de la co-inoculation levures-bactéries. *Revue Française D'Oenologie* **2004**, *207*, 24–28.
65. Gerbaux, V.; Briffox, C.; Dumont, A.; Krieger, S. Research Note: Influence of Inoculation with Malolactic Bacteria on Volatile Phenols in Wines. *Am. J. Enol. Vitic.* **2009**, *60–62*, 233–235.
66. Kunkee, R.E. Malolactic fermentation. *Adv. Appl. Microbiol* **1967**, *9*, 235–279.
67. Silva, A.; Lambri, M.; Fumi, M.D. Ochratoxin: A Decontamination by Lactic Acid Bacteria in Wine: Adsorption or Biodegradation? In Proceedings of the Oeno 2007 VIII Symposium International d'Oenologie—Bordeaux, Paris, France, 25–27 June 2007; pp. 24–27.

68. Iorizzo, M.; Testa, B.; Lombardi, A.J.; García-Ruiz, A.; Muñoz-González, C.; Bartolomé, B.; Moreno-Arribas, M.V. Selection and technological potential of Lactobacillus plantarum bacteria suitable for wine malolactic fermentation and grape aroma release. *LWT Food Sci. Technol.* **2016**, *73*, 557–566. [CrossRef]
69. Capozzi, V.; Russo, P.; Ladero, V.; Fernández, M.; Fiocco, D.; Alvarez, M.A.; Grieco, F.; Spano, G. Biogenic amines degradation by *Lactobacillus plantarum*: Toward a potential application in wine. *Front. Microbiol.* **2012**, *3*, 1–6. [CrossRef]
70. Knoll, C.; Divol, B.; Du Toit, M. Genetic screening of lactic acid bacteria of oenological origin for bacteriocin-encoding genes. *Food Microbiol.* **2008**, *25*, 983–991. [CrossRef]
71. Lerm, E.; Engelbrecht, L.; du Toit, M. Selection and Characterisation of *Oenococcus oeni* and *Lactobacillus plantarum* South African Wine Isolates for Use as Malolactic Fermentation Starter Cultures. *S. Afr. J. Enol. Vitic.* **2011**, *32*, 280–295. [CrossRef]
72. Gerbaux, V.; Briffox, C. Influence de l'ensemencement en bactéries lactiques sur l'évolution de la couleur des vins de Pinot noir pendant l'élevage. *Revue des Œnologues* **2003**, *103*, 19–23.
73. Asenstorfer, R.E.; Markides, A.J.; Iland, P.J.; Jones, G.P. Formation of vitisin A during red wine fermentation and maturation. *Aust. J. Grape Wine Res.* **2003**, *9*, 40–46. [CrossRef]
74. Burns, T.R.; Osborne, J.P. Loss of Pinot noir wine color and polymeric pigment after malolactic fermentation and potential causes. *Am. J. Enol. Vitic.* **2015**, *66*, 130–137. [CrossRef]
75. Bartowsky, E.; Krieger-Weber, S. Management of malolactic fermentation to enhance red wine colour. *Grapegrow. Winemak.* **2020**, *673*, 66–70.
76. Mtshali, P.S.; Divol, B.T.; Van Rensburg, P.; Du Toit, M. Genetic screening of wine-related enzymes in Lactobacillus species isolated from South African wines. *J. Appl. Microbiol.* **2010**, *108*, 1389–1397. [CrossRef]
77. Spano, G.; Rinaldi, A.; Ugliano, M.; Moio, L.; Beneduce, L.; Massa, S. A β-glucosidase gene isolated from wine *Lactobacillus plantarum* is regulated by abiotic stresses. *J. Appl. Microbiol.* **2005**, *98*, 855–861. [CrossRef]
78. Lucio Costa, O. Acidificón Biológica de Vinos de pH Elevado Mediante la Utilización de Bacterias Lácticas. Ph.D. Thesis, University of Valencia, Valencia, Spain, 2014.
79. Lucio, O.; Pardo, I.; Krieger-Weber, S.; Heras, H.M.; Ferrer, S. Selection of *Lactobacillus* strains to induce biological acidification in low acidity wines. *LWT Food Sci. Technol.* **2016**, *73*, 334–341. [CrossRef]
80. Saerens, S.; Edwards, N.; Soerensen, K.; Badaki, M.; Swieger, J.H. Production of Low Alcohol Fruit Beverage. International Patent Application Number PCT/EP2015/051163, 21 January 2015.
81. Edwards, N.; Saerens, S.; Swieger, J.H. The Use of Lactobacillus plantarum As an Anti-Microbial Agent in the Process of Winemaking. International Patent Application Number PCT/EP2015/051161, 21 January 2015.