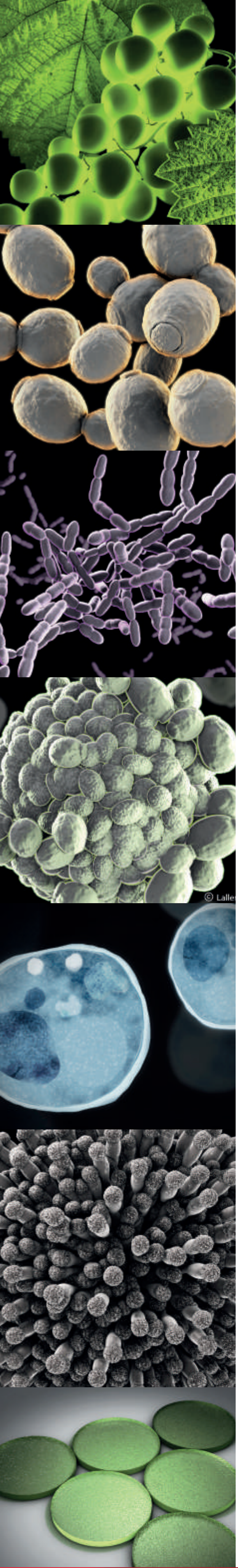

ML PRIME™ A VERSATILE TOOL TO DRIVE MALOLACTIC FERMENTATION

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Even though *Lactobacillus* species are generally avoided in winemaking, *L. plantarum* has much to offer as an option for conducting malolactic fermentation in red and also white wines. It does not produce acetic acid from glucose-fructose metabolism. As a unique robust strain *L. plantarum* ML Prime™ offers an efficient MLF in a range of juice and wine matrices.

Introduction

Lactic acid bacteria (LAB) are important in food and beverage fermentations, including winemaking. In grape vinification LAB conduct the conversion of malic acid to lactic acid, the so-called malolactic fermentation (MLF). This reaction is actually a decarboxylation reaction rather than a fermentation, however the name has remained since historical times.

Four genera of LAB are the principal microorganisms involved in MLF: *Lactobacillus*, *Leuconostoc*, *Oenococcus*, and *Pediococcus*. The population evolution of LAB species during the wine making process is shown in Figure 1. Wine pH is most selective, and, at pH below 3.5, *O. oeni* is probably the best adapted to overcome the harsh environmental conditions and therefore most of the commercial cultures consist of strains from this species. Under more favourable conditions above pH 3.5, species of *Lactobacillus* and *Pediococcus* may conduct the MLF. Ideally table wine pH should be between pH 3.1 and 3.6, but due to global warming wine pH has increased in recent years in almost all wine regions.

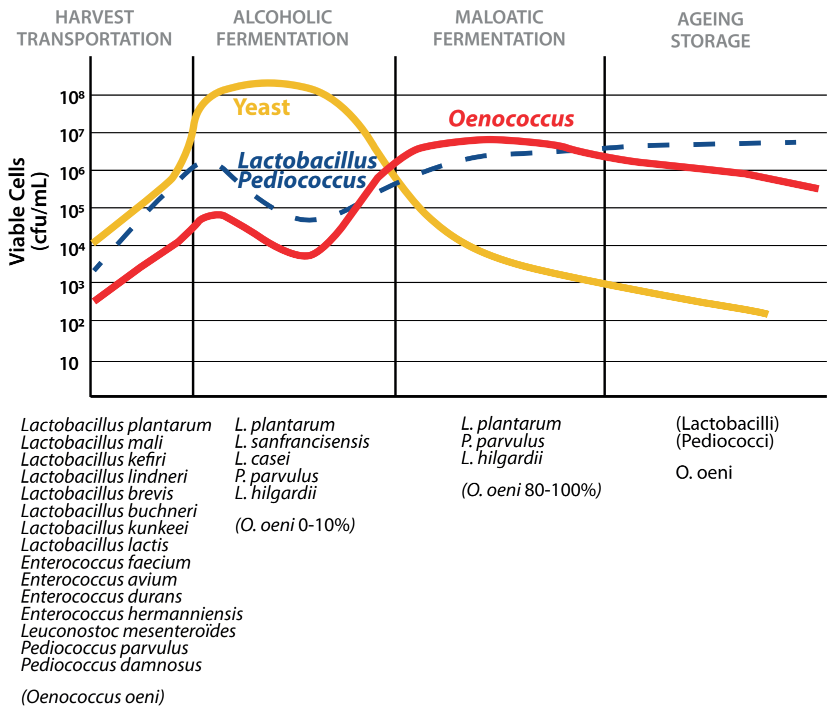

Figure 1. Progression of LAB species populations from harvesting through alcoholic and malolactic fermentations to ageing and storage (adapted from P Lucas, International ML School Toulouse, 2015; Krieger-Weber et al. 2020

Ethanol is also known to be a limiting factor for bacterial growth and survival in wine, however, there have been numerous studies reporting that this is strain dependent with some being able to tolerate up to 16% (v/v) alcohol.

In the past *Lactobacillus* and *Pediococcus* species have been considered spoilage bacteria of grape and wine fermentation. However, with careful strain selection it is possible to identify strains with potential to complete MLF without undesirable spoilage characteristics but give a positive contribution to the wine quality.

Lactobacillus

The *Lactobacillus* is the largest genus amongst all LAB and comprises over 100 species and was separated into at least 16 subspecies. There is a broad growth temperature range (2 to 53 °C), with an optimum of 30–40 °C and an optimum pH range of 5.5–6.2 (Dicks and Endo 2009). When examined under a microscope, you will see regular elongated cells that are 0.5–1.2 μm by 1.0–10 μm in dimension which tend to be long rod-like forms that are often assembled in pairs or chains of varying lengths.

The *Lactobacillus* genus has undergone a recent taxonomic review based on whole genome sequences and has now been reclassified into 25 genera. These reclassifications also impact grape and wine lactobacilli species–*Lactobacillus plantarum* is now known as *Lactiplantibacillus plantarum* (Zheng et al. 2020).

Potential of *L. plantarum* in winemaking

Among the LAB species *L. plantarum* strains have shown most interesting results under hot climate conditions, not only for their capacity to induce MLF, but also for their homo-fermentative properties towards hexose sugars, which makes them suitable for induction of MLF in high pH and high alcohol wines, when inoculated at the beginning of alcoholic fermentation. The first *L. plantarum* strain was introduced with it to be used pre-AF with non-proliferating cells conducting MLF (Prahl et al. 1988). In the early 2000's (Bou and Krieger 2004) proposed a strain for use during alcoholic fermentation.

Sugar fermentation

Many winemakers are hesitant to consider using *Lactobacillus* species for MLF in their wines as they are concerned of potential high acetic acid production by the strain while growing in juice. However, *Lactobacillus* species exhibit different sugar metabolism traits.

Grape juice and wine have various sugars present; different monosaccharides, mainly pentoses and hexoses as well as disaccharides. The major sugars in wine are glucose, fructose, arabinose and trehalose. There are clear species and strain differences in sugar metabolism.

There are two main pathways for hexose metabolism by LAB; the homofermentative fermentation pathway (Embden–Meyerhof–Parnas (EMP) pathway) results in the fermentation of glucose to produce lactic acid and ATP. The second is the heterofermentative pathway (also known as the pentose phosphate pathway, the phosphoketolase pathway or the 6-phosphogluconate/ phosphoketolase pathway) results in the formation of other end products besides lactic acid, including acetic acid and ethanol (Figure 2). Some *Lactobacillus* sp are facultative heterofermentors, meaning that they are homofermentative for hexoses and heterofermentative for pentoses (Dicks and Endo 2009). *L. plantarum* fits into this latter group and as such will not form acetic acid when metabolising hexose sugars.

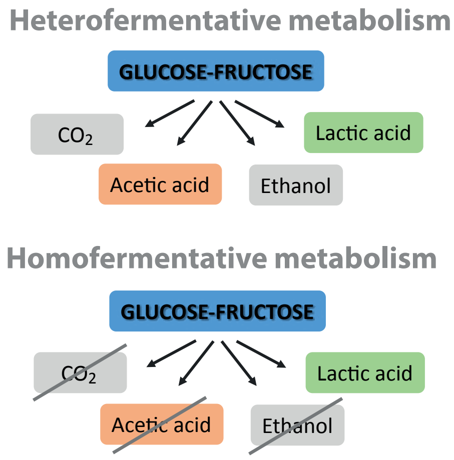

Figure 2. Major difference between homo- and heterofermentation of hexoses is the production of either only lactic acid or a mix of products, respectively.

Enzymes

L. plantarum has an expansive suite of enzymes and metabolic activities which can be exploited for positive wine sensory attributes (Matthews et al. 2004; du Toit et al. 2011). The presence of glycosidases enables the release of monoterpenes and norisoprenoids to bring floral notes to wine. The synthesis and hydrolysis of esters will impact the fruity characters of red wine (Costello et al. 2011) (Figure 3).

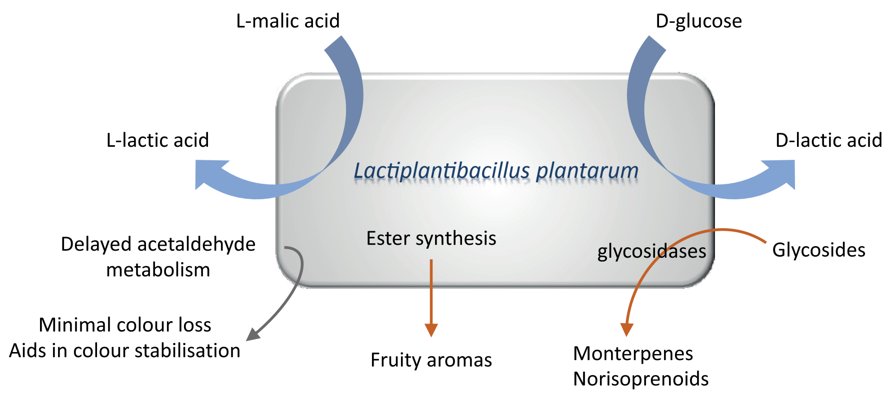

Figure 3. *L. plantarum* had a range of different metabolic activities which will impact wine sensory..

Colour stabilisation in red wine is a key feature and acetaldehyde is known to play an integral role. Acetaldehyde is a microbial metabolite and can be degraded by LAB. The use of micro-oxidation (MOX), timing of MLF inoculation and selection of ML strain and species can aid in overall wine colour management in Pinot noir (Bartowsky and Krieger-Weber, 2020). Recent studies have shown that *L. plantarum* strain ML Prime™ degrades acetaldehyde very slowly (Burns and Osborne 2015). As this *L. plantarum* strain achieves a faster MLF than *O. oeni*, the content of acetaldehyde in this wine is higher and allows an early natural wine colour stabilization. Comparing the two practices, colour intensity using ML Prime™ (co-inoculation) gives better results while remaining easier and faster than the usual combination of AF/MOX/ sequential inoculation with *O. oeni* (Figure 4).

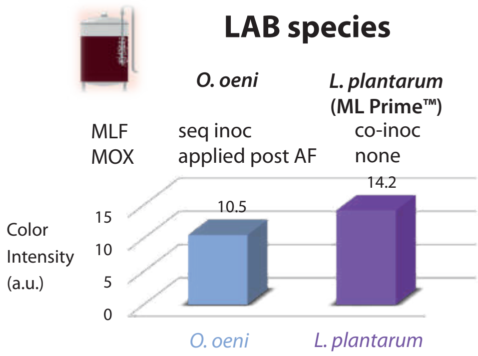

Figure 4. Timing of MLF, LAB species and microoxidation (MOX) can impact wine colour (Cabernet Sauvignon; IFV Val de Loire, France, 2020).

A versatile *L. plantarum* strain for different applications winemaking

While acid reduction is a more important winemaking consideration in the cooler climate regions, microbial stability and sensory contribution as a result of the activity of malolactic bacteria (ML) will play a more important role in the warmer climates. Wines from these climates generally have high pH and alcohol levels, and they require the use of high levels of sulphur dioxide (SO_2). Among the LAB species *L. plantarum* strains have shown most interesting results under hot climate conditions.

The *L. plantarum* strain ML Prime™ exhibits a range of versatile properties. It is a highly concentrated starter culture prepared using a specially optimized process that promotes very high malolactic activity at inoculation into grape must. Due to the very high malolactic enzymatic activity and early inoculation (24 hours after yeast inoculation) into the fermenting must, MLF is completed quickly (usually within 3–7 days) during alcoholic fermentation; wines can be stabilized early and retain their sensory integrity (Figure 5).

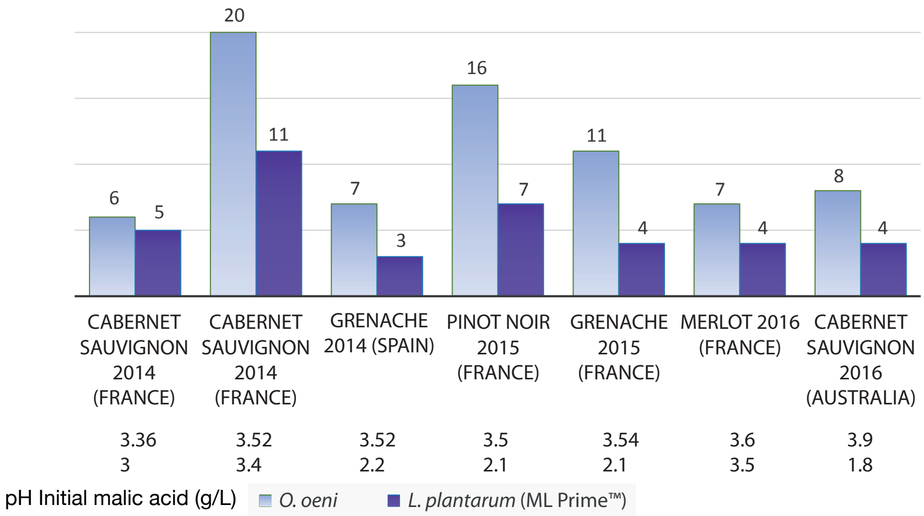

Figure 5. Duration of MLF in different red matrices when inoculated with *O. oeni* or *L. plantarum* (ML Prime™).

Using *L. plantarum* in white winemaking

Many of the early applications of this *L. plantarum* strain have been directed at high pH red winemaking with a focus in use as a co-inoculation, however, the resilience of this strain has demonstrated that it can have broader applications including in white winemaking with low pH.

A partial MLF in case of white wine vinification

In white winemaking, MLF is not always used for complete malic acid degradation. Often a portion of white wine is put through MLF and then blended with the remaining wine batch to reach the desired level of acidity for the wine style. Another approach is to use a partial MLF to modulate the wine acidity, however stopping the ML strain mid through MLF is not always easily done.

L. plantarum (ML Prime™) when applied as co-inoculation and used appropriately in low pH white juices, its high malolactic enzyme activity can greatly shorten the lag phase, the malic acid degradation will initiate immediately, and will often stop before the end of alcoholic fermentation. The percentage of malic acid metabolised during a co-inoculation is not too much influenced by the pH, as illustrated in the Chenin Blanc example in Figure 6. Due to its specific metabolism, the wines have a good sensory quality without any risk of increase of volatile acidity and without diacetyl production.

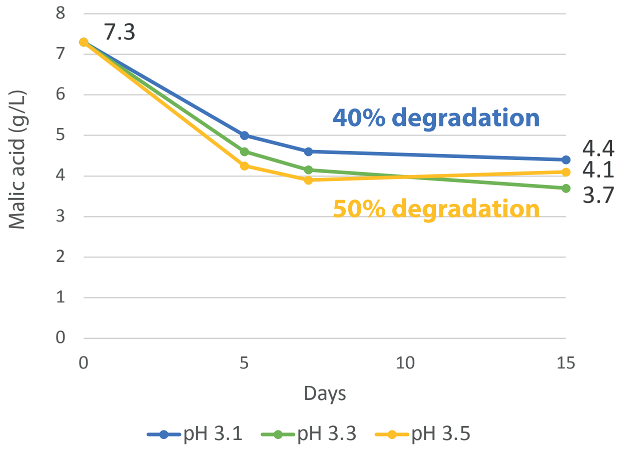

Figure 6. Initial pH can influence the percentage of malic acid metabolised; Chenin blanc with co-inoculation using *L. plantarum* (ML Prime™) with an initial malic acid 7.3 g/L and TA 10.1 g/L.

Using *L. plantarum* (ML Prime™) in challenging wine conditions

ML Prime™ is an exceptional *Lactiplantibacillus plantarum* wine bacteria selected by Universita Cattolica del Sacro Cuore (Piacenza, Italy), with interesting microbiological and oenological properties for high pH red wines. Trials on Spanish white and red varieties have shown the ability of this *L. plantarum* strain to complete MLF under challenging wine composition conditions such as high malic acid (5.27 g/L) where increasing L-lactic acid can be inhibitory to the ML strain, or low malic acid (0.65 g/L) where MLF initiation can be difficult to initiate (Figure 7).

Initial analysis	
Malic acid (g/L)	5.27
Lactic acid (g/L)	0
VA (g/L)	0.2
pH	3.05
F/T SO_2 (mg/L)	0/18
Alcohol (%)	12

Initial analysis	
Malic acid (g/L)	0.65
Lactic acid (g/L)	1.24
VA (g/L)	0.54
pH	3.49
F/T SO_2 (mg/L)	5/17
Alcohol (%)	14.5
G+F (g/L)	0.1

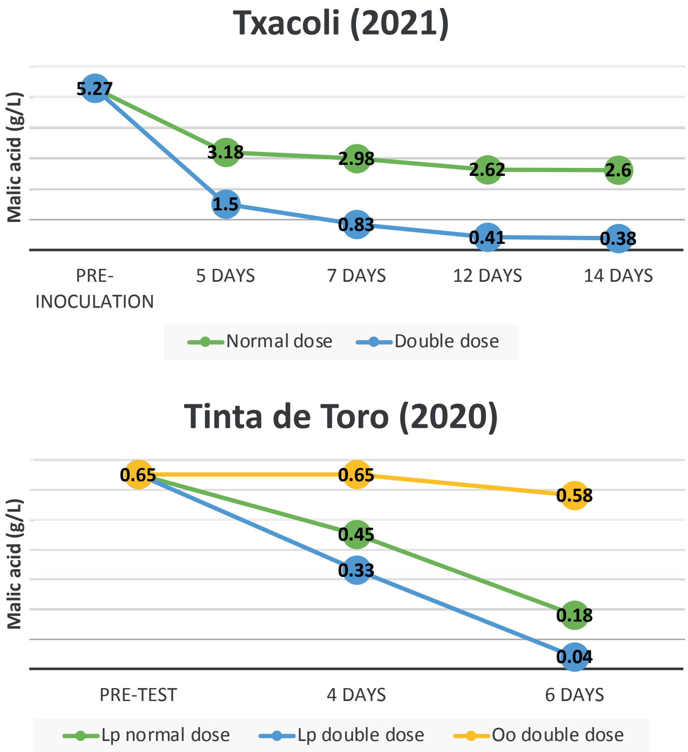

Figure 7. Examples of how L. plantarum (Lp – ML Prime™) is able to complete MLF in very difficult wine conditions; Txacoli wine with very high malic acid concentration, Tinta de Toro with very low malic acid (stuck MLF).

Ensuring that challenging wine composition will support MLF

Fortunately the majority of wines support MLF and the malic acid is degraded efficiently in both co-inoculation and sequential inoculation strategies. However, there often is a batch of wine or parcel of fruit that is difficult to initiate or reinitiate MLF. In these scenarios it is beneficial to conduct a pre-test to ensure the wine will support MLF before investing time and expense to get wines with challenging composition through MLF in a timely manner.

Conclusions

Amongst the diverse *Lactobacillus* species and strains associated with the wine environment *Lactiplantibacillus plantarum* has shown the most potential as a starter culture for the induction of malolactic fermentation. A major feature is its homo-fermentative properties for the metabolism of hexose sugars, which minimizes the risk of acetic acid production. Initially proposed for the management of MLF in processing high pH red grape must this unique *L. plantarum* strain (ML Prime™) has demonstrated its versatility in completing MLF in a broad range of grape juice/ must and wine matrices. 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) enabling an early stabilisation of wine. Even under limiting white wine conditions, a complete or partial malolactic fermentation can be induced. Additionally, this strain has shown to be an excellent option for restarting stuck or sluggish MLFs.

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