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WINEMAKING UPDATE

Number 2 - 2015

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NEWS FLASH

❖ As soon as alcoholic fermentation (AF) is complete, wine becomes very sensitive to oxygen. Oxidation mechanisms are responsible for the loss of fruit aromas and the appearance of heavy notes. **PURE-LEES™ LONGEVITY** is a specific inactivated yeast developed in collaboration with INRA Montpellier (France). Its high dissolved oxygen uptake capacity helps wine resist oxidation during storage and aging.

The new **Lallemand** Wine web site is now officially launched, along with the new **Lallemand** App—two essentials tools for winemakers who want to find out more about our products. You can visit the new web site at the same address www.lallemandwine.com. We've updated our **Lallemand** APP to include all winemaking countries. Visit the iTunes store and look for the free Lallemand Wine App.

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WINEMAKING UPDATE is published by Lallemand to inform oenologists and winemaking staff about the latest news and applications arising from research. To request previous issues, or to send your questions or comments, contact us at:

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A new concept of selected *Lactobacillus plantarum* for high pH wines

Malolactic fermentation (MLF) can occur during or after alcoholic fermentation (see *The Wine Expert on co-inoculation 2012*) and is carried out by one or more species of lactic acid bacteria (LAB). Four genera were identified as the principal organisms involved in MLF: *Lactobacillus*, *Leuconostoc*, *Oenococcus* and *Pediococcus* (du Toit et al., 2011). *Oenococcus oeni* is the predominant species, and a well-known, effective micro-organisms for overcoming difficult wine conditions. It is available in the form of starter cultures that nowadays are very reliable. A few *Lactobacillus* spp. have also shown that they can perform very efficiently in winemaking conditions and that they possess many favorable characteristics for MLF, especially in high pH wines, as was demonstrated in the patented application (EP1631657). This edition of the *Winemaking Update* presents the latest findings on the use of *Lactobacillus plantarum*, based on new concepts for achieving MLF and for limiting the growth of indigenous flora and possible harmful wine microbes.

1. *Lactobacillus plantarum* - a new 'old' friend for MLF

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 those pH levels, we can observe very fast growth of various indigenous micro-organisms, some of which are spoilage bacteria that can cause loss of wine quality. The pH of the wine determines which species of LAB are present. Values above pH 3.5 favor the growth of *Lactobacillus* and *Pediococcus* spp., whereas *O. oeni* tends to dominate at lower pH values (Henick-Kling 1993).

Some selected *L. plantarum* strains (Fig.1) have shown interesting results for their capacity to induce MLF under high pH conditions, and unlike *Oenococcus oeni*, *L. plantarum* has a facultative heterofermenta-

tive metabolism that prevents acetic acid production from hexose sugars. Strains within this species can also have a more complex enzymatic pool that can have a very positive impact on the fruity sensory profile of wines. The selection of a "good" oenological *Lactobacillus plantarum* is not easy. As early as 1988, *L. plantarum* were selected with mixed success. Since 2005 however, some *L. plantarum* isolated at University Catolica del Sacro Cuore in Italy resulted in a very effective *L. plantarum*, adapted to high pH wines. The new starter culture, called ML Prime™, is a pure *Lactobacillus* with a facultative heterofermentative metabolism. It converts glucose or fructose only to lactic acid, but not into acetic acid, therefore avoiding increased production of volatile acidity during MLF. Due to its specific optimized production process, ML Prime™ expresses very high malolactic enzymatic activity on musts, resulting in a fast MLF during alcoholic fermentation and in wines with interesting oenological characteristics.

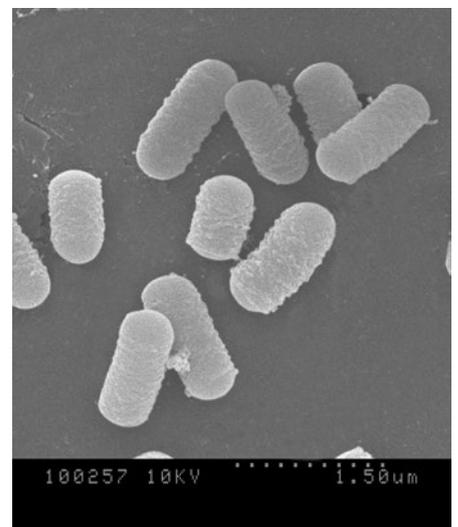


Figure 1. *Lactobacillus plantarum*

2. A fast reliable MLF in high pH wines (pH ≥3,4)

The winemaker's main concern when initiating malolactic fermentation is its timely completion with positive impact on the wine and lower volatile acidity. Co-inoculation is one way to achieve these goals without risk. It allows the winemaker to control MLF because the selected bacteria compete more effectively against indigenous flora. Although, some winemakers still consider co-inoculation with *Oenococcus oeni* to be risky because of its obligatory hetero-fermentative metabolism, ML Prime™ is a very safe option for winemakers because there is no risk of volatile acidity from hexose sugars during MLF.

ML Prime™ has been studied extensively in order to guarantee a fast, secure MLF that takes 3 to 15 days, depending on the conditions. Due to its very great vitality and high concentration, the lag phase is very short. This allows it to quickly dominate the indigenous flora, leading to more rapid onset of MLF.

For example, a trial conducted on a Grenache (Spain, 2014), in a must at pH 3.7, with an alcohol potential of 14% and initial malic content of 2g/L, compared *L. plantarum* ML Prime™ to two different strains of *O. oeni* in co-inoculation and spontaneous MLF. *L. plantarum* ML Prime™ completed the MLF in three days after inoculation, versus one week with *O. oeni* used in co-inoculation. Spontaneous fermentation took longer than 12 days. Furthermore, VA production remained very low in all cases (Figure 2) except for the spontaneous fermentation.™

Table 1. Optimum conditions for the use of *L. plantarum* ML Prime™

Types of wines	Reds – traditional vinification (short or medium maceration – thermovinification (liquid phase) initial sugars /potential alcohol : up to 260g/L - 15,5% V/V
Timing of bacteria inoculation	Only co-inoculation Addition of ML Prime™, 24 hours after adding yeast
SO ₂ addition on grapes/must	≤5 g/hL
pH Acid malic content	≥ 3.4 maximum 3 g/L
Temperature during AF	20° to 26°C

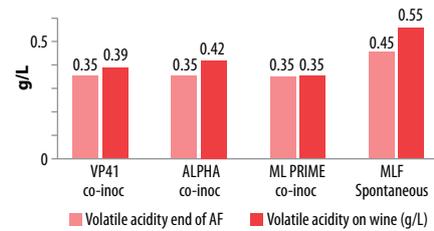


Figure 2. Volatile acidity measured on Grenache must from Spain at high pH (3.7) with ML Prime™ and two strains of *O. oeni* in co-inoculation compared to spontaneous MLF

Studies were carried out to determine the best procedures and conditions in which ML Prime™ could perform successfully. Temperature, pH, SO₂ concentration, inoculation, timing of additions, initial malic acid content and sugar/alcohol concentrations were studied with this bacteria.

The best conditions for successful MLF with *Lactobacillus plantarum* ML Prime™ were established as described in Table 1.

3. Control Microbial contamination – under the care of ML Prime™

A 2014 OIV regulation (OIV-Oeno-264-2014) on good vitivinicultural practices for controlling *Brettanomyces* specifies that “co-inoculation of selected yeast and selected wine bacteria may help to reduce the lag phase between alcoholic fermentation and malolactic fermentation and consequently the development of *Brettanomyces*.” It also states that the use of malolactic starters is a good way to limit the development of *Brettanomyces*.

Because of the high initial vitality of *L. plantarum* ML Prime™ an immediate onset of malolactic fermentation can be

observed and malic acid is degraded during alcoholic fermentation. In Figure 3, the Syrah from Languedoc (INRA, Pech Rouge 2014) was fermented at two different pHs (3.5 and 3.9) where typical microbial contamination is a risk, and the ML Prime™ completed the MLF in 9 days at both pHs. This way, wines can be stabilized early and protected from further contamination and thus retain their sensory integrity. It is important to note that the final VA in those wines did not increase during MLF, but remained at 0.17 g/L.

The trials where implantation controls had been applied, showed that *L. plantarum* ML Prime™ achieved 100% implantation in the must during co-inoculation, and consequently the indigenous contaminating lactic acid bacteria was under control and under the detection threshold of 2x10³ cfu/mL. This allows for a more stable wine, where the risk of spoilage contaminants such as acetic acid bacteria and also undesirable strict hetero-fermentative *Lactobacillus* strains or *Pediococcus* is greatly reduced.

Strong dominance by ML Prime™ is a good way to suppress the growth of undesirable indigenous bacteria. It is well-known that these spoilage bacteria can develop negative metabolites which affect the quality of the wines, either directly by producing bad aromas (mousy taste, volatile phenols, volatile acidity) or masking the fruity characters of the wines due to the production of compounds as biogenic amines and diacetyl or acetaldehyde. Since ML Prime™ is also “phenol negative,” it does not possess the cinnamyl esterase enzyme and cannot produce precursors for volatile phenol production by *Brettanomyces bruxellensis* (please see Winemaking Update #1 – 2014 on this topic).

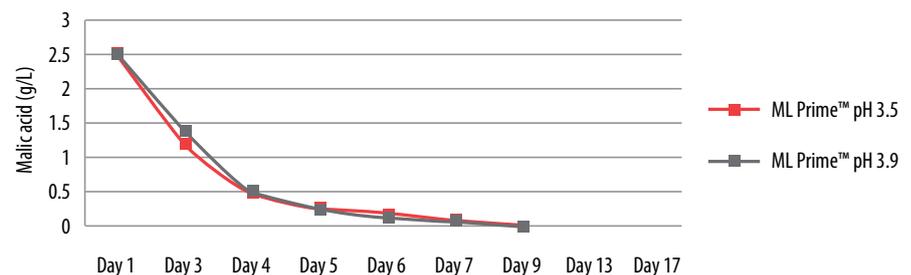


Figure 3. Malic acid degradation in Syrah wines from Languedoc (France) (co inoculation 24 Hours after yeasts addition n at two different ph 3,5 - initial sugars 220g/L - temp: 24°C - thermovinification (liquid phase)

TO SUMMARIZE...

The tendency to harvest higher maturity grapes, resulting in higher pH and alcohol wines, seems more favorable to the development of indigenous bacteria. To limit the development of unknown indigenous flora, which can lead to the production of undesirable sensory deviations, co-inoculation is an interesting winemaking option and using selected *Lb. plantarum* in the freeze-dried form for direct inoculation appears to be a very safe winemaking option in combination with the co-inoculation strategy. *L. plantarum* ML Prime™ offers several advantages: good implantation, fast, effective dominance in co-inoculation and very fast, complete MLF under high pH conditions. In addition, its facultative heterofermentative characteristics makes ML Prime™ a safe choice for preventing the formation of acetic acid (volatile acidity) from hexose sugars.

For more information on this topic, contact your Lallemand representative. References available upon request.