



Editorial



Technical



Innovations



Summary



## Editorial

### Climate change: A threat to wine identity

As they do every first Thursday of the month, a group of wine aficionados gets together for a friendly tasting. This month's finds? A ravishing Pinot Noir from Sweden and a Syrah from Burgundy—2060 was a very good year. This may seem a bit farfetched at first, but is in fact taken quite seriously by the CCEF, the National Committee of French Foreign Trade Advisors, in their report “Wine in the World as We Approach 2050.”

Some still quibble over climate change, but the facts are indisputable—temperatures in France have risen 0.9°C over the last hundred years, a 20 to 30 percent decline in summer rainfall has resulted in increasingly frequent water stresses, and the vine's growth cycle has grown shorter and shorter. In the Côtes du Rhône region, the official date of the harvest has been moved up by a month over the last 50 years.

According to the Intergovernmental Panel on Climate Change (IPCC), an increase of one degree in temperature is equivalent to moving 160 km northwards. The expected temperature increase, 1.4°C to 4.8°C over the course of the century, would leave the map of world vine production unrecognizable.

Climate change might have some positive effects on wine, such as a reduction in vegetative notes caused by pyrazines, or diminished acidity. But if the trend continues, might wines be at risk of losing their distinct identities, their ability to express the terroir? Increased sugar content, reduced acidity, and changes in grapes' secondary metabolism might seriously impact every wine's profile.

Anticipating such risks while responding to changes in consumption patterns will be a key issue for the winegrowers and winemakers of tomorrow. Lallemand stands beside you in facing these challenges, with natural tools tailored to whatever comes your way.

In this new Oenomag issue you'll learn about a new ML Prime™ bacteria for co-inoculating high-pH red wines. As reducing SO<sub>2</sub> dosages becomes another increasingly important concern, the column “Inside wine” explores what happens to sulphur in fermentation.



# 1 The best ways to optimize use of SO<sub>2</sub> during fermentation

Consumer awareness of SO<sub>2</sub> content in wine, particularly since the label “Contains sulphites” was made mandatory in 2005, has fed a trend toward reduction of this compound. Reducing SO<sub>2</sub> content, both added and total residual dose, is now a serious technical and commercial issue for wine producers. This article looks at the antimicrobial role SO<sub>2</sub> plays and explores possible ways to use SO<sub>2</sub> more efficiently and reduce final concentrations.

## The forms of SO<sub>2</sub> and its role in winemaking

SO<sub>2</sub>, which has often been used unwittingly since ancient times, revolutionized winemaking and oenology. As an antioxidant, it preserves flavour, bouquet, and colour and increases the wine shelf-life, while as an antiseptic it reduces microbiological contamination to prevent certain wine diseases and keep the wine from degrading early in the vinification process. Throw in its oxydasic and solvent properties and the result is an extremely useful and hard-to-replace molecule. Recent strides in oenological research however are now suggesting alternative by taking advantage of other mechanisms and tools found in nature. In this article we will focus on SO<sub>2</sub>'s antimicrobial function and how to get the most out of what we use, reducing final concentrations by using microbiological alternatives.

Bear in mind that the SO<sub>2</sub> in wine exists in more than one form. When added to must or wine, one fraction binds with aldehydes (mainly acetaldehyde), sugars, and ketones. This is known as bound SO<sub>2</sub>. The remaining fraction, known as free SO<sub>2</sub>, and it is the one of interest to winemakers. Total SO<sub>2</sub> is the sum of both fractions. Part of the free fraction, known as active or molecular SO<sub>2</sub>, is more active than the rest. How much of the free SO<sub>2</sub> is active depends on pH, temperature, and alcohol level.

The active fraction of SO<sub>2</sub> naturally increases along with the free fraction, as well as in more acidic (lower-pH) conditions, at higher alcohol concentrations, and at increased temperatures.

Clearly one main issue for winemakers seeking to reduce final SO<sub>2</sub> will be to keep the levels of compounds

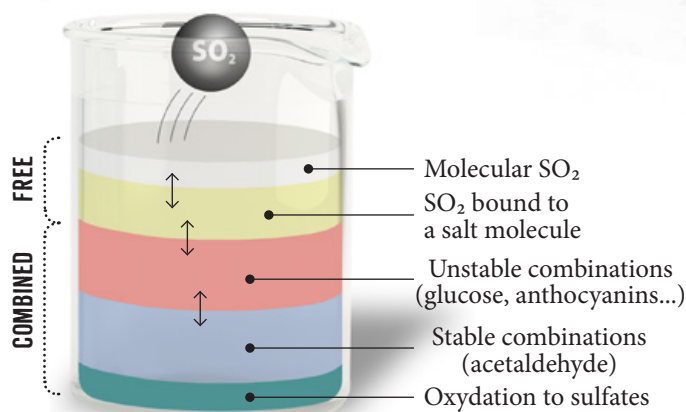


Figure 1. The different forms of SO<sub>2</sub>

that bind SO<sub>2</sub> as low as possible, increasing the free fraction to get the biggest bang for the buck.

## SO<sub>2</sub> in the fermentation stages: whence it comes and where it goes

Banal as it may seem, the best way to reduce final SO<sub>2</sub> content in wine is to minimize the sulphites added. Added SO<sub>2</sub> is the main source of sulphur and how much of it there is has a lot to do with the final SO<sub>2</sub> concentration of the wine—the remaining SO<sub>2</sub> comes from the yeast during alcoholic fermentation. As shown in Figure 2, the metabolism of the yeast and environmental conditions influence how much SO<sub>2</sub> is synthesized from sulphur in the must.

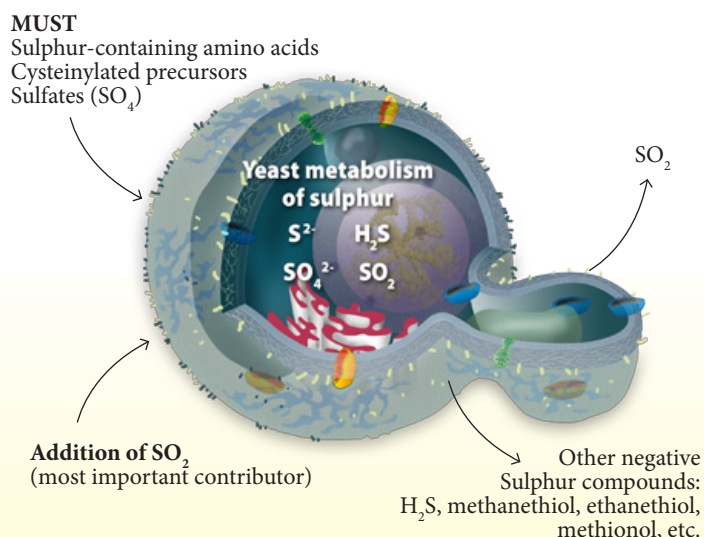


Figure 2. Sulphur metabolism during alcoholic fermentation





## 2 The best ways to optimize use of SO<sub>2</sub> during fermentation

There are many sources of sulphur in a wine, including SO<sub>2</sub> added to the harvest, sulphur amino acids, cysteinylated precursors, sulphates, and others. It is important to rationalize SO<sub>2</sub> use:

- By limiting sources of sulphur: Consider biological controls, control indigenous populations, manage yeast inoculation carefully, and bring in auxiliary solutions and SO<sub>2</sub> alternatives.
- By minimizing yeast-generated SO<sub>2</sub>: Reduce environmental stress through well-managed yeast inoculation and nutrition, cut down on initial sulphites (which can influence the metabolism of some yeast and make them produce more SO<sub>2</sub>), and choose a yeast metabolically incapable of generating SO<sub>2</sub>.
- By controlling compounds that bind SO<sub>2</sub> so you can reduce the SO<sub>2</sub> added at the end of the process: Select low acetaldehyde-producing yeast, use co-inoculation for MLF management (which reduces acetaldehyde concentration at the end of MLF).

Yeast is clearly at the heart of the SO<sub>2</sub> reduction system and a linchpin when you're looking for lower concentrations. That's why Lallemant, the ICV and the INRA (Supagro Montpellier) sponsored a research from 2008 to 2011 on the genetic foundations of sulphur production by yeast to identify the molecular determinants controlling yeast metabolism of SO<sub>2</sub>.

### An SO<sub>2</sub> and acetaldehyde-free yeast today!

The first phase of the project was to find the metabolic pathways and genetic basis of SO<sub>2</sub>, acetaldehyde, and H<sub>2</sub>S production in yeast. This was done by crossing a yeast that produced high levels of SO<sub>2</sub> with another that produced low levels. The resulting yeasts were then subjected to phenotypic analysis (measuring the amount of SO<sub>2</sub> produced by each individual) and by genotype (mapping out the parental origins of their genomes). Comparing the data revealed two regions of the genome that directly influence SO<sub>2</sub>, H<sub>2</sub>S, and acetaldehyde production. This kind of genomic region is known as a QTL (quantitative trait locus) (Figure 3).

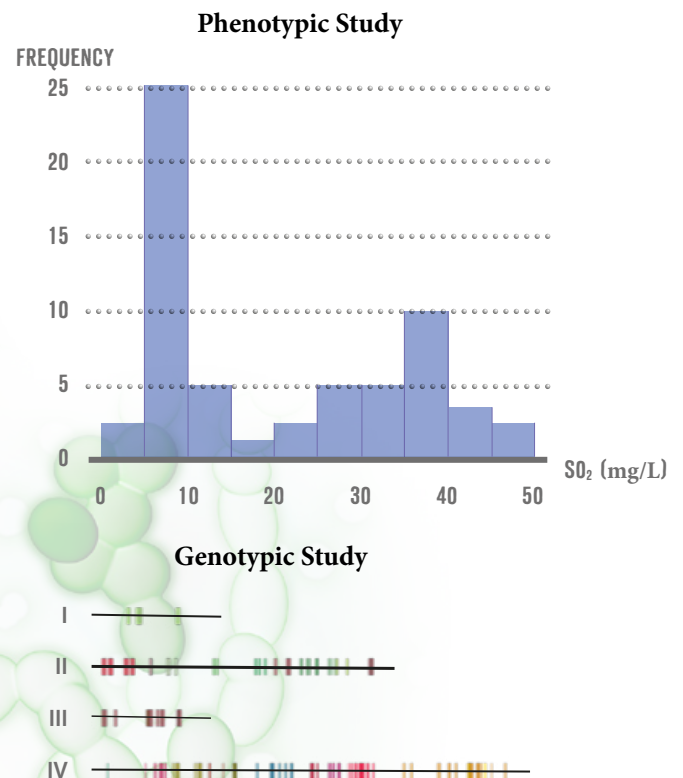
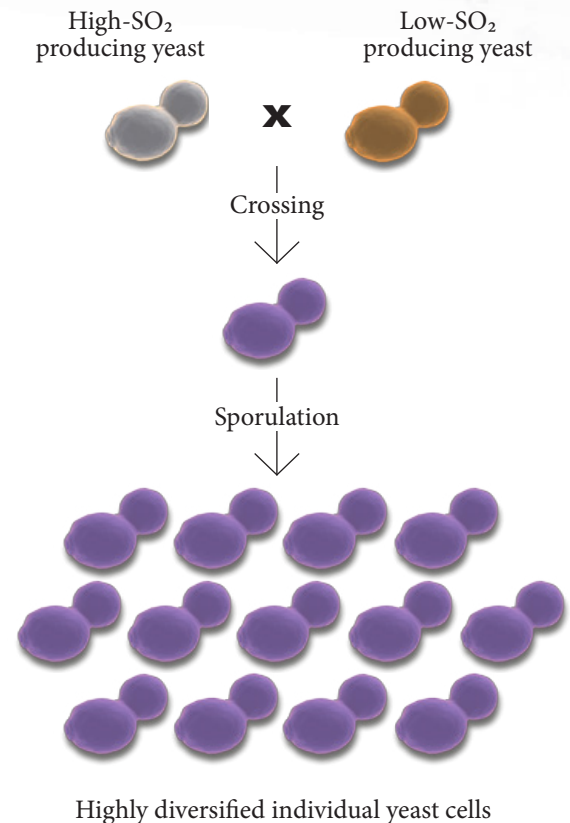


Figure 3. Simplified method to identify the QTL



### 3 The best ways to optimize use of SO<sub>2</sub> during fermentation

When the desired trait (non-production of SO<sub>2</sub>, acetaldehyde and H<sub>2</sub>S) was identified, it was naturally transferred to another yeast that was chosen for its fermentation and other oenological qualities. Transferring the trait involved repeated crosses (backcrossing) between the low-SO<sub>2</sub> yeast and the target yeast. This is a non-GMO technique that can occur naturally with yeasts (Figure 4).

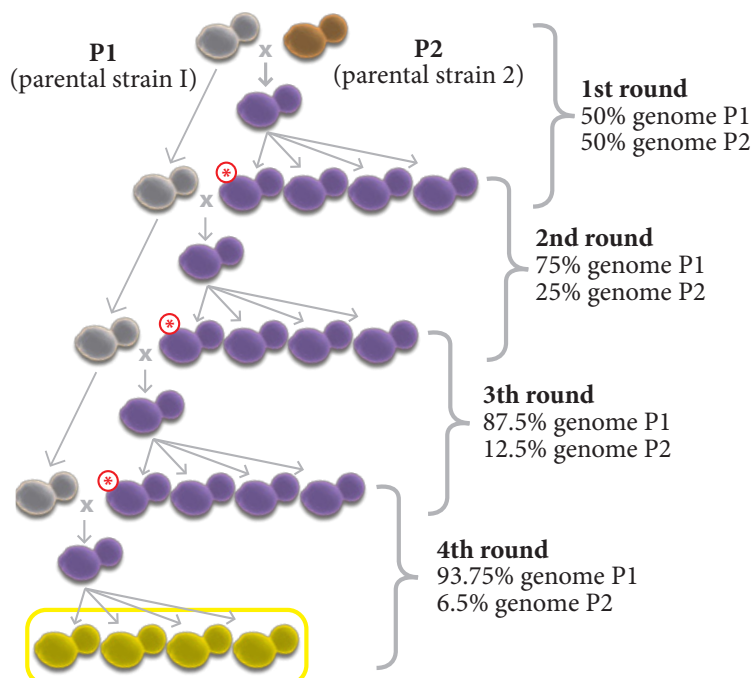


Figure 4. Yeast obtained with backcrossing, assisted by QTL markers \*

This groundbreaking new selection technique (patent application PTC/IB220131050623) resulted in a new yeast—Lalvin ICV OKAY®—that combined a specific metabolic response to sulphur and acetaldehyde with outstanding fermentation.

The new yeast is unable to produce undesired sulphur compounds (SO<sub>2</sub>, H<sub>2</sub>S) and acetaldehyde. This yeast is thus an important addition to winemakers for SO<sub>2</sub> management. Final concentrations of SO<sub>2</sub> will reflect only what is added during vinification, since there is no generation of SO<sub>2</sub>. The amount added late in the process can also be reduced because the lower acetaldehyde content cuts down on production of bound SO<sub>2</sub> compared to classic yeasts and the added sulphites are more efficiently used (Figure 5).

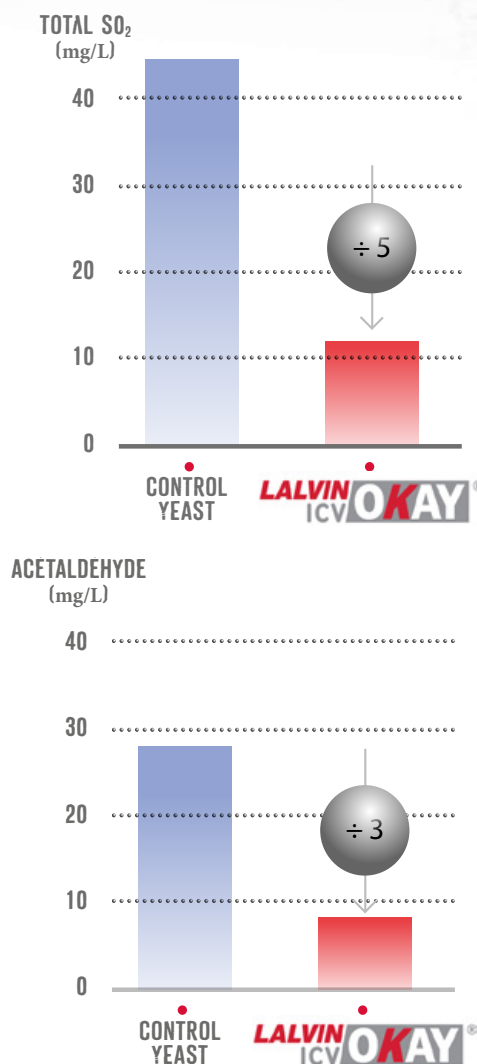


Figure 5. Reduction of SO<sub>2</sub> and acetaldehyde production by Lalvin ICV OKAY®

Consider the example of a wine containing 40 mg/L total SO<sub>2</sub> and 10 mg/L free SO<sub>2</sub> that we want to adjust to 20 mg/L. If the acetaldehyde level is 20 mg/L, 3 g/hL of sulphiting is needed, but if acetaldehyde level is 50 mg/L it takes 7 g/hL! Acetaldehyde management is clearly critically important in rationalizing SO<sub>2</sub> dosage.

#### Co-inoculation, a valuable tool in the management of SO<sub>2</sub> and acetaldehyde

Malolactic fermentation (MLF) is also to the management of SO<sub>2</sub>. There are several aspects to consider:





## 4 The best ways to optimize use of SO<sub>2</sub> during fermentation

- Inoculation with selected wine bacteria as early as possible in the vinification process can reduce the critical gap between the end of alcoholic fermentation and the start of malolactic fermentation.
- Reducing SO<sub>2</sub> dosage allows microorganisms to develop much more easily, including undesirable ones. Inoculation with selected wine bacteria is thus important in preventing contaminants from proliferating. Getting them established is also much easier in a reduced-SO<sub>2</sub> medium.
- Bacteria degrade acetaldehyde during MLF, reducing SO<sub>2</sub> binding situations.

Bacterial degradation of acetaldehyde can also be enhanced, further reducing SO<sub>2</sub> binding and promoting the free fraction, through yeast and bacteria co-inoculation. Research conducted by Ramón Mira de Orduña (Figure 6) has shown that co-inoculation yields lower final MLF acetaldehyde levels when compared to sequential bacterial inoculation (after alcoholic fermentation) under the same conditions. This is directly reflected (Figure 6) in lower concentrations of bound SO<sub>2</sub> compounds with co-inoculation.

There are of course other ways to improve sulphite management and reduce dosages. Adjusting the fermentation environment to reduce yeast stress as much as possible is one, or the use of SO<sub>2</sub> alternatives for microbiological stabilization (such as fungal chitosan, such as No Brett Inside™). There are also alternatives for replacing SO<sub>2</sub> for antioxidation, including ascorbic acid and specific use of tannins, inactivated yeast strains to consume dissolved oxygen (brand name Pure-Lees Longevity™), glutathione-rich inactivated yeast, and others.



Final Value		pH 3.2	pH 3.35	pH 3.5	pH 3.65
Acetaldehyde mg/L	Sequential inoculation	29.6 ± 0	30.4 ± 0.5	16.0 ± 4	12.6 ± 0
	Co-inoculation	19.0 ± 1	12.5 ± 0.1	15.4 ± 0.1	7.3 ± 0.4
Combined SO <sub>2</sub> mg/L	Sequential inoculation	71.5 ± 15	84.5 ± 11	64.5 ± 4	64 ± 2
	Co-inoculation	59.5 ± 7	57 ± 7	59 ± 4	45 ± 6

Figure 6. Impact of co-inoculation on the reduction of acetaldehyde and combined SO<sub>2</sub>



# 1 MLPrime™

An all-new concept in selected wine bacteria for controlling bacterial contaminants during co-inoculation—with no risk of increased volatile acidity

The trend toward reduced SO<sub>2</sub> in winemaking, popular for a number of years now, has brought with it an upsurge in microbiological problems that can put wine quality at risk if not brought under control. High pH (>3.4) wines further aggravates the problem, fostering the growth of undesirable bacteria early in vinification. Lallemand now has a breakthrough solution, ML Prime™—a very high malolactic activity wine bacteria that gets established quickly to fight contamination naturally, completing malolactic fermentation (MLF) faster with no risk of increasing volatile acidity.

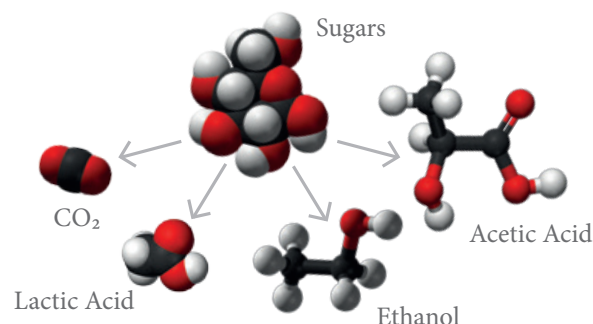
**Forget what you know about bacteria  
ML Prime™ has a new way of working!**

So many things about ML Prime™ are unlike any other wine bacteria.

**No increase in volatile acidity during MLF**

ML Prime™ is a *Lactobacillus plantarum* selected in partnership with the Italian university UCSC in Piacenza (Università Cattolica del Sacro Cuore). It has a facultative heterofermentative metabolism, a type of metabolism specific to bacteria like ML Prime™ that acts like a homo-fermentative metabolism in its response to sugars. Unlike classic wine bacteria, which use sugars to produce acetic acid (hence the classic increase in volatile acidity [VA] during MLF), ML Prime™ produces only lactic acid—it is metabolically incapable of producing acetic acid.

## OBLIGATORY HETEROFERMENTATIVE METABOLISM (STANDARD METABOLISM OF SELECTED WINE BACTERIA)



## FACULTATIVE HETEROFERMENTATIVE METABOLISM (ML PRIME™ METABOLISM)

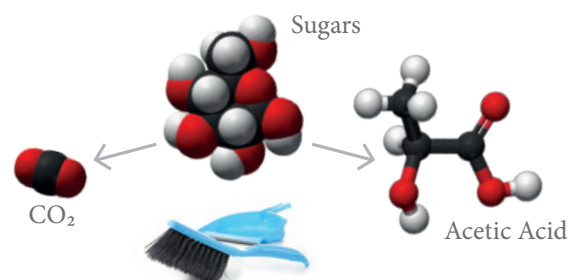


Figure 1. Wine bacteria Metabolism

In practice, this means that there is no increase in volatile acidity during malolactic fermentation with ML Prime™, regardless of sugars or conditions in the juice.

	VOLATILE ACIDITY AT THE END OF ALCOHOLIC FERMENTATION (AF)	VOLATILE ACIDITY AT BOTTLING
<b>Trial 1</b> ( <i>O.oeni</i> A in co-inoculation)	<b>0.39</b> (MLF finished at the end of AF, MLF on skins)	<b>0.43</b>
<b>Trial 2</b> ( <i>O.oeni</i> in sequential)	<b>0.29</b> (MLF no yet initiated)	<b>0.37</b> (MLF done after racking Inoculation)
<b>Trial 3</b> ML Prime™ in co-inoculation	<b>0.29</b> (MLF finished at the end of AF, MLF on skins)	<b>0.31</b>





## 2 ML Prime™

This very specific mode of action has been confirmed in numerous test runs under many conditions and has real value for the management of VA, particularly for co-inoculation in high-pH must. The results in Table 1 show volatile acidity at the end of MLF with ML Prime™ (Method 3) to be identical to the VA in Method 2 before MLF, coming in at 0.29. ML Prime™ thus produced no VA during MLF. Note as well that the increase in VA for co-inoculated and well-managed selected *Oenococcus oeni* (Method 1) was relatively small.

**Outstanding malolactic activity: Almost no lag phase, and MLF in record time**

ML Prime™ is the result of a new production process that optimizes bacterial biomass to obtain direct-inoculation freeze-dried bacteria with a very high level of malolactic activity. This particularly high activity level means that the wine bacteria gets established very quickly, drastically reducing the lag phase so it can degrade malic acid in record time. Malolactic fermentation may actually occur within 24 hours of ML Prime™ inoculation.

All the malic acid is then consumed within 3 to 15 days of inoculation.

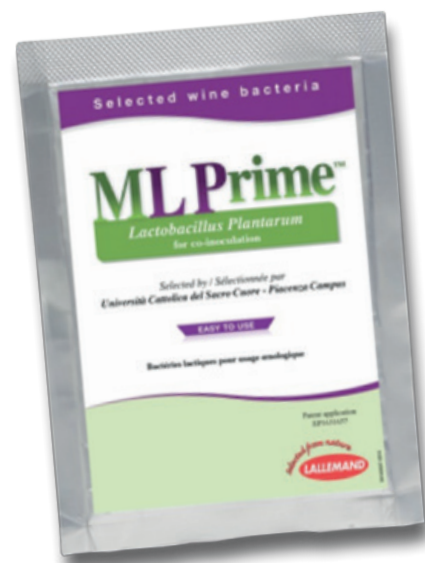
This rapid establishment and completion of MLF leaves no time for indigenous flora to develop, which makes ML Prime™ a valuable biological control tool, microbiologically taking over the must to prevent any of the contamination or spoilage that often occurs under high-pH, low-sulphite conditions.

Other traits make ML Prime™ an outstanding choice for MLF and wine quality management: It produces no biogenic amines, is phenol negative (no cinnamoyl esterase activity, hence no production of volatile phenol precursors), and delays citric acid degradation (very low production of diacetyl, the substance responsible for buttery notes). It all makes ML Prime™ a perfect fit for the vinification of red wines in the modern world.

## Innovations

### Relatively broad optimal conditions

ML Prime™ was developed for co-inoculation (occurring 24 hours after yeasting) for red wine with short to medium maceration periods or liquid-phase wine-making processes (such as thermovinification or *Flash Détente*). It is particularly suitable for hot climate type conditions (pH  $\geq$  3.4; malic acid content  $\leq$  3 g/L; up to 15.5% alcohol content, i.e. roughly 260 g/L of must sugars). ML Prime™ has a limited SO<sub>2</sub> tolerance so addition of over 5 g/hL should be avoided at vatting. The ideal MLF temperature range for ML Prime™ is between 20°C and 26°C.





## Summary

### TO CONTACT US

**Lallemand France/Switzerland/China**  
**Lallemand SAS**  
[fb.france@lallemand.com](mailto:fb.france@lallemand.com)  
 Tel: +33.5.62.74.55.55

**Lallemand Italia**  
[fb.italia@lallemand.com](mailto:fb.italia@lallemand.com)  
 Tel: +39 (0) 45 51 25 55

**Lallemand Península Ibérica**  
[fb.espana@lallemand.com](mailto:fb.espana@lallemand.com)  
 Tel: (+34) 91 4415053

**Lallemand Germany, Austria, Greece, Hungary, Israel, Cyprus, Malta, Poland**  
[fb.eurocenter@lallemand.com](mailto:fb.eurocenter@lallemand.com)  
[kburger@lallemand.com](mailto:kburger@lallemand.com)  
 Tel/Fax: (+43) 27 35 80 147

**Ferment Croatia, Slovenia, Macedonia, Romania, Russia, Serbia, Moldavia, Ukraine**  
[nmaslek@lallemand.com](mailto:nmaslek@lallemand.com)  
 Tel: (+385) 98 30 24 62

**Lallemand North America, Mexico, Japan, Taiwan**  
[gspecht@lallemand.com](mailto:gspecht@lallemand.com)

**Lallferm S.A. Chile, Argentina, Uruguay, Brazil, Ecuador, Colombia**  
[pcarriles@lallemand.com](mailto:pcarriles@lallemand.com)  
 Tel: +54 (261) 425 67 89

**Lallemand Australia, New Zealand**  
[australiaoffice@lallemand.com](mailto:australiaoffice@lallemand.com)  
 Tel: 61 (8) 276 1200

**Lallemand South Africa**  
[ploubser@lallemand.com](mailto:ploubser@lallemand.com)  
 Tel: +27 21 913 7555

### Did you know?

#### Glycosides contribute to the perception of flavours.

Do glycosides help us perceive flavors? That's the conclusion of researchers at the Australian Wine Research Institute (AWRI), who conducted an in-depth study of glycosides lasting over a year. The problem is that aromatic glycosides are non-volatile compounds made up of an aromatic bonded to a sugar. Only when this bond is broken down by yeast or bacterial enzymes is the aromatic part is released.

The glycosides of monoterpene flavour compounds can however contribute significantly to sensory perceptions in the mouth, and the effects can be very persistent.

These findings suggest possible ways to intensify wine flavour and persistence, such as increasing glycoside levels through viticulture or winemaking practices.

**“The best wine isn't necessarily the most expensive—the best one is the wine that is shared.”**

Georges Brassens

### Should selected yeast and bacteria be considered wine additives?

Daniela Shelton's essay on this question won her the 2015 Lallemand Scholarship. The Lallemand Scholarship is open to students from the Masters of Wine Institute and has been awarded since 2010. This year's recipient received an invitation to the Lallemand seminar, where the latest breakthroughs in the field are presented to an audience of seasoned oenologists. Daniela is “delighted to have the opportunity to talk to some of the most influential winemaking consultants and viticulture scientists in the business, like Sam Harrop, MW, and Dr. Bruno Blondin.”

Daniela worked for a number of vineyards in Portugal and South Africa, then took a position as a consultant with the eminent wine critic Robert Joseph. She is currently based in London where she remains a wine consultant while energetically promoting her ideas about wine and food through social media and on her blog.



Daniela Shelton