

Q&A

MALOLACTIC FERMENTATION

A crucial step in the winemaking process, malolactic fermentation, has a huge influence on the stability of a wine. By seeding the wine's microbial ecosystem with a selected, active, and controlled microorganism, you help prevent the development of undesirable yeast and bacteria at the end of the alcoholic fermentation process.

1. Which wine parameters have an effect on MLF success?

Temperature

Lactic acid bacteria prefer temperatures above 15°C (60°F) with an ideal zone between 18°C to 24°C (65°F to 75°F). Temperature acts on membrane fluidity: an excessively high temperature will liquefy the cell membrane; too low it becomes rigid. Ethanol narrows these parameters even further.

Alcohol

Any wine above 13% ABV is considered a difficult environment for lactic acid bacteria, however, some commercial strains are equipped to tolerate up to 17% ABV.

pH

All living cells are dependent on specific pH parameters to function. Lactic acid bacteria used in winemaking require pH levels typically above 3.3, while some strains like Lactoenos B16 can tolerate much lower pH levels.

SO₂ levels

It is important to keep Total SO₂ levels below 60 mg/L so as to not interfere with LAB population survival. Commercial strains are designed to complete MLF quickly, then be eliminated easily with an SO₂ addition.

Malic acid

Below 1 g/L of L-malic acid, it is difficult to trigger MLF as the quantity is not sufficient for the bacteria to activate malic acid enzymes and choose this metabolic pathway. Fermentability is optimal with an L-malic acid content of between 1 and 5 g/L.

Toxins

Besides ethanol, medium chain fatty acids released by yeast in stressful conditions during alcoholic fermentation are known to delay or prevent MLF. The medium chain fatty acids affect membrane fluidity and disrupt normal ML fermentation.

2. What is the dosage rate of an ML culture?

The standard dose is 1 g/hL (10 ppm). Packages are typically sold in hL quantities, for example, 25 hL package weighs 25 grams. Note that malolactic bacteria are susceptible to oxygen. Once opened, the package needs to be used immediately.

3. What is the difference between direct inoculation, PreAc strains, and standard build-up cultures?

Direct inoculation are those strains that may be added directly to your wine without the need to rehydrate. **LACTOENOS® SB3** or **LACTOENOS® B7** are direct inoculation strains.

PreAc strains require less time compared to standard build-up cultures to increase biomass and acclimate before pitching. Thanks to pre-acclimatization during production, **LACTOENOS® 450 PREAC** only requires an overnight build-up (rehydration and time) to build strength before adding to wine.

Standard build-up cultures such as **LACTOENOS® B16** can take up to 3 days to reach optimum viable cell counts. Direct inoculation cultures save time by eliminating extra steps, whereas build-up cultures tend to be more robust and can handle more extreme conditions.

4. Is a specific ML nutrient required for ML fermentation?

Specific ML nutrients are appropriate in wines that have one or more challenging parameters. During a healthy primary fermentation, yeast provide MLF nutrition through natural autolysis that breaks down yeast cells to release essential vitamins, minerals, and peptide fragments. For challenging primary ferments, and especially for uninoculated MLF, a specific nutrient may be necessary. **MALOBOOST®** is a mixture that is rich in peptide fragments and amino acids.

5. In general, what is the average time an ML fermentation should take to complete?

There are several factors that influence the duration of MLF, studies have shown that commercial strains are the most efficient to complete fermentation, while native strains are much more variable. The biggest impacts come from temperature and inhibitory components in the wine.

- Early co-inoculation – 3 days to 2 weeks.
- Late co-inoculation – 1 to 4 weeks.
- Sequential inoculation – 3 to 6 weeks.
- Indigenous MLF – 3 to 11 weeks.
- Using an ML nutrient such as **MALOBOOST®** may reduce MLF by several weeks.

6. What happens if the ice packs melt in shipping?

Frozen bacteria can tolerate a temperature of 25°C (77°F) for a few days without losing their efficiency. This occasionally occurs during transport and is not to be worried about.

The LAFFORT® range of freeze-dried malolactic bacteria can be stored in the refrigerator at 4°C (39°F) for 18 months, and in the freezer at -18°C (0°F) for up to three years from the date of production.

Note that the best option is to always store bacteria in the freezer.

7. Before adding the ML culture, should I detoxify with yeast hulls as a standard step prior to MLF?

In general no, the majority of fermentations create an acceptable environment. If your primary fermentation struggled to finish or stuck, then it is recommended to detoxify the wine with yeast hulls before adding the ML bacteria culture.

Besides ethanol, medium chain fatty acids released by the yeast metabolism are one of the most common inhibitors of lactic bacteria. In case of excessive quantities, their toxic effect can be efficiently eliminated by treating the wine with yeast cell walls (OENOCCELL®) which can adsorb fatty acids and other inhibiting metabolites. Add OENOCCELL® at 20 to 40 g/hL (200 - 400 ppm) and during the 48 hours following the addition, mix anaerobically several times to promote the adsorption and inhibition removal. Bacteria must then be inoculated rapidly or activate the native bacteria with the addition of MALOBOOST® to maximize the clean environment.

8. What are the benefits and risks of co-inoculation with yeast and bacteria?

Early co-inoculation (24-48 hours after the yeast inoculation) saves time as MLF begins before AF is completed. In late co-inoculation (at around 0-4 Brix), the bacteria adapt to the medium while AF is finishing and MLF begins as AF is completed.

Early co-inoculation should be utilized in healthy fermentation conditions: moderate final alcohol, clean fruit, and vineyard blocks which are known to finish AF strongly. With questionable conditions: high potential alcohol, mold, and blocks with historical stuck fermentations, it is best to avoid early co-inoculation. Once you are confident that the AF curve looks good and the wine will finish AF, add the ML starter at around 0-4 Brix.

9. What should I do if my ML fermentation has not started or has started and did not complete the fermentation?

There are many reasons for slow-to-start, or slow-to-finish, or stuck, malolactic fermentations, generally through either microbial or chemical inhibition. First, analyze the microbial status of your wine to assess the health of the malolactic bacteria and the risk of any competing microorganisms, particularly *Brettanomyces*. Second, determine the status of your wine chemistry, alcohol %, glucose-fructose, pH, L-malic acid, free SO₂, and total SO₂.

Other inhibiting metabolites also exist, such as short- and medium-chain fatty acids produced by yeasts during AF, or afterwards by *Brettanomyces*. C8 (octanoic acid), C10 (decanoic acid) and C12 (dodecanoic acid) fatty

acids inhibit MLF bacterial growth and enzyme activity, particularly by disrupting membrane function. An assay of the fatty acids gives an indication of the wine's toxicity for lactic bacteria, and ethanol exacerbates their effect, and these can be removed by detoxification with OENOCCELL®.

Often it can be an interaction of multiple factors, a single one may not be a problem, but a combination can cause an issue.

And remember that occasionally, simply racking the wine may be sufficient to kick off or finish the MLF. In more difficult cases, correct any deficiencies and inoculate with a robust strain that can handle a wide array of conditions such as LACTOENOS® B7, and also see the MLF restart protocol on page 157.

10. What is the easiest way to achieve partial MLF or prevent MLF completely?

Many methods exist to stop or prevent MLF, including SO₂, LYSOZYM, and filtration. Each has advantages and disadvantages. SO₂ added and maintained at over 60 ppm can effectively kill the bacteria but will deplete over time and MLF may restart in the bottle. LYSOZYM degrades the bacteria cell wall to inactivate the cell and has minimal sensory impact. However, it will add heat-labile proteins that need removal with bentonite or may interact with phenolics and reduce color.

Filtration can be used to remove the bacteria, however, keep in mind that recontamination is always a risk. Always consider the end goal before deciding which method is best. With wines that have residual malic acid, it is recommended to sterile filter at bottling time.

11. For low pH wines, what is the best type of ML culture to use?

Strains that require acclimatization typically can handle a wider range of wine conditions including lower pH. LACTOENOS® B16 STANDARD is a 3-day build up strain that can handle wine pH down to 2.9.

12. Can ML bacteria influence the fruit aroma/flavor in a wine?

MLF lactic bacteria are capable of metabolizing sulfur containing amino acids: methionine and cysteine. It is now recognized that sulfanyl-3-methyl propionic acid, a compound derived from this metabolism, positively contributes to red fruit aromas in wine.

It is, in our current state of knowledge, the only compound clearly identified as being implicated in the aromatic impact of lactic bacteria during MLF. Inversely, it is recognized that sluggish MLFs that delay the wine's microbiological stability are detrimental to the fruity aromas derived from alcoholic fermentation. With the indigenous flora, it should be specified that certain compounds such as biogenic amines can mask aromas. Research, led by Professor Gilles de Revel at the University of Bordeaux Oenology Faculty (ISVV), is currently in progress, studying the effect of bacteria and fruitiness in wine. While the initial results show that it is difficult to establish the existence of a bacteria strain effect used during MLF and fruity notes, it appears that certain vinification pathways protect fruity aromas more than others. Co-inoculation is a technique that can shorten the time between end of AF and end of MLF, thereby protecting the fruity aromas.