ALL ABOUT MLF

Malolactic fermentation (MLF) is the conversion of malic acid into lactic acid by lactic acid bacteria (LAB), particularly Oenococcus oeni. Wines that go through MLF become microbiologically stable as malic acid is consumed and can no longer be used by other microorganisms that can alter wine. Additionally, these wines are softer since lactic acid contributes less acidity to wine. MLF also produces organoleptic changes that result in greater aromatic complexity and stabilization of wine color.

When MLF is not desired, how to prevent it?

To keep bright acidity, freshness and to have wines ready to be released early, often MLF is not desired. Preventing MLF to happen and maintaining the wine microbiologically stable through time can be challenging, especially in high pH wines or low SO_2 wines such as base wines for sparkling production when SO_2 is inefficient, not enough, or cannot be used to control Lactic acid bacteria activity.
In these conditions, the use of chitosan is highly recommended. **5-8 g/hL of** **KillBrett** (pure chitosan from *Aspergillus niger*), can be used to reinforce or replace SO_2 antibacterial activity and reduce the number of viable bacteria in wines.

> **KillBrett** has a wide anti-microbial spectrum, it eliminates and prevents the development of *Brettanomyces, Lactobacillus, Pediococcus, Oenococcus, Acetobacter,* and some non-*Saccharomyces* yeast. We recommend using it as preventive, post-fermentation when the microbial population is still low. In this case, a dosage at 5 g/hL of **KillBrett** can be added to wine and remain in it to act as an anti-microbial agent. If it is used as a treatment to remove a higher microbial population, we recommend 8 g/hL followed by a racking a few days after.

What are the parameters impacting the success of MLF?

The main wine compositional factors that determine the success of MLF are alcohol, pH, temperature, and SO_2 concentration (Molecular and Total). Each of these factors has a negative synergistic effect, making the completion of MLF increasingly difficult when combined. Additionally, vineyard sprays, initial malic acid content, yeast strain used for alcoholic fermentation, and wine polyphenol content can be stress factors. Problems can arise when pH <3.3, alcohol >14.5%, wine temperature <65°F or >80°F, total SO_2 >30 mg/L, free SO_2 >10 mg/L and/or molecular SO_2 <0.3. Commercial Lactic Acid Bacteria (LAB), such as **Oeno1,** are typically selected for their resistance to high alcohol, SO_2, low temperature, and have a low pH tolerance.

Spontaneous VS Selected bacteria strains, what are the risks associated?

Certainly, MLF can occur spontaneously, but there can be problems in the resulting wine even if this occurs. Spontaneous MLF is a risk for wine quality and consumer health. Controlling MLF with selected strains of Oenococcus oeni helps control the speed of malic acid degradation and ensures the production of healthy, high-quality wine by preventing the production of biogenic amines and off-flavors.

- Wild MLF fermentation kinetics are often poor when compared with selected LAB, such as **Oeno1** (Figure 1). A longer latency period between alcoholic and malolactic fermentations is an opportunity for spoilage microorganisms to invade due to the absence of SO_2, warm temperature, and low microbial competition. Latency periods and the speed of MLF can be critical (and expensive) factors when considering tank space, labor, time, and heating due to the lower temperatures.
- Commercial Lactic Acid Bacteria (LAB) are typically selected for their resistance to high alcohol, SO_2, low temperature, and have a low pH tolerance. To ensure the completion of MLF in a timely manner, it is highly recommended to use selected LAB strains.
- Indigenous malolactic fermentations typically produce higher levels of diacetyl than selected LAB strains. **Oeno1** has a low ability to produce diacetyl.
- Most indigeneous LAB possess cinnamyl esterase activity, which cleaves the (mainly tartaric) ester precursor cinnamic acid form, allowing *Brettanomyces sp.* to produce vinyl (chemical/medicinal odors) and then ethyl (horse/band-aid odors) phenols. Another reason to inoculate with selected LAB, such as **Oeno1**, is to prevent the production of free cinnamic acids, Brettanomyces substrates.
- Indigenous LAB typically produces higher biogenic amines than selected LAB, such as **Oeno1** (Figure 2). These can generate allergenic reactions in some consumers as well as unpleasant aromas and diminution of wine quality.

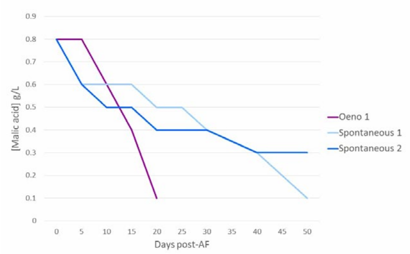

Figure 1: Kinetics of MLF with Oeno1 VS spontaneous MLF

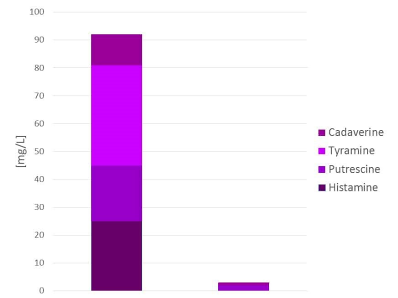

Figure 2: Production of biogenic amines after MLF. Oeno1 VS Indigeneous bacteria

Lamothe-Abiet ML Bacteria: Oeno1

Oeno1 is a freeze-dried *Oenococcus Oeni*, used in direct inoculation for clean and complete MLF. **Oeno1** enhances fresh and fruity aromas, reduces the perception of green characters. **Oeno1** has been selected for its short lag phase, quick conversion of malic acid, strong resistance to difficult conditions, low/no production of VA, biogenic amines, and diacetyl and is free of cinnamyl esterase activity. It also consumes ethylacetate, thus improving the SO_2 efficiency in the later steps of winemaking. **Oeno1** is also doing great when used in co-inoculation, especially with **Excellence XR**.

What are biogenic amines?

Biogenic amines are a group of compounds mostly formed by lactic acid bacteria via decarboxylation of amino acids. Their production is highly dependent on the bacteria strains and their enzymatic activities. Known as a human health threat and to cause allergenic reactions, headaches, and digestion issues, biogenic amines can also be associated with off-aromas in wine such as rotten flesh, algae, and fish food. The main biogenic amines found in wine are putrescine, histamine, tyramine, and cadaverine.

Regarding the production of diacetyl

Diacetyl is produced by LAB, from metabolizing citric acid, typically occurring once the malic acid has all been consumed. At low levels, diacetyl contributes a butter-like character to wine, but at higher levels, this turns to a more rancid aroma buttery notes. Diacetyl is considered by many winemakers to be undesirable. The entire winemaking process impacts the production of diacetyl: a slower MLF speed (with low inoculation rate and/or low temperature) and slightly oxidative environment will increase diacetyl production, while yeast lees contact will break down diacetyl and SO_2 can bind with diacetyl, thus reducing its content in wine. Indigenous malolactic fermentations typically produce higher levels of diacetyl than selected LAB strains.

About co-inoculation

Co-inoculation is the practice of inoculating lactic acid bacteria lactic shortly after yeast inoculation and has many advantages:

- Secure MLF by giving bacteria a favorable environment with lower alcohol concentration, better nutrient fermentations availability, less medium-chain fatty acids (bacteria inhibitors), warmer temperatures, and better acclimation. It is a great technic to use in difficult MLF conditions.
- Limit the risk of microbial contamination and spoilage by eliminating the microbial vacuum.
- Reduce risks of oxidation.
- Lower acetaldehyde concentration, which results in less bound SO_2, more efficient sulfiting, and lower TSO_2 results.
- Produce fresh, fruity, clean, and less buttery wines with better balance and fuller body.
- Save time: blend, stabilize, and age wines earlier.
- Cost-effective: less analysis, less labor, less barrel.

With co-inoculation, it is important to address the concern of a possible production of acetic acid by acid bacteria. The yeast/bacteria couple used will have a strong impact on limiting the risk of stuck/sluggish and the production of acetic acid. Lamothe-Abiet developed a specific yeast/bacteria couple for co-inoculation: **Excellence XR** and **Oeno1** for reds, Excellence TXL and **Oeno1** for whites/rosés.

There are 2 types of co-inoculation: early and late. Early co-inoculation is when the LAB are added just after the start of AF, usually 48 hours after yeast, when there is an active cover of CO_2, important to avoid the production of acetic acid. Late co-inoculation is when the bacterial inoculum is added towards the end of AF, typically at pressing.

Does the yeast strain used for alcoholic fermentation affect MLF?

Yes. Some yeast strains can impact lactic acid bacteria (LAB) development. Several factors should be considered when looking at yeast compatibility with ML bacteria, such as yeast fatty acid production, SO_2 production, nutrient consumption, and fermentation kinetics.

What is the recommended dosage for Oeno1?

The recommended dosage is 1 g/hL. In difficult conditions, low temperature, high malic acid, high alcohol, … we can increase the dosage up to 2 g/hL.

How to prepare and add Oeno1?

Oeno1 is a freeze-dried bacteria for direct addition. You can sprinkle Oeno1 directly into wine, and mix gently without oxygen to homogenize. You can also rehydrate Oeno1. In this case, use 20 times its weight of mineral water at room temperature, mix well, wait 15 minutes and add to wine.

How should Oeno1 be stored?

Store in its original packaging hermetically sealed, in a cool, clean, and dry place without odors. The bacteria may withstand a few days out of the cold (maximum 4 days), at ambient temperature (< 25 °C), without loss of efficacy. Optimal date of use (from the date of production): 36 months at -18°C, 18 months at 4°C.

What if Oeno1 gets warm during shipping?

We recommend that our malolactic bacteria be kept cool, at -18°C/0°F for long-term storage. If, despite our best efforts, the ice is melted and the container is not feeling cold when your bacteria arrive, do not be alarmed. We tested the viability of our ML bacteria, simulating some weeks of shipping. Their viability is not altered and stays excellent even after 1 week at 30°C/86°F. Sealed packets can be delivered and stored for 3 weeks at ambient temperature (<25°C/77°F) without critical loss of viability. So just put the bacteria in the cooler or freezer at reception.