



IMPROVEMENT THROUGH BIOTECHNOLOGY
-SINCE 1963-

Malolactic Bacteria

ML Secondary Fermentation

Malolactic Bacteria

Malolactic fermentation (MLF) is an intricate process in which specific bacteria converts malic acid (two carboxylic group) into lactic acid (one carboxylic group) This translates in less H⁺ being released in the media by lactic, therefore a higher pH. and a lower total acidity.

By inoculating with an AEB starter culture, which contain *O. oeni* as a single strain or mixed cultures, the winemaker can reduce the risk of potential spoilage bacteria or bacteriophages, promote the rapid start and completion of MLF and encourage a positive flavor contribution. AEB Malolact *Oenococcus oeni* strains are best adapted to the harsh wine environment, including conditions of:

- High alcohol
- Low pH
- Presence of sulfur dioxide (50 ppm of SO₂)
- Extreme temperature
- High concentration of phenolic compounds

PRODUCT	NUMBER OF STRAINS	TYPE	CHARACTERISTICS	PACKAGING	DOSAGE
MALOLACT ACCLIMATÉE	3 STRAINS COMBINED, TO BUILD A DOMINANT "COCKTAIL"	DIRECT ADD	FOR CLEAN AND CONSISTENT ML FERMENTATION	2.5 GRAMS (BARREL SIZE), 25 GRAMS, 250 GRAMS, 1 KG	1 GRAM PER 1 HL OR PER 26 GALLONS
MALOLACT ACCLIMATÉE 4R	4 STRAINS COMBINED, TO BUILD A DOMINANT "COCKTAIL" WITH SPECIAL RESISTANCE TO HIGH PHENOLICS.	DIRECT ADD	FOR COMPLICATED ML FERMENTATIONS (HIGH ALCOHOL AND HIGH TANNINS)	2.5 GRAMS (BARREL SIZE), 25 GRAMS, 250 GRAMS	
MALOLACT ACCLIMATÉE F	SINGLE STRAIN	DIRECT ADD	FAST AND CONSISTENT ML FERMENTATION	1 KG	
FERMOPLUS MALOLACTIQUE AF	NUTRIENT FOR MALOLACTIC BACTERIA	YEAST DERIVED NUTRIENT	REINTEGRATES THE FERMENTING MEDIA WITH YEAST DERIVED AMINO-ACIDS AND VITAMINS	500 GRAMS PACKETS AND 5 KG BAGS	200 PPM (ABOUT 1.5 LBS/1,000 GALLONS)



Malolact Acclimatée: frozen culture at -20°C . Multi strain direct inoculum of *Oenococcus oeni* for malolactic fermentations. Malolact Acclimatée is ideal for white, rosé and red wines. It's constituted of three genetically different strains of *Oenococcus oeni*, equally effective in conducting the malolactic fermentation. This combination helps to improve competition in the event of a high population of indigenous bacteria. Malolact Acclimatée has been selected for its capacity to highlight the typical aromas of the cultivar from which the wine was made, integrating them with pleasant fruity nuances. It improves the taste profile of wines enhancing roundness and fullness and prolonged after taste sensations. The appearance of bitter or green nuances, which often characterize wines at the end of spontaneous malolactic fermentation, are absent.

Utilization: remove Malolact Acclimatée from the freezer 30 minutes before use. Malolact Acclimatée works as a direct add, but its activity can be boosted by a simple rehydration for 24 hours (see procedure at the end of this chapter).

Dosage and packaging: add 10 ppm 1g/hL. Comes in pre-dosed packets for 66 gal, 660 gal, 6,600 gal, or 26,400 gal of wine (2.5 grams, 25 grams, 250 grams and 1 kg).

Storage and shelf life: Malolact Acclimatée is stable for two years (with minimal loss of activity), when stored in a freezer (-4°C / -17°C).

Malolact Acclimatée 4R: frozen culture at -20°C . Multi strain direct inoculum of *Oenococcus oeni* for malolactic fermentations. Malolact Acclimatée 4R is a direct add ML inoculum ideal for big red wines and harsh ML conditions.

In this product "coexists" an aggregate of four *Oenococcus oeni* strains, especially selected for working in red wines characterized by high phenolics. When developing this culture, the selection has been mostly focused on having a strain not only resistant to ethanol and SO_2 , but also to high levels of tannins. Malolact Acclimatée 4R has been selected under the following conditions: pH: 3.2; Temperature: 18°C ; Alcohol level: 14.5%; Total SO_2 60 ppm; concentration of polyphenols: 80 (by total polyphenol index).

Utilization: remove Malolact Acclimatée 4R from the freezer 30 minutes before use. It works as a direct add, but its activity can be boosted by a simple rehydration for 24 hours.

Dosage and packaging: add 10 ppm or 1g/hL. Comes in pre-dosed packets for 66 gal, 660 gal, 6,600 gallons of wine (2.5/25/250 grams and 1 kg).

Storage and shelf life: the lactic bacteria's activity of Malolact Acclimatée 4R is stable for two years (with minimal loss of activity) when stored in a freezer (-4°C /-17°C).

Malolact Acclimatée F: vigorous, single strain direct inoculum, selected to focus on the ability of the bacteria to complete the malolactic process in the shortest time possible, given the conditions of the wine. Malolact Acclimatée F has a noticeable tolerance towards low temperatures, sulfur dioxide and high alcohol and is intended to be added directly to the wines. One of the main selection goals of this strain has been the lack of biogenic amine production.

Utilization: Remove Malolact Acclimatée F from the refrigerator 15 minutes before use. Malolact Acclimatée F works as a direct add, but its activity can be boosted by a simple rehydration for 24 hours.

Dosage: dose at 1g/hL. 1 kg pack is good for 26,400 Gallons.

Storage and shelf life: the lactic bacteria's activity of Malolact Acclimatée F is stable for two years (with minimal loss of activity) when stored in a freezer.

Packaging: 1 kg Pre-dosed packets for 26,400 Gallons of wine.

	SIMPLE CONDITIONS	NOT SIMPLE CONDITIONS	DIFFICULT CONDITIONS	EXTREME CONDITIONS
ALCOHOL (%VOL.)	<13	13-15	15-17	>17
PH	>3,4	3,1-3,4	2,9-3,1	<2,9
FREE SO2	<8	10-12	12-15	>15
TOTAL SO2	<30	30-40	40-60	>60
TEMPERATURE (°C)	18-22	LOW: 14-18	LOW: <14	LOW: <10
		HIGH: 18-24	HIGH: >24	HIGH: >29
INITIAL MALIC ACID (G/L)	2-4	HIGH: 4-5	HIGH: 5-7	HIGH: >7
		LOW: 1-2	LOW: 0,5-1	LOW: <0,5

Factors and values that influence Malolactic fermentation

Co-Inoculation yeast/bacteria

Bacteria inoculated in wine at the end of alcoholic fermentation face difficult conditions, such as high ethanol content combined with low pH, these conditions threaten their survival. In contrast inoculation in grape must allows the bacteria to adapt gradually to ethanol before it becomes toxic for the cell. This way ML is substantially facilitated. However, there is still skepticism on co-inoculation due to the risk of increased VA and competition with the yeast.

In most cases, *Oenococcus oeni* is an hetero-fermenter that will create multiple end-products from the utilization of glucose with D-lactic acid and CO₂ being produced in roughly equal amounts to either ethanol or acetate. In reductive conditions, like at the end of fermentation, the third product is usually ethanol, while in slightly oxidative (such as early in alcoholic fermentation or in an un-topped barrel) the bacteria is likely to produce acetate (VA).

Regarding competition for nutrition, Lactic acid bacteria have limited biosynthetic ability, therefore require pre-formed amino-acids and B-vitamins. During co-inoculation, is important to provide amino-acids to the yeast for optimizing biomass formation. An ideal timing for this would be during yeast rehydration, using Fermoplus Energy Glu 3.0 and during fermentation using Fermoplus DAP Free.

Highlights:

- We recommend to wait the end of yeast lag-phase before adding the Malolact of choice.
- Temperature should never reach 30 degrees Celsius.
- Regarding SO₂, the lower the better. 50 ppm might be added, provided that the bacteria addition happens at least 12 hours after KMS addition.
- Depending on the SO₂ level, <50 ppm or >50 ppm, we recommend co-inoculation after 24 or 48 hours respectively.
- Also, the SO₂ produced by the yeast can play a role in slowing bacteria down. The chart: "Selection of AEB yeast for the co-inoculum", shows experiments done with some of our yeasts in co-fermentation with ML bacteria.

YEASTS	SO ₂ PRODUCTION	ML/YEAST CO-INOCULATION
FERMOL ARÔME PLUS	•••	NOT RECOMMENDED
FERMOL ELEGANCE	••	OK
PB 2013	••	OK
GLUTAFERM ONE	•••	OK
FERMOL RED FRUIT	•	RECOMMENDED
FERMOL BLANC	••	OK
FERMOL CRYOFRUIT	•	RECOMMENDED
FERMOL ROUGE	••	OK
FERMOL COMPLET KILLER FRU	••	OK
FERMOL PREMIER CRU	•	RECOMMENDED
FERMOL PB2033	•••	NOT RECOMMENDED
FERMOL CHARMAT	•••	NOT RECOMMENDED
FERMOL SAUVIGNON	••	OK
FERMOL CHARDONNAY	••	OK
FERMOL MEDITERRANEE	••	OK

Selection of AEB yeast for the co-inoculum with ML Bacteria.

How to increase Buttery (Diacetyl) flavor

AEB's Malolact Acclimatée *Oenococcus oeni* bacteria can produce none or considerable Diacetyl depending on conditions. One variable that can boost this buttery aroma component is citric acid paired with oxygen. Biosynthesis of Diacetyl is dependent on the citric acid metabolism and the fact that under partially aerobic conditions, Malolact strains convert citric acid into Diacetyl. Citric acid is first degraded to acetic acid and pyruvic acid. Most of the pyruvic acid is then metabolized to lactic acid with a portion going to diacetyl, acetoin, and 2,3-butanediol. In anaerobic conditions the pathway will favor formation of acetoin and butanediol. This is because the formation of diacetyl requires an oxidative reaction.



- According to our research, additions of 1 gram per liter of Citric acid in partially aerobic conditions, can double the amount of diacetyl in the final wine.
- The bulk of the conversion will start after Malic acid is all depleted and will be diminished by the presence of SO₂. If diacetyl is desired it is better to wait a few days after completions of MLF before SO₂ addition.
- Diacetyl is adsorbed by the lees. The practice of leaving wine on the lees diminishes Diacetyl concentration, both because the lees will adsorb it and because the cell of bacteria that are still viable will convert Diacetyl into acetoin. Adding products like AEB Super-mann, Elevage Glu or Bâtonnage Elevage, can give the same impact of a good sur-lies, without the risk of losing diacetyl.
- If diacetyl is desired do not co-inoculate ML with yeast.

How to save money on ML bacteria by propagation

Direct add bacteria can be added directly pouring the acclimated bag into the wine, or can be propagated in order to increase population and efficiency. Here is a procedure that can benefit all kinds of direct add ML Bacteria:

1. Draw a small portion of the wine to be inoculated.
2. Use 100 liters (26 Gal) for 250 hL (6600 Gal).
3. Add 60 ppm of Fermoplus Malolactique.
4. Adjust pH to 3.5 – 4 and inoculate with the Malolact of choice.
5. Maintain a constant temperature of 24°C (75°F) for 24 hrs.
6. The next day that portion of wine will have a much more aggressive population to quickly start the ML process in the rest of the tank.

ML Nutrition

Fermoplus Malolactique: Malolactic bacteria are typically nutritionally demanding. To grow and develop they need amino acids and vitamins, which are rarely present in the fermented wine, as yeasts tend to deplete them completely. Fermoplus Malolactique re-balances nutritional conditions and reduces the lag-time for the start and completion of the malolactic fermentation. The inoculation of malolactic bacteria with the support of Fermoplus Malolactique, at the end of primary fermentation, helps the onset of MLF. Using Fermoplus Malolactique to improve nutritional conditions results in successful malolactic completion and cleaner wines.

Utilization: dissolve dose in wine to be treated, along with the dose of malolactic bacteria.

Dosage: 50-200 ppm (5-20 grams/hl or 0.5-1.5 lb/1000 gallons). Higher dosage is for when used in co-inoculation early on.

Shelf life and storage: Fermoplus Malolactique is stable at room temperature for at least two years.

Highlights:

Rich in Arginine, which raises the production of ATP (Energy).

Packaging: 500 grams packs and 5 kg bags

Arginine

