

MALOLACTIC FERMENTATION

Malolactic fermentation is the simple process of converting malic acid into lactic acid by bacteria of the species *Oenococcus oeni*. In fact, using the right strain, malolactic fermentation represents the last opportunity to reduce herbaceous notes, enhance fruit aroma, increase aromatic complexity, and improve the balance and structure of wine. Enartis offers a range of bacteria and nutrients suitable for ensuring successful fermentation, even in the most difficult conditions.



enartis

Inspiring innovation.

ML BACTERIA

How to choose ML bacteria strains

Each strain of bacteria performs best within specific environmental parameters. When selecting the appropriate ML bacteria strain, it is important to consider the relative stress conditions of the wine such as pH, SO₂, and alcohol content. ML bacteria can be selected for their effects on wine aroma and mouthfeel. The Enartis bacteria range does not produce biogenic amines.

EnartisML MCW

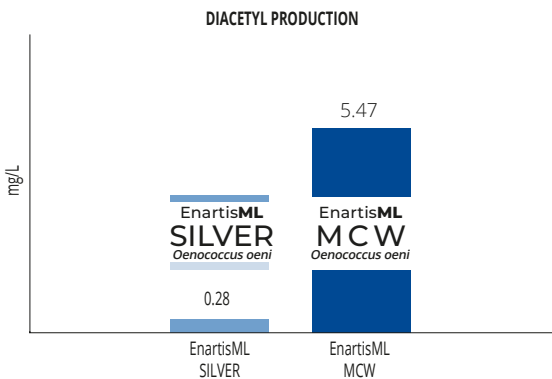


- Freeze-dried form for direct addition after rehydration.
- Isolated from Sonoma County, California.
- Resistant to extreme conditions such as high alcohol and low pH.
- Produces high diacetyl and contributes to creamy, “buttery” characters in wine.

Application: sequential inoculation; co-inoculation; very difficult conditions; increase creamy notes

Package designed for:

- 2.5 hL (66 gal) (Item #30-031-0003)
- 25 hL (660 gal) (Item #30-031-0025)
- 250 hL (6,600 gal) (Item #30-031-0250)



EnartisML MCW produces high amounts of diacetyl which contributes to buttery, creamy notes in wine.

EnartisML SILVER

- Freeze-dried form for direct addition after rehydration.
- Fast and complete malolactic fermentation even under difficult conditions, such as high alcohol and high polyphenol content.
- Respects aromatic characteristics of wine and contributes to fruity and floral notes.

Application: sequential inoculation; co-inoculation; very difficult conditions; increase fruitiness

Package designed for:

- 2.5 hL (66 gal) (Item #35-505-0000)
- 25 hL (660 gal) (Item #35-505-0025)
- 250 hL (6,600 gal) (Item #35-505-0250)
- 1,000 hL (26,400 gal) (Item #35-505-1000)

PROTOCOL FOR ML BACTERIA PREPARATION AND INOCULATION

DIRECT ADDITION, 25 hL

- Rehydrate 25 hL package of EnartisML bacteria in 500 mL of chlorine-free water at 20-25°C (68-77°F). Stir gently and wait 15 minutes.
- Add NUTRIFERM OSMOBACTI to the EnartisML bacteria slurry in order to improve survival rate and activate EnartisML bacteria. Stir gently and let stand for 4 hours at 18-20°C (64.5-68°F).
- Stir the suspension gently and add to wine during pump-over or mixing.

EnartisML UNO

- Freeze-dried form for direct addition after rehydration.
- Provides a quick start and complete malolactic fermentation.
- Production of wines with improved sensory attributes (fruity and varietal notes).

Application: sequential inoculation; co-inoculation; respect wine aroma

Package designed for:

- 2.5 hL (66 gal) (Item #35-501-0002)
- 25 hL (660 gal) (Item #35-501-0025)
- 250 hL (6,600 gal) (Item #35-501-0250)

ENARTIS STRAINS	EnartisML MCW	EnartisML SILVER	EnartisML UNO
SPECIES	<i>Oenococcus oeni</i>		
pH TOLERANCE	>3.1	>3.2	>3.3
TOTAL SO ₂ RESISTANCE (mg/L)	<40	<50	<40
FREE SO ₂ RESISTANCE (mg/L)	<10	<10	<10
ALCOHOL TOLERANCE (%v/v)	>15	>15	<15
CONVERSION SPEED	Moderate	High	High
AROMATIC CHARACTERISTICS	Buttery, “Sweet”	Fruity, Floral	Fruity, Varietal

ML NUTRIENTS

NUTRIFERM ML

- Nutrient specific for ML bacteria rich in amino acids, vitamins, polysaccharides, cellulose, and co-factors.
- Stimulates bacterial growth, ensures domination of inoculated strain over natural flora, improves cell division, and shortens malolactic fermentation time.

Application: nutrition for malolactic bacteria; prevent stuck/sluggish MLF; difficult conditions; increase MLF speed

Dosage: 20-40 g/hL (1.7-3.4 lb/1,000 gal)

1 kg (Item #35-510-0001)

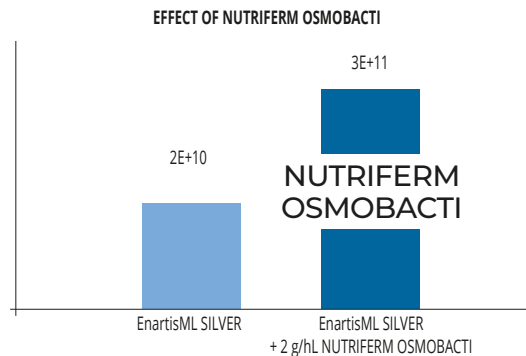
NUTRIFERM OSMOBACTI

- Activator and regulator of osmotic pressure specific for ML bacteria: autolyzed yeast, cellulose, L-malic acid, and bi-ammonium phosphate.
- Improves survival rate of ML bacteria during rehydration and resistance in difficult wine conditions.
- Activates ML bacteria, allowing a faster start and completion of malolactic fermentation.

Application: nutrient during rehydration; difficult conditions; increase the survival rate; accelerates the start of MLF

Dosage: 50 g per 25 hL (660 gal) dose of bacteria

100 g (Item #35-511-0100)



NUTRIFERM OSMOBACTI used during rehydration of ML bacteria increases the cell division and survival rate of the ML bacteria.

KNOW MORE ABOUT MALOLACTIC BACTERIA

MALOLACTIC FERMENTATION BENEFITS

The main role of lactic acid bacteria (LAB) in wine is to conduct malolactic fermentation (MLF): the conversion of malic acid to lactic acid. Additionally, LAB improve wine microbial stability, aroma complexity, mouthfeel, and color stabilization. They also reduce total acidity and bentonite and SO₂ additions due to their ability to break down proteins and degrade acetaldehyde.

WHAT ARE THE PRINCIPAL FACTORS INFLUENCING THE DEVELOPMENT OF LACTIC ACID BACTERIA (LAB)?

At certain levels, factors such as pH, temperature, alcohol, and SO₂ (free and total) can have a negative synergistic effect when combined, making the completion of MLF difficult. 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 3.8 > pH < 3.2, alcohol > 14.5%, malic acid < 1 g/L, wine temperature < 18°C or > 27°C, total SO₂ > 30 mg/L and/or free SO₂ > 10 mg/L.

WHAT HAPPENS IF WINE HAS A LOW CONCENTRATION OF MALIC ACID?

Wines with a malic acid content below 1.0 g/L face greater difficulties starting MLF because there is not enough "food" for the ML bacteria to grow and produce the necessary enzymes to degrade malic acid. The addition of **NUTRIFERM OSMOBACTI** helps start MLF by activating bacteria's enzymes and improving conditions (higher pH and malic acid concentration) to increase the survival rate.

WHAT ARE THE RISKS OF SPONTANEOUS MLF?

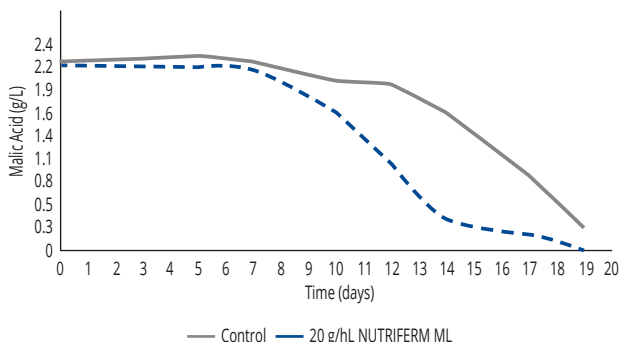
Uncontrolled, spontaneous MLF may increase the risk of spoilage organisms such as *Brettanomyces* sp., as well as the production of undesirable compounds. Volatile acidity, excess of diacetyl, acrolein (bitter), and ropiness are the most common negative sensory characters expected in spontaneous MLF by wild LAB. Spoilage LAB also produce metabolites which are toxic to human health such as ethyl carbamate and biogenic amines (BA). Inoculation with selected *Oenococcus oeni* ensures a rapid onset of MLF and better control over the production of aromas and wine mouthfeel. Enartis bacteria are safe in avoiding BA production.

WHAT ARE BIOGENIC AMINES?

BA are a group of compounds primarily formed by LAB via decarboxylation of amino acids, mostly during ageing. The most common found in wine are putrescine, cadaverine, tyramine, and histamine. Known as a human health threat, BA causes headaches and allergy issues which are enhanced by the alcohol content in wine. Also, they produce irreversible damage to the wine due to the negative sensory impact. Their formation can be prevented by inhibiting indigenous lactic acid bacteria and other spoilage microbes with **EnartisStab MICRO M** and then treat the wine with selected LAB.

HOW TO MANAGE A SUCCESSFUL SEQUENTIAL FERMENTATION IN UNFAVORABLE BACTERIA CONDITIONS

In difficult conditions, it is recommended to add an activator developed to promote the growth of bacteria with necessary nutritional requirements to ensure the total completion of MLF. **NUTRIFERM ML** addition is advised to shorten the length of MLF. It is also useful in cases of stuck MLF as it promotes restart.



DOES THE YEAST STRAIN USED FOR ALCOHOLIC FERMENTATION AFFECT MLF?

Yes. Some yeast strains can negatively impact lactic acid bacteria development by producing toxins and SO₂. All Enartis bacteria have been validated for sequential fermentation and co-inoculation, and none are affected by Enartis yeast strains.

WHEN TO PERFORM CO-INOCULATION

Co-inoculation is the best strategy to shorten MLF duration and obtain a microbiologically stable wine. It is strongly recommended when sequential MLF is compromised by high alcohol content or pH > 3.8 or cellar temperatures are low. The selected bacteria are added 24-48 hours after yeast inoculation or SO₂ addition, taking advantage of alcoholic fermentation conditions: better temperature and nutrition, acclimatizing slowly with the increase of ethanol content. Wines subjected to co-inoculation are fruitier and have a lower diacetyl content, as diacetyl is suppressed by the sugar content during this phase.

HOW TO MONITOR MLF

The most common way to monitor MLF is by tracking malic acid degradation. MLF is considered complete when malic acid is below 200 mg/L.

ABOUT THE PRODUCTION OF DIACETYL

Diacetyl is a compound characterized by buttery notes produced by yeast, but mainly it is LAB during MLF that modulate its final concentration. LAB are responsible for its biosynthesis through citric acid metabolism. **EnartisML MCW** is the bacteria with the highest capacity to produce diacetyl, followed by **EnartisML UNO** and **EnartisML SILVER**. Selected bacteria along with 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. Furthermore, SO₂ can bind diacetyl, thus reducing its content in wine, and co-inoculation practices also can lead to less diacetyl content due to the reductive conditions.

RESTART AND/OR COMPLETE A STUCK ML FERMENTATION - 100 hL

The successful restart of a stuck ML fermentation depends upon three critical factors:

1. Diagnosis of the fermentation arrest causes.
2. Appropriate wine treatment.
3. Proper acclimation of ML bacteria.

1. DIAGNOSIS

Use in-house or outside laboratories to determine the cause(s) of the problem(s) and the degree of fermentation completion.

2. TREAT STUCK WINE BEFORE RESTART - 24 HOURS PRIOR TO ML BACTERIA PREPARATION

- Adjust pH and alcohol.
- Remove spoilage microbes with EnartisStab MICRO M (5 g/hL).
- Absorb toxins with 20 g/hL NUTRIFERM CONTROL.
- Rack off lees 24 hours after treatment.

3. PREPARE AND ACCLIMATE ML BACTERIA

- Rehydrate 4x25 hL pack of EnartisML SILVER in chlorine-free water at 20-25°C (68-77°F) and wait 15 minutes.
- Add 200 g of NUTRIFERM OSMOBACTI to the suspension and wait 2-4 hours.
- Prepare 50 L of wine + 50 L water + 1 kg NUTRIFERM ML and rehydrated ML bacteria.
- At ½ of malic acid depletion, add 200 L of wine to the bacteria culture + 1 kg NUTRIFERM ML.
- At ½ malic acid depletion, add the ML bacteria culture to the remaining wine volume.