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Viniflora® CiNe™ **Inoculation & Usage Protocol**

This document is intended to be used in the winery when managing malolactic fermentation (MLF) in red, white and rosé wines with Viniflora® CiNe™, in either freeze-dried or FroZen™ format. Using CiNe™, wines can be stabilized and overall acidity reduced through MLF, but without the production of any buttery/ creamy flavours. Viniflora® CiNe™ is suggested for wines where freshness and an emphasis on primary fruit character are desired.

Malolactic cultures are living microorganisms so are therefore sensitive to their environment. As wine contains high levels of ethanol and SO₂, has a low pH and is depleted in nutrients, it can be a difficult environment.

It may also contain various compounds inhibiting bacterial growth such as fatty acids (octanoic and decanoic acids, 'C8' and 'C10') or polyphenols. Yeast selection can have a large effect on the presence of these compounds, therefore Viniflora® bacteria are best used in combination with yeast starter cultures designed to facilitate MLF (low to moderate production of SO₂ and C8 & C10 fatty acids). Viniflora® yeast range is recommended for this reason.

In the case of SO₂, the lower the pH is, the higher the amount of free SO₂ will be, and among free SO₂ the most active part: molecular SO₂. SO₂ levels should therefore be kept as low as is feasible.

The physiological parameters required for CiNe™ are:

pH ≥ 3.2

Total SO₂ < 30ppm

Temperature 18-22°C (note that CiNe™ does not ferment malic acid when T is below 18 °C)

Alcohol < 14%

While the recommended inoculation point is either late in alcoholic fermentation (AF)/late co-inoculation or immediately after AF completes. This is to ensure any indigenous bacteria that can produce diacetyl have no opportunity to become established.

The following guidelines are designed to ensure MLF with Viniflora® CiNe™ is completed as reliably, effectively and efficiently as possible, by making certain that all of the factors that can impact upon MLF are controlled appropriately.

3 tools are proposed:

- ① - A check-list per wine tank
- ② - A table with 3 different ways to inoculate Viniflora® bacteria in red, rosé or white wines
- ③ - A reminder from our product information sheets about how to handle Freeze-dried and FroZen™ products: see how convenient is real direct inoculation with Viniflora®!

WINE with VINIFLORA CiNe - INOCULATION PROTOCOL/Oct 2010/1:4

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Check-list for successful malolactic fermentation with Viniflora® CiNe™ per wine tank to be inoculated

| | | | | |
|----------------------------|--|--|---|---|
| GET a PLAN | Consult Viniflora® wine recipes / news for 'application information' | Define your approach regarding A and ML fermentations management. Discuss it with your CH representative | Select your inoculation strategy: early or late co-inoculation, reverse inoculation, sequential inoculation? | Select the right products using the table 'Viniflora® bacteria range' depending: Total and free SO ₂ pH, Ethanol, Temperature, flavor profile expected |
| ACT with CONSISTENCY | Train your team! Secure that inoculations are always achieved the same (right) way to get consistency | Secure product documentation and instructions have been read carefully before use | Viniflora® products are made for direct inoculation: use the correct dosage indicated on the packaging | Only move the cartons, bags or pouches out of their freezer just before inoculation |
| CONTROL | Check grapes' history: ripeness (fatty acids), AF kinetic, yeasts used, Total and free SO ₂ . Use Bactivaaid 2.0 if necessary (see below) | Check inoculation parameters before using the product Total and free SO₂ pH, Ethanol Temperature | Follow the guidelines on the inoculation protocol selected and remember that Viniflora® products are living organisms | Monitor MLF through classical analyses MA, VA, pH, T... ask your CH representative if you have questions |

Special: For must or wine coming from highly ripened grapes, for wine below pH <3.2 or for wine that is highly clarified, Bactivaaid 2.0 should be used prior to Viniflora® inoculation to reduce the amount of fatty acids (octanoic and decanoic acid) and increase the nutrient supply.

Check CiNe™ physiological parameters:

pH ≥3.2

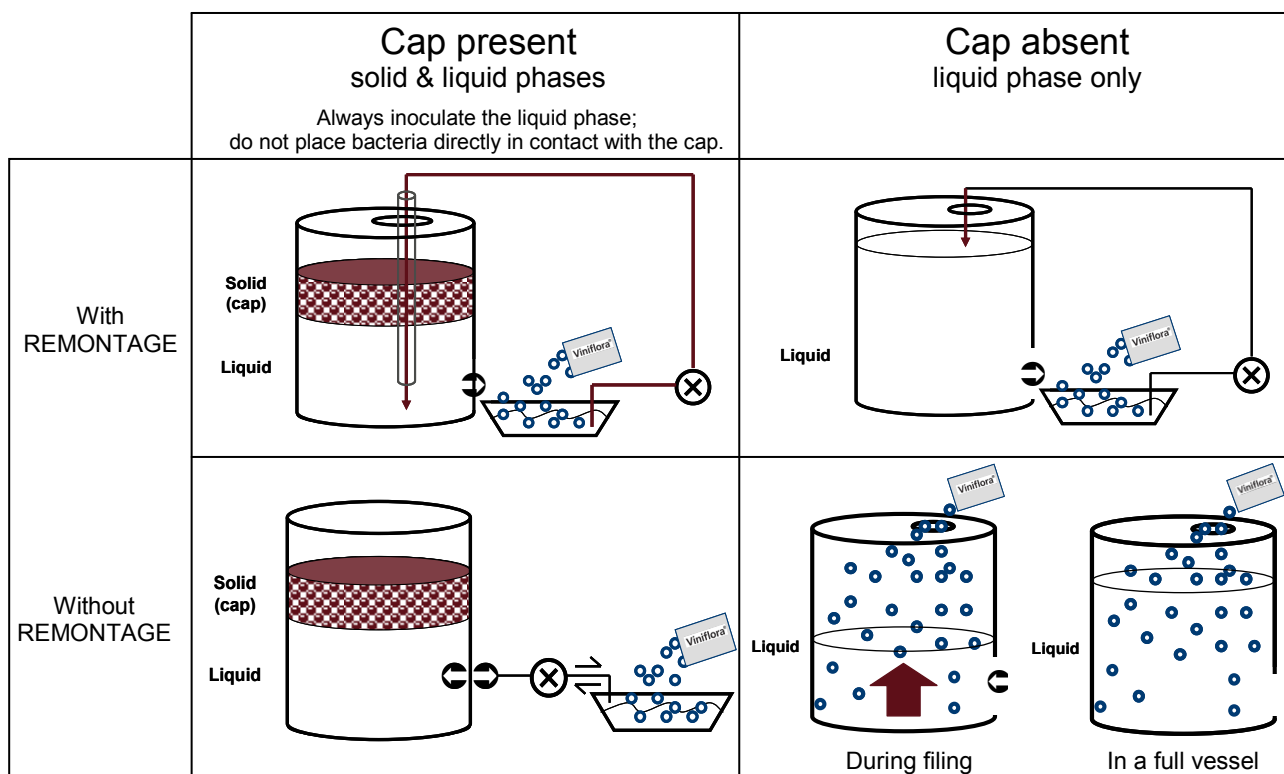
Total SO₂ <30ppm,

Temperature >18°C (if T ≤18°C while all the other parameters are OK, the strain will survive during several weeks without achieving malic acid conversion)

Temperature ≤22°C,

Alcohol <14%

**Depending on cap presence and winemaking choices
four main inoculation options are suggested:**



Inoculating Wine/Must in presence of skins

In the tank, both liquid and solid phases are present. CO₂ pressure has created a cap.

In this case, the best way to inoculate Viniflora® is a ‘bottom direct inoculation’ during a pump-over/remontage, using a clean bucket containing wine. This will supplement the wine with oxygen, which can give a boost to the cultures (this positive effect is useful, but not necessary for successful MLF).

Ideally the inoculum should be pumped through tubing/pipework that penetrates through, and thus bypasses, the cap. If this is not feasible, ensure that the sufficient wine is pumped back into the tank through the cap to avoid the solid phase partially retaining the inoculum. Only inoculate when the wine is below 25°C (and the cap too if not using bypassing the cap). When Viniflora® has been correctly homogenized into the liquid part, a pump-over can then be achieved traditionally with wine dispersed over the cap.

Inoculation after Maceration

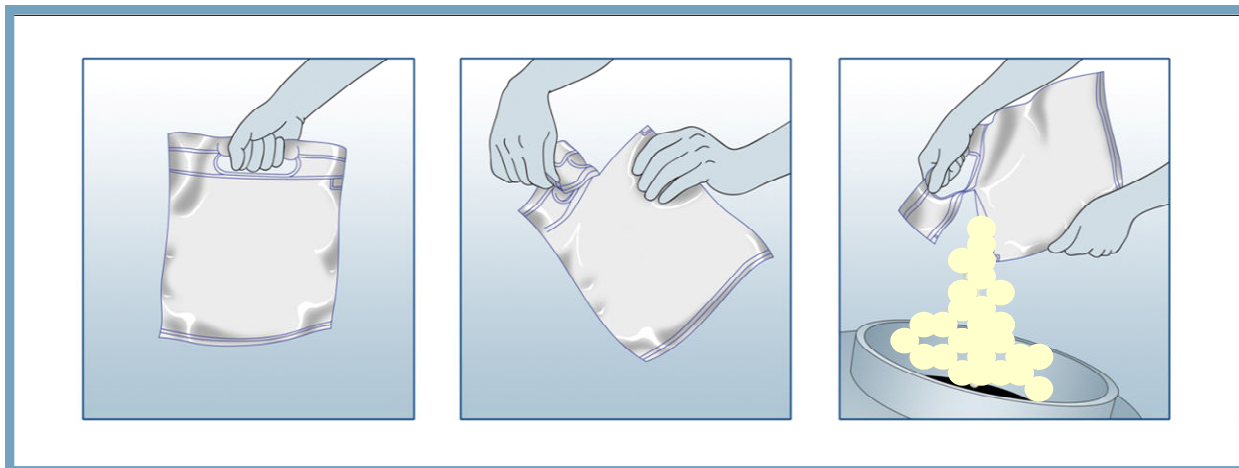
In the vessel, only a liquid phase is present. There is no cap.

In this case, Viniflora® can be inoculated through a ‘top direct inoculation’, followed by mixing to ensure the culture is well incorporated (e.g. by pump-over with/without air or by ‘bottom direct inoculation’ during a pump-over achieved with air).

A further option is to inoculate wine fresh from being pressed off its skins, or from racking, when a vessel is filling, as the action of the filling will help ensure thorough mixing.

Inoculation schemes for Viniflora® products (real direct inoculation)

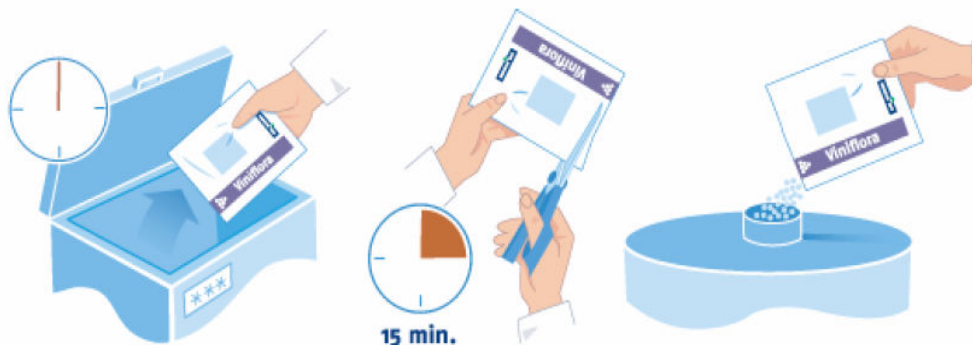
For FroZen™ products (Viniflora® LS) stored in -45;-50°C freezers.



For freeze-dried products (Viniflora®) stored in -18°C freezers or in a 0;+5°C fridges.

Viniflora freeze-dried cultures are adapted for direct inoculation into wine. No rehydration or reactivation is required.

1. Remove the pouch from the freezer 15 min. before use and place at room temperature. Make sure that the dosage complies with the amount of wine to be inoculated.
2. Open the pouch and add the granulated culture directly to wine. The culture can be dissolved in a smaller volume first and added to the total volume right after, if required. Make sure that the culture is completely dissolved in the wine.



Visit our website: <http://www.chr-hansen.com/wine>