A NEW GENERATION OF LYOPHILIZED PREPARATIONS TO MANAGE THE MALOLACTIC FERMENTATION OF WINES WITH HIGH PH.

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INTRODUCTION

Malolactic fermentation (MLF) consists of the enzymatic decarboxylation of malic acid into lactic acid by lactic acid bacteria (LAB). This decreases the wine acidity and gives a softer palate. The MLF does not only just decrease the acidity but also involves the metabolism of other substrates (carbohydrates, organic acids, nitrogen compounds...) by the LAB which then contribute to the aromatic complexity of the wine. The MLF also has a role in the final microbiological stability. Bacterial species found in the wine involved in the MLF belong to the *Pediococcus*, *Leuconostoc*, *Lactobacillus* and *Oenococcus* genera. Throughout the winemaking process, the lactic microflora evolves, not only in number but also in the variety of species. Wine is not a favourable environment for the development of these microorganisms because of its low pH (between 2.9 and 4.0), alcohol level (up to 16%), and relatively low nutrient content and also the presence of inhibitors such as SO_2 or polyphenols.
During the alcoholic fermentation (AF), a natural selection of different LAB happens progressively. *Oenococcus oeni* is the species most resistant to extreme wine conditions. Unlike other wine bacterial species, *O. oeni* is generally not linked to the production of undesirable metabolites nor causes organoleptic faults. Furthermore *Oenococcus oeni* is the only species exploited in malolactic starters that are currently on the market to start the MLF in wines. For many reasons, it would be of interest to examine other species in order to select new malolactic starters, particularly some *Lactobacillus plantarum* species.

SELECTION AND PRODUCTION OF *LACTOBACILLUS PLANTARUM* STRAINS RESISTANT TO ALCOHOL FOR USE IN WINES WITH PH GREATER THAN 3.5

❑ An adapted response in wines with high pH

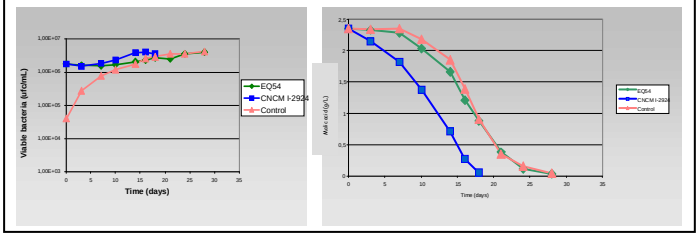
During the last few years, a general increase in wine pH has been observed. Red wines with high pH, greater than 3.5-3.6, are more and more common. At this pH level, bacterial growth is favourable and the development of indigenous bacteria at the expense of *Oenococcus oeni* often occurs.
For these wines of medium to high pH, it is possible to select and produce lyophilized bacterial strains belonging to other species than *Oenococcus oeni* to induce the MLF. The selected strains must be resistant to alcohol and capable of inducing a complete MLF once introduced directly into the wine. They also must not produce undesirable compounds (biogenic amines) nor degrade glycerol or tartaric acid (1). The example given here shows the results obtained in Spain in 2002 on Tempranillo wines, upon bacterial inoculation after the AF. 3 treatments were evaluated:

-inoculation with a *L.plantarum* strain (lyophilized, direct seeding)
-inoculation with an O.*oeni* strain (lyophilized, direct seeding)
-no inoculation

Malic acid degradation in a Tempranillo wine

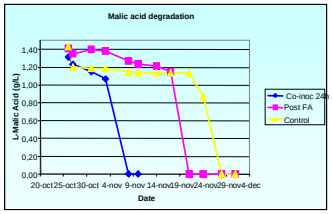
Comparison *O.oeni* / *L.plantarum* (Spain – 2002)

T=18°C; Alcohol=11.98 (%vol); pH=3.9; SO_2 total=11 mg/L; SO_2 free=4 mg/L



During the *L.plantarum* CNCM I-2924 seeding trial, the malic acid degradation starts immediately. Whereas, 8 additional days are needed to observe a decrease in malic acid in wines inoculated with *O.oeni* EQ54 as well as in the non inoculated trial. Significant differences in the kinetics of the last two trials were not observed; for the lot inoculated with *O.oeni*, the indigenous bacteria most likely multiplied rapidly at the expense of the selected *O.oeni* strain. In this case, due to the high pH, inoculation with **a selected *L.plantarum* strain could be an effective solution to suppress the growth of the indigenous flora.**

Malic acid degradation in Sangiovese must/wine
Comparison of different inoculation times (Italy – 2006)



Temperature	21°C
Alcohol (%)	13.3
pH	3.25
total SO_2 (mg/L)	35
free SO_2 (mg/L)	10

❑ *L.plantarum* = Homofermentative strain: a must for inoculation during the AF

In cases where the wine conditions are favorable for the development of LAB, early inoculation of malolactic starters (24 hours after the yeast = "co-inoculation" or during the AF) is an interesting solution to limit or prevent the indigenous bacteria development. Contrary to *O.oeni*, *L.plantarum* is a **homofermentative** species; this means it is incapable of producing volatile acidity from the sugars in the must. This means that even if there is a stuck AF, the risk of lactic acid spoilage is avoided.
In this example, 3 treatments are compared:

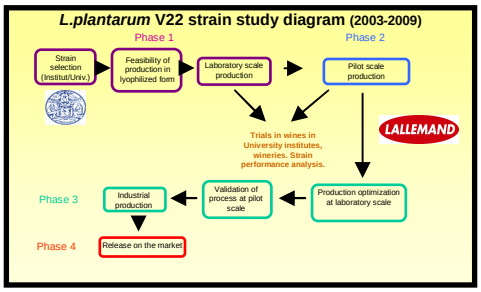
- inoculation with a *L.plantarum* strain (lyophilized, direct seeding in co-inoculation 24 h)
- inoculation with the same *L.plantarum* strain (lyophilized, direct seeding after AF)
- no inoculation

Beyond preventing the development of the indigenous flora, the first treatment also allows for considerable time saving**, :** 13 days and 20 days is the time needed for a complete MLF for treatments 2 and 3, respectively.

❑ A strain with interesting properties: V22

The *Lactobacillus plantarum* V22 strain was isolated during a selection of bacteria strains capable of **degrading ochratoxin A**, at the Sacro Cuore University of Piacenza (Italy) (2). Since **2003** this strain has followed the study diagram shown here. After verifying the feasibility, this strain was lyophilized to permit direct seeding in wines. Since 2004, various seeding trials in wines were completed with this strain both in the laboratory and in partner wineries, in different countries (Italy, Germany, Spain, USA, Chile). It has been shown that the V22 strain is capable of **surviving after direct inoculation and can induce the MLF in wines with the following characteristics: ethanol <15.5%; pH >3.5, total SO2 <50 mg/L, temperature >17 °C.** Furthermore, this strain **does not produce any biogenic amines** and as previously explained, given the **homofermentative** character of *L.plantarum* it can be seeded without waiting for the end of the AF. Recently a study was completed to discover the genes coding for the enzymes involved in the different metabolic pathways of V22 and other *O.oeni* starter cultures on the market (3). It is interesting to notice that several genes not present in most of *O.oeni* starters are found in the genome of this strain, suggesting a higher potential for sensory contribution.

***L.plantarum* V22 strain study diagram (2003-2009)**



CONCLUSION / PERSPECTIVES

During the last few years, the chemical and physical characteristics of wines have evolved and seem to be more favourable to the development of indigenous bacteria. Under **high pH** conditions, *O.oeni* species do not necessarily dominate at the end of the AF. **The selection and production of *L.plantarum* in lyophilized form** seems to be an interesting solution in order to limit the development of the indigenous flora that can cause the production of undesirable compounds or organoleptic deviations. In fact, using this new generation of starters such as the **V22 strain**, can give several advantages: **a good establishment and rapid development after direct seeding, a complete MLF, homofermentative character, no biogenic amine production and potentially greater aromatic potential than that with *O.oeni*.**

REFERENCES

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