MANAGING WINE QUALITY WITH A NEWLY SELECTED, ROBUST AND ORIGINAL WINE BACTERIA

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1. Introduction

The quality of wine is the main objective of winemakers. With this in mind, the use of selected wine bacteria is a tool that lets winemakers master the malolactic fermentation (MLF) process. Over a four-year period, the Institut Français de la Vigne et du Vin (IFV), in partnership with Lallemand, developed a robust and versatile new lactic bacteria which, when integrated into the winemaking process, supports the qualitative efforts made upstream from MLF. This newly selected wine bacteria, O-MEGA™, is very resistant and increases the security of having a safe and complete MLF, as well as offering numerous advantages in terms of effectiveness and sensory impact.

The main steps involved in the selection process are presented, followed by the oenological and sensory benefits of this new wine bacteria.

2. Methodology: The Selection Process

2.1 Selecting a wine bacteria with specific criteria

The objective of the selection process is to develop a new wine bacteria that meets the following specifications:

- Rapid onset and realization of MLF within a wide range of physicochemical conditions, including pH and alcohol
- Low production of volatile acidity (VA), associated with limited degradation of citric acid
- Capable of being produced in the lyophilized form, for use in direct inoculation without rehydration.

2.2 Building a collection of wine bacteria strains

First, the IFV in Beaune put together a collection of lactic bacteria. Corresponding to the specifications initially established with Lallemand, the bacteria were sampled during MLF in white, rosé and red wines, in a variety of regions in France, and over different vintages. To avoid duplicates, only one or two strains were isolated from each of the vats sampled. The samples were stored at -80°C. This collection formed the basis for the research and included 208 lactic bacteria strains from the *Oenococcus oeni* species.

2.3 Selecting the five best-performing strains that are genetically distinct

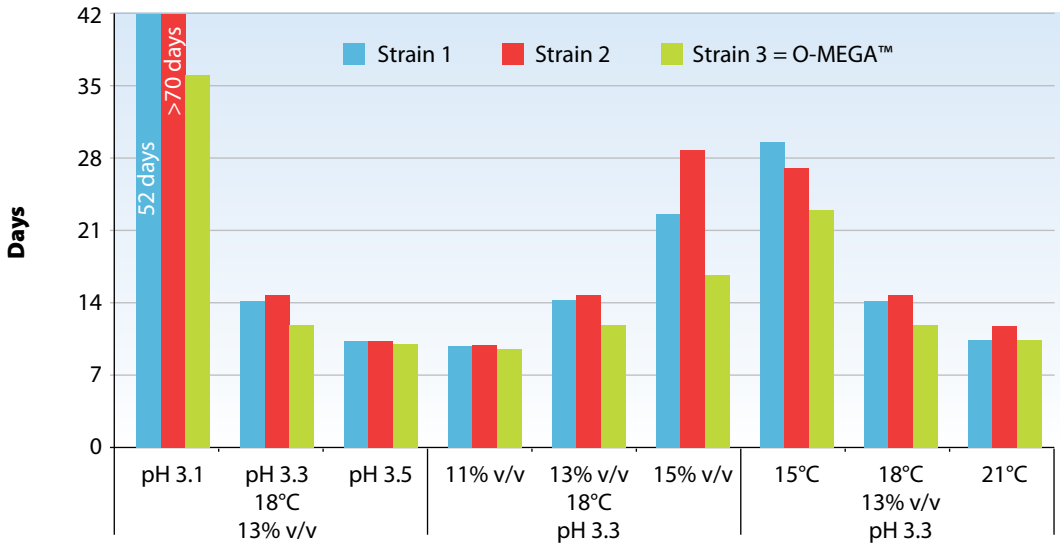
The selection process was carried out in the laboratory on rosé wine with the pH adjusted to 3.2 and the wine was refrigerated after alcoholic fermentation (AF), then divided into 125 mL aliquots and inoculated with the lactic bacteria. The MLF activity of each of the 208 selected bacteria was determined, as well as for four commercially selected bacteria used as the control, for comparison purposes. At the end of MLF, the key parameters were analyzed, including acetic acid level, colour and aroma. Combined with the kinetics of MLF, this analysis led to selecting the six best-performing *O. oeni* strains in the collection. They are all distinct genetically, both among themselves and compared to other selected bacteria already on the market. Production trials (e.g., testing

resistance to lyophilization, yield, etc.), carried out by Lallemand, eliminated two of the strains. The wine bacteria 1, 2 and 3 were then produced in the lyophilized form in a pilot production to test their oenological capacities.

2.4 Performance testing and final selection

The IFV then carried out laboratory trials on rosé wine for these three new biomasses. The wine was divided among 750 mL bottles and the different physicochemical properties (e.g., pH, alcohol and temperature) were adjusted. Each biomass was rehydrated in mineral water and inoculated with the wine bacteria at a rate of 2 million cells per millilitre. As show in figure 1, in each of the limiting conditions the bacteria strain 3 performed the best compared to strains 1 and 2. Selection 3 showed the fastest kinetics at pH 3.1 and tolerated the highest degree of alcohol (15% by volume) and the lowest temperature (15°C). Upon analysis, selection 3 also presented the lowest level of citric acid degradation (15% of the initial level) and the lowest level of VA at the end of MLF (0.21 g/L of H_2S0_4). It was therefore chosen for continued testing, and the new semi-industrial production was called O-MEGA™.

Figure 1. Time required to achieve 90% malic acid degradation



3. Oenological Interest for this New Selection

3.1 A particularly robust selection

The oenological attributes of O-MEGA™ were first defined in the laboratory by the IFV on a red wine and on a rosé adjusted to different physicochemical conditions (pH, alcohol, temperature and SO_2 level). For a given wine must, the only parameter to vary was the wine bacteria used to inoculate it after AF. O-MEGA™ was compared to the other final strains selected, selections 1 and 2.

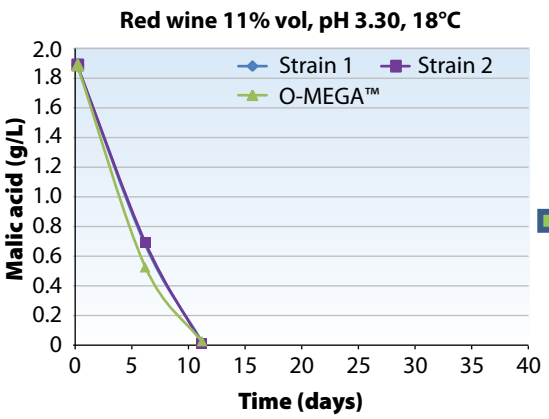
Figures 2 and 3 present the trials on red and rosé wines, where the only parameter to vary was "degree of alcohol." It appears that O-MEGA™ is:

- Little affected by the concentration of ethanol in a range of 11% to 15% by volume
- **Capable of carrying out MLF, even in extreme levels of ethanol** (17% by volume).

Figure 3 shows that the duration of MLF is lengthened by only five days when the degree of alcohol rose from 13% to 15% volume. In addition, O-MEGA™ completed MLF in less than two months (56 days) even in conditions that are very limiting for lactic bacteria (17% by volume).

FIGURES 2A, 2B AND 3. Effect of ethanol content on achievement of malolactic fermentation

2A



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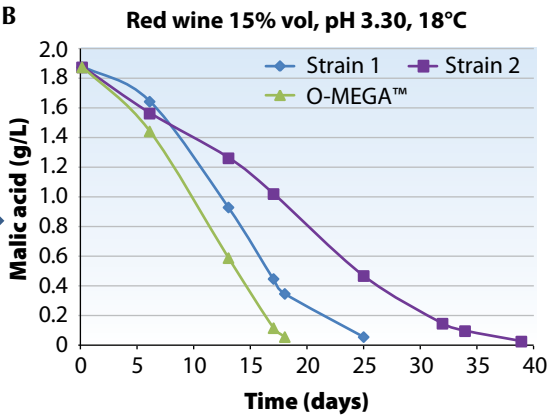

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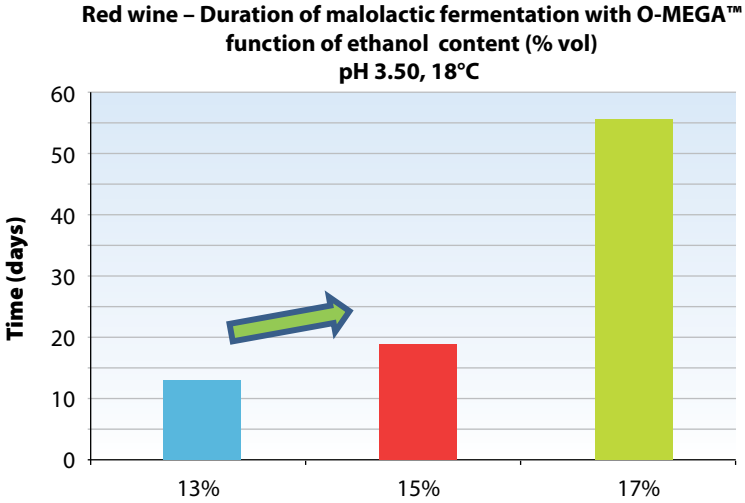
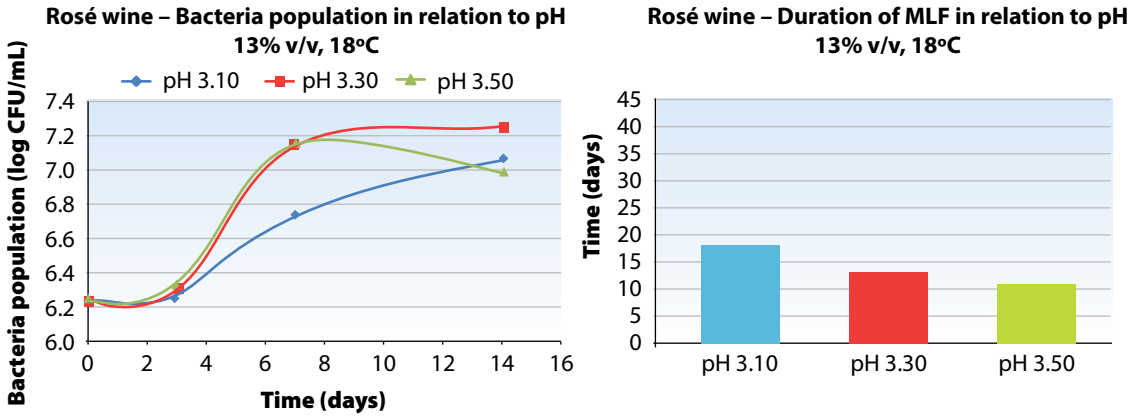


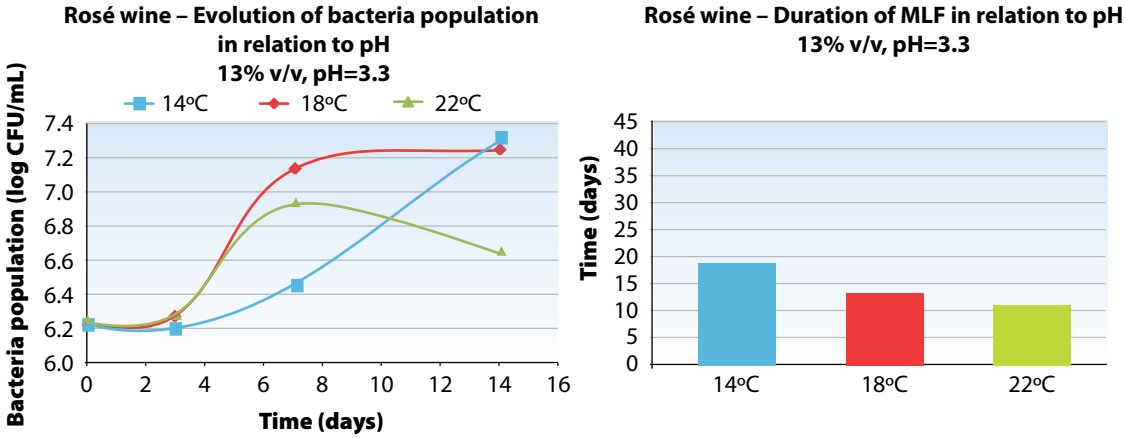
O-MEGA™ also demonstrates **effectiveness in low pH conditions** as shown in figure 4. In a trial conducted on rosé wine, the results show the good implantation of the biomass, even with a pH of 3.1. The MLF kinetics are also little affected by these conditions, with O-MEGA™ completing MLF in 18 days, compared to 11 days with a pH of 3.5.

FIGURE 4. Effect of pH on bacteria implantation and duration of malolactic fermentation in rosé wine



This new selection also demonstrates **very good tolerance to low temperatures**. Figure 5 shows a trial on rosé wine conducted by the IFV at three temperatures. O-MEGA™ is capable of multiplying and carrying out MLF within a short time with the three variables. At a cool 14°C, it took only 19 days to complete MLF.

FIGURE 5. Effect of temperature on malolactic fermentation kinetics in rosé wine

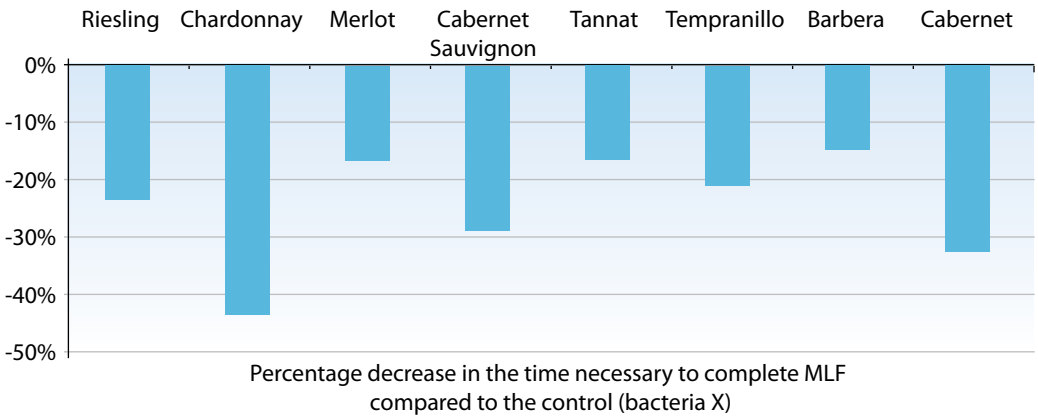


The characterization of this strain at the laboratory level allowed us to see the performance of the O-MEGA™ wine bacteria in a wide range of conditions. Trials were conducted in wineries by Lallemand and its partners on white, rosé and red wines in varied and limiting conditions.

Figure 6 presents different trials and shows the percentage decrease in the time required to complete MLF compared to a control bacteria. The bacteria X (the control) utilized varies according to the trial, but was precisely selected for its robustness. In each case, O-MEGA™ presents faster kinetics, terminating MLF up to 20 days sooner.

TABLE 1 AND FIGURE 6. Results of some field trials in Germany, South Africa, France, Spain, Italy and the United States

	Riesling	Chardonnay	Merlot	Cabernet Sauvignon	Tannat	Tempranillo	Barbera	Cabernet
Country	Germany	South Africa	France	France	France	Spain	Italy	USA
Timing of inoculation	Co-inoculation	Co-inoculation	Post AF	Post AF	Post AF	Post AF	Co-inoculation	Co-inoculation
pH	**3.25**	**3.20**	**3.26**	3.31	3.67	3.53	3.00	3.60
Malic acid	**5.8 g/L**	2.4 g/L	2.0 g/L	2.9 g/L	3.5 g/L	**4.6 g/L**	**5.5 g/L**	**3.0 g/L**
Ethanol	12.1%	**14.4%**	12.3%	11.9%	14.9 %	**14.9%**	**14.8%**	13.5%
Total SO_2/ Free SO_2	15 / <5 mg/L	45 / 17 mg/L	30 / <5 mg/L	39 / <5 mg/L	**36 / 15 mg/L**	25 / <5 mg/L	33 / <5 mg/L	<20 / <8 mg/L
Temperature	17°C then 20°C	**15°C** then 18°C	**15°C**	**15°C**	20°C	17°C	25°C	23°C
Duration of MLF (days) (O-MEGA™)	25	18	39	48	19	37	11	4
Volatile acidity (O-MEGA™)	N/A	0.53 g/L	0.19 g/L	0.32 g/L	N/A	0.39 g/L	0.55 g/l	0.33 g/L



The diversity of these winery trials:

- Confirms the effectiveness of O-MEGA™ in limiting conditions, such as acid pH, high ethanol levels and low temperature
- Completes and defines the **robustness of the selection when initial malic acid concentrations are high** (over 5 g/L) and **on varietals reputed to be difficult for MLF** (e.g., Merlot/Tannat).

Another interesting characteristic of this wine bacteria lies in the weak degradation of citric acid, resulting in low VA and diacetyl (milky and buttery notes) production.

3.2 Low volatile acidity production associated with limited citric acid degradation

The production of the acetic acid measured after MLF is directly related to the metabolism of citric acid, as is the production of diacetyl. Through the action of citrate lyase, this pathway is initiated: the oxaloacetic acid produced is decarboxylated into pyruvate, from which different reactions begin (see figure 7).

- A small portion can lead, through reduction, to D-lactate, but it is weak as it needs nicotinamide adenine dinucleotide (NADH).
- Another pathway via ethanal-thiamine pyrophosphate (TPP) and acetyl-coenzyme A (acetyl-CoA) leads to the synthesis of fatty acids that are themselves used for the phospholipid membrane pathway. This is predominant in optimal growth conditions (when the pH and temperature are favourable, notably).
- Pyruvate is also used for the synthesis of acetoinic molecules, including diacetyl, the most oxidized of these molecules that can be successively reduced to acetoin then butanediol. In limiting growth conditions, this pathway is favoured because the formation of these molecules is considered to be a cell detoxification process that must eliminate excess pyruvate. It is involved in multiple intracellular pH-regulation mechanisms.
- For every citric acid molecule consumed, it is accepted that an average of at least 1.2 acetic acid molecules are formed. This is behind the increase in VA, which is produced during MLF. Citric acid degradation generally begins during MLF and continues after MLF; the other pathway for VA production is the metabolism of residual sugars, which depends on pH and occurs only after the end of MLF (Lonvaud et al. 2010).

FIGURE 7. Citric acid metabolism in *Oenococcus oeni* (Bartowsky 2004)

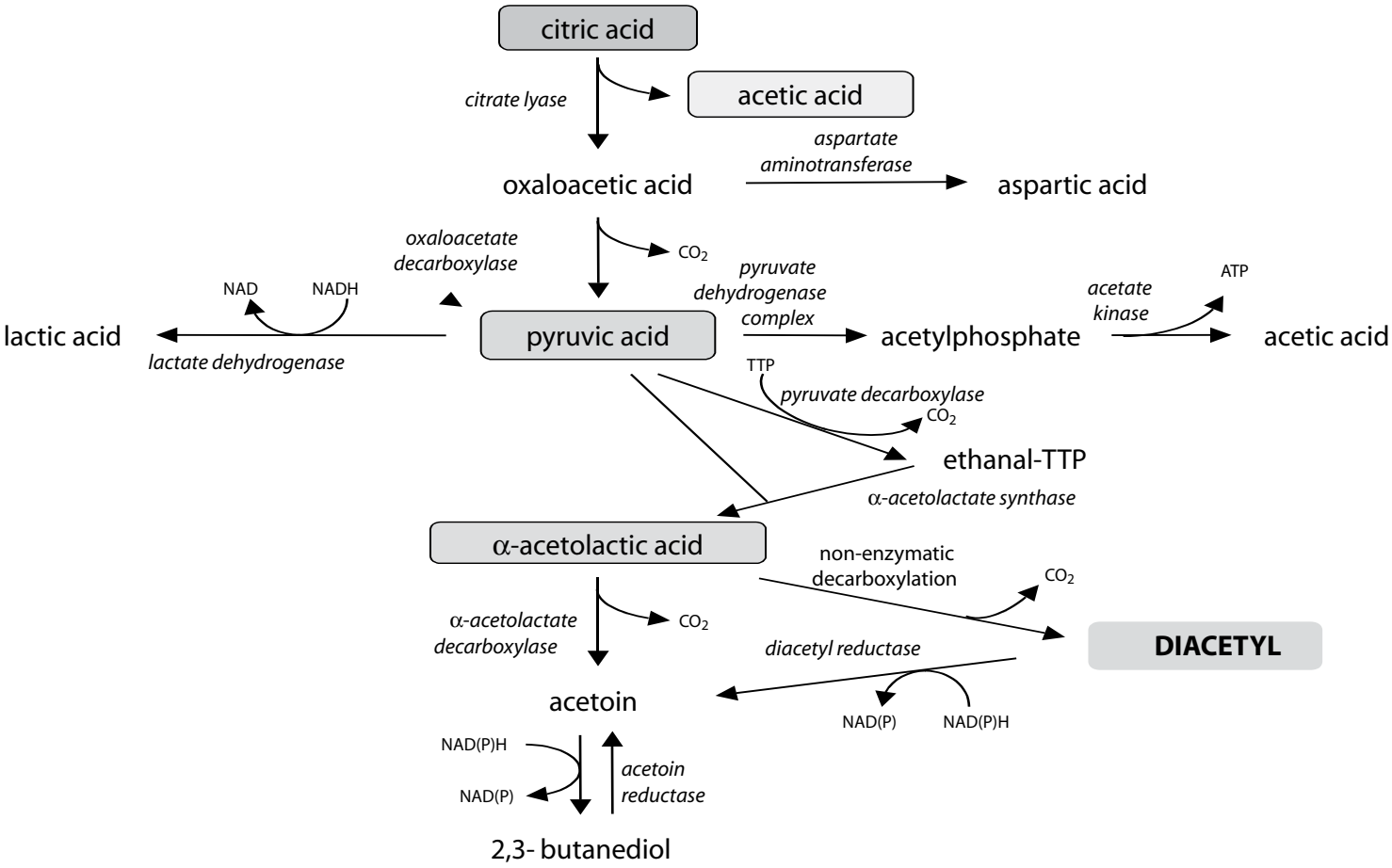
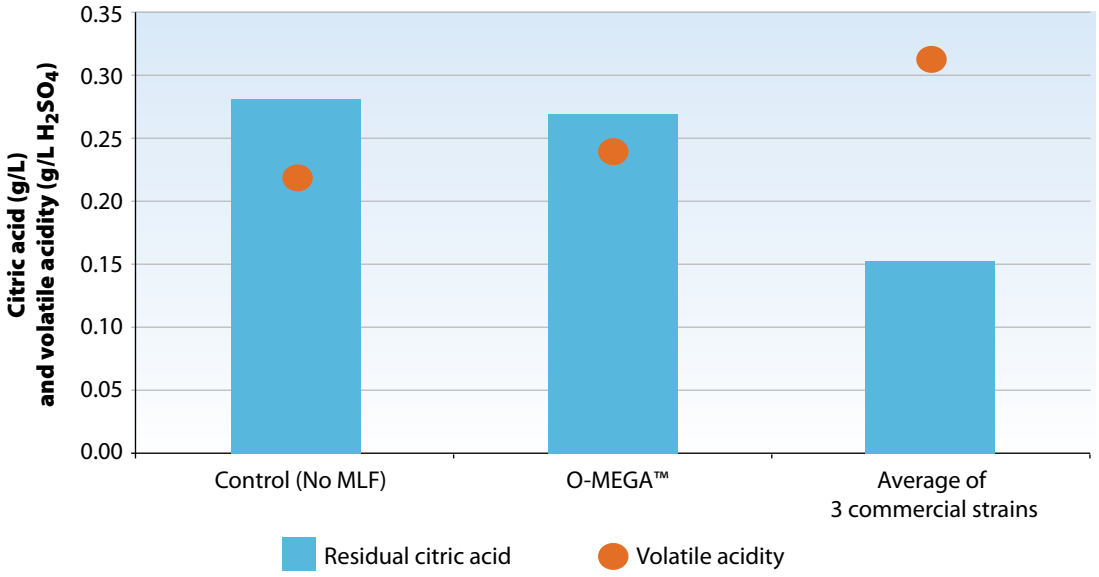


FIGURE 8. Citric acid metabolism of O-MEGA™ compared with 3 commercial strains in a Pinot Noir wine (initial level of citric acid 0.35 g/L; analysis before bottling)



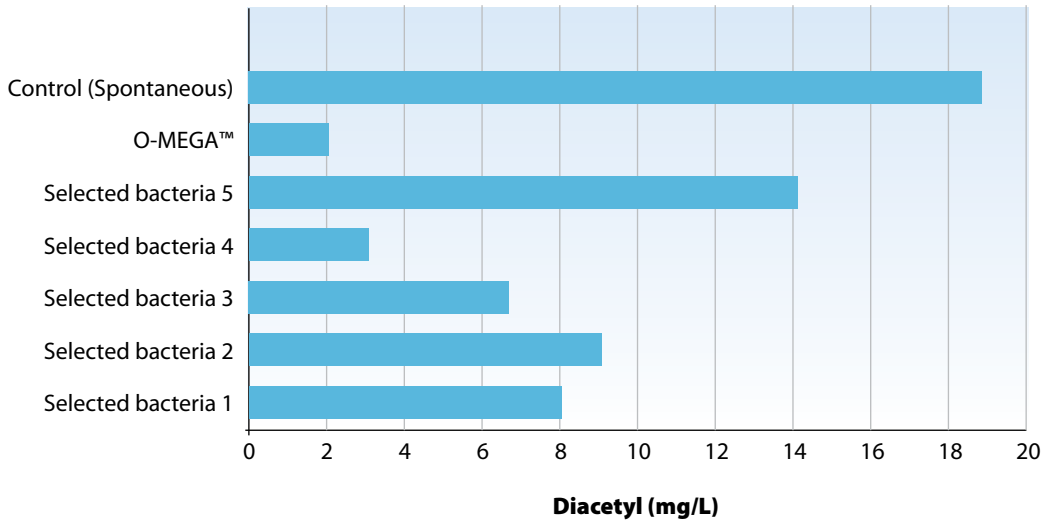
Usually, selected wine bacteria can consume more or less citric acid, more or less early. That can therefore impact the production and final quantity of diacetyl and acetic acid. A limited degradation of citric acid was part of the specifications for the new IFV / Lallemand selection. This oenological property was verified over the various trials.

As we can see in figure 8, the consumption of citric acid by O-MEGA™ is nearly zero, as the residual citric acid level was equivalent to when MLF was blocked. As for the control bacteria, they consumed nearly half of the citric acid present in the medium – the same behaviour as the other three strains studied (the average is shown on the graph). The consequence of this very low level of citric acid degradation is the very low level of acetic acid production, and therefore very low VA levels. Figure 8 shows that the wine that carried out MLF with O-MEGA™ has a VA level equivalent to the wine that did not carry out MLF, while the wines where the selected wine bacteria carried out MLF present a significantly higher level of VA.

The winery trials confirm this property in difficult conditions. As shown in figure 6a, the VA levels at the end of MLF in the wine inoculated with O-MEGA™ are low in all four trials. For the trials on Chardonnay and Barbera, the VA levels are higher, but remain within broadly acceptable levels for wines with ethanol levels above 14%, and lower than those obtained with the control strain (0.56 and 0.80 respectively). We do not have the analyses for two of the trials.

Because of the limited degradation of citric acid, this new selection is a low producer of both VA and diacetyl. In the trial carried out in Italy with the Barbera varietal (described in figure 6a), six selected bacteria strains were compared in terms of kinetics for malic acid degradation, and for diacetyl production. The results of the diacetyl concentrations at bottling are presented in figure 9.

Figure 9. Results of diacetyl analyses in a comparative trial with six different selected bacteria and a control (spontaneous) on Barbera. Italy, 2013



The choice of wine bacteria is therefore a key parameter for managing the diacetyl content in a wine. O-MEGA™ lets the winemaker avoid these buttery and milky notes.

The work on the characterization of O-MEGA™ also confirmed that this wine bacteria does not have the capacity to produce *p*-coumaric acid, a precursor for ethylphenols. Indeed, Burn and Osborne (2013) reported that certain strains of *O. oeni* have the capacity to degrade hydroxycinnamic acids and their tartaric esters, which increases the quantity of ethylphenol precursors in the wine and can lead to higher levels in the presence of *Brettanomyces bruxellensi*. It is therefore essential to select a new wine bacteria to ensure that the strain does not have the capacity to increase the level of these precursors and can thus be qualified as "phenol negative".

4. Conclusion

O-MEGA™ is a robust and versatile new wine bacteria, capable of carrying out malolactic fermentation in a short time in a very broad spectrum of actions. Numerous trials carried out in the laboratory and in the winery show its **remarkable capacity in a broad range of uses, including white, rosé and red wines:**

- pH ≥3.1
- Degree of alcohol <16% by volume, even 17% vol if the other factors are not limiting
- Total SO_2 ≤50 mg/L
- Temperature ≥14°C.

This new wine bacteria also appears to be resistant to polyphenols and has the capacity to degrade important levels of malic acid (6 g/L) like lower levels (1 g/L).

Easy-to-use O-MEGA™ is ready for **direct inoculation with no rehydration** (it is an MBR® wine bacteria) and implants with no difficulty whatever the time of inoculation: in co-inoculation, early inoculation or sequential inoculation. It also offers major sensory advantages because it degrades very little citric acid, producing **little volatile acidity and diacetyl.**

Furthermore, in regards to rosés and red wines O-MEGA™ has **little impact on wine colour**. The first trials have shown the new IFV selection would in fact limit the loss of colour in rosé wine often observed during MLF, and it preserved the colour in red wines more than other selected bacteria.

References

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Lonvaud-Funel, A., V. Renouf, and P. Strehiano. 2010. *Microbiologie du vin - Bases fondamentales et applications*. Tec et Doc, Lavoisier.