MLF co-inoculation – how it might help with white wine

Malolactic fermentation (MLF) is an important process in red winemaking and is also increasingly used in white and sparkling wine production. It is a biological process characterised by the conversion of L-malic acid to L-lactic acid and carbon dioxide, and is predominantly carried out by *Oenococcus oeni*, one of several lactic acid bacteria (LAB) species which can survive and grow in the harsh wine environment. MLF can be used in a range of white wine varieties and styles, with its overall desirability relating to reduction in wine acidity and modifications in sensory properties (e.g. butteriness, complexity and mouth-feel). For example, when fruit is sourced from cool regions, MLF can reduce wine acidity and improve balance. However, MLF is less commonly conducted in whites from hot areas where it can cause the wine to lose freshness. A feature of MLF which can be used in white winemaking is the regulation of buttery aroma and flavour, which comes from diacetyl formed during citric acid metabolism (AWRI publication #795).

Despite the potential to use MLF to shape the sensory properties of white wines, its induction in white wines can often be more challenging than in red wines and MLF failures can occur. This is predominantly due to low wine pH (typically less than pH 3.3), and the presence of sulfur dioxide (SO_2) from additions made in the vineyard (especially with machine-harvested fruit) or at the crusher. Other inhibitory factors associated with white wines include lower fermentation temperatures and lower availability of essential nutrients for the fastidious MLF bacteria. Over recent decades, improvements in MLF efficiency have been gained through use of selected bacterial starter cultures. However, conducting MLF in white wines can still be an obstacle for winemakers, and early inoculation can be a useful strategy to help overcome such difficulties.

Timing options for MLF inoculation

MLF inoculation can be undertaken at several stages during winemaking. Inoculation after the completion of alcoholic fermentation (known as sequential inoculation) is the most common option. More recently there has been a trend to inoculate earlier in the fermentation process, a practice that can greatly reduce the overall time for vinification (AWRI publication #1301) or assist with adapting the bacterial strain to particularly harsh wine conditions. Under this approach, known as co-inoculation, MLF bacteria are usually inoculated 24–48 hours after yeast addition, which helps to alleviate the effects of any free SO_2 arising from harvesting/crushing additions.

Previous work has shown that early inoculation for MLF can substantially reduce overall vinification in time red wine (AWRI publication #1376). This article presents results from

two recent MLF trials in Chardonnay to demonstrate how co-inoculation can be used in white winemaking to obtain a successful MLF.

Trial 1. Difficult grape must/wine composition – high SO_2 content and low pH

The first trial investigated differences between co-inoculation and sequential inoculation strategies. Winemaking was conducted in triplicate at pilot-scale (29 L stainless steel kegs) in Chardonnay juice (2012, Eden Valley; 12.2°Be, pH 3.25, TA 5.4 g/L, YAN 177 mg/L, malic acid 2.1 g/L, FSO_2/TSO_2 13/57 mg/L) at ~ 15°C using a commercial, compatible pair of *Saccharomyces cerevisiae* and *O. oeni* strain preparations. Yeast were rehydrated and inoculated at the recommended rate, and alcoholic fermentation (AF) was completed within two weeks. The presence of bacteria during AF did not hinder the rate of sugar metabolism (Figure 1, top panel). The *O. oeni* strain was prepared and inoculated at the recommended rate, either 24 hours post yeast inoculation (co-inoculation) or Day 16 (sequential MLF). Bacteria viability was monitored throughout the trial (Figure 1, bottom panel).

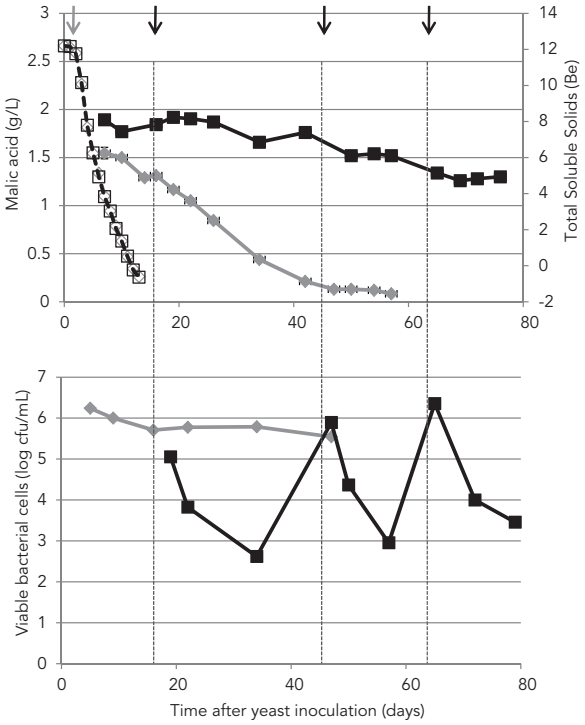

Figure 1. Malic acid metabolism (closed symbols) and alcoholic fermentation progress (open symbols) (upper graph) and bacteria viability (lower graph) after co-inoculation (gray) or sequential inoculation (black) of *O. oeni* into Chardonnay juice/wine. Bacteria were inoculated 24 hours after yeast for the co-inoculation MLF. Sequential MLF was inoculated on Day 16 and then re-inoculated on Days 44 and 64 (arrows indicate time point of inoculation). Each point is the average of triplicate measurements.

In the co-inoculation treatment the bacterial population initially declined, most likely due to the difficult conditions of high SO_2 content coupled with the low pH of the grape juice. However, cell viability stabilised at 5.8×10^5 cfu/mL which was adequate to ensure a slow, yet steady reduction of malic acid. Under these conditions, MLF required about two months to complete.

In contrast, in the sequential inoculation treatment the *O. oeni* population was more severely impacted by the wine conditions, with bacterial cell viability dropping significantly (from 2×10^6 to 6×10^2 cfu/mL) within the first week, resulting in minimal reduction in malic acid concentration. The wine was then reinoculated at Day 44; a similar decline of viable cell population was observed over the following two weeks (from 10^6 to 10^3 cfu/mL) with only 0.5 g/L of malic acid metabolised. Bacteria were inoculated for a third time (Day 64) with another similar population decline observed within two weeks. Sequential MLF inoculation of this Chardonnay wine, after almost 80 days of vinification, had only reduced the malic acid concentration by 35%.

Thus, under harsh white wine conditions, co-inoculation enabled bacteria to retain sufficient viability to successfully complete MLF. It is likely that the low initial alcohol content in the fermenting juice at the time of inoculation was a key to the bacteria being able to survive and adapt to the high SO_2 content and low pH of the Chardonnay juice, and ultimately completely metabolise the malic acid.

Trial 2. The importance of yeast and bacteria combination for successful MLF

In addition to the four key wine parameters that affect MLF (pH, alcohol, SO_2 and temperature), compatibility between yeast and bacterial strains is another significant consideration (AWRI publication #773). Furthermore, such yeast-bacteria interactions can be an important factor influencing the effectiveness of different timings of bacterial inoculation. In this second study, the effects of different timings of bacterial inoculation under stressful white wine conditions (moderate SO_2 content and low pH) were examined using combinations of two different yeast strains and five *O. oeni* strains. Analytical results for the initial Chardonnay juice (2011, Barossa Valley) and subsequent wines are shown in Table 1. This trial was conducted at 5-litre scale in an 18°C temperature controlled room.

Two commercial yeast strains (Y1 and Y2) were separately inoculated at the recommended rate into the Chardonnay juice. Five *O. oeni* strains (ML1 – ML5) were pre-cultured and inoculated either 48 hours after each yeast or at the end of alcoholic fermentation at a rate of 2×10^6 cells/mL. The MLFs were conducted in triplicate. Progress of the MLFs is shown in Figure 2 and bacterial cell viability over the course of the fermentations is shown in Figure 3.

Alcoholic fermentation with *S. cerevisiae* Y1 took approximately three weeks to complete (< 2 g/L G+F), whereas alcohol fermentation with *S. cerevisiae* Y2 was slower and retained some residual sugar (7.2 g/L).

Table 1. Analysis of Chardonnay (2011) juice and wine (no MLF) and time to complete alcoholic and malolactic fermentation with a co-inoculation or sequential strategy.

			Wine	
		Juice	**Y1**	**Y2**
Brix / Alcohol	° / %	21.6	12.6	12.5
Glu + Fru	g/L		1.1	7.2
pH		3.26	3.4	3.38
TA pH 7.0	g/L	5.2	4.9	5.3
TA pH 8.2	g/L	5.4	5.1	5.6
YAN	mg/L	272	93	85
SO_2 (free)	mg/L	12	< 4	< 4
SO_2 (total)	mg/L	43	50	51
L-malic acid	g/L	3	2.79	2.87
Total time for AF+MLF				
Co-inoculation			4 weeks	3-8 weeks
Sequential			6 weeks	No MLF

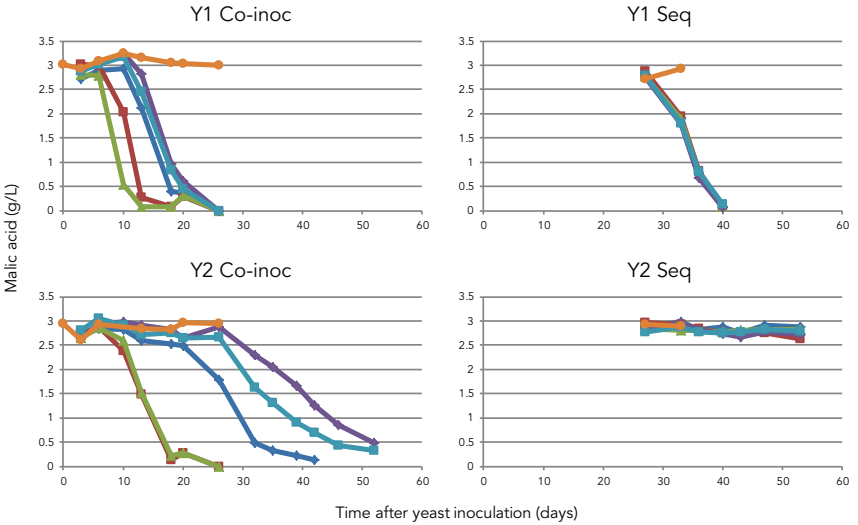

Figure 2. Malolactic fermentation in Chardonnay juice/wine (2011) inoculated with five different *O. oeni* strains (ML1 [blue], ML2 [red], ML3 [green], ML4 [purple], ML5 [aqua], no MLF [orange]) either as co-inoculation (co-inoc) or sequential (seq) with two different commercial *S. cerevisiae* yeast strains (Y1 and Y2). Each point is the average of triplicate measurements

The results of the co-inoculation treatment revealed that the bacteria strains were able to complete MLF in wines fermented by both yeast strains (Figure 2). Two *O. oeni* strains (ML2 and ML3) completed fast MLFs in both Y1 and Y2 wines (within 2–3 weeks) and were not affected by *S. cerevisiae* strain. The other three *O. oeni* strains exhibited slower malic acid metabolism, a reflection of a decline in cell viability in the first two weeks with *S. cerevisiae* Y1 wines and three weeks in *S. cerevisiae* Y2 wines (Figure 3).

The sequential MLF treatments clearly highlighted the importance of yeast strain choice. Wine fermented with *S. cerevisiae* Y1 was compatible/friendly towards each of the malolactic bacteria, with MLF completed by all five strains within two weeks of bacterial inoculation (40 days after yeast inoculation) (Figure 2). However, wine fermented by *S. cerevisiae* Y2 revealed a different story. From the co-inoculation results, it was evident that MLFs conducted by several *O. oeni* strains (ML1, ML4 and ML5) were significantly slower in wines after co-inoculation with *S. cerevisiae* Y2 (> 40 days after yeast inoculation), while MLFs with *O. oeni* strains ML2 and ML3 were only slightly slower (18 days after yeast inoculation). Most importantly, however, it was found that sequential inoculation with wine prepared from *S. cerevisiae* Y2 did not support MLF activity with any of the five *O. oeni* strains over the four weeks of the trial. By tracking the cell viability of the bacteria after inoculation into the Chardonnay wine fermented by *S. cerevisiae* Y2, it is clear the wine was inhibitory towards the bacterial population as it declined quickly (Figure 3).

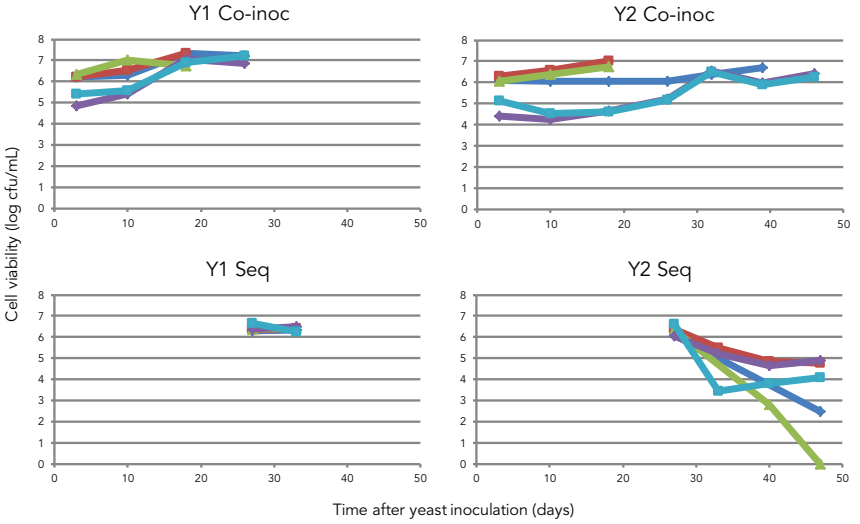

Figure 3. Cell viability in Chardonnay juice/wine (2011) inoculated with five different *O. oeni* strains (ML1 [blue], ML2 [red], ML3 [green], ML4 [purple], ML5 [aqua] either as co-inoculation (co-inoc) or sequential (seq) with two different commercial *S. cerevisiae* yeast strains (Y1 and Y2). Each point is the average of triplicate measurements.

From this trial it appears that *S. cerevisiae* Y2 produced inhibitory components which, coupled with the stressful conditions of relatively high SO_2 content and low pH, rendered the wine hostile towards the malolactic bacteria following sequential inoculation. Further, in this case, co-inoculation was the only method by which MLF could be induced. In contrast, wines fermented with *S. cerevisiae* Y1 were conducive to MLF with either inoculation strategy.

Summary

Co-inoculation is a winemaking strategy that may be used to enable malolactic bacteria to effectively acclimatise to more 'difficult' wine MLF parameters, such as those occurring in white juice. These Chardonnay trials also highlighted the importance of selecting yeast strains that are compatible for wines destined for MLF induction. Further experimentation is needed to investigate and better understand potential problematic MLF induction associated with wines experiencing sluggish/ stuck AF ferments (as observed in Chardonnay wine with yeast strain Y2, trial 2).

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For further information on co-inoculation and sequential MLF in red and white wines contact Eveline Bartowsky.

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