Evaluation of Malolactic Fermentation Cultures for Sparkling Wines

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In the production of sparkling wines in Australia many winemakers choose to have some or all of their base wines undergo a malolactic fermentation (MLF). The decision whether or not to have MLF is based on the style of wine to be produced and the characteristics of the base wine. These characteristics can be related to variety, region, climate, season, etc., and include both flavour and acidity.

During MLF lactic acid bacteria convert malic acid to lactic acid, resulting in a reduction in total acidity and an associated rise in pH. Many other chemical changes occur, some components being removed while others are added, resulting in flavour modification.

Fruit for sparkling wine base is often harvested early, particularly in warmer climates, to reduce the excessively varietal and forward flavours that develop as the fruit ripens. These characteristics are usually not desired in sparkling wine bases. However, the result is often very acidic base wine with a potentially narrow flavour profile. MLF can be very advantageous to these wines, softening the palate by reducing the acidity. Palate hardness is also reduced by the replacement of malic acid by less aggressive lactic acid.

Although high malic acid and a low pH help acid life and freshness to sparkling wine, some winemakers may wish to make a wine which has less sharpness and varietal character and more complexity. The flavour modifications brought about by MLF may result in some loss of freshness, but are said to contribute depth, richness, complexity, roundness and often a creamy texture.

It has been suggested that the rise in pH resulting from MLF may also lead to a faster rate of yeast autolysis during ageing, resulting in added complexity earlier.

The use of MLF may offer an advantage during the settling of tirage wine.

It is possible that lactic acid bacteria will grow spontaneously in a non-MLF base wine during ageing on lees. These organisms are difficult, if not impossible, to remove by remuage. If this problem is to be avoided base wines and yeast cultures must be entirely free from lactic acid bacteria prior to tirage, something which may not always be easy to achieve.

Base wines which have completed MLF have the advantage of being less likely to support the growth of endemic lactic acid bacteria during ageing.

Having decided that MLF is desirable for sparkling wine production, the winemaker must ensure that it happens with a high degree of control. Spontaneous MLF can be quite unpredictable, particularly in the pH-limiting conditions of many sparkling wine bases. Many winemakers are choosing to inoculate their wines with selected cultures to ensure that the desired fermentation outcome is achieved and to minimize the chance of spoilage.

Starter cultures are used to encourage rapid onset and completion of MLF and achieve a dominance over indigenous lactic acid bacteria.

Unlike the commonplace use of dried yeast, by both large and small wineries, advances in the production and use of dried malolactic cultures have been much slower. Starter cultures are often maintained on agar slopes and propagated by complex procedures. A great deal of technical input and fine control is required to produce and maintain suitably active cultures. Commercially prepared malolactic cultures which are reliable and easy to use have been sought for some time. Such cultures are particularly attractive to the small winemaker, who has no propagation equipment or expertise and requires relatively small quantities of organisms.

The organisms used in malolactic starter cultures must be of a suitable strain to rapidly and completely carry out MLF in wine conditions and to result in desirable flavour modification. In addition, the culture must be prepared in such a manner as to acclimatize the organisms, and an adequate number of actively growing organisms must be inoculated into the wine.

The desirable attributes of a commercial starter culture would be ease of storage, ease of preparation and use, and reliability.

In the last 18 months, two products have been released onto the Australian market.

The first is Bitec Vino, produced by Condimenta in Germany and distributed in Australia by Monbat Pty Ltd. The preparation consists of two strains of Leuconostoc oenos selected for their ability to survive in wine and their rapid malolactic activity. More recently, Hansen’s Laboratories in Denmark have produced Viniflora Oenos, a pure culture of malolactic bacteria selected for efficiency and sensory properties.

At Yalumba winery some trials were carried out on 1993 vintage wines to evaluate these preparations and to compare their malolactic ability with an in-house culture.

Preparation of cultures

Bitec Vino is not difficult to prepare for inoculation into wine. A single reactivation mixture is prepared using wine, juice, water and a sachet of reactivation medium which is provided with the culture. pH adjustment is required. The bacteria are rehydrated in warm water and added to the medium. After 2-4 days at 22°C the culture should be ready to inoculate into the wine. A 10-15g sachet is sufficient for 1,000-7,000 litres of wine, and a 100 g sachet for 10,000-70,000 litres of wine. The only equipment required is a facility to heat the juice and wine for pasteurization purposes, some means of keeping the culture warm, and access to a pH meter. Most wineries would be able to prepare the culture with minimal difficulty.

Viniflora Oenos requires no preparation at all. The dried organism is added directly to the wine and mixed well to disperse them.

The manufacturer of Bitec Vino has also issued a protocol for direct inoculation using their organisms. The culture requires only a rehydration step but the seeding rate is increased at least ten-fold. This method was not used for the trial: Bitec Vino was reactivated as previously described.

The Yalumba culture is prepared along more traditional lines. It is a mixture of three strains of lactic acid bacteria selected for their desirable malolactic characteristics. The organisms...
are maintained separately in a freeze-dried state in small glass ampoules. They are propagated through a series of laboratory and cellar stages beginning with a nutrient-rich medium. Successive stages have increasing alcohol concentration and decreasing pH. The time taken to build up to a suitably active stage is several weeks. Each year a fresh batch of freeze-dried ampoules must be prepared to maintain stocks of the organisms.

**Storage of cultures**

Storage conditions for the commercially dried cultures are extremely important. As the storage temperature increases the viability of the organisms can be severely affected.

The makers of *Viniflora Oenos* state that unopened packets will maintain full viability for 18 months if stored at -18°C (normal household freezer); 5°C is suitable for shorter term storage. Exposure to temperatures above 20°C and any humidity will markedly reduce the viability of the product. They stress that packets should be removed from cold storage only just before inoculation and opened immediately before use (i.e., while standing by the tank). Opened packets should not be stored for later use.

*Bitec Vino* can be stored for up to six weeks at 2–4°C. Longer term storage should be in a freezer below -7°C. The manufacturer does not seem to be as cautious about the use of previously opened packets, but warns that they must be very carefully resealed to exclude air and moisture and used within a short time.

**Inoculation conditions**

The manufacturers of both products recommend inoculation at the end of primary yeast fermentation. The *Yalumba culture* has been successfully inoculated late in primary fermentation, near the end or once it has finished. For this trial, all cultures were inoculated at the same time (i.e., at the end of yeast fermentation).

It is well known that malolactic bacteria are inhibited by low pH, high SO₂, high alcohol and low temperatures. Both manufacturers state these limiting conditions quite clearly and suggest that culture activity may be reduced if inoculated under unfavourable circumstances. Table 1 shows limiting conditions as stated by the manufacturers.

For *Bitec Vino* two sets of conditions have been stated. Ideal conditions are those which are considered to be the most suitable for conducting a successful MLF with this culture. Limiting conditions have also been listed, under which MLF may be difficult to induce. However, the reactions may occur until 6 days later. Even though a measurable increase in cell numbers did not occur until 20 days, malolactic activity began at about day 20 and lactic acid concentrations began to increase about 10 days, and malolactic activity began at about day 20 when the cell count had risen to about 10⁵ cfu/mL. The Bitec Vino organisms showed a slow increase in numbers beginning after inoculation. However, the conditions in these two cultures are more severely affected than *Bitec Vino*. For these two cultures a slow increase in numbers began after about 10 days, and malolactic activity began at about day 20 when the cell count had risen to about 10⁵ cfu/mL. The *Bitec Vino* organisms showed a slow increase in numbers beginning after about 10 days, and malolactic activity began at about day 20 when the cell count had risen to about 10⁵ cfu/mL. The *Yalumba culture* organisms were more severely affected than Bitec Vino. For these two cultures a slow increase in numbers began after about 10 days, and malolactic activity began at about day 20 when the cell count had risen to about 10⁵ cfu/mL. The *Bitec Vino* organisms showed a slow increase in numbers beginning after about 10 days, and malolactic activity began at about day 20 when the cell count had risen to about 10⁵ cfu/mL.

**Table 1. Conditions for use of commercial malolactic preparations**

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<thead>
<tr>
<th></th>
<th>Bitec Vino</th>
<th>Viniflora Oenos</th>
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<tbody>
<tr>
<td><strong>Temperature °C</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 15</td>
<td>&gt; 18</td>
<td>17-25</td>
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<tr>
<td><strong>pH</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 3.1</td>
<td>&gt; 3.2</td>
<td>&gt; 3.1</td>
</tr>
<tr>
<td><strong>Total SO₂ mg/L</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 30</td>
<td>0</td>
<td>&lt; 30</td>
</tr>
<tr>
<td><strong>Alcohol % (v/v)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 13.5</td>
<td>&lt; 12.5</td>
<td>&lt; 14</td>
</tr>
<tr>
<td><strong>Inoculation level (cfu/mL)</strong></td>
<td>7 x 10⁶</td>
<td>1 x 10⁶</td>
</tr>
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</table>

The wine used for the trial was a sparkling wine made from Pinot Noir grapes and fermented to dryness prior to inoculation for MLF.

A analysis prior to MLF was for TS, FSO₂, TSO₂, SO₂, TA 9.7 g/L, pH 3.19; alcohol 12% v/v; malic acid 4.2 g/L; lactic acid 0.3 g/L; acetic acid 0.5 g/L. MLF was carried out in steam-sterilized 300 litre barrels. Five-year-old oak was chosen to minimize oak influence on the flavour of the wine. There were three replicates for each organism and three control barrels. The barrels were kept in a warm room and the temperature range during MLF was 16–21°C.

The wines were sampled every second day for the first 3–4 weeks and then twice each week until completion. These samples were analysed for pH, lactic, acetic and acetic acids, and bacterial cell count.

pH was measured using a Mettler pH meter/titrator (Model No. DL40GP1). Organic acids were measured by HPLC. Bacterial populations were enumerated by plating serial dilutions of wine onto MRS agar (Oxoid) with 20% apple juice and 50 mg/L cycloheximide. The plates were incubated at 25°C in a CO₂-enriched atmosphere for 5–7 days.

At the end of MLF the wines underwent sensory evaluation by winemakers to assess for any changes in flavour and/or style.

**Results and discussion**

The results of the analysis for pH, cell count and lactic and acetic acids are presented graphically in Figures 1 to 4.

The results of malolactic acid analyses shown in Figures 1 and 2, respectively, indicate that MLF has occurred over a similar time frame for the three inoculated treatments. There was a lag time of around 20 days before any malolactic activity was evident. MLF was complete at day 39 in all three cases. There was no evidence of malolactic activity in the control wines. The lactic acid levels attained after MLF were similar for all the inoculated wines.

The results of pH measurements (Figure 3) show that similar pH changes during MLF were obtained for the three inoculated treatments. Note that the control wines exhibited very little change in pH over the same time period.

All three organisms showed significant dieback soon after inoculation (Figure 4). The *Viniflora Oenos* and *Yalumba cultures* were more severely affected than *Bitec Vino*. For these two cultures a slow increase in numbers began after about 10 days, and malolactic activity began at about day 20 when the cell count had risen to about 10⁵ cfu/mL. The *Bitec Vino* organisms showed a slow increase in numbers beginning after about 10 days, and malolactic activity began at about day 20 when the cell count had risen to about 10⁵ cfu/mL.

The manufacturers of these products state that their organisms are capable of bringing about MLF much quicker than occurred in this trial and without such large decreases in cell populations after inoculation. However, the conditions in the wine chosen for this trial were outside the limiting conditions stated by both manufacturers. The pH was at or below the lower limit, and the TSO₂ was above the upper limit. While the alcohol at 12% v/v was inside the upper limit, it could be considered to be relatively high, and in combination with the low pH and high TSO₂, may have contributed to an overall hostile environment. It is thought that these factors would have been the probable cause of the lag time in cell growth and malolactic activity.

As an aside, similar trials have been carried out on a red wine and a white table wine. The red wine had a pH of 3.68 and a
T<sub>SO<sub>2</sub></sub> of 20 mg/L. All three bacterial cultures completed MLF in 22 days at a temperature of 13–15°C. In the white table wine however, the T<sub>SO<sub>2</sub></sub> was 50 mg/L (similar to the sparkling wine in this trial) and MLF was delayed by several weeks.

From observations of our in-house culture under a variety of conditions we have seen that it too is sensitive to limiting conditions as was demonstrated here, and it is not unusual for MLF to be delayed under these circumstances.

Analysis for acetic acid after MLF showed that this compound was not produced by any of the organisms. Tasting analysis showed that there were quite apparent sensory differences between the wines. All tasters noted the presence of malolactic characters in all of the treatments except for the control wines, but differences were observed in intensity and aroma profiles.

The Bitec Vino organisms gave less intense malolactic characters, allowing the fruit flavours to remain evident and the wine to appear finer in structure. Some subtle buttery, malt and creamy fragrant characters were noted. The MLF reactions produced by the Viniflora Oenos and Yalumba organisms either masked or modified most of the fruit flavours in the wine. The flavours produced by the Yalumba organisms were described as quite complex buttery, beefy, creamy and cheesy. Flavours produced by the Viniflora Oenos organism also added complexity and were described as nutty, buttery and cheesy but also had some slightly leesy, aldehydic and stale characters.

The Yalumba culture was thought to give MLF characters of greater intensity.

For the particular house style of this wine, the types of

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**Legend for Figures 1 to 4**

- Yalumba
- Viniflora
- Bitec Vino
- Control

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**Figure 1. Results of analysis for malic acid. Mean of three repetitions for each treatment.**

**Figure 2. Results of analysis for lactic acid. Mean of three repetitions for each treatment.**

**Figure 3. Results of analysis for pH. Mean of three repetitions for each treatment.**

**Figure 4. Results of analysis for bacterial cell count. Mean of three repetitions for each treatment.**
flavours given by the Yalumba culture were preferred. The more neutral flavours produced by the Bitec Vino organisms were thought to be more suitable for a fruit-driven style of wine. While the tasters described some similar flavours in the Viniflora Oenos and Yalumba cultures, the Yalumba culture was preferred because of its intensity and cleaner flavours.

**Cost of malolactic starter preparations**

Table 2 shows some cost calculations associated with the use of these starter cultures. When considering the cost of commercially prepared cultures it is important to take into account convenience and the saving in time and equipment. The cost of using these cultures can be more expensive on a per-litre basis than an in-house culture. On the other hand, it is necessary to take into account the advantages associated with the direct access to equipment and technical skills already in place. The costs calculated for the in-house culture account mainly for time and consumables.

The commercially prepared cultures vary in their costs depending on the inoculation rates used, and this will be related to the conditions of the wine. As the conditions become more limiting, the amount of culture required to initiate MLF increases, thereby increasing the cost per litre. Although the makers of Viniflora Oenos do not suggest adding more culture as the conditions become more difficult, this would probably apply. With Bitec Vino there is an additional cost in time and materials to prepare the culture. However, as mentioned earlier this is easily carried out with minimal equipment requirements.

If direct inoculation is required, a much larger quantity of Bitec Vino must be used making Viniflora Oenos a considerably less expensive option.

**Conclusions**

When deciding on which culture to choose, the decision will be based on several factors, such as performance, cost, convenience, equipment and technical expertise available and flavour modifications brought about by the MLF. The preparation procedures for cultures must take account of the wine conditions. Many sparkling wine bases would have conditions similar to the wine used in this study. Note that although very little sulfur dioxide was added during processing it seems that a significant amount had been produced by the yeasts during primary fermentation. This occurrence is not uncommon, and combined with the effect of low pH makes it difficult to induce MLF in sparkling wine bases. Such wines would often have a pH value as low as 3.0–3.2, and although this makes MLF difficult to initiate, it is often for this reason that MLF is required. Once initiated, all cultures effected complete malic acid degradation in an acceptable time frame.

The use of prepared cultures is more convenient and requires less time, equipment and technical skill than using in-house propagated cultures. When it comes to cost, Bitec Vino may be cheaper, depending on the quantity of culture required to overcome limiting conditions, and if equipment and time are available for its preparation. If a direct inoculation is required, Viniflora Oenos is less expensive.

The choice of which preparation to use for flavour contribution will be influenced by the wine style aimed at. There is a place for malolactic characters which modify the wine flavour and make a significant contribution to the style and there is also a place for more neutral, delicate flavours allowing for acid reduction to occur without obvious flavour changes. Bitec Vino has in fact been used in Germany for acid reduction in a Riesling without significantly altering the flavours of the wine.

Overall, these commercial preparations are very welcomed, especially for many small winemakers. In this trial the commercially available cultures were shown to be effective and convenient. Further developments and a wider choice of strains can only improve their adaptability to limiting conditions and the flavours they produce.

**Acknowledgements**

I would like to acknowledge the contributions of the following towards this paper:

- Chr. Hansen's Laboratory
- Condimenta
- Monbat Pty Ltd
- S. Smith & Son Pty Ltd.

**Table 2. Costs involved with the use of malolactic starter cultures**

**Bitec Vino**

1. Prepared culture
   - $38 to inoculate 1,000–7,000 litres (3.8–0.5 cents/L)
   - $292 to inoculate 10,000–70,000 litres (2.5–0.4 cents/L)
   - Plus preparation time (approximately 2 hours), equipment and medium.

2. Direct inoculation
   - $38 to inoculate 100–230 litres (38–16 cents/L)

**Viniflora Oenos**

- $65 to inoculate 2,500 litres of wine (2.6 cents/L)

**Yalumba culture**

- Including the costs of maintaining and storing the culture and propagating it through laboratory and cellar stages, the approximate cost is less than 1 cent/L.

<table>
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