Mixed bacterial starter culture launched

In the following article, Anchor Yeast introduces the development of a mixed malolactic fermentation starter culture of *Oenococcus oeni* and *Lactobacillus plantarum,* and the benefits of its use in co-inoculation with Anchor *Saccharomyces cerevisiae* NT 202.

E. Lerm, L. Malandra, P. Pellerin and M. du Toit

MALOLACTIC FERMENTATION IS a secondary fermentation that usually follows the completion of alcoholic fermentation (AF) by the yeast culture and can be defined as the conversion of L-malic acid to L-lactic acid and CO_2.

The reaction takes place as a result of the metabolic activity of lactic acid bacteria (LAB), of which wine-related species usually belong to the genera of *Oenococcus*, *Pediococcus*, *Lactobacillus* and *Leuconostoc*. This fermentation holds several benefits: a decrease in overall acidity and a moderate increase in pH, increased microbial stability and flavour modifications to the final product.

Similar to active dried yeast cultures, there are commercial MLF starter cultures available for inoculating wine. These starter cultures mainly consist of *Oenococcus oeni* as the primary bacteria culture, although recent research focus has shifted towards the use and development of *Lactobacillus* species in these commercial starter cultures. There are two main inoculation strategies for these cultures, both with implications for successful MLF and aroma modifications of the final commercial product:

- sequential inoculation entails inoculation for MLF after the completion of AF
- co-inoculation refers to the addition of the MLF starter culture during the initial 24 hours after the addition of the selected yeast.

There are various factors – including the choice of bacteria species and strain, when to inoculate and possible flavour modifications – to take into consideration when selecting a starter culture for MLF. The NT 202 Co-Inoculant starter culture for MLF from Anchor Yeast was developed in order to address all of these factors in one efficient package to ensure a simple, secure and speedy fermentation.

The research, characterisation and development of this commercial mixed MLF starter culture took place from 2008 to 2010 at the Institute for Wine Biotechnology, Stellenbosch University in South Africa. There are various novel concepts involved:

- the use of a starter culture containing a mixture (the first commercial product of its kind) of two LAB species (*O. oeni* S5 and *Lactobacillus plantarum* 56)
- a culture specifically developed for co-inoculation and a starter culture being promoted in combination with a specific yeast culture, *Saccharomyces cerevisiae* NT 202 (Anchor Wine Yeast).

Over the course of the research and development (procedure shown in Figure 1), this starter culture was investigated for its compatibility with various commercially available yeast cultures, malic acid degradation capability, volatile acidity production, as well as into its aroma contribution to the overall wine aroma during MLF.

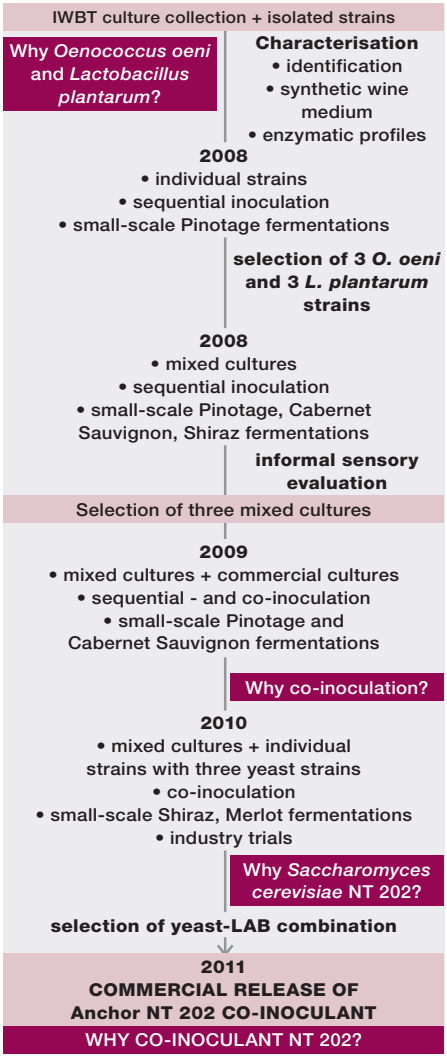

Figure 1. Developmental procedures

In addition, the strains were also selected for their inability to contribute to the biogenic amine content of the wine. This product was commercially launched in the 2011 harvest season as a MLF starter culture for co-inoculation with *S. cerevisiae* NT 202 (Anchor Wine Yeast).

Table 1. The differences in the enzymatic profiles of the two LAB strains in the mixed Anchor NT 202 Co-Inoculant starter culture (Lerm *et al*. 2011)

Enzyme	*L. plantarum 56*	*O. oeni S5*	Significance
β-D-glucosidase	+	-	Release of glycosidically bound aroma compounds
Phenolic acid decarboxylase	+	-	Metabolism of phenolic acids
Proline iminopeptidase	+	-	Release of free amino acids as aroma precursors
Citrate lyase α-subunit	+	-	Diacetyl production
Arginine deiminase	-	+	Ethyl carbamate production

Why a mixed culture of *Oenococcus oeni* and *Lactobacillus plantarum*?

There is a reason why *O. oeni* is the primary LAB species selected for use in MLF starter cultures. It has the best ability to adapt to the harsh environment created by the wine matrix, which includes the presence of ethanol, sulfur dioxide and low pH (Lonvaud-Funel 1999, Lerm *et al.* 2010). As mentioned, recent research has shifted to other species that could also be implemented for MLF inoculation.

Of the *Lactobacillus* species, *L.plantarum* has emerged as the most obvious choice. Not only is it one of the dominant *Lactobacillus* species found in the grape must/wine environment, it can also survive the challenging wine conditions (Du Plessis *et al.* 2004, Du Toit *et al.* 2010). An added benefit, supported by findings in our study, is the more complex enzymatic profile (with the emphasis on aroma-contributing enzymes) of *L. plantarum* strains compared with that of *O.oeni*. The enzymes for which differences exist between the two LAB strains present in the NT 202 Co-Inoculant, are listed in Table 1.

The presence of the β-D-glucosidase gene in *L. plantarum*, indicated with a (+), suggests this strain has the ability to cleave the bond between the grape-derived non-aromatic compound and a sugar molecule (usually glucose), producing a volatile aroma compound that can contribute to the wine aroma (D'Incecco *et al.* 2004).

During the course of developing this product, it was found that the mixed cultures – all consisting of an *O. oeni* and a *L. plantarum* strain – were able to successfully complete MLF without any significant increase in volatile acidity, resulting in distinctly different and favourable aroma profiles.

The focus of developing a mixed culture to inoculate for MLF was to create a product that would not only degrade malic acid, but could also add dimension and depth to the wine aroma profile.

Why use the mixed culture for co-inoculation?

There are various advantages and disadvantages associated with both sequential and co-inoculation strategies. Sequential inoculation results in less risk of adverse interaction between the yeast and bacteria cultures compared with co-inoculation (Costello 2006). This, as well as other possible disadvantages associated with co-inoculation, can be overcome if the correct yeast and bacteria strain combination is selected by the winemaker (Alexandre *et al.* 2004). If you inoculate after the completion of AF, yeast autolysis favours LAB growth and concomitant MLF activity due to the release of vitamins, amino acids, polysaccharides and proteins (Henick-Kling 1993). On the other hand, co-inoculation has the advantage of a matrix that has not been depleted by yeast growth yet. In addition, the yeast has not had sufficient time to produce inhibitory compounds, including ethanol and medium-chain fatty acids, or sufficient time to deplete the nitrogen/nutrient content (which could result in loss in LAB viability) when co-inoculation is implemented (Larsen *et al.* 2003, Zapparoli *et al.* 2009). These are all possible risks that could be associated with sequential inoculation.

A possible advantage of sequential inoculation is the reduced acetic acid production associated with this strategy, but the strains in the mixed culture were selected for their insignificant contributions to volatile acidity.

In addition, none of the studies found a significant increase in volatile acidity during co-inoculation, when compared with sequential inoculation (Lerm *et al.* 2011).

There are additional advantages associated with co-inoculation. Besides allowing for more efficient MLF in 'challenging' (high ethanol, nutrient depleted) wine environments, co-inoculation reduces the overall fermentation time, which makes the wines immediately available for stabilisation with sulfur dioxide (SO_2), racking and fining (Jussier *et al.* 2006). During the development of the Anchor NT 202 Co-Inoculant, no interactions between the yeast or bacteria culture had a negative impact on the fermentation success of either of the microbiological cultures.

In addition, the inoculation strategy has a direct effect on the aroma profile of the final product. Co-inoculated wines tend to be better structured, more complex, less 'buttery' and more fruity (Jussier *et al.* 2006). Fermentation-derived esters are the compounds responsible for the fruity characters in young wines. We found that the Anchor NT 202 Co-inoculant starter culture produced significantly higher concentrations of total esters during co-inoculation, compared with sequential inoculation (Figure 2).

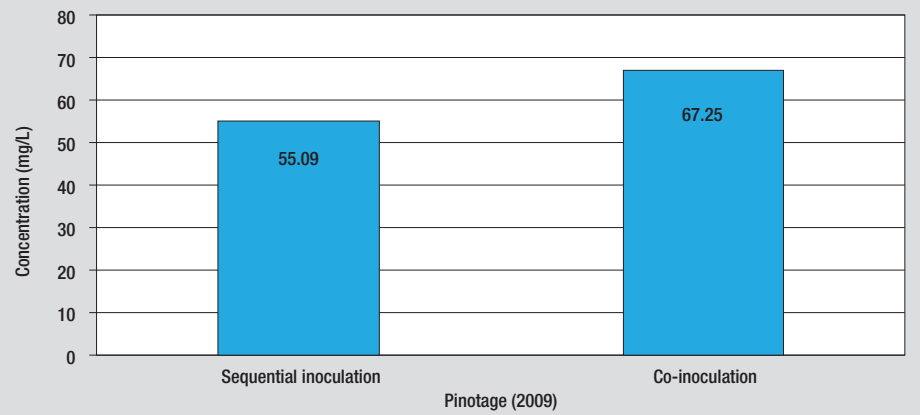

Figure 2. A comparison of the total ester (excluding ethyl acetate) production of the Ancor NT 202 Co-Inoculant during sequential – and co-inoculation.

The use of the co-inoculant with yeast strain Anchor NT 202 is the optimal yeast/bacteria combination for co-inoculation is a critical choice. In order to ensure compatibility between the yeast and bacteria strains, the winemaker needs to consider all the factors that could impact on the performance of each of these microbiological cultures. This selection has been simplified with the commercialisation of Anchor NT 202 Co-Inoculant. Both the yeast and bacteria cultures were selected to ensure there were no negative interactions that could have a detrimental effect on either AF or MLF.

The Anchor NT 202 Co-Inoculant was developed keeping the following selection criteria in mind: growth under standard winemaking conditions of pH, ethanol and sulfur dioxide

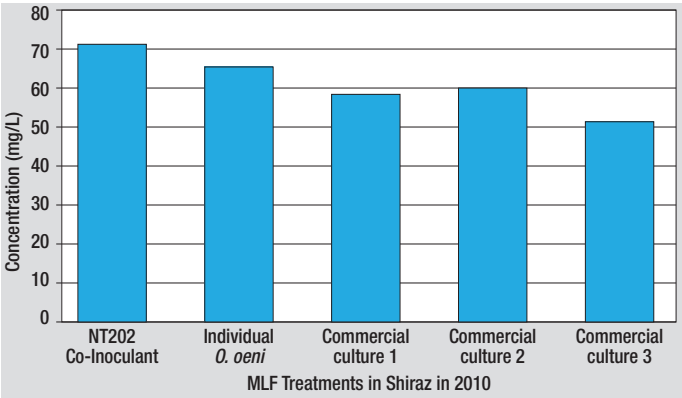

Figure 3a. The total ester production (excluding ethyl acetate) of the Anchor NT 202 Co-Inoculant compared with the individual *O. oeni* strain present in the mixed culture and various commercially available MLF starter cultures.

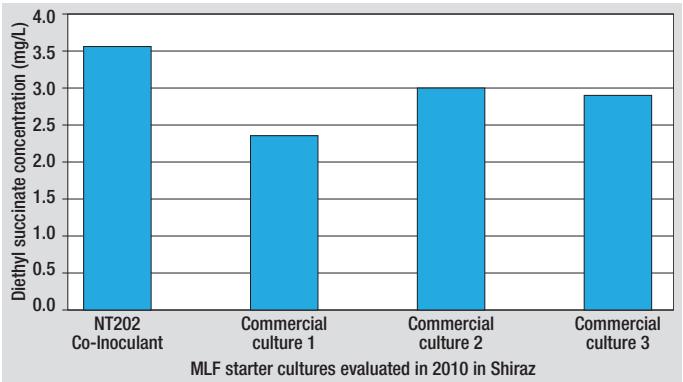

Figure 3b. A comparison of the diethyl succinate production of the Anchor NT 202 Co-Inoculant and various commercial cultures during co-inoculation with *S. cerevisiae* NT 202 Anchor Wine Yeast.

concentrations, successful completion of MLF, the inability to act as a major contributor to volatile acidity, compatibility with various commercial *S. cerevisiae* yeast cultures, lack of biogenic amine or off-odour production, as well as the production of compounds that favourably contribute to the wine aroma profile (Figure 1) (Lerm *et al.* 2010). The genetic potential of the strains with regards to the production of aroma and health-impacting compounds has been determined.

In addition, the Anchor NT 202 Co-Inoculant has been tested in the experimental winery of the University of Stellenbosch and successfully completed MLF in various cultivars, over a number of vintages and in co-inoculation. In 2011, the average time to complete MLF was assessed for 110 wines from 40 South-African wineries. These results are displayed in Table 2.

The total ester production of the Anchor NT Co-Inoculant was compared with that of a commercial culture in the 2008 vintage in Pinotage, Cabernet Sauvignon and Shiraz. The mixed culture consistently, in all three cultivars, produced significantly larger total ester concentrations compared with the commercial culture.

In 2010, in addition to comparing the Anchor NT 202 Co-Inoculant with various commercial *O. oeni* cultures with regards to the production of aroma impacting compounds (diethyl succinate and total esters), the mixed culture was also compared with the individual *O. oeni* strain present in the Anchor NT 202 Co-Inoculant mixture.

Diethyl succinate is one of the most important esters associated with MLF and contributes fruity/melon aromas to the wine.

Table 2. The average time (in days) taken by the Anchor NT 202 Co-Inoculant starter culture to complete MLF (AF completed on average on day seven) during fermentation trials.

Vintage	Cultivar	Inoculation	Total duration (incl. AF and MLF)
2009	Pinotage, Cabernet Sauvignon	Co-inoculation	9
2010	Shiraz	Co-inoculation	26
2011	Pinotage, Cabernet Sauvignon, Shiraz, Merlot	Co-inoculation	22

Once again, the mixed culture resulted in significantly larger concentrations of total esters and diethyl succinate being produced (Figure 3a and 3b).

The take-home message

The Anchor NT 202 Co-Inoculant and Anchor Yeast NT 202 ensure a simple, secure and speedy AF and MLF.

It offers simplicity by being able to inoculate yeast and bacteria at the same time in the juice. Speed comes when MLF is completed zero to 14 days after alcoholic fermentation under optimal conditions. Security is found in the co-inoculation of yeast and bacteria, which offers several advantages over inoculation after alcoholic fermentation:

- the heat of the fermentation favours bacterial growth
- the bacteria is inoculated into 0% alcohol and has time to adapt to rising alcohol levels
- ample nutrient supply
- the wine can be sulfured sooner and is thus protected against microbial spoilage (*Brettanomyces*). GW

This article was written for and submitted by Anchor Yeast. **E. Lerm** and **M. du Toit** are based at the Institute for Wine Biotechnology, Department of Viticulture and Oenology, University of Stellenbosch, Private Bag X1, Matieland (Stellenbosch), South Africa; **L. Malandra** works for Anchor, South Africa and **P. Pellerin** works for Oenobrands, France.

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Accolade wines expands into China

ACCOLADE WINES HAS moved to strengthen its presence in China, buying a majority stake in a Shanghai-based wine distribution business.

In a media statement, Accolade Wines chief executive officer Troy Christensen said the acquisition of Shanghai CWC Wine Trading Co Ltd would strengthen Accolade Wines' platform in China and provide a springboard for Accolade Wines' expansion into this rapidly developing market. CWC has been a long-time distributor of Accolade wines in China and Mongolia, with the acquisition giving the company permanent offices in Shanghai and Beijing.

Accolade Wines commercial general manager, Asia, Freddie Choong said Accolade now had a solid platform from which to rapidly build its market position.

"As Chinese wine consumers are now rapidly exploring the world of wine, this acquisition ensures we are well-placed to provide an exciting suite of wines from icons through to premium and commercial wines," he said.

Minority shareholder and current manager Bong Ha said Accolade Wines' diverse portfolio meant the company had the capacity to offer its existing wines to Chinese consumers but to develop wines specifically for the Chinese market.

Accolade Wines is headquartered at Reynella, South Australia, and now has offices in Guildford (Surrey, UK), Bristol, Sydney, Brisbane, Perth, Melbourne, Singapore, Stellenbosch, Tokyo, Moscow, Amsterdam, Shanghai and Beijing.