Emerging trends in the application of malolactic fermentation

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Abstract

Deacidification of wines by malolactic fermentation (MLF) is an essential step in the production of most red, many white and some sparkling base wines. While this secondary fermentation can occur spontaneously, the majority of winemakers, particularly in new world winemaking regions, prefer to minimise the risk of a failed or sluggish MLF by inoculating with a reliable, commercially available starter culture. This review focuses on the scientific literature underpinning growing trends in the application of MLF starters. It considers the literature on co-inoculation regimes, where MLF bacteria are inoculated into a ferment prior to completion of alcoholic fermentation and what benefits this might bring relative to sequential inoculation, when bacteria are added after completion of primary fermentation. It also considers the benefits of the growing trend of using bacteria other than the traditional *Oenococcus oeni* for induction of MLF, and of developing starter cultures from regional isolates of MLF bacteria to enhance regional identity of wines.

Keywords: *co-inoculation in wine*, *lactic acid bacteria*, *malolactic fermentation*, Oenococcus, *regionality*

Introduction

Malolactic fermentation (MLF) is the bacterial-driven decarboxylation of L-malic acid to L-lactic acid and carbon dioxide. This deacidification reaction is essential in the production of most red wines and desirable in some white and sparkling base wines. Its main function is to 'soften' (i.e. increase the pH of) wine, but it also contributes to microbial stability through the removal of a potential microbial carbon source, and it impacts on the flavour profile of finished wines (Davis et al. 1985, 1988).

Several members of the lactic acid bacteria (LAB) group can undertake MLF. Of the LAB species, particularly *Oenococcus oeni* is the most suited to the harsh conditions of wine. The MLF bacteria have in common the capacity to import malate from their environment into the cytoplasm via a specific (malate) permease, and they produce cytoplasmic malolactic enzyme, which is responsible for the decarboxylation reaction (Lonvaud-Funel 1995). Their capacity, however, to deliver a successful MLF is highly strain dependent and can be compromised by many factors, including a high concentration of alcohol and sulfur dioxide, extremely low pH and poor nutritional status of the wine (Lonvaud-Funel 1999, Bartowsky 2005).

These complications make MLF one of the most capricious and precarious stages in winemaking. This is particularly the case when the indigenous microflora in a wine ferment are relied upon to conduct spontaneous MLF; initiation and completion are unpredictable and can take many weeks or even months to complete leaving wine susceptible to spoilage as sulfur dioxide (SO_2) cannot be added to stabilise the wine until MLF is complete (Sponholz 1993, Bartowsky 2009). In addition, the impact of indigenous MLF bacteria on wine quality is not predictable. This has led over the past 20+ years to the development of commercially available MLF bacterial starter cultures, which have assisted considerably in improving MLF efficiency and reliability (Nielsen et al. 1996). Mostly these starter cultures use *O. oeni*, but there is a growing interest in other species of LAB.

Nevertheless, even with access to robust starter cultures of malolactic bacteria, the successful induction of MLF, particularly in difficult wine conditions, continues to pose a major challenge to winemakers. In order to improve on this, greater knowledge of options for MLF induction is required.

In addition, there is growing interest in MLF research and development to introduce strategies that can be used to enhance the regional identity of wines. The provenance or 'sense of place' of a wine is becoming increasingly important for product differentiation in an overcrowded market. Thus, enhancing the regional identity of a wine will potentially increase its value in the marketplace.

Over recent years, there have been several reviews on MLF, the bacteria that are involved, and the aroma and flavour impacts of bacterial metabolism during MLF on wine (Bartowsky 2005, 2009, Lerm et al. 2010, Sumby et al. 2010, 2014, Bartowsky and Borneman 2011, Styger et al. 2011). The current review will complement these publications by focusing on emerging trends in the application of MLF in winemaking and the research that underpins these trends. Specifically, the review will concentrate on inoculation strategies and types of MLF starter cultures, including: research on inoculation regimes and how this is informing current practices in the winery to improve efficiency and reliability of MLF; the adoption of LAB species other than *O. oeni* for MLF, and how these increase winemakers' options; and the practice of isolating indigenous (autochthonous) MLF bacteria to develop starter cultures that can be used to enhance the regional identity of wines.

Inoculation regimes: the emerging trend of yeast–bacteria co-inoculation

Since the introduction of malolactic starter cultures for improving the induction of MLF, there has been considerable research and development aimed at optimising inoculation regimes to further enhance MLF efficiency. A major consideration has been to determine the optimal time point for inoculation. Starter cultures can be co-inoculated with yeast (at the beginning or towards the end of alcoholic fermentation), or sequentially (after alcoholic fermentation) (Kunkee et al. 1964, Edwards and Beelman 1989). Inoculation prior to yeast inoculation (pre-fermentation) with certain strains of *Lactobacillus plantarum* has

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also received some attention. At least for *O. oeni* starter cultures the practice in industry has largely been to use sequential inoculations, but there is a growing interest in co-inoculation strategies (Abrahamse and Bartowsky 2012a).

From a historical perspective, impacts of the timing of bacterial inoculation were first observed in the 1950s and 1960s [see e.g. Peynaud and Domercq (1959), Webb and Ingraham (1960)]. Studies at this time indicated that, for a successful MLF, it is important to inoculate for MLF before the primary fermentation is complete. Findings in subsequent studies (Kunkee et al. 1964, Kunkee 1974), however, contradicted this, with data indicating that there was no advantage to be gained from early inoculation. Such discrepancies between different studies may be related to variation in grape composition (Kunkee 1974) or methods of starter culture preparation. Nevertheless, up until the end of the 1970s it was not clear from the research literature which inoculation strategy was best; MLF inoculation towards the end (Kunkee 1974), during or after (Gallander 1979) alcoholic fermentation were all viable options.

By the 1980s, however, it became apparent that there were potential advantages to using co-inoculation; generally, relative to sequential inoculation, co-inoculation reduces overall vinification time [see Edwards and Beelman (1989)]. This has important ramifications for the wine industry: speeding up vinification rate leads to more rapid wine stabilisation. This reduces the risk of spoilage and frees up winery resources (e.g. tank space), thereby minimising bottlenecks in processing.

It is thought that the advantages of inoculating MLF starter cultures simultaneously with yeast relate to the more conducive conditions for bacterial growth and metabolism, specifically, there is greater availability of nutrients in grape must than in wine and there is less alcohol and other potential yeast-derived inhibitors (Davis et al. 1985, Edwards and Beelman 1989, Edwards et al. 1990). These conditions are thought to enable malolactic bacteria to acclimatise to ethanol as the concentration increases, thereby improving MLF performance (Zapparoli et al. 2009, Azzolini et al. 2010).

Nonetheless there has been some reluctance by industry to adopt co-inoculation as a practice. One possible explanation for this is that *O. oeni* is heterofermentative (Kandler 1983). This means that under certain conditions, one of the products of its sugar metabolism is acetic acid. Thus it might be assumed that *O. oeni* has the potential to produce wines with elevated volatile acidity. However, it has been demonstrated by several laboratories that, at least under winemaking conditions and with careful management of fermentations, this is not the case (Semon et al. 2001, Rosi et al. 2003, Jussier et al. 2006, Zapparoli et al. 2009, Pan et al. 2011, Abrahamse and Bartowsky 2012b). It is thought that *O. oeni* does not produce acetic acid when grown in grape juice at low pH; under these conditions, it preferentially utilises organic acids (malic and citric acids) rather than sugars (Cox and Henick-Kling 1989). As the pH increases, there is a shift to a preference for sugar utilisation, thus increasing the risk of acetic acid accumulation (Arnink and Henick-Kling 1993, Henick-Kling 1995, Ramos et al. 1995).

Another concern regarding the use of simultaneous MLF is its potential negative impact on yeast growth and vitality, which could lead to stuck or sluggish alcoholic fermentation (Semon et al. 2001). This was demonstrated in a study by Muñoz et al. (2014). These authors compared two *Saccharomyces cerevisiae* yeast strains (Lalvin ICVD80 and Fermicru UY4) in co- and sequential inoculations with *O. oeni* VP41. All sequential fermentations went to completion. In contrast, although co-inoculation resulted in a much shorter duration of MLF (6–7 days) compared with sequential inoculation (14–20 days) and did not affect the duration of alcoholic fermentation, co-ferments that used ICVD80 with early inoculation of VP41 did not go to completion (3.9 g/L residual fructose) and had elevated volatile acidity (0.56 g/L). It is important, however, to note that co-ferments using the other yeast strain (Fermicru UY4) were completed.

Thus it is important to choose the correct combination of yeast and bacterial strains in co-inoculated ferments; not all combinations are equally compatible. This, however, is not peculiar to simultaneous fermentations, as sequential inoculations are well known to be influenced by yeast–bacteria interaction phenomena [see Henick-Kling and Park (1994), Alexandre et al. (2004), Arnink and Henick-Kling (2005)]. Moreover, Muñoz et al. (2014) suggest that specific yeast–bacteria interactions may differ between different timings of bacterial inoculation. Overall, while the choice of yeast strain is an important consideration for successful vinification, there is growing evidence that optimal combinations may indeed differ for co- and sequential fermentations.

With the caveat mentioned earlier in mind regarding judicious choice of yeast and bacterial strain combination, simultaneous inoculation can provide a major advantage for MLF induction, particularly in more difficult must/wine conditions. In one study (Guzzon et al. 2013), five pairs of commercial yeast and bacterial strains were tested in four red grape musts (Cabernet Sauvignon, Merlot, Teroldego and Marzemino) with low nitrogen content (<70 mg/L readily available nitrogen). Compared with sequential inoculations, co-inoculations for MLF induction were found to be more reliable in that the majority went to completion whereas most of the sequential MLFs did not. Similar advantages of simultaneous inoculation techniques have been reported for red wine with high alcohol concentration (Zapparoli et al. 2009), and white wine with low pH (Knoll et al. 2012).

While the majority of research trials comparing inoculation regimes have been conducted at laboratory scale, there are examples of winery-scale trials that also demonstrate a clear advantage in co-inoculation for efficient vinification. Azzolini et al. (2010) compared inoculation regimes using *O. oeni* VP41 for industrial scale (6.5 and 20 kL vinification of Valpolicella wine—a blend of Corvina and Rondinella cultivars). Co-inoculated ferments completed MLF during alcoholic fermentation, whereas the sequentially inoculated ferments took 33 days longer to complete vinification. Similarly, Abrahamse and Bartowsky (2012a,b) compared inoculation regimes in Australian Shiraz at both 1.5-kg laboratory and 9-kL winery scale. In both cases simultaneous inoculation resulted in faster completion of MLF (by 6–12 weeks), leading to earlier wine stabilisation. Antalick et al. (2013) also reported greater efficiency with simultaneous compared with sequential inoculation (1.6- to 2.8-fold reduction in total fermentation time) in five Merlot wines vinified under winery conditions (>300 L) in France and Switzerland.

As a result of the above research there has been a growing interest internationally in trialling and using co-inoculation in the production of many red and some white wines. Co-inoculation strategies have been found to benefit production of: Pinot Noir (Krieger 2002, Christen and Mira de Orduña 2010), Shiraz (Abrahamse and Bartowsky 2012b), Cabernet Sauvignon (Guzzon et al. 2013), Tannat (Muñoz et al. 2014), Merlot (Cañas et al. 2012, Antalick et al. 2013), Cabernet Franc (Cañas et al. 2015), Tempranillo (Cañas et al. 2012), Riesling (Knoll et al. 2011), Teroldego and Marzemino (Guzzon et al. 2013), Malbec (Massera et al. 2009, Mendoza et al. 2011), Amarone (Zapparoli et al. 2009), Nero di Troia (Garofalo et al. 2015). Co-inoculation has also been used in Pinot Noir in conjunction with microwave maceration (a novel winemaking

process) to shorten vinification time, which in this trial was 37 days from harvest to stable wine (Carew et al. 2015). In fact, co-inoculated MLF is also being utilised in the production of cherry wine (Sun et al. 2013), pear wine using *Lactobacillus acidophilus* (Zhang et al. 2011), cider (Sánchez et al. 2014) and cachaça (Duarte et al. 2011).

In addition to improving MLF efficiency, the wine sensory profile following co-inoculation of bacteria with wine yeast can differ from that of sequential inoculation. A sensory study of Malbec produced using co- and sequential inoculation demonstrated that co-inoculation led to a higher rating for fruity aroma descriptors and a reduction in bitterness and astringency perception (Massera et al. 2009). The fermentation-derived, volatile compound profiles of Shiraz wines produced either through co-inoculation or sequential MLF were found to be distinctly different; more fruity compounds were noted with co-inoculation than sequential MLF (Abrahamse and Bartowsky 2012a,b). Similarly, in Chardonnay, co-inoculated wines tended to be fruitier than wines produced using sequential inoculation (Jussier et al. 2006), and in a German Riesling study, co-inoculated wines had a higher concentration of volatile compounds that contribute to fruity sensory characters compared with that of sequential inoculated wines (Knoll et al. 2011). Antalick et al. (2013) also reported the effect of timing of inoculation on modifying the metabolic and aroma profile of Merlot wines vinified under winery conditions. This study found, however, that co-inoculation did not always favour fruity expression, and lactic aroma intensity could either increase or decrease. These authors concluded that the lack of any clear trends in the effect of inoculation regime on metabolic and sensory impacts reflected the complex interactive effects of yeast and bacteria strains used in fermentation.

It can be concluded that, with appropriate choice of compatible yeast/bacterial strain combinations, compared with sequential inoculation, simultaneous alcoholic fermentation and MLF leads to more rapid and reliable vinification. This is particularly the case under harsh conditions. In addition, this approach has the potential to influence wine style by modifying the profiles of wine volatiles and sensory properties.

Alternatives to *O. oeni* for bacterial MLF starter cultures

While *O. oeni* has been the bacterium of choice for MLF starter cultures over the past 20+ years, other wine LAB are capable of conducting MLF and may prove beneficial in some winemaking contexts (Spano and Massa 2006). These non-*O. oeni* LAB, however, in particular, certain species and strains of *Lactobacillus* and *Pediococcus*, have largely been avoided in the past because they have generally been associated with the formation of negative attributes in wine (Davis et al. 1985, Sponholz 1993). For example, *Lactobacillus* species can cause a range of wine spoilage, including mousy off-flavour and excessive acetic acid from residual wine sugars, and have also been implicated in causing spoilage during sluggish/stuck alcoholic fermentations (Davis et al. 1985, Edwards et al. 1998, 1999, 2000, Costello and Henschke 2002, Bartowsky 2009). Furthermore, some strains of *Pediococcus damnosus* are well known for their potential to produce ropy wines (Lonvaud-Funel and Joyeux 1988, Lonvaud-Funel 1999).

Nevertheless, more recent research in this area has demonstrated that some strains of *Lactobacillus* and also *Pediococcus* lack such negative traits and are indeed suitable for MLF induction. Commercial development of these strains has expanded the biodiversity of MLF starter cultures available to winemakers.

Applications of L. plantarum *in MLF*

L. plantarum is commonly found in wine (Edwards et al. 1993, Beneduce et al. 2004, G-Alegria et al. 2004, Ruiz et al. 2010a, du Toit et al. 2011), and has been associated with spontaneous MLF in, for example, Patagonian red wines (Valdés La Hens et al. 2015). This bacterium is homofermentative for hexoses such as glucose (Fugelsang and Edwards 2007); it will produce only lactic acid and not acetic acid when it metabolises glucose, thus eliminating any potential risk of contributing to volatile acidity. In addition, *L. plantarum* has a preference for malate as an energy source at low pH, even in the presence of glucose (Guerzoni et al. 1995, du Toit et al. 2011), making it suitable for malate decarboxylation in a co-fermentation or even pre-alcoholic fermentation, when it can begin decarboxylating malate before a yeast starter culture has been added. The reader is directed to a recent review on the application of *L. plantarum* to MLF by du Toit et al. (2011).

In addition *L. plantarum* produces a broader range of extracellular enzymes, including glycosidases and esterases, than *O. oeni* (Guerzoni et al. 1995, Grimaldi et al. 2005, Pozo-Bayón et al. 2005, Matthews et al. 2006, Mtshali et al. 2010). Extracellular enzymes, particularly glycosidases and esterases, play an important role in the development of wine sensory properties through the release of flavour molecules from inactive precursors (Williams et al. 1989, Sefton et al. 1993). Thus it is possible that *L. plantarum* may enhance wine sensory properties to a greater extent than *O. oeni*, however, further research is required to test this assumption.

Interestingly, the application of a *Lactobacillus* spp. as a starter culture is not particularly novel. Indeed, as reported in the previous section, the *Lactobacillus* strain ML-30 was successfully used in inoculation timing trials in Pinot Noir in the early 1960s (Kunkee et al. 1964), and a commercial *L. plantarum* strain (Viniflora plantarum, CHR Hansen) was promoted in the late 1980s (Henschke 1989) for inoculation prior to alcoholic fermentation (Prahl 1988). While suppliers of *L. plantarum* starters recommend pre-alcoholic inoculation of starter cultures of this bacterium, there is no peer-reviewed literature comparing this inoculation regime with co- or sequential inoculation.

A commercial starter culture of *L. plantarum*, V22, was released to market in around 2010 by Lallemand Inc., and is recommended for use in high pH red wines (Fumi et al. 2010, du Toit et al. 2011). Following an extensive screening of LAB for their ability to reduce ochratoxin A (OTA) in must and wine, this *L. plantarum* strain was selected for its ability to reduce OTA and it efficiently conducted MLF at high pH (Fumi et al. 2010). Other studies have shown that this *L. plantarum* strain conducts efficient MLF in Cabernet Sauvignon wine (pH 3.5) and produces 'berry-fruity' sensory characters (Bartowsky et al. 2012). It was also found to be as efficient at MLF as commercial *O. oeni* strains in the same red wine.

Chr Hansen has recently released another *L. plantarum* strain (NoVA) to the market (Saerens et al. 2015). This strain was isolated from a screening undertaken in collaboration with Professor Maret du Toit at Stellenbosch University in South Africa (http://www.chr-hansen.com). As with their previous *L. plantarum* product (Viniflora plantarum), it is recommended that this starter culture be inoculated into grape must prior to alcoholic fermentation and it is promoted as not producing volatile acidity.

A recent screening characterised 53 *L. plantarum* strains isolated from Patagonian Pinot Noir wines with the aim of developing commercial starter cultures (Bravo-Ferrada et al. 2013). The screening for tolerance to different wine stress factors (high ethanol, pH, SO_2), glucosidase and tannase activity, citrate utili-

sation, absence of biogenic amine genes (*hdc*, *tdc* and *ptc*) and malolactic performance resulted in the isolation of two *L. plantarum* strains, which are to be tested further for performance in a winery.

Testa et al. (2014) have undertaken a similar study examining the biodiversity of *L. plantarum* strains from traditional Italian wines. The aim of this work was not only to identify *L. plantarum* strains suitable for MLF inoculation, but also to select *L. plantarum* strains as suitable candidates for MLF starter cultures to enhance wine regional identity, as discussed later.

An additional potential benefit of using *L. plantarum* strains for MLF is that they have the ability to degrade biogenic amines (Capozzi et al. 2010, García-Ruiz et al. 2011), many of which have negative health implications (Bartowsky and Stockley 2011).

Applications of Pediococcus *spp. in MLF*

Several different *Pediococcus* spp. have been isolated from wine, including strains of *P. damnosus*, *P. parvulus*, *P. inopinatus* and *P. cerevisiae* (Back 1978, Wibowo et al. 1985, Edwards et al. 1994). As with *Lactobacillus* spp. the proliferation of *Pediococcus* spp. is generally associated with wines of reasonably high pH (3.5–4.0) (Davis et al. 1988) and historically has been considered detrimental to wine quality because of its potential risk of causing wine spoilage, including ropiness and a high level of diacetyl (Davis et al. 1988, Walling et al. 2005, Bartowsky 2009). Members of this genus also have the potential to produce biogenic amines such as histamine (Landete et al. 2005). Despite this, recent research is revealing that some strains exhibit potential for use in MLF induction in high pH red and white wines.

Edwards and Jensen (1992) and Edwards et al. (1994) were perhaps the first to highlight that the presence of *Pediococcus* spp. in wine was not necessarily associated with spoilage. These researchers found that one strain of *P. parvulus* (WS-9), out of 10 tested, was capable of inducing MLF in a high pH Cabernet Sauvignon wine (pH 3.98), and no *P. parvulus* isolates completed MLF in Chardonnay wine (pH 3.66). Edwards et al. (1994) also reported that while the growth of *P. parvulus* could modify the bouquet characteristics of Cabernet Sauvignon wines without previous MLF, the precise nature of such aroma differences caused by *P. parvulus* remained unclear.

Recent studies by Strickland (2012) provided further insight into the effect of different isolates of *Pediococcus* spp. on the chemical and sensory properties of Oregon Pinot Noir wine. A common metabolic trait amongst isolates was the ability to convert *p*-coumaric acid to 4-vinyl phenol, which as a consequence, facilitated an accelerated rate of 4-ethylphenol (i.e. Brett character) production by *Brettanomyces bruxellensis* in a model system. There was also considerable variation between isolates in their capacity to affect the concentration of other wine components. These included L-malic acid degradation (varied between about 20–100%), the production of D-lactic acid (up to 264 mg/L), diacetyl production (<0.5 to >15 mg/L) and the reduction of red wine colour (by up to more than 10%) and polymeric pigment (by up to nearly 30%) in association with a reduction in acetaldehyde content. None of the strains produced a high level of biogenic amines, with only one strain (*P. inopinatus* OW8) producing a measurable amount (3.3 mg/L histamine) and none of the strains degraded glycerol in Pinot Noir wine. Strickland (2012) also reported considerable variation amongst *Pediococcus* spp. isolates in their abilities to impact the sensory properties of Pinot Noir wine, with a higher intensity of butter, plastic and vegetal aromas, and lower perceived astringency produced by some strains compared with that of non-MLF control wines.

Nonetheless, because of the potential that some *Pediococcus* spp. isolates have shown, there is interest in looking to this genus to find suitable strains from which starter cultures can be developed. For example, Juega et al. (2013) report the successful application of two autochthonous strains of *P. damnosus*, isolated from Caiño wine, in inducing MLF in Albariño and Caiño white wines (pH 3.51 and pH 3.71, respectively) where a strain of *O. oeni* failed. MLF was successfully induced in both wines by inoculation with a starter culture comprising a mix of the two autochthonous *P. damnosus* strains (C5 and C8). Importantly, the authors found that genes responsible for the production of ropy-causing exopolysaccharide (*dps*) and biogenic amines (*hdc*, *tdc* and *odc*) were absent from these strains. Sensory analysis of the resultant wines also indicated that MLF treatment with the *P. damnosus* strains had a significant effect on the intensity of several common attributes, including an increase in yellow-golden highlights in Albariño wine, with increased honey aroma, and a decrease in aromatic herbs aroma and acidity in Caiño wine. There was also an increase in body and softness in Albariño, and development of a more mature wine in Caiño. The disappearance of some fruity aromas, such as pear and citrus, and the appearance of other attributes including vanilla or caramel following MLF was also noted.

Recent patents relating to the application of selected strains of *Pediococcus* spp. for MLF induction in some wine types gives some indication of the potential of these bacteria for use as malolactic starter cultures. For example, further to the work of Juega et al. (2013), a patent relating to the use of two strains of *P. damnosus* (DSM 25074 and DSM 25075) for the induction of MLF has been filed (Carrascosa Santiago et al. 2013). In addition, a patent relating to the use of alcohol-tolerant strains of *Lactobacillus* spp. and *Pediococcus* spp. for MLF induction in wines of moderate to high pH was filed by Bou and Krieger (2012). In this patent, the use of a direct inoculation starter culture preparation of *P. acidilactici* (CNCM MA 18/5M) is described either alone or in combination with specified *L. plantarum* strains. Compared with *O. oeni* and indigenous microflora these starter cultures are reported to rapidly initiate and conduct MLF in high pH conditions (Bou and Krieger 2012). Rapid initiation of MLF with these products is claimed to avoid uncontrolled growth and conduct of MLF by undesirable bacteria, which is a significant risk in wines of high pH.

In conclusion, even though using *Pediococcus* spp. for MLF may be counter-intuitive for many winemakers, there are clearly some strains that are potentially useful for producing wines from musts that have a pH above 3.5. In addition the different array of enzyme activities displayed by this bacterium compared with *O. oeni* may provide the opportunity to develop novel wine styles.

Enhancing regionality of wine through MLF

There is evidence that regional branding is an effective means of producing higher returns for wine companies, providing a clear point of difference for marketing purposes (Easingwood et al. 2011). Many well-known 'old world' premium wines are strongly associated with the region they come from (e.g. Burgundy, Barolo, Mosel, Champagne), with each region having a characteristic wine style determined by grape cultivar, geology, climate, and viticultural and winemaking practices. There is also mounting evidence that the local, indigenous, bacterial microflora contribute to a wine's terroir [see e.g. Gilbert et al. (2014), Zarraonaindia et al. (2015)].

It is not surprising therefore that research has been conducted on regional microbial isolates and their potential application in winemaking. For example, selected indigenous yeast strains have been shown to be well adapted to the specific

environmental conditions of the wine region they were isolated from (Ruiz et al. 2010b), and in other research native yeast isolates were found to preserve the typicity of a given cultivar and region (Blanco et al. 2014, Tufariello et al. 2014). Regional yeast isolates have been trialled in the production of Apulian Primitivo wines (Grieco et al. 2010).

Similarly there is growing interest in the application of regional malolactic bacteria (Capozzi and Spano 2011). Analysis of the genetic variation in indigenous LAB communities in Tempranillo wines that underwent spontaneous MLF, from ten different La Rioja wineries over three vintages, suggested that there is an endemic microbiota for the region (González-Arenzana et al. 2013). Similar observations were made for Tempranillo wines produced in Castilla-la-Mancha wineries over three vintages (González-Arenzana et al. 2015); again there was a microbiota endemic to the region. In addition, the genome of five autochthonous *O. oeni* isolated from the same terroir (spontaneous MLF in Nero di Troia wine) has been sequenced (Capozzi et al. 2014). This highlights the importance that is more recently being placed on wine regional identity.

Ruiz et al. (2010b) isolated an indigenous *O. oeni* strain, C22L9, from a Tempranillo wine produced in the Castilla-La-Mancha region, and the authors reported that this bacterium imparted the sensory characteristics of wines made in this region. Subsequently, the bacterium was prepared as a freeze-dried product by a commercial microbial starter culture company and trialled in co- and sequential inoculations in Tempranillo and Merlot at a 100-kg scale with two different wine yeasts (Cañas et al. 2012). The strain performed well in these trials, particularly when co-inoculated with the yeast.

A screening of indigenous *O. oeni* strains from Yueqiannian (in the Changli region of China) red wine resulted in the isolation of two *O. oeni* strains that grew at low pH and high alcohol concentration with efficient malic acid metabolism (Liu et al. 2014). The authors propose that these strains are candidates for starter cultures. In this case, however, the use of the native isolates was perhaps less about regionality and more associated with the performance of the strains in wines of the Yueqiannian region.

To date, most regional isolates of malolactic bacteria have focused on *O. oeni*. As mentioned previously in this review, however, Testa et al. (2014) screened *L. plantarum* strains sourced from different artisanal wineries located in various areas of Southern Italy to select potential *L. plantarum* strains as candidate starter cultures with the aim of enhancing regional character.

Conclusions

This review focuses on applications of MLF starter cultures in winemaking, particularly with regard to emerging practices. Inoculating with a starter culture for MLF has traditionally relied on tried and tested strains of *O. oeni* that are added post-alcoholic fermentation, with no consideration of the geographic provenance of the strain being used. This review uses the scientific literature to assess the merit of variations to these practices. This literature shows that there is considerable merit in inoculating for MLF at an earlier stage in vinification rather than waiting until primary fermentation is complete; that starter cultures of bacteria other than *O. oeni* can bring benefits, particularly when dealing with a relatively high pH must; and that regional isolates of malolactic bacteria work well and potentially contribute to regional character and, thereby, identity of a wine.

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