International Journal of Food Microbiology 243 (2017) 16–27

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Review

Linking wine lactic acid bacteria diversity with wine aroma and flavour

Maria Stella Cappello [a,*], Giacomo Zapparoli [b,*], Antonio Logrieco [c], Eveline J Bartowsky [d,1]

[a] CNR, Institute of Science of Food Production (ISPA), Prov.le Lecce-Monteroni, 73100 Lecce, Italy
[b] Department of Biotechnology, University of Verona, Strada le Grazie 15, 37134 Verona, Italy
[c] CNR, Institute of Science of Food Production, Via G. Amendola, 122/0, 70126 Bari, Italy
[d] The Australian Wine Research Institute, PO Box 197, Glen Osmond, SA 5064, Australia

ARTICLE INFO

Article history:
Received 6 April 2016
Received in revised form 9 November 2016
Accepted 27 November 2016
Available online 30 November 2016

Keywords:
Wine
Lactic acid bacteria
Aroma
Flavour
Biodiversity

ABSTRACT

In the last two decades knowledge on lactic acid bacteria (LAB) associated with wine has increased considerably. Investigations on genetic and biochemistry of species involved in malolactic fermentation, such as *Oenococcus oeni* and of *Lactobacillus* have enabled a better understand of their role in aroma modification and microbial stability of wine. In particular, the use of molecular techniques has provided evidence on the high diversity at species and strain level, thus improving the knowledge on wine LAB taxonomy and ecology. These tools demonstrated to also be useful to detect strains with potential desirable or undesirable traits for winemaking purposes. At the same time, advances on the enzymatic properties of wine LAB responsible for the development of wine aroma molecules have been undertaken. Interestingly, it has highlighted the high intraspecific variability of enzymatic activities such as glucosidase, esterase, proteases and those related to citrate metabolism within the wine LAB species. This genetic and biochemistry diversity that characterizes wine LAB populations can generate a wide spectrum of wine sensory outcomes. This review examines some of these interesting aspects as a way to elucidate the link between LAB diversity with wine aroma and flavour. In particular, the correlation between inter- and intra-species diversity and bacterial metabolic traits that affect the organoleptic properties of wines is highlighted with emphasis on the importance of enzymatic potential of bacteria for the selection of starter cultures to control MLF and to enhance wine aroma.

Contents

1. Introduction

The production of wine involves numerous microbial transformations comprising a complex succession of various yeast and bacterial species. The alcoholic fermentation (AF) (primary fermentation) carried out by yeast (often separated into *Saccharomyces* and non-

* Corresponding authors.
E-mail addresses: maristella.cappello@ispa.cnr.it (M.S. Cappello), giacomo.zapparoli@univr.it (G. Zapparoli).
[1] Present address: Lallemand Australia, PO Box 210, Edwardstown, SA, 5039, Australia.

http://dx.doi.org/10.1016/j.ijfoodmicro.2016.11.025
0168-1605/

Saccharomyces species) generally followed by malolactic fermentation (MLF) (secondary fermentation) conducted by bacteria.

MLF involves the conversion of L-malate to L-lactate and CO_2 via malate decarboxylase, also known as the malolactic enzyme (MLE), resulting in a reduction of wine acidity, providing microbiological stabilization and modifications of wine aroma (Davis et al., 1988; Liu, 2002; Lonvaud-Funel, 1999). MLF is conducted by lactic acid bacteria (LAB), mainly represented by species of *Oenococcus*, *Lactobacillus*, *Pediococcus*, *Leuconostoc* and *Weissella* (Bae et al., 2006; Dicks and Endo, 2009; Lonvaud-Funel, 1999). Even though malic acid conversion can be conducted by any of the wine associated bacteria, *O. oeni* is the main species associated with MLF because of its tolerance to the harsh wine conditions (such as high ethanol concentration, low pH and nutrient content) (Ribéreau-Gayon et al., 2006). In view of the fact that MLF usually occurs after AF, the proliferation of wine LAB and efficiency of MLF will be affected by the composition of the wine. Production and release of compounds by yeasts, such as ethanol, SO_2, fatty acids, peptides, polysaccharides and other macromolecules will affect wine bacteria growth and MLF. In addition, yeast strains vary in their nutrient demands which can also influence MLF; low nutrient demanding yeast tend to favour efficient MLF (Guilloux-Benatier et al., 1998).

MLF and wine LAB metabolism have been extensively studied over the years (Bartowsky, 2005; Davis et al., 1988; Liu, 2002; Lonvaud-Funel, 1999; Swiegers et al., 2005). In addition to understanding phenotypic expression, molecular techniques have been used to examine the genetic diversity of wine LAB (Bilhère et al., 2009; Cappello et al., 2010; Valdés La Hens et al., 2015).

The onset of MLF can be controlled through the inoculation of a selected *O. oeni* or *Lb. plantarum* strain, however, under favourable conditions, MLF can occur spontaneously. The growth of the indigenous wine LAB population is related to their diverse ability to utilize sugars, amino acids and to tolerate multiple stresses, including acidity, SO_2, high concentrations of ethanol, and low temperatures (Borneman et al., 2012a; Ribéreau-Gayon et al., 2006).

Apart from the two crucial fermentation reactions in grape vinification, a myriad of other changes occur to complete the transformation of grape juice to wine. A wide range of secondary bacterial driven metabolic modifications are important for the taste, flavour improvement and stability of wine, and these include metabolism of citrate, amino acids, polysaccharides, and polyols, catabolism of aldehydes, hydrolysis of glycosides, synthesis and hydrolysis of esters, and degradation of phenolic acids, lipolysis, proteolysis and peptidolysis (Bartowsky, 2005; Liu, 2002; Matthews et al., 2004). These numerous reactions rely on the hydrolytic action of enzymes and the metabolites formed as a result of enzymatic activity of wine LAB. Although the presence of a broad range of enzymes in wine LAB have been documented (Liu, 2002; Matthews et al., 2004; Matthews et al., 2007; Ugliano et al., 2003;), information on the role of these bacterial enzymes activities including their potential use in winemaking is still limited. Moreover, very few studies have investigated the genetic potential of wine LAB with respect to the genes coding for enzymes of interest in winemaking.

Taking into account the increasing knowledge of wine LAB and their role in aroma and flavour formation in wine, acquired over the last 20 years, and the more recent progress on biochemistry and genetic characterization of LAB associated with wine, this review examines some of these interesting aspects as a way to elucidate the link between LAB diversity with wine aroma and flavour. In particular, it highlights the correlation between the inter- and intra-species diversity and bacterial metabolic traits that affect the organoleptic properties of wines with emphasis on the importance of enzymatic potential of bacteria for the selection of starter cultures to enhance wine aroma.

2. Lactic acid bacteria associated with wine

The wine-associated LAB belong to the phylum *Firmicutes*, class *Bacilli*, order *Lactobacillales*, within the family *Lactobacillaceae* and *Leuconostocaceae* (Garrity et al., 2004). The combination of different methods of phylogenetic analysis and different models of phylogenetic inference allowed the recognition of a number of wine associated genera and phylogenetic groups (Makarova et al., 2006).

The more recent availability of complete genome sequences for all major branches of *Lactobacillales* has allowed a more definitive analysis of their evolutionary relationships (Borneman et al., 2012b; Makarova et al., 2006; Makarova and Koonin, 2007), particularly with respect to adaptation to specific ecological niches. Despite the importance of LAB populations in characterizing wine quality, our current knowledge on the spatiotemporal distribution of LAB populations in grapes and in musts during the malolactic fermentation is still limited. Different and dynamic is the distribution of the vineyard- and winery-associated microbial system. Typically, LAB species diversity associated with grape surfaces is rather limited, mainly due to their nutritional requirements (Bae et al., 2006; Ribéreau-Gayon et al., 2006). The LAB species recovered in grape must disappear progressively during the AF. During spontaneous MLF, *O. oeni* is the major bacterial species found, however, several species can be occasionally detected, mainly *Lactobacillus*, *Pediococcus* and *Leuconostoc* (Dicks and Endo, 2009; du Toit et al., 2011).

Among the genera that are capable of MLF, *O. oeni* is the preferred species for winemaking as species of *Lactobacillus* and *Pediococcus* tend to produce undesirable sensory characteristics. *O. oeni* has been the only species within the *Oenococcus* genus until the mid-2000s when *O. kitaharae* was identified in composting distilled shochu residue (Endo and Okada, 2006). Over centuries of selective pressure, *O. oeni* has honed and perfected various adaptive strategies that enable it to outcompete with other potential MLF bacteria, during the later stages of vinification and thus to dominate in wine (Ribéreau-Gayon et al., 2006). *O. oeni* and *O. kitaharae* differentially encode several carbohydrate utilization and amino acid biosynthesis pathways which have resulted in adaptation to their individual ecological niches (Borneman et al., 2012b). Recently a third *Oenococcus* species has been identified, *O. alcoholitolerans* isolated from Brazilian cachaça (Badotti et al., 2014). Even though a draft genome sequence is available, what strategies are employed by *O. alcoholitolerans* to survive in its specific niche have not yet been elucidated. Unlike *O. kitaharae*, *O. alcoholitolerans* appears to have a functional malolactic enzyme, however what advantage this may provide is yet to be revealed.

Lactobacillus is an important genus involved in grape fermentation and among wine LAB this genus is represented approximately by 30 species (Dicks and Endo, 2009). *Lactobacillus oeni* is the most recent novel species recognized by the descriptive characterization of ten isolates from wine (Manes-Lazaro et al., 2009). Generally, the most frequent lactobacilli isolated from wine belongs to *Lb. plantarum*, *Lb. brevis*, *Lb. buchneri*, *Lb. hilgardii* and *Lb. fructivorans*, although, their occurrence, and that of others species (i.e. *Lb. kunkeei*, *Lb. fermentum*, *Lb. mali*, *Lb. vini*), can be very variable in different grape varieties and typologies of wines (du Toit et al., 2011). Among all species *Lb. plantarum* is certainly the most important in wine because it is found frequently on grapes and in wine, and is often involved in spontaneous MLF. This versatile bacterium tolerates ethanol up to 14% v/v and has similar SO_2 tolerance of *O. oeni*. Moreover, *Lb. plantarum* has a more diverse array of enzymes and can potentially exert positive effects on organoleptic properties of wine (du Toit et al., 2011; Matthews et al., 2006). Due to these characteristics, selected strains of *Lb. plantarum* are currently being commercialized to induce MLF in wine (Bartowsky et al., 2015; du Toit et al., 2011; Sumby et al., 2014).

Pediococcus genus is represented by four species (*P. damnosus*, *P. parvulus*, *P. pentosaceus* and *P. inopinatus*) associated with grape and wine (Dicks and Endo, 2009). The most investigated species are *P. parvulus* and *P. damnosus* due to their detrimental effects on wine quality; in particular production of biogenic amine and mediator of ropiness (Lonvaud-Funel, 1999; Walling et al., 2005). However, recently there has been a study in which MLF conducted by *P. damnosus* produced fault-free Albariño wine (Juega et al., 2013).

Other wine LAB previously described are *Leuconostoc mesenteroides*, *Weissella paramesenteroides* and *W. uvarum*; this latter was recently isolated from Greek wine grapes (Nisiotou et al., 2014).

Among wine LAB it is noteworthy the isolation of *Enterococcus faecium* from wines (Capozzi et al., 2011). The presence of isolates of this genus is not common in wine and their occurrence, even if for a short time, should be taken into account due to its potential to produce biogenic amines. Finally, Nisiotou et al. (2015) detected *Lc. lactis* on grapes, a species that is quite scarce on grapes and destined to disappear rapidly at the beginning of fermentation.

3. Techniques for identification of wine lactic acid bacteria

There are various molecular techniques available to aid in the characterization of wine LAB and add to the knowledge of these bacteria and their role in the winemaking process. These techniques enable the identification of wine associated bacteria, differentiate wine bacteria from each other as well as distinguish between different strains within the same species (Table 1).

Species delimitation of wine LAB can be generally obtained by gene sequencing and PCR-based techniques, that have replaced molecular ribotyping based on DNA hybridization, an expensive and time-consuming technique.

The sequencing of 16S rDNA has played a crucial role in the accurate identification of wine LAB isolates as well as in phylogeny analysis (Bartowsky and Henschke, 1999; Zapparoli et al., 1998). The sequencing of other genes such as phenylalanyl-tRNA synthase alpha subunit (*phe*S) and the RNA polymerase alpha subunit (*rpo*A) have been used to attribute the species to several wine LAB isolates (Naser et al., 2007).

PCR-based techniques include species-specific PCR targeting 16S rRNA genes or genes encoding the MLE (Zapparoli et al., 1998; Bartowsky and Henschke, 1999), PCR reactions with species-specific sequence characterized amplified regions (SCAR) primers (Petri et al., 2013) and molecular fingerprinting method based on the amplification of specific gene sequences, namely amplified polymorphic DNA-polymerase chain reaction (SAPD-PCR) (Sebastian et al., 2011) that allows the identification of many LAB species.

The identification of species among wine LAB isolates has also been achieved by enzyme restriction of the 16S RNA gene (ARDRA), and more recently *rpo*B gene (Claisse et al., 2007; Rodas et al., 2003). Moreover, PCR-denaturing gradient gel electrophoresis (PCR-DGGE) utilizing the 16S RNA and *rpo*B genes for designing primers allowed a reliable discrimination of different species of wine LAB (González-Arenzana et al., 2013; Renouf et al., 2006; Valdés La Hens et al., 2015). PCR-DGGE has been also used as a culture-independent method to monitor microbial populations in several kinds of fermented food including wine (Cocolin et al., 2013).

Strain discrimination within species of wine LAB is generally performed using molecular techniques that include pulse-field gel electrophoresis (PFGE), PCR-based fingerprinting and multilocus sequence typing. In the past PFGE had been widely utilized for genetic typing of wine LAB strains, however, gradually it has been replaced by easier and less time consuming PCR-based fingerprinting techniques. Randomly amplified polymorphic DNA (RAPD) is an extensively used technique, as it is a rapid, sensitive and inexpensive method, despite, converse to the previous, it can have low reproducibility across laboratories; however, it does have good reproducibility within a research group. Both techniques have been applied to investigate strain diversity

Table 1
Main molecular techniques used for studies of identification of microorganisms in the wine.

Technique	Application	Comments	Reference
Species-specific PCR (MLE gene)	- Specific PCR primers target and amplify either 16S rRNA genes or genes encoding the MLE - Distinguish LAB genera	Good discrimination at species level. Identification of individual species by species-specific PCR only; otherwise only group level identification	Zapparoli et al. (1998) Bartowsky and Henschke (1999)
Randomly amplified polymorphic DNA (RAPD) analysis (a PCR-based technique)	- Quick and sensitive discrimination of LAB strains - Follow *O. oeni* population changes during MLF	RAPD could discriminate between the type strains within a species	Zapparoli et al. (2000) Bartowsky et al. (2003) López et al. (2008)
PCR-denaturing gradient gel electrophoresis (PCR-DGGE)	- Identify and distinguish LAB - Monitor spoilage microorganisms during fermentation	PCR-DGGE has allowed discrimination at the species level	Renouf et al. (2006) Cocolin et al. (2013) González-Arenzana et al. (2013) Valdés La Hens et al. (2015)
Multilocus Sequence Typing (MLST)	- It allows to evaluate the genetic diversity of the isolates	The profiles obtained by random amplification of polymorphic DNA (MLST) allow to assess the genetic diversity among strains with the same allelic profile concatenated	Bilhère et al. (2009) González-Arenzana et al. (2014)
Multiple locus variable number of tandem repeat analysis (VNTR)	By VNTR, based on five polymorphic loci of the genome, allows to type *O. oeni* with highly discriminating manner	VNTR typing of *O. oeni* is highly discriminating, faster, and more reliable than the PFGE or MLST methods	Claisse and Lonvaud-Funel (2012) Claisse and Lonvaud-Funel (2014)
Restriction analysis of the amplified 16S rDNA or *rpo*B gene (PCR-ARDRA)	- Identification of species of LAB	Good method to discriminate different species within a genus, based on similar band pattern	Rodas et al. (2003) Claisse et al. (2007)
Pulsed-field gel electrophoresis (PFGE)	- Patterns of digested chromosomal DNA used to differentiate closely related *O. oeni* strains - Most reliable for strain differentiation. - Produces unique DNA fingerprint for individual strains	PFGE patterns of digested chromosomal DNA have been used to differentiate closely related strains of *O. oeni* and other wine LAB	Zapparoli et al. (2000) López et al. (2008)
DNA sequencing, amplified fragment length polymorphism (AFLP)	- Allows identifying and distinguishing differences of strain within a species	This technique can be applied to analyze the genomes of different genera of various microorganism	Cappello et al. (2008) Cappello et al. (2010)
Whole genome sequencing	- Determination of genetic variation between strains	Enables to determine what specific genetic differences there are between strains	Mills et al. (2005) Borneman et al. (2012a, 2012b) Campbell-Sills et al. (2015) Sternes and Borneman (2016)

of several species of wine LAB such as *O. oeni* and *Lb. plantarum* (Bartowsky et al., 2003; López et al., 2008; Zapparoli et al., 2000). Besides RAPD, other PCR-based techniques such as repetitive extragenic palindromic PCR (Rep-PCR), triplicate arbitrary primed PCR (TAP-PCR), enterobacterial repetitive intergenic consensus (ERIC)-PCR or polytrinucleotide $(GTG)_5$-PCR have been used to strain discrimination within several LAB species including those found on grapes and wine (Ruiz et al., 2014). Amplified fragment length polymorphism (AFLP) is an additional approach that utilizes enzymatic restriction of PCR amplified products and displayed highly reliability for typing within *O. oeni* species (Cappello et al., 2008, 2010).

Multilocus sequence typing (MLST) has been developed to identify different genotypes among the wine LAB populations of *O. oeni* and has been shown to be very efficient for typing. MLST resulted more or slightly less discriminatory power than PFGE according to Bilhère et al. (2009) and González-Arenzana et al. (2014), respectively. This incongruence is most likely due to the different rates of genetic changes that each technique is able to reveal. Nevertheless, MLST is a reliable molecular tool suitable for investigation of population and evolution of LAB as demonstrated by Bilhère et al. (2009) analyzing eight targeted loci in 43 *O. oeni* strains.

Multiple-locus variable number of tandem repeat analysis (VNTR) is a recent approach used to investigate genomic diversity among *O. oeni* (Claisse and Lonvaud-Funel, 2012, 2014). Its discriminatory power was higher than the PFGE and MLST methods, and the targeted VNTR markers were found to be stable, not changing for clones of the same strain. This technique is easy and fast to perform and is recommended for typing closely related strains as an alternative to existing typing methods for LAB strains. Moreover, VNTR is a promising tool for investigating phylogenetic relationship among populations of strains belonging to the same species as previously demonstrated (Claisse and Lonvaud-Funel, 2012).

In light of the above, the use of molecular tools has greatly improved the knowledge of taxonomy and ecology of wine LAB. In particular PCR-techniques have facilitated the investigations of LAB populations during the winemaking processes being, for most of them, easy and rapid to perform. Moreover, it has been demonstrated that the use of culture-independent methods such as PCR-DGGE allows detection of LAB species at very low cell concentration in wine (González-Arenzana et al., 2013). The introduction of so-called next-generation sequencing (NGS) technologies has allowed the identification of different species of the lactic acid bacterial consortium inhabiting grapevine and wine. The first use of NGS sequencing to analyze the atypical LAB community of a botritized wine was carried out by Bokulich et al. (2012). Beside the more typical *Lactobacillus* and *Pediococcus*, taxa such as *Leuconostoc*, *Lactococcus*, *Weissella* and *Streptococcus* were detected, while notably *Oenococcus oeni* was entirely absent. Analysis of other bacterial grape and wine communities demonstrated the great potential of these new methods on explore these microbiomes and specifically the correlation between LAB diversity and wine aroma (Piao et al., 2015; Portillo et al., 2016; Zarraonaindia et al., 2015). Hence, these last tools are promising for studies in complex microbial ecosystems like wine despite that they do not always yield quantitative or qualitative information. In fact, to have a full profile on the progress of the biodiversity of such ecosystems, the culture-independent methods should be combined with culture-dependent techniques.

4. Enzymatic activities that play a role in organoleptic properties

Wine LAB have been found to possess an extensive array of enzymatic activities, many of which have the potential to influence wine composition and therefore the processing, organoleptic properties, and quality of wine (Bartowsky and Borneman, 2011). Some of the enzymes of major interest in wine aroma include glycosidases, β-glucosidases, esterases, phenolic acid decarboxylases and citrate lyases (Grimaldi et al., 2005a, 2005b; Liu, 2002; Matthews et al., 2004) (Fig. 1).

An example of unravelling the enzyme-encoding genes and their translation to wine aroma is the buttery character in both red and white wines due to the formation of diacetyl via the metabolism of citrate (Guymon and Cromwell, 1965). Bartowsky and Henschke (2004) describe that altering winemaking conditions during MLF can affect the buttery character of wine. Furthermore, the expression of citrate utilization genes in *O. oeni* PSU-1 strain was examined under simulated winemaking conditions, and it was shown that ethanol, more so than wine pH, affected their transcription (Olguìn et al., 2009).

Wine aroma has been shown to be enhanced through *O. oeni* glycosidase enzymatic activities that are dependent on wine conditions such as pH, ethanol and residual sugar content (Grimaldi et al., 2000, 2005b; Ugliano et al., 2003). Most commercial glycosidase preparations are crude extracts prepared from fungi rather than bacteria.

However, there has recently been renewed interest in using wine LAB as potential sources of these enzymes; it is expected that the bacterial enzymes could act efficiently under high ethanol and acid conditions, encountered during winemaking, since the bacteria from which they were extracted are well adapted to these growth conditions (Bartowsky and Borneman, 2011).

Moreover, changes in ester concentrations have the potential to influence wine quality (Sumby et al., 2010; Swiegers et al., 2005). Recent studies demonstrated that wine LAB exhibit two different ester-synthesizing activities that may increase the ethyl ester content of wine thus modulating the fruity-berry character of red wine (Costello et al., 2013).

4.1. Glycosidases

A very important pool of compounds that can contribute to wine aroma is found in grapes and wine as odorless non-volatile glycosides (Günata et al., 1988; Maicas and Mateo, 2005). These aroma contributing compounds can be released from their sugar-bound form either through acidic or enzymatic hydrolysis.

In general, the aroma compounds are conjugated in the first instance to glucose as β-D-glucopyranosides or form more complex disaccharides with glucose being further conjugated with a second sugar unit of α-L-arabinofuranose, α-L-rhamnopyranose, β-D-xylopyranose, or β-D-apiofuranose (Günata et al., 1988). The hydrolysis of monoglucosides only requires the action of a β-glucosidase, while hydrolysis of disaccharide glycosides requires two enzymes acting sequentially, where an appropriate *exo*-glycosidase removes the outermost sugar molecule followed by a β-glucosidase to remove the remaining glucose. Therefore, specific glycosidases are needed to release the aroma compounds from the sugars and *O. oeni* has been shown to possess these activities (Bartowsky and Borneman, 2011; D'Incecco et al., 2004).

Many microorganisms possess glycosidases, including filamentous fungi, yeasts and bacteria. *Saccharomyces cerevisiae*, the main yeast involved in AF, does not have many glycosidases, thus most of the glycosides remain intact in wine (Matthews et al., 2004). Even though it has been known that bacteria have a suite of glycosidases, these have only been studied more recently. Glycosidase activities that can affect wine aroma have been detected in wine strains of *Oenococcus*, *Lactobacillus* and *Pediococcus* (Grimaldi et al., 2000, 2005a, 2005b). Numerous potential glycosidases have been identified in the genome sequence analysis of *O. oeni* strains (Borneman et al., 2010) and a gene coding for a β-glucosidase enzyme has been identified in several LAB (Spano et al., 2005). The first study on the expression of an *O. oeni* β-glucosidase gene (*bgl*) under winemaking conditions was carried out by Olguín et al. (2011), highlighting that ethanol concentration is an important factor, but also wine matrix and *O. oeni* strain.

The β-glucosidase activity in wine LAB, mainly *O. oeni*, was observed in a synthetic media by Guilloux-Benatier et al. (1993) and was further confirmed by Grimaldi et al. (2000), who found readily detectable activity of this enzyme in 11 commercial preparations of *O. oeni*.

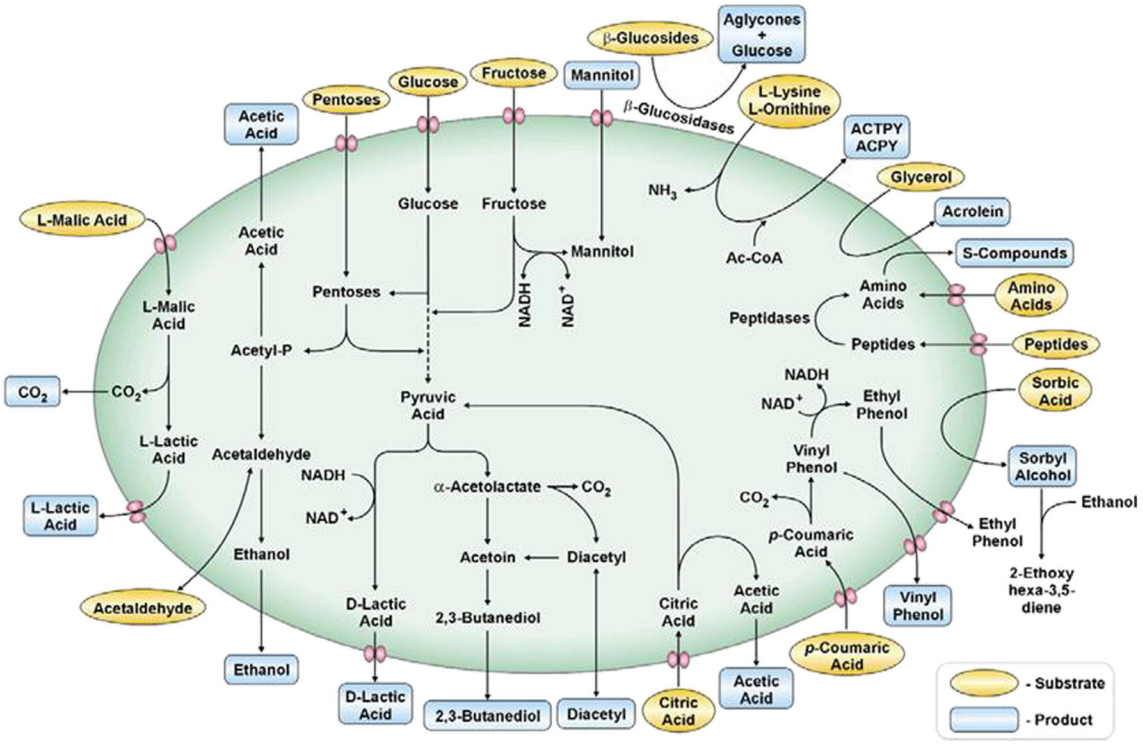

Fig. 1. A schematic representation of the production and modification of flavour-active compounds by malolactic bacterium, *Oenococcus oeni*, reprinted with permission from Swiegers et al. (2005).

The detection of β-glucosidase activities by *O. oeni* under oenological conditions provided important insights on the role of this bacterium to contribute to wine aroma modification during MLF. The β-glucosidase activity on synthetic glycoside substrates is dependent on wine composition (pH, ethanol and residual sugar content) (Grimaldi et al., 2000, 2005b). Investigations of the nature and extent of glycosidase activity of 22 *O. oeni* strains demonstrated that strains do not necessarily hydrolyse all substrates tested, but rather were grouped according to substrate specificity (Grimaldi et al., 2005b). This study also examined the influence of potential inhibitory parameters such as pH, temperature and selected wine components (ethanol, glucose and fructose), both singularly and in combination on the activity of five glycosidases. This work represents the most comprehensive investigation of this type in terms of numbers of strains and particularly, substrates and impacting parameters. Variability of glycosidase activity on synthetic substrates within *O. oeni* species have also been reported by other research groups (Bloem et al., 2008; Cappello et al., 2010; Gagné et al., 2011; Hernandez-Orte et al., 2009; Pérez-Martìn et al., 2012).

The degree of release of glycosidically bound aroma compounds appears to be very much strain-related with synthetic as well as natural substrates. Ugliano et al. (2003) described large difference in the extent of hydrolysis of native wine aroma precursors such as linalool, α-terpineol, nerol and geraniol (monoterpenes) by four commercial *O. oeni* strains. Hernandez-Orte et al. (2009) reported different ability of five *O. oeni* strains in the release and formation of volatile compounds in synthetic wine enriched in glycosidic precursors extracted from grapes; strain-variations were the most evident in concentration of compounds such a benzenoids, terpenes and norisoprenoids.

Effect of MLF on glycosylated and free aroma compounds in Tannat wine also resulted in significant *O. oeni* strain-related changes in formation of compounds including 2-ethylphenol, C_{13}-norisoprenoids and terpineols (Boido et al., 2002). In addition, the polysaccharides derived from *O. oeni* were shown to have the capacity to bind to released aroma compounds thus influencing the final wine aroma (Boido et al., 2002). The limitation of the release of free C_{13}-norisoprenoids by the absorption and occlusion phenomena of *O. oeni* polysaccharides was also suggested by Antalick et al. (2012) who observed only a slightly variation of their concentration in red wine following MLF undertaken by different *O. oeni* strains.

As well as *O. oeni, Lactobacillus* and *Pediococcus* strains can affect wine aroma by glycosidase activity. Grimaldi et al. (2005a) investigated the magnitude of β- and α-D-glucopyranosidase activities, assessed in several strains of these two genera, and their response to the physicochemical winemaking parameters (ethanol, sugars, temperature and pH) demonstrating that they varied in a strain-dependent manner. Spano et al. (2005) reported that the expression of the β-glucosidase gene in *Lb. plantarum* is regulated by abiotic stresses such as temperature, ethanol and pH. Olguín et al. (2011) deduced that the *bgl* gene expression in *O. oeni* may be induced under certain growth conditions (e.g. moderate ethanol concentration). Nevertheless, the relationship between β-glucosidase activity and gene expression in these wine LAB is still unclear and more investigations are needed.

Michlmayr et al. (2010b) identified an intracellular enzyme from *Lb. brevis* which displayed functional similarities to a β-glucosidase previously isolated from *Le. mesenteroides* (Gueguen et al., 1997). Based on the protein sequence of the enzyme from *Lb. brevis* a similar sequence in the genome of *O. oeni* ATCC BAA-1163 was identified. Further, the heterologous expression of this enzyme in *E. coli* and its initial characterization has been described (Michlmayr et al., 2010a). Finally, the glycosidase activities of several wine related species under different growth conditions have been reported (Matthews et al., 2006), including the glycosidic activity of the newly described species *O. kitaharae* (Endo and Okada, 2006).

The effects of glycosidase activity on aroma compounds among different wine LAB species were investigated by several research groups. Michlmayr et al. (2012) reported that *O. oeni* glycosidase treatment in wine produced significantly higher linalool and citronellol + nerol content than *Lb. brevis* glycosidase treatment. Differences in C_{13}-norisoprenoides and monoterpenes concentration were observed among wine bacteria species (*O. oeni*, *Lb. brevis* and *Lb. casei*) when analyzing the volatile composition of synthetic wine containing glycosidic precursors extracted from non-floral grapes post MLF (Hernandez-Orte

et al., 2009). Notably, among monoterpenes, linalool and linalool acetate, the concentration was half and four-fold lower in wine fermented by one *Lb. brevis* strain than wines fermented by three *O. oeni* and one *Lb. casei* strain, respectively. The γ-butyrolactone concentration in Tempranillo wine fermented with two *Lb. plantarum* strains was much lower (~50%) with respect to the same wine fermented with two *O. oeni* strains (Pozo-Bayon et al., 2005).

The importance of wine LAB interaction with wood during MLF in wine has been demonstrated with the conversion of glycoconjugated oak precursors to vanillin by bacterial glycosidases (Bloem et al., 2007). As well, Gagné et al. (2011) analyzed the release of volatile compounds by 15 *O. oeni* strains from untoasted or toasted oak wood extracts and demonstrated the release of whisky-lactone, phenolic aldehydes (including vanillin) and phenylketones. Interestingly, the capability of this bacterium to hydrolysate glycoconjugate aroma precursors is strain-dependent and variable depending on the substrate. The practical relevance of this study highlights the capability of *O. oeni* to release volatile oak-derived compounds demonstrating that when MLF is conducted in barrels, it can augment oak characters in wine.

The recent increasing investigations on the role of bacterial glycosidases on wine aroma have improved the understanding of the mechanisms for the generation of various sensory properties (e.g. floral and flowery notes) from grape glycosides. Moreover, the use in these studies of different species and strains allowed to evaluate their specific impact on flavour formation, correlating the glycosidase activities and released molecules. Hence, the modulation of the level of glycosides in wine through the management of MLF appears to be possible. Nevertheless, because the great variability of viticulture and winemaking factors that affect the final aroma nuances further investigations should better clarify the effective contribution of LAB during the winemaking on the aroma formation related to the presence of specific glycoside precursors.

4.2. Esterases

Esterases are defined as enzymes that catalyse the hydrolysis of esters of organic acids, regulating the equilibrium between esters and free acids. Esters are an important group of volatile compounds that can contribute to wine flavour and changes in their concentration have the potential to influence wine quality (Swiegers et al., 2005). They are responsible for the desirable, fruity aroma of young wines (Lambrechts and Pretorius, 2000), although, as with many compounds, they can also have a detrimental effect on wine aroma when present in excessive concentrations. The accumulation of esters in wine during fermentation is known to be the result of the balance of the enzymatic synthesis and hydrolysis reactions involving esterases (EC 3.1.1.1) and synthesis reactions involving alcohol acetyltransferases (EC 2.3.1.84) (Matthews et al., 2007).

The esterases of wine yeasts have been extensively studied (Sumby et al., 2010) and only in the last decade have wine LAB esterases and their effects on wine aroma been more extensively investigated. The importance of bacterial esterases on changing the concentration of individual esters during MLF have been demonstrated; increases in ethyl acetate, isoamyl acetate, and ethyl lactate levels have been observed following MLF in numerous studies (Delaquis et al., 2000; Gambaro et al., 2001; Maicas et al., 1999). Like the esterases of dairy bacteria, those of wine LAB are involved in both the synthesis and hydrolysis of esters (Matthews et al., 2004).

Analysis of esterase activity on synthetic substrates of wine LAB revealed variability at species and strain level (Cappello et al., 2008; Matthews et al., 2007). Matthews et al. (2007) examined the activity of nine strains of *O. oeni*, *Lactobacillus* spp. and *Pediococcus* spp. under various wine conditions; strains were found to have greater activity towards short-chained esters (C_2–C_8) compared with long-chained esters (C_{10}–C_{18}) and these different substrate specificities have the potential to produce important aroma compounds derived from fatty acids, higher alcohols and organic acids. Pérez-Martín et al. (2013) found great variability of esterase activity among 243 wine LAB strains isolated from wines of different red grape varieties. The majority of strains of all genera displayed a trend to more readily hydrolyse ester substrates from C_8 and longer.

By and large, ethyl fatty acid esters tend to increase post MLF rather than acetate esters. Trials in Aglianico wine reaffirmed other observations that final C_4-C_8 ethyl fatty acid ester concentration was dependent on the *O. oeni* strain used for MLF (Ugliano and Moio, 2005). Similarly, the content of some ethyl esters has been found significantly different among wines fermented by *O. oeni* and *Lb. plantarum* strains (Lee et al., 2009; Pozo-Bayon et al., 2005).

The extent and diversity of the impacts of MLF on wine chemical and sensory properties are directly influenced by the choice of bacterial strains, pre-MLF pH and wine matrix (Costello et al., 2012). In addition, the ability to increase ethyl esters that contribute to the berry fruit character of red wine (Pineau et al., 2009), such as ethyl butanoate, ethyl hexanoate and ethyl 2-methylbutanoate, was also strain dependent (Costello et al., 2012).

Further evidence of the central role of bacterial esterases on wine aroma modification has been noted by Antalick et al. (2012). Variation of esters involved in fruity aroma in numerous red wines following MLF was confirmed in sensory studies (Antalick et al., 2012; Costello et al., 2012). Metabolism by *O. oeni* of sulphur-containing compounds has also been linked with cystathionine β/γ-lyase activity (Knoll et al., 2011; Pripis-Nicolau et al., 2004). Furthermore, esterases appear to be associated with the metabolism of the sulphur-containing compounds (Antalick et al., 2012).

There are less data on the esterase activity of *Pediococcus* compared to *Lactobacillus* and *Lactococcus*. Esterase activity with a greater affinity for acetate substrate was observed in dairy *P. pentosaceus* strains but not *P. acidilactici* (Bhowmik and Marth, 1989). The limited number of studies of wine *Pediococcus* strains suggest variable esterase activity within this genus; one study found four of five *P. parvulus* strains exhibited activity (Davis et al., 1988), but another study found only one of 11 *Pediococcus* strains examined displaying significant esterase activity, compared to the *Lactobacillus* and *O. oeni* strains (Matthews et al., 2006).

Ester synthesis by dairy bacteria in an aqueous environment is thought to be mainly due to a transferase reaction (alcoholysis), in which the fatty acyl group of a glyceride is transferred to an alcohol (Liu et al., 2003). This mechanism is particularly relevant to wine, not only because of the aqueous environment, but also because higher ethanol availability increases transferase activity (Holland et al., 2005). In addition, recently alcohol acyltransferase activity has been identified in *O. oeni* and *Lb. plantarum* as a mechanism to increase ester concentrations and subsequently impact on the fruity aromas of red wine (Costello et al., 2013).

In silico analysis performed on the genome of *O. oeni* strain PSU-1, showed that there are at least four potential esterase genes in *O. oeni*. These genes are most closely related to *Est*A from *Lc. lactis* subsp. *lactis* (B1014), *Est*B from *Lb. casei* and *Est*C from *Lb. casei*. A putative acyltransferase with homology to *EstC* from *Lb. casei* was also identified (Sumby et al., 2010).

Studies performed by Mtshali et al. (2010) on wine *Lactobacillus* strains, isolated from South African grape and wine samples, demonstrated that 60% of tested strains possessed genes coding for esterases, although enzyme activity was not measured in any of these strains. This suggests that strains within this genius may also be involved in ester synthesis and / or hydrolysis and contribute to wine aroma and flavour (Liu, 2002; Matthews et al., 2004). In addition, *est*A gene has been detected in *Le. mesenteroides* subsp. *mesenteroides* isolates originating from South African grape and wine samples (Mtshali et al., 2012).

Evidence of esterase activity in *O. oeni* is demonstrated through a number of reports showing changes in the volatile aroma composition

of wines after MLF with *O. oeni* (Costello et al., 2012). Sumby et al. (2013a) cloned and characterized two related intracellular esterases from *O. oeni* and *Lb. hilgardii* under wine-like conditions. Both esterases were stable and retained activity under conditions that would be encountered in wine, having the potential to reduce short-chain ethyl esters such as ethyl acetate. Subsequently, Sumby et al. (2013b) demonstrated that two purified esterases (EstA2 and EstB28) of *O. oeni* have dual activity (synthetize and hydrolyze) on ethyl butanoate, ethyl hexanoate and ethyl octanoate to varying degrees. Moreover, it has been demonstrated that esters are either hydrolysed or synthesized in a strain-dependent manner, despite that the final ester balance is also dependant on the component of wine that may be affecting this activity tested. Therefore, wine style composition and the desired outcome of the wine are important criteria for the correct choice of *O. oeni* strain for MLF.

Recently, the first arylesterase was described in a wine LAB, *Lb. plantarum* WCFSI (Esteban-Torres et al., 2014). Initially, the gene lp_1002 encoding this putative lipase/esterase was cloned from *Lb. plantarum* WCFSI and overexpressed in *E. coli* BL21(DE3). The expressed Lp_1002 showed high hydrolytic activity on phenyl acetate and also the ability to hydrolyze most of the esters assayed, including relevant wine aroma compounds. Importantly, this enzyme exhibited hydrolytic activity at winemaking conditions, although optimal catalytic activity was observed at 40 °C and pH 5–7.

4.3. Enzymes related to citrate metabolism

In wine, citrate utilization leads to the production of compounds, such as diacetyl, acetoin, butanediol and acetate which are important to wine aroma and complexity (Bartowsky and Henschke, 2004; Olguìn et al., 2009). Diacetyl is the most important aroma compound from LAB and its production and modulation during MLF has been well documented (Bartowsky and Henschke, 2004). However, this compound can also be regarded as an off-flavour when present in wine at higher concentrations.

Citrate lyase is a key enzyme of citrate metabolism since it cleaves citrate into acetate and oxaloacetate, and this latter is decarboxylated to pyruvate by an oxaloacetate decarboxylase, leading onto the formation of diacetyl, which can be further metabolized through to 2,3-butanediol. The gene encoding citrate lyase enzyme (*cit*E) has been investigated in various wine LAB species (Mills et al., 2005). Olguìn et al. (2009) have investigated the expression of this gene in *O. oeni* strain PSU-1 to further understand the role of this metabolic pathway in the adaptation to wine environment and its impact on organoleptic qualities. Interestingly, an inter-strain comparison of the transcriptional levels of this gene and other two genes involved in citrate metabolism (acetate kinase *ack*A, α-acetolactate decarboxylase gene *als*D) revealed that strains can have different metabolic traits (Olguìn et al., 2009). Thus *O. oeni* strains have different potential to impart desirable aromatic features related to citrate catabolism, such as buttery aroma, in wine. The strain-dependent aroma impact could be great with the occurrence of lactobacilli and *Leuconostoc* in wine. Interestingly, recently it has been reported that some strains of *Lb. plantarum*, *Lb. hilgardii* and other *Lactobacillus spp.*, as well as all *Le. mesenteroides*, did not possess citrate lyase complex genes (Mtshali et al., 2010, 2012). It is feasible that wines that undergo MLF dominated by strains without citrate lyase or with low transcriptional levels of this gene will have very low diacetyl concentrations. Further studies are needed to elucidate the link between expression levels of citrate metabolism genes and the formation of related flavour compounds during winemaking.

5. Other enzymes of enological interest

Additional enzyme activities, which are important for aroma and flavour development in wine, include those which act upon amino acids and proteins.

Proteases have been studied extensively in dairy bacteria (McSweeney and Sousa, 2000). By comparison to dairy LAB, little work has focused on the proteolytic activity of wine-related strains. Two exoproteases identified from *O. oeni* strains isolated in Argentina (Rollan et al., 1993, 1995) were able to release amino acids from red and white wines. Their expression was detected at different stages during growth (Rollan et al., 1993) and was induced by nutrient and energy deprivation (Rollan et al., 1998). The presence of a protease (*prt*P) has been demonstrated in several wine lactobacilli and further studies have shown it to be distributed across numerous species of wine bacteria. Among the lactobacilli examined, *prt*P was present mainly in *Lb. plantarum*, *Lb. paraplantarum* and *Lb. hilgardii* (Mtshali et al., 2010). The presence of *prt*P in wine bacteria has implications on the genetic capability of these strains to release amino acids from peptides which could, in addition to bacteria growth, influence development of wine flavour, as it has been reported for dairy bacteria (Hynes et al., 2003). In silico analysis of *O. oeni* PSU-1 strain did not reveal any Prt-P-like cell-envelope proteases and no peptidase genes with a well-supported signal peptide were identified. It remains to be determined which, if any, of the PSU-1 proteases correspond to the activities previously examined (Rollan et al., 1993; Rollan et al., 1995; Rollan et al., 1998).

Some peptidases are able to release sulphur-containing amino acids from peptide degradation (Kunji et al., 1996). Pripis-Nicolau et al. (2004) were the first to demonstrate the ability of wine LAB to metabolize methionine to produce volatile sulphur compounds during MLF. The wine strains examined were able to degrade methionine to methanethiol, dimethyl disulphide and 3-(methylsulphanyl)-propan-1-ol (also known as methionol) and 3-(methylsulphanyl)-propionic acid. 3-(methylsulphanyl)-propan-1-ol is quantitatively the most significant volatile sulphur compound in wines; it is known to contribute to the aromatic complexity of the wine, but at high concentrations also contributes to reductive wine flavour. Methanethiol and 3-(methylsulphanyl)-propan-1-ol are characterized by putrid faecal-like aroma and cooked cabbage descriptors, respectively, while 3-(methylsulphanyl)-propionic acid has chocolate and roasted aromas. However, at low concentrations, these compounds may well contribute to wine aroma complexity, rather specifically off-flavour.

Antalick et al. (2012) reported that MLF affects the content of 3-sufanylhexanol and dimethyl sulfide (DMS), very odorant sulphur-containing compounds that contribute to the fruity aroma in red wine. Notably, the increase of the former associated with an increase of ethyl fatty acid esters which led to significant enhancement of fruity aroma. Hence, wine bacteria seem to influence the intensity of this note in red wine by modulation of 3-sufanylhexanol. It has been proposed that the increase of this compound during MLF could be derived by cleavage of cysteinylated grape precursors by cystathionine β-lyase of *O. oeni*. Similarly, wine bacteria should be able to synthetize DMS, although at low levels, contributing in association with the increase of ethyl fatty acid esters, to increase or enhance the fruity note of wine following MLF. The probable pathways in wine bacteria involved in the synthesis of DMS could be the catabolism of amino acid and dimethyl sulfoxide reduction by reductase activities, but this has yet to be confirmed. Moreover, the production of these volatile sulphur compounds are also strain dependent as well as genus dependent, with *O. oeni* having a higher capacity for producing these compounds, compared to *Lactobacillus* species (Pripis-Nicolau et al., 2004).

There are various factors that influence the production of these volatile sulphur compounds, including the presence of methionine as precursor and the growth phase of the bacteria. Vallet et al. (2008) found that production of methionol occurred during the exponential growth phase of the wine bacteria, while the production of 3-(methylthio) propionic acid was during both the exponential and stationary growth phases. These authors also found that methionol and 3-(methylthio) propionic acid production only occurs in the presence of methional, which implies that this compound is an important precursor in their production.

Furthermore, an enzyme that degrades sulphur-containing amino acids was cloned and characterized, from two *O. oeni* strains (Knoll et al., 2011). This enzyme has been shown to catalyse several reactions that produce different compounds, such as cysteine, α-ketobutyrate, ammonia, homocysteine, pyruvate and hydrogen sulfide. Furthermore, the ability to demethiolate methionine into methanethiol, an unfavourable volatile sulphur compound in terms of wine aroma, was observed. This suggests that *O. oeni* appears to play a minor role in the production of volatile sulphur compounds during the vinification process.

The *met*K and *met*B/*met*C genes that encode enzymes involved in the conversion of methionine to diverse volatile sulphur compounds have been detected in grape and wine isolates of *Le. mesenteroides* (Mtshali et al., 2012) and in *O. oeni* strains (Cappello et al., 2014).

In addition to these metabolites described thus far, there are further aroma contributing metabolites produced by different metabolic pathways during the fermentation (Pripis-Nicolau et al., 2004; Swiegers et al., 2005).

Phenolic acids (substituted cinnamic acids) are important aromatic acids and natural constituents of plant cell walls. These acids (particularly ferulic, *p*-coumaric and caffeic acids) are generally esterified with tartaric acid (for example, in grape must and wine) and can be released as free acids during winemaking by some cinnamoyl esterase activities (Dugelay et al., 1993).

Most often, free phenolic acids are metabolized by different microorganisms into 4-vinyl derivatives and then are eventually reduced into 4-ethyl derivatives. Some of these volatile phenols, particularly vinyl and ethyl guaiacol (generated from ferulic acid), are useful aromatic chemicals or contribute naturally to aroma in wine (Ribéreau-Gayon et al., 2006). Other volatile phenols, such as ethyl and vinyl phenols (from *p*-coumaric acid), are most often considered phenolic off-flavours and are responsible for alterations in organoleptic properties. The production of volatile phenols in wine is usually associated with *Dekkera bruxellensis* spoilage (Brett character) (Ribéreau-Gayon et al., 2006), however some *Lactobacillus* species have been shown to be able to produce these compounds, albeit in lower quantities (Couto et al., 2006; Knockaert et al., 2012).

The conversion of phenolic compounds to vanillin, a powerful aroma compound, by wine bacteria has been demonstrated by Bloem et al. (2007). *O. oeni*, *Lactobacillus* spp. and *Pediococcus* spp. are able to produce vanillin from ferulic acid, albeit at low yield. Ferulic acid catabolism by *Lactobacillus* spp. leads to the formation of traces of vanillin and greater amounts of 4-vinylguaiacol, while *O. oeni* could transform 4-vinylguaiacol to vanillin by a decarboxylation pathway.

Mtshali (2011) have demonstrated the existence of a putative *pad* gene encoding phenolic acid decarboxylase (PAD) in two *O. oeni* strains (out of 27 strains tested) of South African wine origin. This is the first study to report on the presence of a putative (novel) *pad* gene in *O. oeni* strains. Sequence verification and comparative sequence analysis revealed that the *pad* gene from the two strains share 74–80% identity to other closely related species of the genera *Lactobacillus* and *Pediococcus*. This gene was not found in any of the three fully sequenced *O. oeni* strains (PSU-1, ATCC BAA 1163 and AWRI B429). Its presence in only two strains also reinforces the notion that this pathway is rare in *O. oeni* and is not essential for *O. oeni* survival. However, this study should be followed by functional enzymatic characterization in order to assess whether the genetic potential translates to functional enzymatic activity, particularly with regards to aroma production under oenological conditions. In another study, using PCR enzyme-specific primers, 120 South African wine *Lactobacillus* strains were screened (including *Lb. plantarum*, *Lb. hilgardii*, *Lb. brevis*, *Lb. pentosus*, *Lb. paracasei*, *Lb. sakei*, *Lb. florum* and *Lb. paraplantarum*) for the *pad* gene; more than 70% strains possessed this gene (Mtshali et al., 2010). The presence and absence of gene encoding for PAD in *Lb. plantarum* and *O. oeni* strains, respectively, isolated from South African wine was reported also by Lerm et al. (2011).

6. Contribution of wine lactic acid bacteria to sensory properties of wine

Investigations in the 1980s of the effects of MLF on sensory wine quality indicated that malolactic activity of bacteria can alter significantly the sensory characteristic of wine (Davis et al., 1988). Through comparisons of wine that did or did not undergo MLF, it was possible to determine that the contribution of bacteria to the wine aroma may be species and/or strain specific (Bartowsky and Borneman, 2011; Sumby et al., 2014). Further investigations confirmed the importance of bacterial activity during MLF in enhancing wine sensory quality and new insights have been provided correlating volatile wine composition with sensory panel test results (Bartowsky et al., 2002; Bartowsky and Henschke, 1995; Costello et al., 2012; Liu, 2002; Swiegers et al., 2005).

First of all, the microbial stability due to MLF activity has indirect consequences on sensory properties of wine because the depletion of L.-malic acid, citric acid, acetaldehyde and the reduction of the availability of essential nutrients inhibit the growth of potential fastidious microorganisms. The importance of arginine catabolism by *O. oeni* at the end of MLF to reduce the risk of spoilage bacteria was described by Tonon and Lonvaud-Funel (2000). Since arginine and other amino acids promote the anaerobic growth of *Dekkera bruxellensis* (Blomqvist et al., 2012), their depletion by the growth of wine LAB during MLF could have inhibitory effects on spoilage yeasts. This fact is congruent with Gerbaux et al. (2009) that observed as wines in which the MLF did not occur showed increase of volatile phenols. Although lactobacilli such as *L. hilgardii* and *L. buchneri*, that include spoilage strains, seem to have lower nutrient requirements than *O. oeni* (Terrade and Mira de Orduña, 2009), the prior depletion of some essential amino acids due the activity of the latter, possibly added as a starter culture, could reduce the risk of subsequent contamination avoiding negative sensory effects.

The contribution of specific starter bacterial cultures to wine sensory attributes, can affect flavour formation depending on wine parameters (i.e. temperature, pH and ethanol) and precursors present in the wine (Costello et al., 2012; Delaquis et al., 2000; Malherbe et al., 2012). The careful selection of strains, for MLF, can thus be used to develop specific wine styles and consumer preferences.

Malolactic fermentation is conducted worldwide in a range of well-known and lesser known red and white grape varieties. From these studies it is evident that MLF influences wine sensory attributes and that these changes are dependent on numerous factors, in particular, grape variety and bacterial strain. This is illustrated in the following examples and in Table 2.

An early study which investigated the sensory impact of MLF in Chardonnay showed that four *O. oeni* (formerly *Leuconostoc oenos*) strains produced wines with different aroma and flavour profiles (Avedovech et al., 1992).

In this study, MLF did not affect the intensity of many fruity and floral aromas, however decreased the intensity of several vegetative aromas such as the urine-like or cooked garlic of 1-octanol and 1-octen-3-ol, while producing buttery, yeasty, oaky and earthy aromas in Chardonnay. In the investigation of Sauvageot and Vivier (1997) Chardonnay wines were perceived higher in hazelnut, fresh bread, and dried fruit aromas due to MLF with two *O. oeni* strains, while the absence of the MLF retained specific aromas such as apple and grapefruit-orange. In another study using a white Sauvignon wine partial MLF conducted by two *O. oeni* strains resulted in a wine with greater complexity, increased buttery, spiced, roasted, vanilla and smoked notes, while a decrease in the intensity of grape variety (de Revel et al., 1999).

In addition, MLF conducted in barrel with two *O. oeni* strains resulted in wines with high concentrations of wood-related compounds (lactones, eugenols and vanilla) (de Revel et al., 1999).

Recently, the sensory effects of MLF were also analyzed in white wines such as Albariño and Caiño (Juega et al., 2013), where MLF decreased the intensity of acidity in both wines. In the Caiño wine

aromatic herb aroma decreased, while increased the intensity of honey. This study is particularly of interest as autochthonous strains of *P. damnosus* and *O. oeni* were used to induce MLF. Thus, even though not a large number of white grape varieties are necessarily put through MLF, those that are, the resultant wines are most often more complex.

The contribution of MLF on sensory quality of red wines is well investigated. Sauvageot and Vivier (1997) showed in a Pinot noir wine, that after MLF the wine lost part of its berry notes in favour of animal and vegetable notes, while the absence of MLF retained specific aromas such as strawberry-raspberry. Improvement of sensory quality of Frankovka wine, a Croatian red wine, MLF lowered acidity and resulted in more complexity, rounded and matured aromas (Herjavec and Tupajić, 1998). Several studies have opposing observations; for example a study in Tannat wine, MLF led to a significant decrease of berry fruit and fresh vegetative descriptors (Gambaro et al., 2001), while in a Cabernet Sauvignon wine demonstrated MLF had a significant effect on dark fruit aroma, viscosity and astringency (Costello et al., 2012). Additionally, the magnitude and divergence of these properties and related chemical properties varied with choice of bacterial strain, pre-MLF pH and the interaction of these variables (Costello et al., 2012). More recent studies have shown that fruity characters of red wine can be influenced by MLF and is *O. oeni* strain dependent. Changes in sensory properties of Merlot and Cabernet Sauvignon wines were described by Antalick et al. (2012). In their study, the fruity note was the most altered by bacterial activity although the variation perceived of the fruity note by the sensory panel between wine with and without MLF was higher than for lactic, vegetal and smoked/toasted descriptors. Notably, smoked/toasted attributes were more affected than the lactic aroma and the authors proposed that the increase of several perceived aromas is linked to the decrease of fruity intensity. The relationships between consumer liking and sensory attributes of Pinotage wines inoculated with different *O. oeni* starter cultures was reported by Malherbe et al. (2013). Significant strain-specific variations were observed in the degradation of citric acid and production of lactic acid during MLF and consequently, compounds directly and indirectly related to citric acid metabolism, such as diacetyl, acetic acid, acetoin, and ethyl lactate, were also affected depending on the bacterial strain used for MLF (Malherbe et al., 2013).

A recent contribution on the impact of *Pediococcus* on Pinot noir wine confirmed that this bacterium can affect positively sensory properties indicating that its growth does not always result in spoiled wine (Strickland et al., 2016).

The effects of MLF on red wines fermented or aging in presence of oak chips or in barrels were described through chemical and sensory analysis and evidenced the importance of bacterial metabolism on modifying the aroma. Spanish wines in contact with oak chips during MLF was characterized by enhanced oak related attributes and lower intensity of fruity and flower notes than the same wine in contact with oak chips during AF (García-Carpintero et al., 2012, 2014). Izquierdo-Cañas et al. (2016) reported that Cabernet franc wine that underwent MLF in barrel was characterized by more aromatic intensity than MLF conducted in tank. It has been observed that such sensory modifications due to contact with wood were linked to the increase of higher furanic compounds responsible for almond, caramel and toasted aromas. These studies, aside that of de Revel et al. (1999), confirm the importance of LAB strain in the release of oak wood volatile compounds suggesting the potential ability of *O. oeni* to modify the woody aroma of wine (Bloem et al., 2008).

Since wine matrix components can affect the odorant volatility, flavour release and overall perceived aroma intensity and quality (Villamor and Ross, 2013), the use of semi-synthetic substrate instead of a natural matrix can facilitate the assignment of correspondence between volatiles and odors nuances. Relevant sensory effect linked to the action of different bacteria (*O. oeni*, *Lb. brevis* and *Lb. casei*) on grape precursors contained in a model wine was described by Hernandez-Orte et al. (2009). The presence of bacteria in the medium had intense effects on some important aromatic nuances; sensory descriptors such as vegetative, spicy, violet and oxidized were linked to the direct action of bacteria in the pool of flavour precursors. Interestingly, these aroma nuances presented higher scores in samples containing bacteria and precursors than in samples containing only bacteria. As seen in other studies, selection of bacterial strain plays an important role in release of aroma compounds and ultimate effect on the wine (Hernandez-Orte et al., 2009).

Previous investigations observed that in wine the interaction of bacteria with yeasts and other parameters rather than species- or strain-specificity of bacteria are more important on conferring sensory attributes to wine. Delaquis et al. (2000) highlighted significant differences in several sensory attributes of Chancellor wines due to the combination of two-way interactions (yeast-bacteria, yeast-temperature, bacteria-temperature) and three-way interaction (yeast-bacteria-temperature). The effect of LAB inoculation time on the metabolic profile of wine were described in several studies (Antalick et al., 2013; Izquierdo Cañas et al., 2012, 2015). It has been demonstrated that the impact of yeast/LAB co-inoculation can be significant from a sensory point of view under winery conditions. Nevertheless, how the contribution of a certain bacterial strain, used in combination with a certain yeast strain, is not so still clear. In fact, under certain conditions the presence of many variables, in particular the co-presence of bacteria and yeasts make it more difficult to link the specific strain to chemical and sensory properties of the resulting wine. Further studies are necessary to understand the metabolic role of a specific bacterial strain used in combination with a specific yeast and to ultimately predict their effect on the flavour composition of wine. The recent recognition of the positive contribution of non-*Saccharomyces* yeasts to enhance the wine aroma complexity (Padilla et al., 2016) and their use as starter cultures in combination with wine LAB should be considered in these further investigations. The wider spectrum of yeast-bacteria combinations accounting non-*Saccharomyces* yeasts could enable a better modulation of wine aroma.

Table 2
Studies that evaluated the effects of MLF carried out by the inoculation of selected LAB cultures on sensory aroma profile of wine.

Wine	ML cultures	References
Chardonnay	Four commercial strains of *O. oeni*	Avedovech et al. (1992)
Chardonnay and Pinot Noir	Two commercial strains of *O. oeni*	Sauvageot and Vivier (1997)
Frankovka	Two commercial strains of *O. oeni*	Herjavec and Tupajić (1998)
Sauvignon	Two commercial strains of *O. oeni*	de Revel et al. (1999)
Chancellor	Two commercial strains of *O. oeni*	Delaquis et al. (2000)
Tannat	A commercial culture of *O. oeni*	Gambaro et al. (2001)
Cabernet Sauvignon	Three commercial strains of *O. oeni*	Costello et al. (2012)
Merlot and Cabernet Sauvignon	Eleven commercial strains of *O. oeni*	Antalick et al. (2012)
Morava Agria	A commercial strain of *O. oeni*	García-Carpintero et al. (2012)
Pinotage	Four commercial cultures of *O. oeni*	Malherbe et al. (2013)
Albariño and Caiño	Three cultures: a commercial strain of *O. oeni* and two strains of *P. damnosus*	Juega et al. (2013)
Bobal	A commercial strain of *O. oeni*	García-Carpintero et al. (2014)
Cabernet Franc	Two commercial strains of *O. oeni*	Izquierdo-Cañas et al. (2016)
Pinot Noir	Six cultures: four strains of *P. parvulus*, a strain of *P. damnosus* and a strain of *P. inopinatus*	Strickland et al. (2016)

Therefore, the aroma and flavour profile of wine is the result of several variables during the vinification, but the choice of the microorganism is very important. Even though it is well known the role that *S. cerevisiae*, and now several non-*Saccharomyces* species, have in affecting wine aroma, flavour, colour and chemical complexity, it is evident that the influence of LAB and MLF on wine aroma and flavour is considerable. Thus, in order to provide an additional tool to produce quality wines, exploiting the possibilities of bacterial diversity to improve the aroma and flavour profile of wine is essential.

7. Conclusions

In the last 20 years, progress in understanding wine LAB, their biology, ecology and impact on aroma compounds has been considerable. It is certain that such knowledge has significantly contributed to the improvement of wine quality through the flow of information from the scientist to winemaker. The use of selected starter cultures for MLF induction is the emblematic step of this knowledge transfer. Advances in the understanding of metabolic mechanisms of LAB involved in the transformation of grape precursors into potent flavour-active compounds has emphasized the important role that bacteria play in developing the final wine aroma profile.

This review has emphasized the variability of bacterial enzyme activity including glycosidase, β-glycosidase, esterases, proteases and other enzymes that can generate a wide spectrum of wine sensory significant compounds. In particular, it has been shown how inter- and intra-specific differences of metabolic traits can influence the genesis of aroma compounds and their stability in wine. With these findings the awareness of the importance of the use of selected bacteria strain for MLF has increased. In particular, recent investigations that reported the release of important flavours such as ethyl ester, volatile sulphur and glycosidically bound aroma compounds is species- or strain-dependent highlights the significance of choice of a specific LAB strain to conduct MLF to achieve the desired wine style.

As genetically modified organisms (GMOs) are not currently permitted, or widely accepted by the consumer, continued research into the biodiversity within wine LAB will assist in identifying unique strains to be used to modulate wine flavour and aroma compounds. The opportunity to use selected LAB strains, particularly *O. oeni*, to contribute to the sensory properties of wine is increasingly becoming a pertinent component influencing the winemaker's decision to conduct MLF in red and white wines. Therefore, ongoing studies are aimed at to understand how the genes encoding these enzymes are regulated under winemaking conditions, and also to evaluate their expression level and enzyme activity under different winemaking parameters. Moreover, these microorganisms may serve as a source for the preparation of enzyme extracts that are better able to function under the harsh and changing environmental conditions of wine fermentation.

Acknowledgements

MSC is supported by the Apulia region grant: CNR for the Midday-Projet CISIA (Integrated Search I for Sustainability and Innovation of Made in Italy Agro-food) (CNR Application reads 191/2009, Tremonti). EJB is supported by the Australian grape growers and winemakers through their investment body Wine Australia with matching funds from the Australian Government (Wine Australia AWR 1303). The Australian Wine Research Institute is a member of the Wine Innovation Cluster in Adelaide

References

Antalick, G., Perello, M.C., de Revel, G., 2012. Characterization of fruity aroma modifications in red wines during malolactic fermentation. J. Agric. Food Chem. 60, 12371–12383.

Antalick, G., Perello, M.C., de Revel, G., 2013. Co-inoculation with yeast and LAB under winery conditions: modification of the aromatic profile of Merlot wines. S. Afr. J. Enol. Viticult. 34, 223–232.

Avedovech, R.M., Mcdaniel, M.R., Watson, B.T., Sandine, W.E., 1992. An evaluation of combinations of wine yeast and *Leuconostoc oenos* strains in malolactic fermentation of Chardonnay wine. Am. J. Enol. Vitic. 43, 253–260.

Badotti, F., Moreira, A.P.B., Tonon, L.A.C., de Lucena, B.T.L., Gomes, F.D.O., Kruger, R., Thompson, C.C., de Morais, M.A., Rosa, C.A., Thompson, F.L., 2014. *Oenococcus alcoholitolerans* sp. nov., a lactic acid bacteria isolated from cachaça and ethanol fermentation processes. Antonie Van Leeuwenhoek 106, 1259–1267.

Bae, S., Fleet, G.H., Heard, G.M., 2006. Lactic acid bacteria associated with wine grapes from several Australian vineyards. J. Appl. Microbiol. 100, 712–727.

Bartowsky, E.J., 2005. *Oenococcus oeni* and malolactic fermentation moving into the molecular arena. Aust. J. Grape Wine Res. 11, 174–187.

Bartowsky, E.J., Borneman, A.R., 2011. Genomic variations of *Oenococcus oeni* strains and the potential to impact on malolactic fermentation and aroma compounds in wine. Appl. Microbiol. Biotechnol. 92, 441–447.

Bartowsky, E.J., Henschke, P.A., 1995. Malolactic fermentation and wine flavour. Aust. Grapegrow. Winemak. 378, 83–94.

Bartowsky, E.J., Henschke, P.A., 1999. Use of a polymerase chain reaction for specific detection of the malolactic fermentation bacterium *Oenococcus oeni* (formerly *Leuconostoc oenos*) in grape juice and wine samples. Aust. J. Grape Wine Res. 5, 39–44.

Bartowsky, E.J., Henschke, P.A., 2004. The 'buttery' attribute of wine diacetyl desirability, spoilage and beyond. Int. J. Food Microbiol. 96, 235–252.

Bartowsky, E.J., Francis, I.L., Bellon, J.R., Henschke, P.A., 2002. Is buttery aroma perception in wines predictable from diacetyl concentration? Aust. J. Grape Wine Res. 8, 180–185.

Bartowsky, E.J., McCarthy, J.M., Henschke, P., 2003. Differentiation of Australian wine isolates of *Oenococcus oeni* using random amplified polymorphic DNA (RAPD). Aust. J. Grape Wine Res. 9, 122–126.

Bartowsky, E.J., Costello, P.J., Chambers, P.J., 2015. Emerging trends in the application of malolactic fermentation. Aust. J. Grape Wine Res. 21, 663–669.

Bhowmik, T., Marth, E.H., 1989. Esterolytic activities of *Pediococcus* species. J. Dairy Sci. 72, 2869–2872.

Bilhère, E., Lucas, P.M., Claisse, O., Lonvaud-Funel, A., 2009. Multilocus sequence typing of *Oenococcus oeni*: detection of two subpopulations shaped by intergenic recombination. Appl. Environ. Microbiol. 75, 1291–1300.

Bloem, A., Bertrand, A., Lonvaud-Funel, A., de Revel, G., 2007. Vanillin production from simple phenols by wine-associated lactic acid bacteria. Lett. Appl. Microbiol. 44, 62–67.

Bloem, A., Lonvaud-Funel, A., de Revel, G., 2008. Hydrolysis of glycosidically bound flavour compounds from oak wood by *Oenococcus oeni*. Food Microbiol. 25, 99–104.

Blomqvist, J., Sànchez Nogué, V., Gorwa-Grauslund, M., Passoth, V., 2012. Physiological requirements for growth and competitiveness of *Dekkera bruxellensis* under oxygen limited or anaerobic conditions. Yeast 29, 265–274.

Boido, E., Lloret, A., Medina, K., Carrau, F., Dellacassa, E., 2002. Effect of beta-glycosidase activity of *Oenococcus oeni* on the glycosylated flavor precursors of Tannat wine during malolactic fermentation. J. Agric. Food Chem. 50, 2344–2349.

Bokulich, N.A., Joseph, C.M.L., Allen, G., Benson, A.K., Mills, D.A., 2012. Next-generation sequencing reveals significant bacterial diversity of botrytized wine. PLoS ONE 7, 1–10.

Borneman, A.R., Bartowsky, E.J., McCarthy, J., 2010. Genotypic diversity in *Oenococcus oeni* by high-density microarray comparative genome hybridization and whole genome sequencing. Appl. Microbiol. Biotechnol. 86, 681–691.

Borneman, A.R., McCarthy, J.M., Chambers, P.J., Bartowsky, E.J., 2012a. Comparative analysis of the *Oenococcus oeni* pan genome reveals genetic diversity in industrially-relevant pathways. BMC Genomics 13, 1–13.

Borneman, A.R., McCarthy, J.M., Chambers, P.J., Bartowsky, E.J., 2012b. Functional divergence in the genus *Oenococcus* as predicted by genome sequencing of the newly-described species, *Oenococcus kitaharae*. PLoS ONE 7, 1–10, e29626.

Campbell-Sills, H., El Khoury, M., Favier, M., Romano, A., Biasioli, F., Spano, G., Sherman, D.J., Bouchez, O., Coton, E., Coton, M., Okada, S., Tanaka, N., Dols-Lafargue, M., Lucas, P.M., 2015. Phylogenomic analysis of *Oenococcus oeni* reveals specific domestication of strains to cider and wines. Genome Biol. Evol. 7, 1506–1518.

Capozzi, V., Ladero, V., Beneduce, L., Fernández, M., Álvarez, M.A., Benoit, B., Laurent, B., Grieco, F., Spano, G., 2011. Isolation and characterization of tyramine-producing *Enterococcus faecium* strains from red wine. Food Microbiol. 28, 434–439.

Cappello, M.S., Stefani, D., Grieco, F., Logrieco, A., Zapparoli, G., 2008. Genotyping by amplified fragment length polymorphism and malate metabolism performances of indigenous *Oenococcus oeni* strains isolated from Primitivo wine. Int. J. Food Microbiol. 127, 241–245.

Cappello, M.S., Zapparoli, G., Stefani, D., Logrieco, A., 2010. Molecular and biochemical diversity of *Oenococcus oeni* strains isolated during spontaneous malolactic fermentation of Malvasia Nera wine. Syst. Appl. Microbiol. 33, 461–467.

Cappello, M.S., De Domenico, S., Logrieco, A., Zapparoli, G., 2014. Bio-molecular characterisation of indigenous *Oenococcus oeni* strains from Negroamaro wine. Food Microbiol. 42, 142–148.

Claisse, O., Lonvaud-Funel, A., 2012. Development of a multilocus variable number of tandem repeat typing method for *Oenococcus oeni*. Food Microbiol. 30, 340–347.

Claisse, O., Lonvaud-Funel, A., 2014. Multiplex variable number of tandem repeats for *Oenococcus oeni* and applications. Food Microbiol. 38, 80–86.

Claisse, O., Renouf, V., Lonvaud-Funel, A., 2007. Differentiation of wine lactic acid bacteria species based on RFLP analysis of a partial sequence of *rpoB* gene. J. Microbiol. Methods 69, 387–390.

Cocolin, L., Alessandria, V., Dolci, P., Gorra, R., Rantsiou, K., 2013. Culture independent methods to assess the diversity and dynamics of microbiota during food fermentation. Int. J. Food Microbiol. 167, 29–43.

Costello, P.J., Francis, I.L., Bartowsky, E.J., 2012. Variations in the effect of malolactic fermentation on the chemical and sensory properties of Cabernet Sauvignon wine: interactive influences of *Oenococcus oeni* strain and wine matrix composition. Aust. J. Grape Wine Res. 18, 287–301.

Costello, P.J., Siebert, T.E., Solomon, M.R., Bartowsky, E.J., 2013. Synthesis of fruity ethyl esters by acyl coenzyme A: alcohol acyltransferase and reverse esterase activities in *Oenococcus oeni* and *Lactobacillus plantarum*. J. Appl. Microbiol. 114, 797–806.

Couto, J.A., Campos, F.M., Figueiredo, A.R., Hogg, T.A., 2006. Ability of lactic acid bacteria to produce volatile phenols. Am. J. Enol. Vitic. 57, 166–171.

Davis, C.R., Wibowo, D., Fleet, G.H., Lee, T.H., 1988. Properties of wine lactic acid bacteria: their potential enological significance. Am. Soc. for Enol. Viticult. 39, 137–142.

de Revel, G., Martin, N., Pripis-Nicolau, L., Lonvaud-Funel, A., Bertrand, A., 1999. Contribution to the knowledge of malolactic fermentation influence on wine aroma. J. Agric. Food Chem. 47, 4003–4008.

Delaquis, P., Cliff, M., King, M., Girard, B., Hall, J., Reynolds, A., 2000. Effect of two commercial malolactic cultures on the chemical and sensory properties of Chancellor wines vinified with different yeasts and fermentation temperatures. Am. J. Enol. Vitic. 51, 42–48.

Dicks, L.M.T., Endo, A., 2009. Taxonomic status of lactic acid bacteria in wine and key characteristics to differentiate species. S. Afr. J. Enol. Viticult. 30, 72–90.

D'Incecco, N., Bartowsky, E.J., Kassara, S., Lante, A., Spettoli, P., Henschke, P.A., 2004. Release of glycosidically bound flavor compounds from Chardonnay by *Oenococcus oeni* during malolactic fermentation. Food Microbiol. 21, 257–265.

du Toit, M., Engelbrecht, L., Lerm, E., Krieger-Weber, S., 2011. *Lactobacillus*: the next generation of malolactic fermentation starter cultures an overview. Food Bioprocess Technol. 4, 876–906.

Dugelay, I., Gunata, Z., Sapis, J.C., Baumes, R., Bayonove, C., 1993. Role of cinnamoyl esterase activities from enzyme preparations on the formation of volatile phenols during winemaking. J. Agric. Food Chem. 41, 2092–2096.

Endo, A., Okada, S., 2006. *Oenococcus kitaharae* sp. nov., a non-acidophilic and non-malolactic-fermenting *Oenococcus* isolated from a composting distilled shochu residue. Int. J. Syst. Evol. Bacteriol. 56, 2345–2348.

Esteban-Torres, M., Barcenilla, J.M., Mancheño, J.M., de las Rivas, B., Muñoz, R., 2014. Characterization of a versatile arylesterase from *Lactobacillus plantarum* active on wine esters. J. Agric. Food Chem. 62, 5118–5125.

Gagné, S., Lucas, P.M., Perello, M.C., Claisse, O., Lonvaud-Funel, A., De Revel, G., 2011. Variety and variability of glycosidase activities in an *Oenococcus oeni* strain collection tested with synthetic and natural substrates. J. Appl. Microbiol. 110, 218–228.

Gambaro, A., Boido, E., Zlotejablko, A., Medina, K., Lloret, A., Dellacassa, E., Carrau, F., 2001. Effect of malolactic fermentation on the aroma properties of Tannat wine. Aust. J. Grape Wine Res. 7, 27–32.

García-Carpintero, E.G., Gómez Gallego, M.A., Sánchez-Palomo, E., González Viñas, M.A., 2012. Impact of alternative technique to ageing using oak chips in alcoholic or in malolactic fermentation on volatile and sensory composition of red wines. Food Chem. 134, 851–863.

García-Carpintero, E.G., Sánchez-Palomo, E., González Viñas, M.A., 2014. Volatile composition of Bobal red wines subjected to alcoholic/malolactic fermentation with oak chips. LWT Food Sci. Technol. 55, 586–594.

Garrity, G.M., Bell, J.A., Lilburn, T.G., 2004. Taxonomic Outline of the Procaryotes. Bergey's Manual of Systematic Bacteriology. second ed. Springer-Verlag, New York.

Gerbaux, V., Briffox, C., Dumont, A., Krieger, S., 2009. Influence of inoculation with malolactic bacteria on volatile phenols in wines. Am. J. Enol. Vitic. 60, 233–235.

González-Arenzana, L., López, R., Santamaría, P., López-Alfaro, I., 2013. Dynamics of lactic acid bacteria populations in Rioja wines by PCR-DGGE, comparison with culture-dependent methods. Appl. Microbiol. Biotechnol. 97, 6931–6941.

González-Arenzana, L., Santamaría, P., López, R., López-Alfaro, I., 2014. *Oenococcus oeni* strain typification by combination of multilocus sequence typing and pulsed field gel electrophoresis analysis. Food Microbiol. 38, 295–302.

Grimaldi, A., McLean, H., Jiranek, V., 2000. Identification and partial characterization of glycosidic activity of commercial strains of the lactic acid bacterium *Oenococcus oeni*. Am. J. Enol. Vitic. 51, 362–369.

Grimaldi, A., Bartowsky, E., Jiranek, V., 2005a. Screening of *Lactobacillus* spp. and *Pediococcus* spp. for glycosidase activities that are important in oenology. J. Appl. Microbiol. 99, 1061–1069.

Grimaldi, A., Bartowsky, E., Jiranek, V., 2005b. A survey of glycosidase activities of commercial wine strains of *Oenococcus oeni*. Int. J. Food Microbiol. 105, 233–244.

Gueguen, Y., Chemardin, P., Labrot, P., Arnaud, A., Galzy, P., 1997. Purification and characterization of an intracellular β-glucosidase from a new strain of *Leuconostoc mesenteroides* isolated from cassava. J. Appl. Microbiol. 82, 469–476.

Guilloux-Benatier, M., Son, H.S., Bouhier, S., Feuillat, M., 1993. Activités enzymatiques: glycosidases et peptidase chez *Leuconostoc oenos* au cours de la croissance bactérienne. Influence des macromolécules de levures. Vitis 32, 51–57.

Guilloux-Benatier, M., Le Fur, Y., Feuillat, M., 1998. Influence of fatty acids on the growth of wine microorganisms *Saccharomyces cerevisiae* and *Oenococcus oeni*. J. Ind. Microbiol. Biotechnol. 20, 144–149.

Günata, Z.Y., Bitteur, S., Brillouet, J.M., Bayonove, C.L., Cordonnier, R.E., 1988. Sequential enzymic hydrolysis of potentially aromatic glycosides from grapes. Carbohydr. Res. 184, 139–149.

Guymon, J.F., Cromwell, E.A., 1965. The formation of acetoin and diacetyl during fermentation, and the levels found in wines. Am. J. Enol. Vitic. 16, 85–91.

Herjavec, S., Tupajić, P., 1998. Changes in acidity, some aroma compounds and sensory properties of Frankovka wine after malolactic fermentation. Food Technol. Biotechnol. 36, 209–213.

Hernandez-Orte, P., Cersosimo, M., Loscosa, N., Cachoa, J., Garcia-Moruno, E., Ferreira, V., 2009. Aroma development from non-floral grape precursors by wine lactic acid bacteria. Food Res. Int. 42, 773–778.

Holland, R., Liu, S.Q., Crow, V.L., Delabre, M.-L., Lubbers, M., Bennett, M., Norris, G., 2005. Esterases of lactic acid bacteria and cheese flavour: milk fat hydrolysis, alcoholysis and esterification. Int. Dairy J. 15, 711–718.

Hynes, E., Bach, C., Lamberet, G., Ogier, J.C., Son, O., Delacroix-Buchet, A., 2003. Contribution of starter lactococci and adjunct lactobacilli to proteolysis, volatile profile and sensory characteristics of washed-curd cheese. Lait 83, 31–43.

Izquierdo Cañas, P.M., Pérez-Martín, F., García Romero, E., Seseña Prieto, S., Palop Herreros Mde, L., 2012. Influence of inoculation time of an autochthonous selected malolactic bacterium on volatile and sensory profile of Tempranillo and Merlot wines. Int. J. Food Microbiol. 156, 245–254.

Izquierdo Cañas, P.M., García Romero, E., Pérez-Martín, F., Seseña Prieto, S., Llanos Palop, M., 2015. Sequential inoculation versus co-inoculation in Cabernet Franc wine fermentation. Food Sci. Technol. Int. 21, 203–212.

Izquierdo-Cañas, P.M., Mena-Morales, A., García-Romero, E., 2016. Malolactic fermentation before or during wine aging in barrels. LWT Food Sci. Technol. 66, 468–474.

Juega, M., Costantini, A., Bonello, F., Cravero, M.C., Martinez-Rodriguez, A.J., Carrascosa, A.V., Garcia-Moruno, E., 2013. Effect of malolactic fermentation on the composition and sensory profile of Albarinò and Cainò white wines by *Pediococcus damnosus*. J. Appl. Microbiol. 116, 586–595.

Knockaert, D., Raes, K., Wille, C., Struijs, K., Van Camp, J., 2012. Metabolism of ferulic acid during growth of *Lactobacillus plantarum* and *Lactobacillus collinoides*. J. Sci. Food Agric. 92, 2291–2296.

Knoll, C., du Toit, M., Schnell, S., Rauhut, D., Irmler, S., 2011. Cloning and characterization of cystathionine β/γ lyase from two *Oenococcus oeni* oenological strains. Appl. Microbiol. Biotechnol. 89, 1051–1060.

Kunji, E.R.S., Mierau, I., Hagting, A., Poolman, B., Konings, W.N., 1996. The proteolytic systems of lactic acid bacteria. Antonie Van Leeuwenhoek 70, 187–221.

Lambrechts, M.G., Pretorius, I.S., 2000. Yeast and its importance to wine aroma: a review. S. Afr. J. Enol. Viticult. 21, 97–129.

Lee, J.E., Hong, Y.S., Lee, C.H., 2009. Characterization of fermentative behaviors of lactic acid bacteria in grapes wine through ^{1}H NMR- and GC-based metabolic profiling. J. Agric. Food Chem. 57, 4810–4817.

Lerm, E., Engelbrecht, L., du Toit, M., 2011. Selection and characterisation of *Oenococcus oeni* and *Lactobacillus plantarum* South African wine isolates for use as malolactic fermentation starter cultures. S. Afr. J. Enol. Viticult. 32, 280–295.

Liu, S.Q., 2002. A review: malolactic fermentation in wine beyond deacidification. J. Appl. Microbiol. 92, 589–601.

Liu, S.Q., Holland, R., Crow, V.L., 2003. Ester synthesis in an aqueous environment by *Streptococcus thermophilus* and other dairy lactic acid bacteria. Appl. Microbiol. Biotechnol. 63, 81–88.

Lonvaud-Funel, A., 1999. Lactic acid bacteria in the quality improvement and depreciation of wine. Antonie Van Leeuwenhoek 76, 317–331.

López, I., Torres, C., Ruiz-Larrea, F., 2008. Genetic typification by pulsed-field gel electrophoresis (PFGE) and randomly amplified polymorphic DNA (RAPD) of wild *Lactobacillus plantarum* and *Oenococcus oeni* wine strains. Eur. Food Res. Technol. 227, 547–555.

Maicas, S., Mateo, J.J., 2005. Hydrolysis of terpenyl glycosides in grape juice and other grape juices: a review. Appl. Microbiol. Biotechnol. 67, 322–335.

Maicas, S., Gil, J.-V., Pardo, I., Ferrer, S., 1999. Improvement of volatile composition of wines by controlled addition of malolactic bacteria. Food Res. Int. 32, 491–496.

Makarova, K.S., Koonin, E.V., 2007. Evolutionary genomics of lactic acid bacteria. J. Bacteriol. 189, 1199–1208.

Makarova, K., Slesarev, A., Wolf, Y., Sorokin, A., Mirkin, B., Koonin, E., Pavlov, A., Pavlova, N., Karamychev, V., Polouchine, N., Shakhova, V., Grigoriev, I., Lou, Y., Rohksar, D., Lucas, S., Huang, K., Goodstein, D.M., Hawkins, T., Plengvidhy, V., Welker, D., Hughes, J., Goh, Y., Benson, A., Baldwin, K., Lee, J.H., Díaz-Muñiz, I., Dosti, B., Smeianov, V., Wechter, W., Barabote, R., Lorca, G., Altermann, E., Barrangou, R., Ganesan, B., Xief, Y., Rawsthorne, H., Tamir, D., Parker, C., Breidt, F., Broadbent, J., Hutkins, R., O'Sullivan, D., Steele, J., Unlu, G., Saier, M., Klaenhammer, T., Richardson, P., Kozyavkin, S., Weimer, B., Mills, D., 2006. Comparative genomics of the lactic acid bacteria. Proc. Natl. Acad. Sci. U. S. A. 42, 15611–15616.

Malherbe, S., Tredoux, A.G.J., Nieuwoudt, H.H., Du Toit, M., 2012. Comparative metabolic profiling to investigate the contribution of *O. oeni* MLF starter cultures to red wine composition. J. Ind. Microbiol. Biotechnol. 39, 477–494.

Malherbe, S., Menichelli, E., du Toit, M., Tredoux, A., Muller, N., Naes, T., Nieuwoudt, H., 2013. The relationships between consumer liking, sensory and chemical attributes of *Vitis vinifera* L. cv. Pinotage wines elaborated with different *Oenococcus oeni* starter cultures. J. Sci. Food Agric. 93, 2829–2840.

Manes-Lazaro, R., Ferrer, S., Rossello-Mora, R., Pardo, I., 2009. *Lactobacillus oeni* sp. nov., from wine. Int. J. Syst. Evol. Microbiol. 59, 2010–2014.

Matthews, A., Grimaldi, A., Walker, M., Bartowsky, E., Grbin, P., Jiranek, V., 2004. Lactic acid bacteria as a potential source of enzymes for use in vinification. Appl. Environ. Microbiol. 70, 5715–5731.

Matthews, A., Grbin, P.R., Jiranek, V., 2006. A survey of lactic acid bacteria for enzymes of interest to oenology. Aust. J. Grape Wine Res. 12, 235–244.

Matthews, A., Grbin, P., Jiranek, V., 2007. Biochemical characterisation of the esterase activities of wine lactic acid bacteria. Appl. Microbiol. Biotechnol. 77, 329–337.

McSweeney, P.L.H., Sousa, M.J., 2000. Biochemical pathways for the production of flavour compounds in cheese during ripening. Lait 80, 293–324.

Michlmayr, H., Schümann, C., Wurbs, P., Barreira Braz da Silva, N.M., Rogl, V., Kulbe, K.D., del Hierro, A.M., 2010a. A β-glucosidase from *Oenococcus oeni* ATCC BAA-1163 with potential for aroma release in wine: cloning and expression in *E. coli*. World J. Microbiol. Biotechnol. 26, 1281–1289.

Michlmayr, H., Schümann, C., Da Silva, N.M., Kulbe, K.D., del Hierro, A.M., 2010b. Isolation and basic characterization of a β-glucosidase from a strain of *Lactobacillus brevis* isolated from a malolactic starter culture. J. Appl. Microbiol. 108, 550–559.
Michlmayr, H., Nauer, S., Brandes, W., Schümann, C., Kulbe, K.D., del Hierro, A.M., Eder, R., 2012. Release of wine monoterpenes from natural precursors by glycosidases from *Oenococcus oeni*. Food Chem. 135, 80–87.
Mills, D.A., Rawsthorne, H., Parker, C., Tamir, D., Makarova, K., 2005. Genomic analysis of *Oenococcus oeni* PSU-1 and its relevance to winemaking. FEMS Microbiol. Rev. 29, 465–475.
Mtshali, P.S., 2011. Molecular Screening of Lactic Acid Bacteria Enzymes and Their Regulation Under Oenological Conditions. (PhD dissertation). Stellenbosch University (unpublished data).
Mtshali, P.S., Divol, B., du Toit, M., 2010. Genetic screening of wine-related enzymes in *Lactobacillus* species isolated from South African wines. J. Appl. Microbiol. 108, 1389–1397.
Mtshali, P.S., Divol, B., du Toit, M., 2012. PCR detection of enzyme-encoding genes in *Leuconostoc mesenteroides* strains of wine origin. World J. Microbiol. Biotechnol. 28, 1443–1449.
Naser, S.M., Dawyndt, P., Hoste, B., Gevers, D., Vandemeulebroecke, K., Cleenwerck, I., Vancanneyt, M., Swings, J., 2007. Identification of lactobacilli by *pheS* and *rpoA* gene sequence analyses. Int. J. Syst. Evol. Microbiol. 57, 2777–2789.
Nisiotou, A.A., Dourou, D., Filippousi, M.E., Banilas, G., Tassou, C., 2014. *Weissella uvarum* sp. nov., isolated from wine grapes. Int. J. Syst. Evol. Microbiol. 64, 3885–3890.
Nisiotou, A.A., Dourou, D., Filippousi, M.E., Diamantea, E., Fragkoulis, P., Tassou, C., Banilas, G., 2015. Genetic and technological characterisation of vineyard- and winery-associated lactic acid bacteria. Biomed. Res. Int. 2015, 9–17.
Olguìn, N., Bordons, A., Reguant, C., 2009. Influence of ethanol and pH on the gene expression of the citrate pathway in *Oenococcus oeni*. Food Microbiol. 26, 197–203.
Olguín, N., Oriol Alegret, J., Bordons, A., Reguant, C., 2011. β-Glucosidase activity and *bgl* gene expression of *Oenococcus oeni* strains in model media and Cabernet Sauvignon wine. Am. J. Enol. Vitic. 62, 99–105.
Padilla, B., Gil, J.V., Manzanares, P., 2016. Past and future of non-*Saccharomyces* yeasts: from spoilage microorganisms to biotechnological tools for improving wine aroma complexity. Front. Microbiol. 31, 1–20.
Pérez-Martìn, F., Seseña, S., Izquierdo, P.M., Martìn, R., Lianos Palop, M., 2012. Screening for glycosidase activities of lactic acid bacteria as a biotechnological tool in oenology. World J. Microbiol. Biotechnol. 28, 1423–1432.
Pérez-Martín, F., Seseña, S., Izquierdo, P.M., Palop, M.L., 2013. Esterase activity of lactic acid bacteria isolated from malolactic fermentation of red wines. Int. J. Food Microbiol. 163, 153–158.
Petri, A., Pfannebecker, J., Fröhlich, J., König, H., 2013. Fast identification of wine related lactic acid bacteria by multiplex PCR. Food Microbiol. 33, 48–54.
Piao, H., Hawley, E., Kopf, S., DeScenzo, R., Sealock, S., Henick-Kling, T., Hess, M., 2015. Insights into the bacterial community and its temporal succession during the fermentation of wine grapes. Front. Microbiol. 6, 1–12.
Pineau, B., Barbe, J.C., Van Leeuwen, C., Dubourdieu, D., 2009. Examples of perceptive interactions involved in specific 'Red' and 'Black-berry' aromas in red wines. J. Agric. Food Chem. 57, 3702–3708.
Portillo, M.D., Franquès, J., Araque, I., Reguant, C., Bordons, A., 2016. Bacterial diversity of Grenache and Carignan grape surface from different vineyards at Priorat wine region (Catalonia, Spain). Int. J. Food Microbiol. 219, 56–63.
Pozo-Bayon, M.A., G-Alegria, E., Polo, M.C., Tenorio, C., Matin-Alvarez, P.J., Calvo De La Banda, M.T., Ruiz-Larrea, F., Moreno-Arribas, M.V., 2005. Wine volatile and amino acid composition after malolactic fermentation: effect of *Oenococcus oeni* and *Lactobacillus plantarum* starter cultures. J. Agric. Food Chem. 53, 8729–8735.
Pripis-Nicolau, L., De Revel, G., Bertrand, A., Lonvaud-Funel, A., 2004. Methionine catabolism and production of volatile sulphur compounds by *Oenococcus oeni*. J. Appl. Microbiol. 96, 1176–1184.
Renouf, V., Claisse, O., Lonvaud-Funel, A., 2006. *rpoB* gene: a target for identification of LAB cocci by PCR-DGGE and melting curves analysis in real time PCR. J. Microbiol. Methods 67, 162–170.
Ribéreau-Gayon, P., Dubourdieu, D., Donèche, B., Lonvaud-Funel, A., 2006. Handbook of Enology: The Microbiology of Wine and Vinifications. second ed. Wiley & Sons Chichester. Elsevier Science Publishers B.V., Amsterdam, The Netherlands.
Rodas, A.M., Ferrer, S., Pardo, I., 2003. 16S-ARDRA, a tool for identification of lactic acid bacteria isolated from grape must and wine. Syst. Appl. Microbiol. 26, 412–422.
Rollan, G.C., Farias, M.E., Manca de Nadra, M.C., 1993. Protease production by *Leuconostoc oenos* strains isolated from wine. World J. Microbiol. Biotechnol. 9, 587–589.
Rollan, G.C., Farias, M.E., Manca de Nadra, M.C., 1995. Characterization of two extracellular proteases from *Leuconostoc oenos*. World J. Microbiol. Biotechnol. 11, 153–155.
Rollan, G.C., Farias, M.E., Strasser De Saad, A.M., Manca De Nadra, M.C., 1998. Exoprotease activity of *Leuconostoc oenos* in stress conditions. J. Appl. Microbiol. 85, 219–223.
Ruiz, P., Seseña, S., Llanos Palop, M., 2014. A comparative study ofdifferent PCR- based DNA fingerprinting techniques fortyping oflactic acid bacteria. Eur. Food Res. Technol. 239, 87–98.
Sauvageot, F., Vivier, P., 1997. Effects of malolactic fermentation on sensory properties of four Burgundy wines. Am. J. Enol. Vitic. 48, 187–192.
Sebastian, P., Herr, P., Fischer, U., Konig, H., 2011. Molecular identification of lactic acid bacteria occurring in must and wine. S. Afr. J. Enol. Viticult. 32, 300–309.
Spano, G., Rinaldi, A., Ugliano, M., Moio, L., Beneduce, L., Massa, S., 2005. A beta-glucosidase gene isolated from wine *Lactobacillus plantarum* is regulated by abiotic stresses. J. Appl. Microbiol. 98, 855–861.
Sternes, P.R., Borneman, A.R., 2016. Consensus pan-genome assembly of the specialised wine bacterium *Oenococcus oeni*. BMC Genomics 17, 308.
Strickland, M.T., Schopp, L.M., Edwards, C.G., Osborne, J.P., 2016. Impact of *Pediococcus* spp. on pinot noir wine quality and growth of *Brettanomyces*. Am. J. Enol. Vitic. 67, 188–198.
Sumby, K.M., Grbin, P.R., Jiranek, V., 2010. Microbial modulation of aromatic esters in wine: current knowledge and future prospects. Food Chem. 121, 1–16.
Sumby, K.M., Grbin, P.R., Jiranek, V., 2013a. Characterization of EstCOo8 and EstC34, intracellular esterases, from the wine-associated lactic acid bacteria *Oenococcus oeni* and *Lactobacillus hilgardii*. J. Appl. Microbiol. 114, 413–422.
Sumby, K.M., Jiranek, V., Grbin, P.R., 2013b. Ester synthesis and hydrolysis in an aqueous environment, and strain specific changes during malolactic fermentation in wine with *Oenococcus oeni*. Food Chem. 141, 1673–1680.
Sumby, K.M., Grbin, P.R., Jiranek, V., 2014. Implications of new research and technologies for malolactic fermentation in wine. Appl. Microbiol. Biotechnol. 98, 8111–8132.
Swiegers, J.H., Bartowsky, E.J., Henschke, P.A., Pretorius, I.S., 2005. Yeast and bacterial modulation of wine aroma and flavour. Aust. J. Grape Wine Res. 11, 139–173.
Terrade, N., Mira de Orduña, R., 2009. Determination of the essential nutriment requirements of wine-related bacteria from the genera *Oenococcus* and *Lactobacillus*. Int. J. Food Microbiol. 133, 8–13.
Tonon, T., Lonvaud-Funel, A., 2000. Metabolism of arginine and its positive effect on growth and revival of *Oenococcus oeni*. J. Appl. Microbiol. 89, 526–531.
Ugliano, M., Moio, L., 2005. Changes in the concentration of yeast-derived volatile compounds of red wine during malolactic fermentation with four commercial starter cultures of *Oenococcus oeni*. J. Agric. Food Chem. 26, 10134–10139.
Ugliano, M., Genovese, A., Moio, L., 2003. Hydrolysis of wine aroma precursors during malolactic fermentation with four commercial starter cultures of *Oenococcus oeni*. J. Agric. Food Chem. 51, 5073–5078.
Valdés La Hens, D., Bravo-Ferrada, B.M., Delfederico, L., Caballero, A.C., Semorile, L., 2015. Prevalence of *Lactobacillus plantarum* and *Oenococcus oeni* during spontaneous malolactic fermentation in Patagonian red wines revealed by polymerase chain reaction-denaturing gradient gel electrophoresis with two targeted genes. Aust. J. Grape Wine Res. 21, 49–56.
Vallet, A., Lucas, P., Lonvaud-Funel, A., de Revel, G., 2008. Pathways that produce volatile sulphur compounds from methionine in *Oenococcus oeni*. J. Appl. Microbiol. 104, 1833–1840.
Villamor, R.R., Ross, C.F., 2013. Wine matrix compounds affect perception of wine aromas. Annu. Rev. Food Sci. Technol. 4, 1–20.
Walling, E., Gindreau, E., Lonvaud-Funel, A., 2005. A putative glucan synthase gene *dps* detected in exopolysaccharide-producing *Pediococcus damnosus* and *Oenococcus oeni* strains isolated from wine and cider. Int. J. Food Microbiol. 98, 53–62.
Zapparoli, G., Torriani, S., Pesente, P., Dellaglio, F., 1998. Design and evaluation of malolactic enzyme gene targeted primers for rapid identification and detection of *Oenococcus oeni* in wine. Lett. Appl. Microbiol. 27, 243–246.
Zapparoli, G., Reguant, C., Bordons, A., Torriani, S., Dellaglio, F., 2000. Genomic DNA fingerprinting of *Oenococcus oeni* strains by pulsed-field gel electrophoresis and randomly amplified polymorphic DNA PCR. Curr. Microbiol. 40, 351–355.
Zarraonaindia, I., Owens, S.M., Weisenhorn, P., West, K., Hampton-Marcell, J., Lax, S., Bokulich, N.A., Mills, D.A., Martin, G., Taghavi, D.S., van der Lelie, D., Gilberta, J.A., 2015. The soil microbiome influences grapevine-associated microbiota. MBio 6, 1–10.