Chapter

Wild Yeast and Lactic Acid Bacteria of Wine

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Abstract

Wine is an ancient and popular alcoholic beverage made from fermented grapes. Different yeasts and bacteria strains produce different styles of wines. Over time, the inoculation of *Saccharomyces cerevisiae* strains to produce wine has been the common practice in the wine industry, and the other species of yeasts have been considered undesirable for the alcoholic fermentation. However, in the last decades, the use of wild or indigenous yeasts and lactic acid bacteria strains has significantly increased. Wild yeasts and lactic acid bacteria are interesting microorganisms that contribute to differentiate the wine character of a region. The production of wines by spontaneous or inoculated fermentations by selected wild microorganisms may be an interesting tool to improve the quality of wines. This chapter summarizes relevant aspects of these microorganisms related to this scientific field.

Keywords: biotechnology, fermentation, flavor, microbiota, *Saccharomyces*

1. Introduction

Wine is an ancient and popular alcoholic beverage made from fermented grapes. The wine quality is determined by many factors, including the climate, the soil characteristics, the grape variety and the production processes, such as the viticultural practices, the winemaking techniques and the aging period. Among these factors, the fermentations carried out during winemaking, mainly alcoholic and malolactic fermentation, strongly influence on the wine composition. Different yeasts and bacteria strains produce different styles of wines derived from the biotransformation involved in both fermentations. Generally, the alcoholic fermentation (AF) is conducted by yeasts which convert sugars into ethanol, carbon dioxide and other minor metabolites. On the other hand, the malolactic fermentation (MLF) is conducted by lactic acid bacteria (LAB), which mainly convert malic acid into lactic acid and carbon dioxide. These metabolic processes are complex and sophisticated and sometimes may induce undesirable metabolite production pathways. Accordingly, an adequate selection of the yeast and bacteria strains is an important task for winemakers.

Over time, the inoculation of *Saccharomyces cerevisiae* strains to produce wine has been the common practice in the wine industry, and the other species of yeasts have been considered undesirable for the AF. However, in the last decades, the use and the inoculation of wild, native, autochthonous or indigenous yeasts and LAB strains to conduct the AF and the MLF have been significantly increased.

The isolation, selection and inoculation of the indigenous strains are useful tools to avoid sluggish and stuck fermentations and to increase the microbial diversity, enhancing the wine character [1]. In this way, this chapter pretends to summarize the most relevant aspects of these microorganisms and showing results derived from the studies related to the wild yeast and LAB strains on the wine properties.

2. Wild microorganisms associated to wine fermentations

The biotransformation of grape juice into wine is a complex ecological and biochemical process involving the sequential development of several microbial species such as yeasts, bacteria and fungi. Yeasts are the most important microorganisms involved in this process, being *S. cerevisiae* the main responsible of the AF. Although there are other genera and species present during winemaking, *Saccharomyces* possess a range of singular characteristics that are not found in other genera, such as the high capacity to ferment sugars, the high alcohol tolerance and the great ability to compete with other species and to colonize the wine medium [2]. The non-*Saccharomyces* yeasts are also commonly known by winemakers and wine microbiologists. This term includes many different yeast species. The current taxonomy recognizes around 149 yeasts genera and 1500 species, and more than 40 species have been isolated from grapes and grape juices [3]. *Dekkera* (anamorph form of *Brettanomyces*), *Candida* (anamorph form of *Metschnikowia*), *Cryptococcus*, *Debaryomyces*, *Hanseniaspora* (anamorph form of *Kloeckera*), *Kluyveromyces or Lachancea, Pichia*, *Rhodotorula*, *Saccharomycodes*, *Schizosaccharomyces*, *Torulaspora* and *Zygosaccharomyces* are the well-known non-*Saccharomyces* genera [4]. Generally, the non-*Saccharomyces* yeasts are commonly known as wild yeasts, because they are mostly present in grapevines, grape clusters and berry surfaces. The wild microbiota found in grapes and, therefore, in musts is affected by many external factors, such as the geographical location, the climatic conditions, the grape variety, the stage of maturity, the age of vines, the use of fungicides, the berry physical damages caused by fungi and even the presence of insects and birds [5–7]. Within a winemaking environment, the diversity of yeast species can be influenced by the population of cellar habitats, such as wall surfaces, equipment and oak barrels, among others. Thus, the cleaning and the cellar hygienic practices influence the winery microbiota affecting their diversity, composition and evolution. Nowadays, the actual hygienic practices used in the modern cellars seem to minimize the contamination by the resident cellar flora and, therefore, its diversity [8, 9]. In general, the non-*Saccharomyces* wine-related species have a low fermentation activity and a low SO_2 resistance [3]. However, they have the ability to colonize non-inoculated musts and to start the AF. They play an important role in the wine aroma complexity mainly due to their interesting enzymatic activities (proteases, β-glucosidases, esterases, pectinases and lipases) [10–12]. Many reports showed that the enzymatic activity of yeasts is conditioned by the pH, temperature, as well as the presence of inhibitors (sugars and ethanol) [13]. It seems that approximately 80% of the wild yeasts possesses one or more enzymes with biotechnological interest, being polygalacturonase the most common enzyme, followed by proteases (casein, gelatin) [14]. The β-glucosidase activity was linked to *Metschnikowia pulcherrima* species. A proteolytic activity was observed in *Pichia membranifaciens* and also in *Metschnikowia pulcherrima*. Furthermore, *Hanseniaspora* and *Torulaspora* genera are reported good producers of β-glucosidases, pectinases, proteases and enzymes involved in the xylan degradation [15–20]. *Lachancea thermotolerans* exhibited the activities of four carbohydrolases and three aminopeptidases. So, this strain could be an excellent candidate for improving the color and the turbidity of the red wines. Furthermore,

this strain could even increase the acidity due to its ability to produce lactic acid during the AF [21, 22]. However, the secretion of each enzyme is not characteristic of a particular genus or species and depends on the strains. It is important to notice that although non-*Saccharomyces* populations were not detected at the end of the vinification, their secreted enzymes remained in the fermenting media [16].

LAB are the second important group of wine microorganisms, which are also present in grapes. The LAB of wines, musts and grapes belong to the genera *Oenococcus, Pediococcus, Lactobacillus* and *Leuconostoc* [23]. LAB can be homofermentative and producing exclusively lactic acid and CO_2 from sugars (glucose and/or fructose) or can be heterofermentative and also producing ethanol, acetic acid and CO_2. Generally, the MLF is conducted by *O. oeni*, which presents a heterofermentative metabolism. Other species of the mentioned genera, such as *P. pentosaceus* and *P. damnosus*, have a homofermentative metabolism, while *Lactobacillus casei* and *Lactobacillus plantarum* have been described as facultative heterofermentative. Other *Lactobacillus* species, such as *brevis* and *hilgardii*, are strictly heterofermentative [23, 24]. The acetic acid bacteria are considered spoilage microorganisms during winemaking. Their metabolism is strictly aerobic, and their principal property is that they can oxidize ethanol into acetic acid by the acetaldehyde pathway. Finally, the fungi found in vines, such as *Botryotinia*, *Uncinula*, *Alternaria*, *Plamapara*, *Aspergillus*, *Penicillium*, *Rhizopus*, *Oidium* and *Cladosporium*, can infect and colonize grapes prior to harvest and to be present in musts [25]. *Botryotinia fuckeliana* (or its anamorph form *Botrytis cinerea*), *Aspergillus* spp. and *Penicillium* spp. are able to produce metabolites that can delay the growth of yeasts during the fermentation. Furthermore, the fungi growth on grapes may contribute to the growth of some acetic acid bacteria on the grape surface.

3. Population dynamics

The main important microorganisms present in grapes are yeasts and in a minor proportion LAB, acetic acid bacteria and fungi. The content and diversity strongly depend on the sanitary status of grapes. Although grape musts are relatively complete in nutrients, its low pH and its high sugar content convert them in a selective media in which only a few bacteria and yeasts species can grow. The number of yeasts on the grape berry just before harvest varies from 10^3 to 10^6 cells/mL depending on the abovementioned factors [26]. The predominant wild species on the surface of grape berries are *Candida*, *Hanseniaspora*, *Hansenula*, *Metschnikowia* and *Pichia*. The *S. cerevisiae* population is very low in grapes [27], while the non-*Saccharomyces* could proliferate up to reach about 10^6–10^7 cells/mL populations, although it declines at mid-fermentation. *S. cerevisiae* species are the most alcohol-tolerant yeast and can reach populations of at least 10^7–10^8 cells/mL [26]. Thus, at the last stage of fermentation, they become predominant and complete the process. Besides, some species of *Brettanomyces*, *Kluyveromyces*, *Schizosaccharomyces*, *Torulaspora* and *Zygosaccharomyces* may also be present in wine during fermentation. Some of these species are considered spoilage microorganisms because they produce metabolites with an undesirable impact in wine [8].

Regarding LAB, the population and behavior mainly depend on the pH and the SO_2 content, and they can reach 10^2–10^4 cells/mL populations after grape crushing. In general, an increase on the pH involves higher LAB populations and diversity. At this initial fermentation stage, the four genera abovedescribed can be commonly identified, although the greatest diversity of LAB species is mainly detected during the AF. During the first days of the AF, the LAB population generally increases to a maximum of 10^4 cells/mL and then decreases until 10^2 cells/mL.

At the end of the AF, *O. oeni* is commonly the only species identified and remains in a latent phase waiting to the proper conditions to start the MLF. The MLF starts when their population achieves values around 10^6 cells/mL and the environment conditions are adequate (pH, ethanol, temperature and SO_2 content) [28]. As soon as the malic acid is completely degraded, the bacterial population begins to decline [24, 29].

4. Spontaneous and inoculated fermentations

There are a lot of different species in grapes that can participate on the wine fermentations. In general, the AF is conducted by a mixture of yeasts species [5]. The AF can be conducted spontaneously without inoculating any yeast strains or by the inoculation of the specific strains, commercial or wild. The most common worldwide practice is the use of commercial starters from *S. cerevisiae* to ensure a reproducible, predictable and controlled fermentation. The use of commercial wine yeasts can influence the natural microflora of musts and often leads to its removal. Wines produced under this practice show low variability, complexity and typicity with analytical and sensory properties often similar [1]. In contrast, the spontaneous fermentations have some problems to predict their evolution, due to the variability on the microbiota that comes from the grapes. However, wines produced under this kind of fermentation have greater complexity and present higher differentiating notes and character [30]. In the inoculated fermentations, *S. cerevisiae* is the most common active dry wine yeast (ADWY) used as starter culture since it offers a great control on the fermentation evolution. Currently, a wide commercial ADWY yeast strains and species are available for cellars.

The MLF is not always successful even if it is conducted by inoculated commercial *O. oeni* strains. Some reports showed the presence of different species in spontaneous MLF, although, as mentioned, *O. oeni* has been described as the principal species. The evolution of the MLF and the diversity species of LAB implicated in this process may modulate the composition of wine (pH, the ethanol content, etc.), the fermentation temperature, the winemaking technology used, the geographical region and also the yeast strains employed during the AF [31–35]. As in the case of yeasts, to develop a correct spontaneous MLF, a wild bacterial starter is needed, which is well adapted to the specific producing area and to the cellar conditions. LAB inoculation is recommended in modern and industrial wineries in order to control the evolution of the MLF. Fast and reliable fermentations are essential to obtain a high-quality wine [36]. However, the use of commercial starters shows some controversies because of the homogeneity and standardization of wines, limiting their organoleptic properties [37].

In summary, the use of wild yeast and LAB can be used to define the typicity of the wines of a region. Some authors stated that the microflora diversity is characteristic of a given area and could be considered its microbiological fingerprint [38, 39]. The inoculation of selected wild yeast and LAB species could help to control the development of the AF and MLF and to improve the complexity and could typify the wine of a region [1].

5. Fermentation end-products and wild microorganisms

Numerous fermentation end-products contribute to the aroma and flavor characteristics of wines, which determine their quality and final complexity. As it is known, wine is made up of thousands of aromatic compounds, and a large part

of them are produced or transformed during the AF and MLF [40]. As mentioned above, these processes are carried out by wild or commercial strains. The use of wild strains allows us to obtain wines with a unique expression with representative characteristics of each variety and area. The aromatic profile of wines is determined by varietal aromas (from grapes), fermentative aromas (produced by yeast and LAB during fermentations) and post-fermentative aromas (associated to the aging period). The fermentative aromas clearly influence the final quality of wines, and the strains used during winemaking are responsible for the presence or absence of some flavors and other non-volatile metabolites [41]. Ethanol, carbon dioxide and glycerol are the main fermentative products. Ethanol is the main volatile product of yeasts metabolism, followed by diols, higher alcohols and esters. The ethanol content influences the wine viscosity and contributes on the aroma fastening. Other important metabolites derived from AF, such as pyruvic acid, participate on the formation of secondary products namely diacetyl, keto acids, succinic acid and butanediol [23]. Succinic acid and glycerol are two of the most important by-products affecting the "body" of the wine. Succinic is the main acid produced by yeasts, and its formation is strain dependent. The tartaric acid experiment slightly changes during fermentation, and the malic acid usually decreases during MLF, although the yeasts metabolism can also modify its concentration during the AF. Regarding acetic acid, this compound may reach more than 90% of the volatile acidity, and it is one of the most important by-products that negatively affect sensory profile of wine. This acid is mainly synthetized by acetic acid bacteria but also may be synthetized by yeasts and LAB.

Other volatile acids, such as propionic and hexanoic acids, are also produced by yeasts and bacteria, as a result of the fatty acid metabolic pathway [42]. Another important, but not always desirable, secondary metabolite of wine fermentation is the acetaldehyde. This compound is the product of the decarboxylation of the pyruvate during the AF. The higher alcohols represent another group of secondary products influencing the sensory profile of wines. The concentrations of higher alcohols are influenced by factors such as the yeast strains, the concentration of amino acids (the precursors of higher alcohols), ethanol concentration, fermentation temperature, pH, composition of grape must, aeration, etc. The higher alcohols are also important precursors for the ester formation; both of them are associated with pleasant aromas, although at high concentrations they can be undesirable.

LAB modulate the flavors of wines by modifying their chemical composition and, therefore, its sensory properties. LAB are responsible to maintain the result of the yeasts metabolism and to increase the complexity and microbial stability of the wine [24]. LAB decrease the wine acidity by the decarboxylation of malic acid to lactic acid, and they also contribute to the aroma by metabolizing other acids such as citric acid. The degradation of citric acid produces acetic acid and diacetyl, both of which have an important and undesirable effect on the wine flavor. Other metabolites affected by LAB metabolism, which have an impact on wine flavor, are alcohols, such as glycerol and mannitol, or carbonyls such as acetaldehyde and diacetyl. Finally, esters can also be modified for LAB species, including ethyl acetate, ethyl hexanoate, ethyl lactate and ethyl octanoate [42]. Depending on the species or even on the strains, LAB may be beneficial or detrimental to wine quality [29]. Meanwhile, acetic acid bacteria, as mentioned above, are only spoilage microorganism because they lead to the formation of such major oxidized aromas (acetaldehyde, acetic acid and ethyl acetate).

During the spontaneous AF, the development of many aromatic compounds occurs, mainly those belonging to the families of alcohols, ethyl esters, fatty acids, acetates and carbonyls. Aliphatic esters and alcohols seem to be more influenced than acids and carbonic compounds. In addition, terpenes and norisoprenoids, well-known primary aromas, can be provided by the wild yeasts during

fermentation [43, 44]. The main aromatic descriptors of all of them are the fruity and floral notes, always appreciated in wines. The use of wild yeast to conduct a spontaneous AF may produce higher concentration of alcohols (1- hexanol, phenylethanol), terpenes and other aromatic compounds, such as β-phenyl acetate and γ-nonalactone, compared to wines produced by selected yeasts [45]. The selection of indigenous *S. cerevisiae* in red musts and its effect in their aromatic profile have been studied. The results showed that the produced wines had greater content of aromas and color intensity. These native yeasts synthesized higher content of linalool and citronellol, which exceeded their sensory limits [46].

The most related aromas of the inoculated MLFs are commonly associated with butter, yogurt, sulfur and toasted notes. Moreover, during spontaneous MLFs, the formation of many aromatic compounds is affected. Some studies have demonstrated the biosynthesis of the aromatic compounds produced during this kind of fermentations and its sensory repercussion. A reduction of herbaceous and vegetable aromas has been highlighted, and the appearance of fruity and floral aromas has been reported [47]. The changes produced in the aromatic composition of Tempranillo wines during spontaneous MLF by using wild LAB have been reported, showing significant increase in esters, lactones, terpenes, norisoprenoids and volatile phenols, such as vanillin and furfural [48].

6. Selection of wild yeast and lactic acid bacteria

For the selection of wild yeasts and LAB species of a specific wine region, first, it is needed to conduct a biodiversity study, knowing which species are present in grapes. After that, knowing the species at the different stages of a spontaneous fermentation, at the beginning, middle and end of fermentation, is essential. The first stage is to conduct a spontaneous fermentation. Then, isolate different colonies at each stage of the fermentation to obtain a collection of the different microorganisms implied. The second stage is to identify and typify each colony. The recovery and the molecular characterization of a high number of yeasts and LAB strains should be considered to establish a strain collection of oenological interest.

For the yeast species identification, different techniques could be applied. The restriction analysis of ribosomal gens is the simplest technique, reliable and extended [10, 49]. Nevertheless, several available techniques, such as microsatellites (SSRs), Rapid Amplification of Polymorphic DNA (RAPD-PCR), Pulsed-Field Gel Electrophoresis (PFGE) and DNA array technology, have been used to typify yeast strains. Between all these techniques, the more usual techniques for their simplicity and reproducibility are the restriction analysis of mitochondrial DNA [50] and the amplification of delta elements [51]. PCR-based methods have been already successfully used to identify LAB in different wines. To identify different LAB species, a good technique, fast and reliable, is the Restriction analysis of the amplified 16S-rDNA (ARDRA-PCR) [52, 53]. RAPD-PCR (Random Amplified Polymorphic DNA) is considered to be a suitable method to typify *O. oeni* strains in winemaking [54], such as PFGE (Pulse Field Gel Electrophoresis) of DNA digested with *Sfi*I [24].

Once the wild species and strains are identified and typified, the next step is to characterize each isolated strain, which has different genetic profile between them and between commercial yeast. Performing micro-fermentations with pure inoculations of all the strains with the specific characteristics of wine region must in order to test relevant species starter kits. With a better understanding of the different yeasts properties, the yeast selection procedure can be adapted to acquire strains that could improve the wine quality [55]. The AF and MLF performance by selected strains at winery conditions is the last step of selection.

The wild starter kit can be a single strain of *S. cerevisiae* or a mixture of *S. cerevisiae* and non-*Saccharomyces* species. The main trends in wine biotechnology is the use of different non-*Saccharomyces* species as starter cultures, such as *Torulaspora delbrueckii*, *Pichia kluyveri*, *Lachancea thermotolerans*, *Metschnikowia pulcherrima*, *Hanseniaspora uvarum*, *Schizosaccharomyces pombe*, etc. This practice combines the advantages of recovering features from traditional spontaneous fermentation, with a control of the vinification process, decreasing the risk associated with the microbial spoilage. The different species of non-*Saccharomyces* yeast starters are generally used in either sequential or simultaneous inoculation with *S. cerevisiae* [56]. Several studies have shown that the mixed inoculation starter kit with *S. cerevisiae* and non-*Saccharomyces* species can contribute positively to wine flavor. *M. pulcherrima* decreases volatile acidity [57], *H. uvarum* increases ester content in wine [58] and *Schizosaccharomyces pombe* deacidifies musts and increases the synthesis of glycerol and pyruvic acid [59]. In mixed fermentation, the interactions between the different yeasts composing the starter culture can led the stability of the final product and the analytical and aromatic profile [60].

The LAB selection as starters requires an ecological study and the characterization of useful technological and physiological features of the isolated strains in order to select the ones that are potentially more suitable for industrial applications. The selection of LAB for wine inoculation is essentially based on the survival of this strain and the consumption of malic acid. However, there are other important properties that are required to study the ability to produce biogenic amines and different enzymatic activities related to the final aroma profile. *O. oeni* is the preferred starter species because of its resistance to the alcohol, pH and SO_2 content. The ability to resistance the harsh wine conditions is strictly strain dependent. Furthermore, *O. oeni* ensures control of the time and the rate of MLF, reducing the potential for spoilage microorganisms and, finally, giving positive effects on flavor and aroma [61]. The development of the *Lactobacilli* and *Pediococci* in wine samples was linked to the decrease in wine quality [23]. González-Arenzana et al. demonstrated a high diversity *O. oeni* in spontaneous MLF and the complexity of the ecology involved [24]. They suggested a successful adaptation to winemaking conditions for some strains and also their potential utility for the selection of wild LAB starter cultures as individual or mixed strains.

The selection of microorganisms has been successfully used to improve the technological properties of different wines as well as their sensorial profiles helping in the production of wines without sulfites, reducing the levels of ethanol, increasing the glycerol content, varying the acidity of the must and realizing different aromatic components. In summary, the selection of wild yeast and LAB offers the best way to obtain different species and strains, which could improve the oenological characteristics and sensorial profile of wines, giving tools to the oenologist to direct their wine fermentation process. The exploitation of the microbial diversity that exists in the vineyards and in the cellars with the selection of wild yeast and LAB strains has been considered an interesting approach to overcome the distinctive peculiarities of wines produced in different regions [57].

7. Wild yeasts and lactic acid bacteria from viticultural Spanish regions

This section summarizes several studies carried out in VITEC (Wine Technology Center) from different grape varieties and Spanish regions. The isolation, identification and selection of wild yeasts and LAB were performed in 2016 and 2017 vintages, from grapes and spontaneous fermentations. The grape varieties studied were Verdejo from D.O. Rueda, Albariño from D.O. Rías Baixas and Tempranillo from D.O. Ribera del Duero and D.O.Q. Rioja. In all the cases, different species of

S. cerevisiae, non-*Saccharomyces* species and LAB were identified (**Tables 1** and **2**). **Table 1** shows the different non-*Saccharomyces* species found for each grape variety and region. The results showed that a great variety of yeasts species and strains present in grapes during spontaneous fermentation has been reported. Up to fourteen different strains of *S. cerevisiae* and seven species of non-*Saccharomyces* were identified in Verdejo. Seventy strains of *S. cerevisiae* and nine species of non-*Saccharomyces* were identified in Albariño, only at 2017. Seventy-eight strains of *S. cerevisiae* and ten non-*Saccharomyces* species were identified from Tempranillo in both regions. The non-*Saccharomyces* species were isolated in order to be inoculated together with a selected *S. cerevisiae* strain in mixed cultures. As mentioned, some of the used species are described as interesting wild yeasts, since they are able to led desirable compounds and metabolites to improve the wine quality (*Torulaspora delbrueckii*, *Pichia kluyveri*, *Lachancea thermotolerans*, *Candida/Metschnikowia pulcherrima* and *Hanseniaspora* species). It has been reported that *T. delbrueckii* can produce lower levels of volatile acidity than *S. cerevisiae*. *M. pulcherrima* can produce high concentrations of esters, especially ethyl octanoate; *Starmerella bacillaris* can produce high levels of glycerol and *Hanseniaspora* can improve the aromatic composition [30].

In order to study the influence of fermentation mixtures from wild selected yeasts on the wine properties, several studies were carried out in VITEC. The behavior of all yeast strains were studied, conducting fermentations at laboratory scale and at semi-industrial scale, in pure (*S. cerevisiae*) and mixed inoculations (*S. cerevisiae* and non-*Saccharomyces*). In the case of pure inoculations, significant differences were obtained in all the analyzed parameters (alcoholic degree, volatile acidity, total acidity, sulfur dioxide, glycerol and malic acid), except in the lactic acid content (data not shown). In these studies, firstly, the non-*Saccharomyces* species were inoculated and later the *S. cerevisiae* strains. In these mixed inoculations, the differences obtained depended on the time of the inoculation of the *S. cerevisiae* strain. As later the inoculation of *S. cerevisiae* is done, more differences were obtained. The inoculation time

Non-*Saccharomyces* species	**Verdejo Rueda**	**Albariño Rías Baixas**	**Tempranillo R. del Duero**	**Tempranillo Rioja**
Metschnikowia pulcherrima	√	√		√
Hanseniaspora vineae			√	√
Torulaspora delbrueckii	√	√	√	
Lanchacea thermotolerans			√	√
Hanseniaspora guillermondii	√	√		
Issatchenkia orientalis	√			
Pichia kluyveri	√			
Hanseniaspora uvarum	√	√	√	√
Aureobasidium pullulans			√	√
Rhodotorula glutinis		√		
Cryptococcus flavescens		√		
Cryptococcus magnus		√		
Starmerella bacillaris		√		
Pichia membranifaciens		√		

Table 1.
Identification of non-Saccharomyces species at different grape varieties and Spanish regions.

LAB species	Tempranillo Ribera del Duero	Tempranillo Rioja
Lactobacillus delbrueckii	√	
Lactobacillus helveticus	√	√
Lactobacillus hilgardii	√	√
Lactobacillus fermentum	√	
Lactobacillus pentosus	√	
Lactobacillus collinoides		√
Pediococcus acidilacticii		√
Oenococcus oeni	√	√

Table 2.
Identification of LAB species in Tempranillo grapes.

affected the basic oenological parameters and the aromatic fermentative compounds, including higher alcohols, esters, acetates and acids.

Concerning LAB, the highest diversity was found at the beginning of the AF. Up to eight different wild LAB species and fourteen *O. oeni* strains were identified. The fermentative characteristics of different *O. oeni* strains were studied. The results showed that some of these strains were able to conduct MLFs when the alcoholic degree did not exceed 14.5 vol., both in low and high pH wines (pH ranged from 3.3 to 4).

8. Conclusions

Wild yeasts and lactic acid bacteria are interesting microorganisms that contribute to differentiate the wine character. The suitable use of numerous wild species and strains during winemaking favors the improvement of the complexity and the organoleptic properties of wines. The production of wines by spontaneous or inoculated fermentations using selected wild microorganisms is a remarkable practice for wineries. Further studies should be done in order to deep into the knowledge of the wild microflora of grapes and wines to better understand their behavior and importance. Even more, some drastic physical and chemical winemaking techniques, increasingly used in wineries, could be replaced taking advantage of the biological properties of these microorganisms. Above all, wild yeast and lactic acid bacteria may help to produce modern and new wine styles in a climate change viticultural environment.

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Conflict of interest

We have no conflict of interest to declare.

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