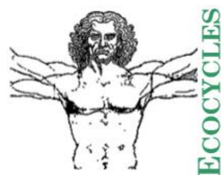
ECOCYCLES
ISSN 2416-2140

OPEN ACCESS
Scientific journal of the
European Ecocycles Society



Ecocycles, Vol. 6, No. 2, pp. 57-72 (2020)
DOI: 10.19040/ecocycles.v6i2.181

REVIEW ARTICLE

Microbiological control of alcoholic fermentation

Albert Mas*, Gemma Beltran, María Jesús Torija

Oenological Biotechnology, Department of Biochemistry and Biotechnology, Faculty of Oenology, University Rovira i Virgili. Marcel·lí Domingo, 1. 43007 Tarragona. Spain.

E-mail address: albert.mas@urv.cat

Abstract – Alcoholic fermentation and the production of wine has accompanied humanity for more than 10000 years. However, it has been only in the last 50 years when the winemakers have had the tools to manage and control the process. The methodology to analyse and monitor the succession of the microorganisms that participate in the process along with the effective use of antimicrobial compounds (for instance sulphur dioxide), the control of the temperature and, above all, the use of cellar-friendly fermentation starters (mostly as Active Dry Wine Yeast) have provided the appropriate conditions for that control. However, the use of a limited number of commercial presentations of the starters has generated an unwanted uniformity of the wines produced. Furthermore, new tendencies in wine making with limited or no human intervention have considered these tools as a negative aspect in the wine quality, although most of these concerns are only philosophical, without clear scientific evidence. We present a revision of the present state of the art in these methodologies where our research group has been working for the last 25 years.

Keywords – Yeast, fermentation starters, sulphur dioxide, molecular methods, spoilage

Received: December 7, 2020 Accepted: December 29, 2020

1. INTRODUCTION

The production of fermented products has been used by humans for at least 10000 years or even more. Among them, the fermented grape vine must, or wine, has been considered one of the most prestigious in many different cultures. Since its appearance in the Caucasian region around 7000 years ago has been found on the tables of kings, nobles, religious authorities and almost all the humanity, especially in Europe and America. It was considered the result of a kind of miracle that is why in some cultures was closely associated to religion and priests. For ancient humans to understand how a sweet liquid started "boiling" without the application of any heat and got transformed into a beverage leading to its disinhibition was much beyond their comprehension. Humanity had a previous experience already, by producing beer, knowing that those fermented products were healthy and the best way to drink water. At that time water was known to be the source of many diseases and drinking water as wine or beer was known to be safe.

The nature of the "miracle" took very long to be understood. Although the chemical transformation was defined at the end of the 18th Century (the transformation of sugar into ethanol and water), it was in the 19th Century when living microorganisms were discovered to be responsible of the process and it was not until the second half of the 20th Century to completely describe and control it. Cagniard de Latour in 1836 mentioned living organisms during the alcoholic fermentation. However, this observation was ignored for 30 years. It was Louis Pasteur, considered the start of the modern biochemistry and microbiology, who devoted several years to understand the production of wine and beer, describing beyond any doubt its microbiological nature. He described a succession of microorganisms that he named 'mycoderma' (defined as "fungi that were growing on the surface"), which were later named yeasts. Additionally, some of those microorganisms were identified the responsible for the wine spoilage and also for the production of vinegar. Some years later, when the microbiological methods were more developed, including the isolation and the study of isolated species and strains, researchers and winemakers understood

enough the process to start to have the appropriate control tools. For instance, in 1899 Hansen started the development of selected starters for the beer production. However, in the wine cellars it took longer to acquire this novelty. Among other reasons, we can consider the importance that the yeast has in the beer characteristics or the concentration of brewers in big companies, although the main cause is that brewing is process, which can be done throughout the whole year, while the wine is a seasonal product. The starters in the form of liquid media, for instance, were not the most appropriate for the wine sector due to their strong demand in a very short period (four-six weeks). The real outbreak of inoculation in wine making came with the development of Active Dry Yeast and the commercial offer available. Although many winemakers have used and still continue to use the commercial presentations, recent movements in wine making have challenged this practice by returning to old fashion and uncontrolled wine making yielding what are self-named "natural wines" (how the inoculation of a living organism, as wine yeasts, domesticated for centuries by humans and coming from such natural habitats as grapes or fermentations turns into "unnatural" or "non-natural"?).

2. Methods of analysis: Classical and molecular methods

The classical microbiological approaches to detect and quantify different wine microorganisms are generally supported by plating and observation under the microscope. Basically, a first approach consists of morphological tests, which are complemented with several physiological tests. Furthermore, the isolation of microorganisms is required to properly identify and quantify the given microorganisms. Barnett et al. (2000) described identification protocols to identify yeasts. One of the hurdles is the number of tests needed for the identification of yeast at species level. Thus, this methodology is time consuming, and the interpretation has to be done by experts with considerable experience.

At bacterial level, initial tests are the Gram stain and Catalase test, which can be used to discriminate between Lactic Acid Bacteria (LAB) and Acetic Acid Bacteria (AAB) present in wines. However, to identify at species level becomes much more difficult and often the physiological tests are not sufficient. The growth of microorganisms in different specific culture media produces colonies with diverse morphologies, which can be useful (Fugelsang and Edwards 2007).

The observation under a phase-contrast microscope is a first step to analyse the microorganisms' morphology. This observation provides information about size, shape, and arrangements of the cells. However, this can be misleading, as the morphology of the microorganisms is age- and culture-dependent.

The monitoring of the density and diversity of the microbiological population gives important information about the evolution of the winemaking process. Oenologists use counting under the microscope and direct plating to have an idea about the population densities. A limitation of microscope counting is the minimal population that is required, although it is a very fast approach. Low microbial population can be tackled by concentration after filtration. Direct plating methods are also a good alternative in these cases, although it lasts longer to get the results. The combination of both methods can be a good approach to those wines presenting low population or viability of the microorganisms. Microscope counting chambers, for instance Neubauer or Thoma, are needed for appropriate quantification. The main limitation can be low detection limits and lack of discrimination between alive and dead cells.

On the other side, counting the colonies grown on different media allows plate quantification of microorganisms. Some non-selective media allow the growth of all microorganisms. However, as there are different species of microorganisms that are mixed, the fastest growing and more prevalent species dominate on the plate, which will not allow the detection of those slower growing or in low proportion.

The use of selective media can circumvent this problem, because these media can limit or impede the growth of the dominant microorganisms. For instance, Lysine agar is a selective medium that reduces the detection of *Saccharomyces cerevisiae* because this species hardly grows with lysine as single nitrogen source. This medium is often used to study the non-*Saccharomyces* yeasts. Another alternative is the addition of antibiotics that inhibit microorganisms. Also, media enriched with different nutrients can favour the growth of different microorganisms are common in studying microorganisms involved in winemaking. Lactic Acid Bacteria are commonly isolated in MRS agar (De Man, Rogosa and Sharpe agar) an Acetic Acid Bacteria in GYC agar (glucose, yeast extract, and calcium carbonate agar). This last medium should also be considered differential medium. AAB produce acid gluconic or acetic, which dissolves the calcium carbonate precipitates and develops a clear halo around the colony. A selective medium can be obtained after changes of temperature, pH, aerobiosis/anaerobiosis condition, etc in a generic medium. Generally, the different conditions are used together for a more efficient enumeration.

The incorporation of DNA analysis methodology has been an important step forward in the identification microorganisms. The application of these methodologies together with isolation after plating has allowed a deep understanding of the ecology of grape and/or wine. The analysis of the polymorphism in the ribosomal RNA coding regions is the most usual method for the identification of wine microorganisms. The ribosomal genes of all living beings are grouped in tandem. These tandems form transcription units that have many copies in the genome. In each transcription unit exist coding regions that express the ribosomal genes (external transcript spaces ETS), the internal transcriber spacers (ITS) and the rRNA codifying genes. The ribosomal genes allow the establishment of the phylogenetic relations and are used to identify species (Kurtzman and Robnett 1998). The ribosomal genes are highly conserved regions and, thus, their sequences can be aligned with the sequences

available in the databases allowing the identification of microorganisms. Instead, the ITS are not coding regions that present higher polymorphism, which allow the differentiation of closely related species that cannot be differentiated by the analysis of the ribosomal genes. A phylogenetic tree is generated by comparison with the sequences available in the databases and used for the identification of microorganisms.

The main regions for sequencing ribosomal genes of yeast are the domain D1 and D2 in the 26S gene (Kurtzman and Robnett 1998). For bacteria, the main gene is 16S rRNA (Cole et al. 2005). In wine these regions have been used to differentiate among yeast species (Montrocher et al. 1998) and bacterial species (Le Jeune and Lonvaud-Funel 1997). However, for routine analysis of large number of samples required in ecological studies, a cheaper alternative has been the Restriction analysis of ribosomal genes (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism, PCRRFLPs). This technique uses specific endonucleases to generate fragments that can be species-specific. The regions used for wine yeast identification are the regions comprised between the 18S and 26S rRNA genes for yeast, which includes the intergenic spacers ITS1 and ITS2, and the 5,8S rRNA gene. The most RFLP used for bacteria is the 16S rRNA gene, which has been denominated Amplified Ribosomal DNA Restriction Analysis (ARDRA). The application to wine species was initiated by Guillamón et al. (1998) and Esteve-Zarzoso et al. (1999) and several studies have used this technique later on (Torija et al. 2001, Beltran et al. 2002). ARDRA has been used to identify LAB (Rodas et al. 2005) and AAB (Poblet et al. 2000, Ruiz et al. 2000, González et al. 2006a, Gullo et al. 2006, Vegas et al. 2010). Additional species discrimination has been done with the 16S - 23S intergenic spacer region (Ruiz et al. 2000, González and Mas, 2011).

Sequencing has become more accessible and affordable after the effort to fulfil the Human Genome made during this last two decades. Nowadays, only sequencing, alignment with sequences in databases and elaboration of genetic trees should be accepted as criteria for the identification of microbial species. However, when a large number of samples is to be processed, grouping through RFLP of the appropriate ribosomal genes or ITS has to be considered an initial step, assuming that all the isolates that present the same identification or banding pattern will belong to the same species. A minimum of two or three representatives of each grouping should be sequenced.

The application of molecular-based methods on plate isolates has allowed also the discrimination at strain level. The polymorphism and repeated sequences along the genome have been used as methods for strain genotyping. The most basic technique is based on the random amplification of genomic DNA with a single primer sequence of 9 or 10 bases of length (RAPD). Each strain present different amplification fragments, in size and number. The amplification is followed by agarose gel electrophoresis, which yields a band pattern that should be characteristic of a given strain. This technique has been used to genotype wine yeasts (Cocolin et al. 2004), LAB strains of *Oenococcus oeni* (Cappello et al. 2008) and AAB strains (Bartowsky et al. 2003). Other methods have used the repetitive elements of the genome, all of them based on the design of oligonucleotides homologous to these repeated sequences that allow the amplification of these regions, obtaining a pattern of electrophoretic bands for each species or strain. For the identification of different wine microorganisms several different techniques for yeast and bacteria have been applied. For instance, microsatellites are tandem repeat units of short DNA sequences, typically 1-10 nucleotide length in eukaryotic cells. The number of repeated sequences along the genome is very variable, making the distances between sequences highly polymorphic in size. Thus, the technique consists in the amplification of the parts of the genome that are flanked by these microsatellites, which yields an amplicon pattern that allows to differentiate strains. The most common oligonucleotides used are $(GACA)_4$, $(GAG)_5$, $(GTG)_5$ and others. *S. cerevisiae* strains were differentiated by Lieckfeldt et al. (1993) and then it was applied to wine strains by Maqueda et al. (2010). Gevers et al. (2001) used of $(GTG)_5$-PCR (also named rep-PCR in bacteria) to differentiate a wide range of food associated lactobacilli and other LAB species. Nowadays, $(GTG)_5$-PCR are extensively used to genotype AAB in wine vinegar production (Hidalgo et al. 2010, Vegas et al. 2010).

Different methods have been used to genotype *S. cerevisiae* as main microorganism in the alcoholic fermentation. For instance, delta elements are conserved sequences that flank transposable Ty elements. The separation distance between these elements is variable and does not exceed 1-2 kb, which determines that are appropriate to amplify the region comprised between them. The separation by size of these bands can be used to differentiate *S. cerevisiae* strains. This method was developed by Ness et al. (1993) and Masneuf and Dubourdieu (1994) to genotype strains of *S. cerevisiae*. The facility to perform the PCR analysis without extraction of the DNA (using directly the colony) has made this technique the most widely used to differentiate *S. cerevisiae* strains. The other main technique to differentiate *S. cerevisiae* strains is the Restriction analysis of mitochondrial DNA (mtDNA-RFLP). The basis of this technique is to use specific restriction endonucleases to fragment the DNA into specific sites, generating fragments of variable size. These fragments are separated on agarose gel showing pattern strain specific. This technique was firstly applied to brewer's yeast and wine strains of *S. cerevisiae* by Aigle et al. (1984) and Dubourdieu et al. (1987), respectively. Querol et al. (1992) simplified the protocol by using a unique characteristic of the mtDNA with high proportion of AT. Then, the restriction pattern DNA with enzymes that target sequences such as GCAT will cut less frequently the mtDNA than the nuclear DNA. So far, this was the most used technique to genotype the strains of *S. cerevisiae* (Torija et al. 2001, Beltran et al. 2002), although it still has the need to extract the DNA and it needs more time consuming than the direct PCR that can be performed with delta elements.

Finally, the most traditional technique for typing is the Pulsed-Field Gel Electrophoresis (PFGE), based on the

electrophoretic separation of the entire set of chromosomes with alternating electrical fields. The chromosomes should change their migration direction, which enables the separation of large fragments of DNA. This technique has been used to genotype wine strains of *S. cerevisiae* (Guillamón et al., 1996), some non-*Saccharomyces* (Esteve-Zarzoso et al. 2001) and *O. oeni* (Vigentini et al. 2009).

However, the main drawback of the methods based on plating is that they only quantify the microorganisms that are able to grow, and thus, the cells that are able to form colonies (colony forming units, abbreviated as CFU). The population enumerated by this method is considered as the "culturable" population, which sometimes is regarded as the representative of the viable population. Despite the extension of its use, this limitation together with the time required for some microorganisms to grow (2-5 days in yeasts, 2-10 days in bacteria) is a main handicap for the wine industry. However, one of the main challenges of the wine microbial ecology is that many microorganisms undergo states that are defined as Viable, But Not Culturable (VBNC, Millet and Lonvaud-Funel 2000). Microorganisms that are VBNC state are those that lose the ability to grow in a culture medium but still maintain some metabolic activity. This is one of the responses of many microorganisms when the environmental conditions are not optimal. The previous assumption was that these microorganisms were dead.

Thus, there are live cells, dead cells, and several cells in transient states in all microbial mixtures, as during the wine making process. These transient cells could be old cells that still retain the ability to grow under optimal conditions; old cells that have impaired the ability to grow on regular plates but still fully viable with active metabolism and finally cells that have already entered the lytic process. The old cells that have lost the ability to grow on plates can often be recovered by providing a very rich medium, normally using liquid medium with strong aeration to resume their growth again (Wang et al. 2016). Thus, culture independent techniques have used the molecular techniques to identify and/or quantify wine microorganisms without previous cultivation of these microorganisms (Rantsiou et al. 2005). These methods provide a better knowledge of the population, avoiding the biases that represent the microorganisms that are absent or not grow well on a plate.

As consequence, most of the consolidated knowledge on wine microbiology has emerged from the use of plating and the analysis of the microorganisms that could be recovered on the plates. However, the enumeration and identification of the microorganisms recovered on plates underwent a strong change from the extension of the molecular biology techniques that targeted DNA as main element, which meant a quick and big step toward the determination of grape and wine ecology. The expansion of these molecular biology techniques for identification and typing allowed a step further: the use of those techniques directly from grapes or wines, without the steps of culturing the microorganisms on plates. These "culture-independent" techniques have been used quite extensively since the beginning of this century and still they are very common. Many of these culture-independent techniques have some limitations, though. If the main target is DNA, this molecule is rather stable with time, and it does not allow the differentiation between live and dead cells. Several alternatives have been proposed to circumvent this limitation: targeting more labile molecules, such as RNA; quantification and identification through hybridisation of non-DNA molecules with short life, etc. For instance, a solution for appropriate differentiation between dead cells, VBNC cells and culturable alive cells has been the use of culture independent techniques with some modifications to eliminate the DNA from dead cells or use RNA. Several studies used RNA instead of DNA to quantify or detect the viable population, since this molecule is rapidly degraded in the dead cells (Cocolin and Mills 2003, Hierro et al. 2006). However, it is very tedious to work with RNA because it is unstable and can be degraded during the purification or analysis. Furthermore, rRNA might be more stable than required (Hierro et al. 2006, Andorrà et al. 2011, Sunyer-Figueres et al, 2018). Successful alternatives to use RNA have been developed with DNA binding dyes that only penetrate in the dead cells (damaged membranes) and block the amplification of this DNA (Rudi et al. 2005, Nocker and Camper 2006). Ethidium monoazide bromide (EMA) and propidium monoazide bromide (PMA) were proposed by Nogva et al. (2003) and Nocker et al. (2006), respectively, to detect bacterial viable cells. Both chemicals penetrate only into dead cells, in fact, into cells with compromised membrane integrity but not into live cells with fully functional cell membrane. Upon binding to the DNA of dead cells, the photo-inducible azide group allows these dyes to be covalently cross-linked by exposure to bright light and precipitate the DNA (Nocker and Camper 2006). Thus, only the DNA from live cells will be detected and quantified after the treatment with these dyes. This methodology has been applied successfully to wine microorganisms (Andorrà et al. 2010a).

The control of wine making process requires the identification of the microorganisms present as well as the quantification of each species in the different stages. The quantification is based on the correlation of the amount of the target molecules with the amount of biomass. This is true for DNA, but it is not completely valid for other molecules such as RNA or proteins, as they are more related to the physiological statuses of the cells, which present strong changes during wine making. In fact, almost all the relevant microorganisms in wine making undergo complete life and growing cycles during the process.

3. Grape microbiome

Grapes support microorganisms that are mostly epiphytes (that grow on the grape surface). The substrates that allow the growth of microorganisms are normally the exudates from grapes, rich in saccharides. The yeast population on sound grapes can go from 10^2 cfu/berry to 10^5 cfu/berry depending on the ripening state (Renouf et al. 2005). Interestingly, population quantity also changes during ripening of grapes, being the highest at the end of ripening (Renouf et al. 2005).

The increased population at the harvest time is mostly due to the increased nutrient availability, because the berry cuticle becomes soft and might have some microfissures not easily visible (Barata et al. 2012). At full ripening, grape musts obtained from healthy grapes contain yeast populations varying from 10^4 to 10^6 cfu/ml (Beltran et al. 2002, Padilla et al. 2016). Damaged grape berries can sustain growth of many microorganisms, increasing considerably the population at least one log cycle of population (to 10^6 or 10^8 cfu/berry) due to nutrient availability (Barata et al. 2012).

The yeasts present on the grape surface are mostly *Ascomycetous* moulds (yeast-like), *Basidiomycetous* and *Ascomycetous*. As main species of the *Ascomycetous* moulds, *Aureobasidium pullulans* is the most common yeast-like mould occupying grape surface. *Basidiomycetous* yeasts are also abundant on grape surface and the most frequent species are from genera *Cryptococcus*, *Rhodotorula* and *Sporodiobolus*. Although *Ascomycetous* yeasts generally colonize intact grape berries, a great diversity is found in the worldwide surveys. Common *Ascomycetous* yeasts on grape surface include the genera *Hanseniaspora*, *Candida* (most of those found on grapes have been later reclassified within *Starmerella)*, *Issatchenkia*, *Debaryomyces*, *Metschnikowia* and *Pichia*. Species diversity of *Ascomycetous* yeasts is even higher depending on a series of variations (climatic conditions, vineyard treatments, biotic factors, geographic location and vineyard factors including size, age, variety of grape and vintage year) (Barata et al. 2012). However, some species from *Ascomycetous* have been found worldwide such as *Hanseniaspora uvarum*, *Metschnikowia pulcherrima, Issatchenkia terricola* and *Issatchenkia orientalis*. *Saccharomyces cerevisiae* has hardly been found on sound grape berries, similar to some spoilage species such as *Zygosaccharomyces bailii*. However, damaged or rotted berries can provide more nutrients to favour the growth of *Ascomycetous* yeast. When whole bunch is harvested, some damaged berries may yield high numbers of the *Ascomycetous* yeast. Therefore, the isolation of *S. cerevisiae* and other spoilage species from grape berry is suspected to be related with grape health and sampling approach (Barata et al. 2012). *Ascomycetous* moulds and *Basidiomycetous* yeasts are considered residents on grape berries. These oligotrophic residents are thought to be adapted to the environment with poor nutrient availability (Loureiro et al. 2012). However, *Ascomycetous* yeasts are classified as copiotrophic opportunists, because they are rarely detected on immature grape berries but detected on grape berries with high nutrient availability (veraison, harvest or damaged grape berries). This is supported by the uneven distribution of *Ascomycetous* yeasts: microcolonies gather around the sites with most likely nutrient leaking from the berries (Loureiro et al. 2012). Although all *Ascomycetous* yeasts are opportunist, it is difficult to isolate some species on sound berries even at harvest time and the classical representative is *S. cerevisiae*. *S. cerevisiae* and its close relatives (other *Saccharomyces* yeast species) reside primarily in tree barks and soils as spores, where they are detected all year long. Only in the two months with grape growing from veraison to harvest or decay, the spores are dispersed onto grape berries by some vectors such as insects (Loureiro et al. 2012).

4. The succession of microorganisms during alcoholic fermentation: Yeast interactions

Yeasts on grape berries could survive and grow in grape must during alcoholic fermentation. Yeasts metabolize the main nutrients (sugars) to ethanol but also to other volatile compounds giving the wine its particular character. According to their fermentation capacity, competitiveness and contribution to wine, two main types of yeast can be considered in spontaneous wine fermentation: non-*Saccharomyces* yeasts and *Saccharomyces* yeasts. Non-*Saccharomyces* yeasts have lower fermentative capacity and are less competitive than *Saccharomyces* yeasts. However, today they are considered to increase wine complexity (Jolly et al. 2014, Mas et al. 2016).

The transformation of grape must into wine is a complex process that involves the sequential development of microbial species: mostly fungi, yeast, LAB and AAB. The microorganisms present in the berry surfaces are mainly yeasts. The microbiota associated to grapes varies constantly in response to grape variety, climatic conditions, viticultural practices, stage of ripening, physical damage (caused by moulds, insects and birds) and fungicides applied to vineyards (Pretorius et al. 1999). Although grape must is rather complete in nutrient content, its low pH and high sugar content, yields a selective media where only a few bacteria and yeast species can grow. Furthermore, the oenological practice of adding sulphur dioxide as antioxidant and antimicrobial preservative supposes an additional selection. This practice is meant to limit the growth of undesirable oxidative microbes and to prevent oxidation of grape must. Another important factor derives from the anaerobic conditions created during fermentation, especially at the start due to massive production of carbon dioxide (Henschke 1997). As a result, the alcoholic fermentation of grape juice into wine can be regarded as a heterogenous microbial process. The number of yeasts on the grape berry and grape must change depending on the geographical situation of the vineyard, climatic conditions, sanitary state of the berries and pesticide treatments of the vineyard (Beltran et al. 2002, Romano et al. 2006, Padilla et al. 2016). At harvest time, the yeast population is quite complex and the major fermenting yeast, *S. cerevisiae*, is not very abundant (Beltran et al. 2002, Torija et al. 2001). Therefore, the non-*Saccharomyces* population is expected to be dominant in the early stages of grape must processing. Thus, non-*Saccharomyces* yeasts predominate during the early stages of wine fermentation (Fleet 2003), and finally the *S. cerevisiae* yeast species, the most alcohol tolerant yeast, dominates the fermentation. Besides, some species of non-*Saccharomyces* may also be present during fermentation and in wine. Some of these yeast species should be considered as spoilage microorganisms because they produce metabolites with an undesirable impact (Pretorius 2000).

4.1. Non-Saccharomyces yeasts

The term of non-*Saccharomyces* has no taxonomical significance. According to Jolly et al. (2014), only yeast with a positive role in wine production is included in this description whereas spoilage yeasts such as *Dekkera*/*Brettanomyces* should not be included in this denomination. However, this is not a widespread concept and many authors refer to all species regardless their effects as non-*Saccharomyces*. In fact, many of those species considered as having a positive role in wine fermentation may have spoilage activity if their activity is prolonged during wine fermentation. Non-*Saccharomyces* yeasts are commonly known as wild yeasts, because they are mostly present in grapes and at the beginning of the fermentation (Fugelsang and Edwards 2007).

There are around 15 non-*Saccharomyces* yeast genera involved in wine fermentation. These are: *Dekkera* (anamorph *Brettanomyces*), *Candida/Starmerella*, *Cryptococcus*, *Debaryomyces*, *Hanseniaspora* (anamorph *Kloeckera*), *Kluyveromyces/Lachancea*, *Metschnikowia*, *Pichia*, *Rhodotorula*, *Saccharomycodes*, *Schizosaccharomyces*, *Torulaspora* and *Zygosaccharomyces* (Pretorius et al. 1999). Most of the non-*Saccharomyces* wine-related species show limited oenological aptitudes, such as low fermentation activity and low SO_2 resistance (Ciani et al. 2010). However, these species play an important role in the metabolic impact and aroma complexity of the final product. Furthermore, these species contribute to the enzymatic reactions, the main enzymatic activities described for some non-*Saccharomyces* species are protease, β-glucosidase, esterase, pectinase and lipase (Esteve-Zarzoso et al. 1998). Thus, the metabolic activities of various non-*Saccharomyces* yeast species during alcoholic fermentation have been matter of interest. Some yeast species such as *Torulaspora delbrueckii*, *Metschnikowia pulcherrima*, *Pichia kluyveri* and *Lachancea thermotolerans* are currently sold as commercial starters for wine production. The assessment of *Hanseniaspora uvarum*, *Starmerella bacillaris* (previously *Candida zemplinina*) and other species are still on the way to balance their positive contribution and negative impact on wine (Masneuf-Pomarede et al. 2016). Another species, *Hanseniaspora vineae* has been successfully used in wines from Uruguay and Spain (Lleixà et al. 2016, Martín et al. 2016), although it is not present as commercial product yet.

The negative impact of non-*Saccharomyces* is mainly the low fermentative activity and high level of undesirable flavours. The low fermentative activity can be overcome by mixed fermentation with *Saccharomyces* yeasts. The undesirable flavours are solved by olfactive perception experiments to screen acceptable or neutral strains (Bely et al. 2013). The genetic and phenotypic performance of 115 *Hanseniaspora uvarum* strains were fully assessed by Albertin et al. (2016), as well as 63 *Starmerella bacillaris* strains by Englezos et al. (2015), both being designed for exploitation of the two common non-*Saccharomyces* yeast species isolated in wine fermentation.

4.2. Saccharomyces yeasts

Saccharomyces is the most useful and widely exploited yeast genus at industrial level. The taxonomy of the genus *Saccharomyces* has undergone many revisions and reclassifications. In fact, many species considered as non-*Saccharomyces* were initially classified as *Saccharomyces*. According to Barnett et al. (2000) and Naumov et al. (2000), *Saccharomyces* yeasts were taxonomically separated into three groups: *Saccharomyces sensu stricto* group, containing *S. cerevisiae*, *S. bayanus*, *S. paradoxus*, *S. pastorianus*, *S. cariocanus*, *S. mikatae* and *S. kudriavzevii*, *Saccharomyces sensu lato* group, including *S. dairensis*, *S. exiguus*, *S. unisporus*, *S. servazzi* and *S. castelli* and the third group with only *S. kluyveri*. Later, *Saccharomyces* genus involved four species isolated from natural habitats, *S. cariocanus, S. kudriavzevii*, *S. mikatae* and *S. paradoxus* and three species associated with industrial fermentation processes, *S. bayanus*, *S. cerevisiae* and *S. pastorianus* (Barrio et al. 2006). Nowadays only *S. arboricolus* (not a wine species), *S. eubayanus* and *S. uvarum* are considered pure species, and the other "species" are considered hybrids (Borneman and Pretorius, 2015).

Physiological tests are not useful to differentiate the species of *Saccharomyces* and only their DNA sequences are reliable (Ribéreau-Gayon et al. 2006). In fact, the *Saccharomyces* species of oenological interest are *S. cerevisiae* and *S. bayanus. S. cerevisiae* is the main species in alcoholic fermentation, responsible for the metabolism of grape sugar to alcohol and carbon dioxide, but also important in the formation of secondary metabolites and conversion of grape aroma precursors to varietal wine aromas. *S. bayanus* has been used for alcoholic fermentation at low temperature since they are cryotolerant (Tamai et al. 1998); *S. bayanus* var. *uvarum* (synonym *S. uvarum*) is proved to be a good starter culture due to its reduced ethanol production, psychrophilism and acetate ester production (Masneuf-Pomarede et al. 2010, Bely et al. 2013, Csernus et al. 2014). In addition to these species, it is important to remember that haploid cells or spores from the *Saccharomyces sensu stricto* species are able to mate with each other resulting in viable hybrids (Querol et al. 2003). Hybrid strains of *S. bayanus* and *S. cerevisiae* and of *S. cerevisiae* and *S. kudriavzevii* have been isolated in alcoholic fermentations (González et al. 2006b). This phenomenon is a great possibility for the development of new species or strains. However, it is a source of taxonomic confusion due to the molecular and phenotypic classification analysis. For example, *S. cerevisiae* and *S. bayanus* are thought to be either two separate species, or the same species, that differ slightly from physiological aspects (Fugelsang and Edwards 2007). It is also known the physiological instability of strains belonging to *Saccharomyces sensu stricto* group (Ribéreau-Gayon et al. 2006).

Saccharomyces genus possesses series of unique characteristics that are not found in other genera. *Saccharomyces* yeasts have the ability to produce and accumulate ethanol even under aerobic conditions (Crabtree effect) (Marsit and Dequin 2015). Also, they have a high capacity to ferment

sugars quickly and efficiently. This ability allows them to colonize sugar-rich media and efficiently overgrow other yeasts, which are not so tolerant to alcohol (Barrio et al. 2006). However, the competition between *Saccharomyces* and non-*Saccharomyces* is more complex than the production of ethanol. In fact, there are many interactions, among them probably the most relevant cell-to-cell contact, nutrient limitation or the secretion of antimicrobial peptides (Wang et al. 2016). Although most of these mechanisms of interactions have been shown by analysing the growth on plates, recent findings relate that they induce the VBNC states that can end with the cell death (Branco et al. 2015, Wang et al. 2016). Nissen and Arneborg (2003) described also cell-to-cell contact as a possible inducer of lack of cultivability, although the reported mechanism seems to be limited to S101 *S. cerevisiae* strain, as other strains did not show the same mechanism (Wang et al. 2015).

4.3. Population dynamics of wine yeasts during spontaneous fermentation

The contribution of yeasts to wine is affected by their participation during the alcoholic fermentation (Comitini et al. 2011). Yeast species commonly found in spontaneous fermentation can be divided into three groups: aerobic yeast (*Pichia*, *Debaryomyces*, *Rhodotorula*, *Candida/Starmerella*, *Cryptococcus*), apiculate yeast (*Hanseniaspora*) and fermentative yeast (*Kluyveromyces*, *Torulaspora*, *Metschnikowia*, *Zygosaccharomyces* and *Saccharomyces*). Generally, the succession of yeast involves the initial domination of aerobic and apiculate yeasts which are present on grape surface, their decrease and then the increase of fermentative yeasts during fermentation, and finally the domination of the *Saccharomyces* yeasts (Schütz and Gafner 1993, Torija et al. 2001, Beltran et al. 2002). The main yeast species isolated at the beginning of the fermentation generally belong to *Hanseniaspora*, *Metschnikowia* and *Starmerella* genera.

The dominance of *S. cerevisiae* is needed to finish the alcoholic fermentation (Jolly et al. 2014). However, distinct fermentation dynamics are the result of the fermentation conditions and the relative levels of the main yeast species present. For instance, *Hanseniaspora* persists longer in fermentations at low temperature (Andorrà et al. 2010b); *Zygosaccharomyces bailii* leads botrytis-affected spontaneous fermentation (Nisiotou et al. 2007); *Pichia kudriavzevii* emerges along with *Saccharomyces* when relative low ethanol (9%) was obtained at the end of fermentation (Wang and Liu 2013); *Starmerella (Candida)* has been reported to codominate at late stages of fermentation (Llauradó et al. 2002) or to finish alcoholic fermentation (Clemente-Jimenez et al. 2004).

Furthermore, to the succession of different yeast species during wine fermentation, a dynamic change of strains within each species is also evident, based on molecular techniques for strain differentiation (Fleet 2003). For *S. cerevisiae*, some dominant or codominant strains have been found (Sabate et al. 1998, Torija et al. 2001), and in some cases where a single strain dominates the killer phenotype may be present (Schuller et al. 2005). Strain diversity of non-*Saccharomyces* species has also been reported but focused on their oenological interest rather than in the dynamic changes (Capece et al. 2005, Masneuf-Pomarede et al. 2015, Albertin et al. 2016).

5. Control of fermentation: from spontaneous to inoculated fermentations

Winemakers have traditionally seen non-*Saccharomyces* yeast as a source of wine spoilage. The main way for microbiological control in fermentations is the use of starter cultures. In winemaking, the most common yeast used as starter culture is *S. cerevisiae.* The development of cellar-friendly Active Dry Wine Yeast (ADWY) has extended its use in wine production, helping the winemaker to control the fermentation. The selection of yeast to be used as starter cultures has been developed using different tests and criteria. Nowadays, many different ADWY are commercially available. These yeasts are meant to increase aromatic expression, resistance to ethanol, low or high temperature, etc., but all of them with good fermentation potential and generally sufficient to complete the alcoholic fermentation.

Furthermore, yeasts not only lead the alcoholic fermentation, but also have an important role in wine quality. The activity of different yeast species and strains has an important effect on the organoleptic profiles of wine increasing its complexity and sensory richness (Ribereau Gayon et al. 2006). Presently, wine producers use commercial starters of *S. cerevisiae* to ensure the control of fermentation and produce a predictable and reproducible wine. A side effect of the widespread practice is the elimination of the participation of native microbiota. This limited participation might result in wines with similar sensory and analytical properties, depriving them from the, complexity, variability and personality, which define the typicality of a wine (Fleet 1993). Thus, the use of indigenous or native yeasts can be a tool to protect the authenticity, since it has been presented that microbial diversity is distinctive for a given area (Bokulich et al. 2014, Setati et al. 2015). The microbial population characteristic of a given area can be defined as the ‘microbial fingerprint’.

This microbial population will develop a distinctive character in the wine, measurable by the various components (molecules) that each microorganism leaves that we can define as the ‘microbial footprint’. The different microbial footprints will be related to the presence of these microorganisms during the winemaking process. The knowledge on the evolution of yeast populations during alcoholic fermentation has been going on, since the microbiology got the appropriate methods. Obviously, as techniques have evolved, knowledge has been completed. Despite the fact that the populations of *Saccharomyces* are very low in grapes (Beltran et al. 2002), their development during the alcoholic fermentation and the extensive use of ADWY have turned *S. cerevisiae* as the most common “cellar-resident yeast” (Beltran et al. 2002, Bokulich et al. 2014). Thus, the populations associated with the grapes vary through contact with the cellar environment (presses, pumps, tanks), where they join the resident microbiota. This

microbiota is not usually found in new wineries with equipment without previous use (Constanti et al. 1997).

In spontaneous fermentations, the native microbiota proliferates for several days and produce various compounds that could improve the organoleptic quality of the wines or at least give the wines a specific flavour. When the activities of these yeasts have been analysed, it has been detected the presence of enzymatic activities of great interest: esterases, beta-glucosidase, pectinases, etc. (Jolly et al. 2014). Additionally, they may cause ethanol reduction (Gonzalez et al. 2013, Contreras et al. 2014), which has been proposed as a key objective in the current winemaking due to the increased concentration of sugars, among other effects, derived from climate change (Mira de Orduna 2010). Despite these favourable aspects, the traditional bias of winemakers against non-*Saccharomyces* yeast has limited their use. However, in recent years, there is an increasing interest in selecting non-*Saccharomyces* yeasts to be used with *S. cerevisiae*. Thus, the key role of *S. cerevisiae* during alcoholic fermentation has been challenged (Fleet 2003, Jolly et al. 2014).

The positive effects on wine quality are the main goal for the selection of non-*Saccharomyces* yeast. These include either the production of new aromas or the removal of detrimental compounds that would decrease the wine quality. *Torulaspora delbrueckii* reduces the volatile acidity that is normally produced during winemaking (Renault et al. 2009) and has proved appropriate for the fermentation of botrytised grapes (Bely et al. 2008). Nowadays, it is possible to find various commercial preparations of this yeast. Another commercially available non-*Saccharomyces* yeast is *Metschnikowia pulcherrima*, which is recommended for the production of some aromas based on thiols and terpenes in white wines (González-Royo et al. 2015). Finally, another yeast available is *Lachancea thermotolerans*, for its production of lactic acid and glycerol (Gobbi et al. 2013). Although there are still few commercial preparations of non-*Saccharomyces* yeasts, they will probably increase in the near future. These include *Starmerella bacillaris* that produces large amounts of glycerol (Ciani and Ferraro 1996) and also because of its fructophilic character, which favours the end of fermentation (Soden et al. 2000). Other non-*Saccharomyces* species that can be expected in commercial preparations are the typical apiculate yeasts from the *Hanseniaspora* genus, such as *H. uvarum* (Andorrà et al. 2010c), *H. vinae* (Medina et al. 2013) and *H. guilliermondii* (Moreira et al. 2008). Other species that can have some oenological interest are species of the genera *Hansenula, Pichia, Schizosaccharomyces, Zygosaccharomyces,* etc., although its possible commercial development seems unlikely (Jolly et al. 2014). Nevertheless, pure culture fermentations with non-*Saccharomyces* wine yeast generally increase metabolite contributions to noticeable negative levels and poor fermentation activities that generally exclude their use as single starter cultures. The most important spoilage metabolites produced by non-*Saccharomyces* yeast are acetic acid, acetoin, acetaldehyde and ethyl acetate (Ciani et al. 2010).

However, the use of non-*Saccharomyces* yeast in the production of wine has the goal to increase some characteristics of the final product, yet it does not solve the main problem induced by the massive use of ADWY: the uniformity observed in inoculated wines. Some winemakers have eliminated or reduced the amount of starter cultures used in the production of "natural" wine to increase the effect of the native microbiota. This practice increases the risks of uncontrolled fermentations, which may lead to economic losses as these wines may have much higher risks of presenting different levels of spoilage that will not be acceptable for the consumer. The recommended solution to fight this uniformity is to exploit indigenous yeasts. Some years ago, different yeast producers developed commercial "local selection" yeasts in an attempt to protect the genuineness and authenticity of wines. However, in all cases the focus was on strains of *S. cerevisiae*. This solution defends the policy of *terroir* and typicality by using these starter cultures from local selection. Therefore, the use of oenologically competent indigenous yeasts as suitable inocula for the production of conventional or organic wines can achieve this goal.

6. Spoilage microorganisms in winemaking

In the wine industry, where alcoholic fermentation is conducted by many microorganisms, it is difficult to distinguish between beneficial fermenting activity and spoilage activity. Microorganisms can spoil wines at several stages during production. Any inappropriate grow of microorganisms may produce undesirable flavours.

Wine that is exposed to air may develop fermentative or oxidative yeasts on its surface, usually species of *Candida* and *Pichia* (Fleet 2003). These species oxidise ethanol, glycerol and acids, giving wines with unacceptably high levels of acetaldehyde, esters and acetic acid. Other wines can also be spoiled by fermentative species of *Zygosaccharomcyes*, *Dekkera* (anamorph *Brettanomyces*), *Saccharomyces* and *Saccharomycodes*. In addition to causing excessive carbonation, sediments and haze, these species produce estery and acid off-flavours (Sponholz 1993).

The winemaker's most feared spoilage yeast is *Dekkera /Brettanomyces*. This yeast produces off-flavours due to the synthesis of tetrahydropyridines and volatile phenols (4-ethylguaiacol and 4-ethylphenol). Generally, the production of these phenolic off-odours is noticed under a broad range of descriptors such as “barnyard-like, mousy, horsey, leather and pharmaceutical” (Grbin and Henschke 2000, Du Toit and Pretorius 2000). Among the species of this genus, *Dekkera bruxellensis* is the most representative in wines (Rodrigues et al. 2001). Furthermore, it has been found that other species are able to produce volatile phenols, such as *Pichia guilliermondii*, which has the ability to produce 4-ethylphenol with efficiencies as high as those observed in *D. bruxellensis* (Dias et al. 2003). *Pichia anomala*, *Metschnikowia pulcherrima* and *H. uvarum* are known for producing high levels of ethyl acetate and acetic acid before and during initial fermentation steps, leading to serious wine

deterioration (Romano et al. 1992, Plata et al. 2003). Spoilage species of LAB and AAB may grow in different stages of wine making, wines during storage in the cellar and after bottling (Sponholz 1993, Fuselsang 1997, Fleet 1998, Du Toit and Pretorius 2000). LAB can spoil wine during winemaking or during maturation and bottle aging. In the first case, bacteria can start performing the malolactic fermentation too early, before all the sugars have been consumed by yeasts. The fermentation of these carbohydrates by LAB leads to the production of lactic acid as major metabolite, but acetic acid, ethanol and CO_2 are also produced. Ideally during wine aging, no yeasts or bacteria should survive in wine. Not all the strains spoil wine, most depreciations and diseases are related to lactobacilli and pediococci, but they are normally destroyed during wine production. However, some strains demonstrate abnormal tolerance to the medium, especially to the ethanol concentration. Other undesirable compounds which are consequence of the LAB metabolism are the biogenic amines and ethylcarbamate (Lonvaud-Funel 1999). These metabolites do not have an impact on the aroma of the wine, but they are considered as pernicious for the health of the wine consumer.

The AAB can also spoil wines at many stages during the winemaking process. AAB that are naturally occurring in grape can survive in winemaking processes, depending on the environmental conditions and the technological practices carried out. Moreover, equipment and instruments used during wine making could be a good vehicle of AAB to contaminate the product in which the hygienic conditions are disregarded. The AAB isolated from grapes of different origins include the species of *Acetobacter, Ameyamaea, Asaia, Gluconobacter* and *Komagataeibacter* genus (Joyeux et al. 1984, González et al. 2005, Prieto et al. 2007, Valera et al. 2011, Barata et al. 2012, Mateo et al. 2014). On the other hand, the present view of microbial species associated with grapes, must and wines is much more complex than it has been previously described in early studies based on culture-dependent methods (Portillo and Mas 2016). The AAB species found on grapes or in grape must show differences from those in wine, depending on the differences in environmental conditions. Recent studies based on next generation sequencing technologies suggest that AAB are more abundant than previously thought during wine fermentations, independently of the grape variety (Portillo and Mas 2016). AAB that are usually involved in the wine spoilage are strains belonging to the genera *Acetobacter, Gluconobacter, Gluconacetobacter, Komagataeibacter* and *Asaia*.

Finally, filamentous fungi can also impact on wine production at several stages: spoilage of the grapes in the vineyard, production of mycotoxins in grapes and their transfer to wines, production of metabolites that enhance or inhibit the growth of wine yeast and malolactic bacteria, and cause the earthy, corky taints in wines after grow in grapes, corks and wine barrels (Fleet 2003). In order to prevent wine spoilage, hygienic conditions should be controlled during wine production. Although high hygienic conditions lead to limit the contaminant microorganisms, additional applications are mostly necessary to decrease the risk of spoilage. Sulphur dioxide (SO_2) is the one of the most efficient additives used for the prevention of wine spoilage. The effects of SO_2 depend on the kinds of organism to be suppressed and also pH value and sugar content of wine. 75 to 200 ppm sulfur dioxide is enough to inactivate spoilage microorganisms in must, while low concentrations of sulfur dioxide have minimal effect on *A. pasteurianus* strain (Du Toit et al. 2005). On the other hand, some metabolites synthesized by AAB, such as acetaldehyde from ethanol and dihydroxyacetone from glycerol, bind SO_2 and reduce the antimicrobial effect of this compound (Ribéreau-Gayon et al. 2000, Valera et al. 2017). In recent years, there has been a growing interest to develop emerging preservation technologies that can replace or complement the action of SO_2, since it might cause negative effects on health. These alternatives include the addition of antimicrobial agents (silver nanoparticles, bacteriocins, polyphenols etc.) and the application of physical methods (high pressure, low electric current, pulsed electric field, pulsed light, ultrasound, UV and e-beam irradiation, etc.) (García-Ruiz et al. 2015, Morata et al. 2017).

ACKNOWLEDGEMENTS

This manuscript has been prepared a within the framework of the SUMCULA and OENOBIO Erasmus + Programme Projects (2017-1-SE01-KA203-034570 and 2018-1-FR01-KA203-047839 respectively).

REFERENCES

Aigle, M., Erbs, D. and Moll, M. (1984). Some molecular structures in the genome of larger brewing yeast. American Society of Brewing Chemists 42: 1-7.
https://doi.org/10.1094/ASBCJ-42-0001

Albertin, W., Miot-Sertier, C., Bely, M. and Mostert, T.T. (2016). *Hanseniaspora uvarum* from winemaking environments show spatial and temporal genetic clustering. Frontiers in Microbiology 6: 1569.
https://doi.org/10.3389/fmicb.2015.01569

Andorrà, I., Esteve-Zarzoso, B., Guillamón, J.M. and Mas, A. (2010a). Determination of viable wine yeast using DNA binding dyes and quantitative PCR. International Journal of Food Microbiology 144: 257-262.
https://doi.org/10.1016/j.ijfoodmicro.2010.10.003

Andorrà, I., Landi, S., Mas, A., Esteve-Zarzoso, B. and Guillamón, J.M. (2010b). Effect of fermentation temperature on microbial population evolution using culture-independent and dependent techniques. Food Research International 43: 773-779.
https://doi.org/10.1016/j.foodres.2009.11.014

Andorrà, I., Berradre, M., Rozés, N., Mas, A., Guillamón, J.M. and Esteve-Zarzoso, B. (2010c). Effect of pure and

mixed cultures of the main yeast species on grape must fermentations. European Food Research and Technology 231: 215-224. https://doi.org/10.1007/s00217-010-1272-0

Andorrà, I., Monteiro, M., Esteve-Zarzoso, B., Albergaria, H. and Mas, A. (2011). Analysis and direct quantification of *Saccharomyces cerevisiae* and *Hanseniaspora guilliermondii* populations during alcoholic fermentation by fluorescence in situ hybridisation, flow cytometry and quantitative PCR. Food Microbiology 28:1483-1491. https://doi.org/10.1016/j.fm.2011.08.009

Barata, A., Malfeito-Ferreira, M. and Loureiro, V. (2012). Changes in sour rotten grape berry microbiota during ripening and wine fermentation. International Journal of Food Microbiology 154:152-161. https://doi.org/10.1016/j.ijfoodmicro.2011.12.029

Barnett, J. A., Payne, R.W., Yarrow, D. (2000). Yeasts: Characteristics and Identification (3rd ed.). Cambridge University Press, Cambridge, UK. https://doi.org/10.1046/j.1525-1470.2001.1862020a.x

Barrio, E., González, S.S., Arias, A., Belloch, C. and Querol, A. (2006). Molecular Mechanisms Envolvedin the Adaptative Evolution of Industrial Yeasts. In: Yeast in Food Beverages (Ed. A. Querol and G. H. Fleet). Springer, Berlin, pp. 153-174. https://doi.org/10.1007/978-3-540-28398-0_6

Bartowsky, E.J., Xia, D., Gibson, R.L., Fleet, G.H. and Henschke, P.A. (2003). Spoilage of bottled red wine by acetic acid bacteria. Letters in Applied Microbiology 36: 307-14.

https://doi.org/10.1046/j.1472-765x.2003.01314.x.

Beltran, G., Torija, M.J., Novo, M., Ferrer, N., Poblet, M., Guillamon, J.M., Rozes, N. and Mas, A. (2002). Analysis of yeast populations during alcoholic fermentation: a six year follow-up study. Systematic and Applied Microbiology 25: 287-293. https://doi.org/10.1078/0723-2020-00097

Bely, M., Stoeckle, P., Masneuf-Pomarède, I. and Dubourdieu, D. (2008). Impact of mixed *Torulaspora delbrueckii-Saccharomyces cerevisiae* culture on high-sugar fermentation. International Journal of Food Microbiology 122: 312-320. https://doi.org/10.1016/j.ijfoodmicro.2007.12.023

Bely, M., Renault, P., da Silva, T., Masneuf-Pomarede, I., Albertin, W., Moine, V., Coulon, J., Sicard, D., de Vienne, D. and Marullo, P. (2013). Non-conventional yeasts and alcohol level reduction. Conference "Alcohol Level Reduction in Wine" 3-37. http://www.observatoriova.com/wp-content/uploads/2013/12/oenoviti-actas-primer-simposio.pdf

Bokulich, N.A., Thorngate, J.A., Richardson, P.M. and Mills, D.A. (2014). Microbial biogeography of wine grapes in conditioned by cultivar, vintage, and climate. Proceedings of the National Academy of Sciences 111(1): 139-148. https://doi.org/10.1073/pnas.1317377110

Borneman, A.R. and Pretorius, I.S. (2015). Genomic insights into the *Saccharomyces sensu stricto* complex. Genetics, 199: 281-291. https://doi.org/10.1534/genetics.114.173633

Branco, P., Viana, T., Albergaria, H. and Arneborg, N. (2015). Antimicrobial peptides (AMPs) produced by *Saccharomyces cerevisiae* induce alterations in the intracellular pH, membrane permeability and culturability of *Hanseniaspora guilliermondii* cells. International Journal of Food Microbiology 205: 112-118. https://doi.org/10.1016/j.ijfoodmicro.2015.04.015

Capece, A., Fiore, C., Maraz, A. and Romano, P. (2005). Molecular and technological approaches to evaluate strain biodiversity in *Hanseniaspora uvarum* of wine origin. Journal of Applied Microbiology 98: 136-144. https://doi.org/10.1111/j.1365-2672.2004.02434.x

Cappello, M.S., Stefani, D., Grieco, F., Logrieco, A. and Zapparoli, G. (2008). Genotyping by Amplified Fragment Length Polymorphism and malate metabolism performances of indigenous *Oenococcus oeni* strains isolated from Primitivo wine. International Journal of Food Microbiology 127: 241-245. https://doi.org/10.1016/j.ijfoodmicro.2008.07.009

Ciani, M. and Ferraro, L. (1996). Enhanced glycerol content in wines made with immobilized *Candida stellata* cells. Applied and Environmental Microbiology 62: 128-132. https://doi.org/10.1128/AEM.62.1.128-132.1996

Ciani, M., Comitini, F., Mannazzu, I. and Domizio, P. (2010). Controlled mixed culture fermentation: a new perspective on the use of non-*Saccharomyces* yeasts in winemaking. FEMS Yeast Research 10: 123-133. https://doi.org/10.1111/j.1567-1364.2009.00579.x

Clemente-Jiménez, J., Mingorance-Cazorla, L., Martínez-Rodríguez, S., Heras-Vázquez, F.J.L. and Rodríguez-Vico, E. (2004). Molecular characterization and oenological properties of wine yeasts isolated during spontaneous fermentation of six varieties of grape must. Food Microbiology 21: 149-155. https://doi.org/10.3389/fmicb.2015.01563

Cocolin, L. and Mills, D.A. (2003). Wine yeast inhibition by sulfur dioxide: a comparison of culture-dependent and independent methods. American Journal of Enology and Viticulture 54(2): 125-130. https://agris.fao.org/agris-search/search.do?recordID=US201301958663

Cocolin, L., Pepe, V., Comitini, F., Comi, G. and Ciani, M. (2004). Enological and genetic traits of *Saccharomyces*

cerevisiae isolated from former and modern wineries. FEMS Yeast Research 5: 237-245. https://doi.org/10.1016/j.femsyr.2004.08.005

Cole, J.R., Chai, B., Farris, R.J., Wang, Q., Kulam, S.A., McGarrell, D.M., Garrity, G.M. and Tiedje, J.M. (2005). The Ribosomal Database Project (RDP-II): sequences and tools for highthroughput rRNA analysis. Nucleic Acids Research 33: 294-296. https://doi.org/10.1093/nar/gki038

Comitini, F., Gobbi, M., Domizio, P., Romani, C., Lencioni, L., Mannazzu, I. and Ciani, M. (2011). Selected non-*Saccharomyces* wine yeasts in controlled multistarter fermentations with *Saccharomyces cerevisiae*. Food Microbiology 28: 873-882. https://doi.org/10.1016/j.fm.2010.12.001

Constantí, M., Poblet, M., Arola, L., Mas, A. and Guillamón, J.M. (1997). Analysis of yeast populations during alcoholic fermentation in a newly established winery. American Journal of Enology and Viticulture 48: 339-344.

Contreras, A., Hidalgo, C., Henschke, P.A., Chambers, P.J., Curtin, C. and Varela, C. (2014). Evaluation of non-*Saccharomyces* yeasts for the reduction of alcohol content in wine. Applied and Environmental Microbiology 80(5): 1670-1678. https://doi.org/10.1128/AEM.03780-13

Csernus, O., Pomázi, A. and Magyar, I. (2014). Isolation, characterization, and selection of wine yeast strains in Etyek-Buda wine district, Hungary. Acta Alimentaria 43(3): 489-500. https://doi.org/10.1556/aalim.2014.1111

Dias, L., Dias, S., Sancho, T., Stender, H., Querol, A., Malfeito-Ferreira, M. and Loureiro, V. (2003). Identification of yeasts originated from wine related environments and capable of producing 4- ethylphenol. Food Microbiology 20: 567-574. https://doi.org/10.1016/s0740-0020(02)00152-1

Du Toit, M. and Pretorius, I.S. (2000). Microbial spoilage and preservation of wine: Using weapons from Nature's own arsenal — A Review. South African Society for Enology & Viticulture 21: 74-96. https://doi.org/10.21548/21-1-3559

Du Toit, W.J., Pretorius, I.S. and Lonvaud-Funel, A. (2005). The effect of sulphur dioxide and oxygen on the viability and culturability of a strain of *Acetobacter pasteurianus* and a strain of *Brettanomyces bruxellensis* isolated from wine. Journal of Applied Microbiology 98: 862-871. https://doi.org/10.1111/j.1365-2672.2004.02549.x

Dubourdieu, D., Sokol, A., Zucca, J., Thalouarn, P., Datee, A. and Aigle, M. (1987). Identification the souches de levures isolées de vins par l'analyse de leur DNA mitochondrial. Conn Vigne Vin 4: 267-278. https://doi.org/10.20870/oeno-one.1987.21.4.1286

Englezos, V., Rantsiou, K., Torchio, F., Rolle, L., Gerbi, V. and Cocolin, L. (2015). Exploitation of the non-Saccharomyces yeast *Starmerella bacillaris* (synonym *Candida zemplinina*) in wine fermentation: physiological and molecular characterizations. International Journal of Food Microbiology 199: 33-40. https://doi.org/10.1016/j.ijfoodmicro.2015.01.009

Esteve-Zarzoso, B., Manzanares, P., Ramón, D. and Querol, A. (1998). The role of non-*Saccharomyces* yeasts in industrial winemaking. International Microbiology 1: 143-148.

Esteve-Zarzoso, B., Belloch, C., Uruburu, F. and Querol, A. (1999). Identification of yeasts by RFLP analysis of the 5.8S rRNA gene and the two ribosomal internal transcribed spacers. International Journal of Systematic Bacteriology 49: 329-337. https://doi.org/10.1099/00207713-49-1-329

Esteve-Zarzoso, B., Peris-Torán, M.J., Ramón, D. and Querol, A. (2001). Molecular characterisation of *Hanseniaspora* species. Antonie Van Leeuwenhoek 80: 85-92. https://doi.org/10.1023/A:1012268931569

Fleet, G.H. (1993). Wine microbiology and biotechnology. Harwood, Chur, Switzerland.

Fleet, G.H. (1998). Microbiology of alcoholic beverages. In: Microbiology and Fermented Foods (Ed. B. J. Wood). Blackie Academic & Professional, London, England, pp. 217-262. https://doi.org/10.1007/978-1-4613-0309-1_9

Fleet, G.H. (2003). Yeast interactions and wine flavour. International Journal of Food Microbiology 86: 11-22. https://doi.org/10.1016/S0168-1605(03)00245-9

Fugelsang, K.C. (1997). Wine Microbiology. Chapman & Hall, New York, USA. https://link.springer.com/book/10.1007/978-1-4757-6970-8

Fugelsang, K.C. and Edwards, C.G. (2007). Wine microbiology. Practical Applications and Procedures. Springer Science Business Media, LLC, New York, USA. ISBN 978-0-387-33349-6

García-Ruiz, A., Crespo, J., Lopez-de-Luzuriaga, J.M., Olmosi M.E., M. Monge, M., Rodríguez-Alfaro, M.P., Martín-Alvarez, P.J., Bartolome, B. and Moreno-Arribas, M.V. (2015). Novel biocompatible silver nanoparticles for controlling the growth of lactic acid bacteria and acetic acid bacteria in wines. Food Control 50: 613-619.

Gevers, D., Huys, G. and Swings, J. (2001). Applicability of rep-PCR fingerprinting for identification of *Lactobacillus* species. FEMS Microbiology Letters 205: 31-36. https://doi.org/10.1111/j.1574-6968.2001.tb10921.x

Gobbi, M., Comitini, F., Domizio, P., Romani, C., Lencioni, L., Mannazzu, I. and Ciani, M. (2013). *Lachancea thermotolerans* and *Saccharomyces cerevisiae* in simultaneous and sequential cofermentation: a strategy to enhance acidity and improve the overall quality of wine. Food Microbiology 33: 271-281. https://doi.org/10.1016/j.fm.2012.10.004

González, A., Hierro, N., Poblet, M., Mas, A. and Guillamón, J.M. (2005). Application of molecular methods to demonstrate species and strain evolution of acetic acid bacteria population during wine production. International Journal of Food Microbiology 102: 295-304. https://doi.org/10.1016/j.ijfoodmicro.2004.11.020

González, A., Guillamón, J.M., Mas, A. and Poblet, M. (2006a). Application of molecular methods for routine identification of acetic acid bacteria. International Journal of Food Microbiology 108: 141-146. https://doi.org/10.1016/j.ijfoodmicro.2005.10.025

González, S.S., Barrio, E., Gafner, J. and Querol, A. (2006b). Natural hybrids from *Saccharomyces cerevisiae, Saccharomyces bayanus,* and *Saccharomyces kudriavzevii* in wine fermentations. FEMS Yeast Research 6: 1221-1223.

González, A., Mas, A. (2011) Differentiation of acetic acid bacteria based on sequence analysis of 16S-23S rRNA gene internal transcribed spacer sequences. International Journal of Food Microbiology, 147, 217-222. https://doi.org/10.1016/j.ijfoodmicro.2011.04.005

Gonzalez, R., Quiros, M. and Morales, P. (2013). Yeast respiration of sugars by non-*Saccharomyces* yeast species: A promising and barely explored approach to lowering alcohol content of wines. Trends in Food Science and Technology 29: 55-61. https://doi.org/10.1016/j.tifs.2012.06.015

González-Royo, E., Pascual, O., Kontoudakis, N., Esteruelas, M., EsteveZarzoso, B., Mas, A., Miquel Canals, J. and Zamora, F. (2015). Oenological consequences of sequential inoculation with nonSaccharomyces yeasts (*Torulaspora delbrueckii* or *Metschnikowia pulcherrima*) and *Saccharomyces cerevisiae* in base wine for sparkling wine production. European Food Research and Technology 240: 999-1012. https://doi.org/10.1007/s00217-014-2404-8

Grbin, P.R. and Henschke, P.A. (2000). Mousy off-flavour production in grape juice and wine by *Dekkera* and *Brettanomyces* yeasts. Australian Journal of Grape and Wine Research 6: 255-262. https://doi.org/10.1111/j.1755-0238.2000.tb00186.x

Guillamón, J.M., Barrio, E. and Querol, A. (1996). Characterization of wine yeast strains of the *Saccharomyces* genus on the basis of molecular markers: relationships between genetic distance and geographic or ecological origin. Systematic and Applied Microbiology 19: 122-132. https://doi.org/10.1016/S0723-2020(96)80019-1

Guillamón, J.M., Sabate, J., Barrio, E., Cano, J. and Querol, A. (1998). Rapid identification of wine yeast species based on RFLP analysis of the ribosomal internal transcribed spacer (ITS) region. Archives of Microbiology 169: 387-392. https://doi.org/10.1007/s002030050587

Gullo, M., Caggia, C., De Vero, L. and Giudici, P. (2006). Characterization of acetic acid bacteria in ‘‘traditional balsamic vinegar’’. International Journal of Food Microbiology 106: 209-212. https://doi.org/10.1016/j.ijfoodmicro.2005.06.024

Henschke, P.A. (1997). Yeast Sugar Metabolism. In: Wine yeast (Ed. K. D. Entian). Zimmermann FK, Technomic Publishing: Lancaster, Pennsylvania, USA, pp. 527-560.

Hidalgo, C., Vegas, C., Mateo, M., Tesfaye, W., Cerezo, A.B., Callejón, R.M., Poblet, M., Guillamon, J.M., Mas, A. and Torija, M.J. (2010). Effect of barrel design and the inoculation of *A. pasteurianus* in wine vinegar production. International Journal of Food Microbiology 141: 56-62. https://doi.org/10.1016/j.ijfoodmicro.2010.04.018

Hierro, N., Esteve-Zarzoso, B., González, A., Mas, A. and Guillamón, J.M. (2006). Real-time quantitative PCR (QPCR) and reverse transcription-QPCR (RT- QPCR) for the detection and enumeration of total yeasts in wine. Applied and Environmental Microbiology 72: 7148-7155. https://doi.org/10.1128/AEM.00388-06

Jolly, N.P., Varela, C. and Pretorius, I.S. (2014). Not your ordinary yeast: non-*Saccharomyces* yeasts in wine production uncovered. FEMS Yeast Research 14: 215-237. https://doi.org/10.1111/1567-1364.12111

Joyeux, A., Lafon-Lafourcade, S. and Ribéreau-Gayon, P. (1984). Evolution of acetic Acid bacteria during fermentation and storage of wine. Applied and Environmental Microbiology 48: 153-156. https://aem.asm.org/content/aem/48/1/153.full.pdf

Kurtzman, C.P. and Robnett, C.J. (1998). Identification and phylogeny of ascomycetous yeast from analysis of nuclear large subunit 26S ribosomal DNA partial sequences. Antonie Van Leeuwenhoek 73: 331-371. https://doi.org/10.1023/A:1001761008817

Le Jeune, C. and Lonvaud-Funel, A. (1997). Sequence of DNA 16S/23S spacer region of *Leuconostoc oenos* (*Oenococcus oeni*): application to strain differentiation. Research in Microbiology 148: 79-86.

Lieckfieldt, E., Meyer, W. and Börn, T. (1993). Rapid identification and differentiation of yeasts by DNA and PCR fingerprinting. Journal of Basic Microbiology 33: 413-426. https://doi.org/10.1002/jobm.3620330609

Llauradó, J., Rozés, N., Bobet, R., Mas, A. and Constantí, M. (2002). Low temperature alcoholic fermentations in high sugar concentration grapemusts. Journal of Food Science 67: 268-273. https://doi.org/10.1111/j.1365-2621.2002.tb11396.x

Lleixà, J., Martín, V., Portillo, M.C., Carrau, F., Beltran, G. and Mas, A. (2016). Comparison of fermentation and wines produced by inoculation of *Hanseniaspora vineae* and *Saccharomyces cerevisiae*. Frontiers in Microbiology 7: 338. https://doi.org/10.3389/fmicb.2016.00338

Lonvaud-Funel, A. (1999). Lactic acid bacteria in the quality improvement and depreciation of wine. Antonie Van Leeuwenhoek 76: 317-331. https://doi.org/10.1023/A:1002088931106

Loureiro, V., Ferreira, M.M., Monteiro, S. and Ferreira, R.B. (2012). The microbial community of grape berry. In: The Biochemistry of the grape berry (Ed. H. Gerós, M. M. Chanves and S. Delrot). Bentham Science publishers, Sharjah, United Arab Emirates, pp. 241-268. https://doi.org/10.2174/97816080536051120101

Maqueda, M., Zamora, E., Rodríguez-Cousiño, N. and Ramírez, M. (2010). Wine yeast molecular typing using a 209. https://doi.org/10.1016/j.fm.2009.10.004

Marsit, S.M. and Dequin, S. (2015). Diversity and adaptive evolution of *Saccharomyces* wine yeast: a review. FEMS Yeast Research 15: fov067. https://doi.org/10.1093/femsyr/fov067

Martín, V., Mas, A., Carrau, F., Dellacasa, E. and Boido, E. (2016). Effect of yeast assimilable nitrogen on the synthesis of phenolic aroma compounds by *Hanseniaspora vineae* strains. Yeast 33: 323-328. https://doi.org/10.1002/yea.3159

Mas, A., Padilla, B., Esteve-Zarzoso, B., Beltran, G., Reguant, C., Bordons, A. (2016). Taking advantage of natural biodiversity for wine making: The WILDWINE Project. Agriculture and Agricultural Science Procedia, 8: 4-9. https://doi.org/10.1016/j.aaspro.2016.02.002

Masneuf, I. and Dubourdieu, D. (1994). Comparaison de deux techniques d'identification des souches de levures de vinification basées sur le polymorphisme de l'ADN génomique: réaction de polymérisationen chaine (PCR) et analyse des caryotypes (electrophorese en champ pulsé). Journal International des Sciences de la Vigne et du Vin 28: 153-160. https://doi.org/10.20870/oeno-one.1994.28.2.1150

Masneuf-Pomarede, I., Bely, M., Marullo, P., Lonvaud-Funel, A. and Dubourdieu, D. (2010). Reassessment of phenotypic traits for *Saccharomyces bayanus var. uvarum* wine yeast strains. International Journal of Food Microbiology 139: 79-86. https://doi.org/10.1016/j.ijfoodmicro.2010.01.038

Masneuf-Pomarede, I., Juquin, E., Miot-Sertier, C., Renault, P., Laizet, Y.H., Salin, F., Alexandre, H., Capozzi, V., Cocolin, L., Colonna-Ceccaldi, B., Englezos, V., Girard, P., Gonzalez, B., Lucas, P., Mas, A., Nisiotou, A., Sipiczki, M., Spano, G., Tassou, C., Bely, M. and Albertin, W. (2015). The yeast *Starmerella bacillaris* (synonym *Candida zemplinina*) shows high genetic diversity in winemaking environments. FEMS Yeast Research 15(5): fov045.

Masneuf-Pomarede, I., Bely, M., Marullo, P. and Albertin, W. (2016). The genetics of non-conventional wine yeasts: current knowledge and future challenges. Frontiers in Microbiology 6:1563. https://doi.org/10.3389/fmicb.2015.01563

Mateo, E., Torija, M.J., Mas, A. and Bartowsky, E.J. (2014). Acetic acid bacteria isolated from grapes of South Australian vineyards. International Journal of Food Microbiology 178: 98-106. https://doi.org/10.1016/j.ijfoodmicro.2014.03.010.

Medina, K., Boido, E., Fariña, L., Gioia, O., Gomez, M.E., Barquet, M., Gaggero, C., Dellacassa, E. and Carrau, F. (2013). Increased flavour diversity of Chardonnay wines by spontaneous fermentation and co-fermentation with *Hanseniaspora vineae*. Food Chemistry 141: 2513-2521. https://doi.org/10.1016/j.foodchem.2013.04.056.

Millet, V. and Lonvaud-Funel, A. (2000). The viable but nonculturable state of wine micro-organisms during storage. Letters in Applied Microbiology 30: 136-141. https://doi.org/10.1046/j.1472-765x.2000.00684.x.

Mira de Orduna, R. (2010). Climate change associated effects on grape and wine quality and production. Food Research International 43: 1844-1855. https://doi.org/10.1016/j.foodres.2010.05.001

Montrocher, R., Verner, M.C., Briolay, J., Gautier, C. and Marmeisse, R. (1998). Phylogenetic analysis of the *Saccharomyces cerevisiae* group based on polymorphisms of rDNA spacer sequences. International Journal of Systematic and Evolutionary Microbiology 48: 295-303. https://doi.org/10.1099/00207713-48-1-295

Morata, A., Loira, I., Vejarano, R., Gonzalez, C., Callejo, M.J. and Suarez-Lepe, J.A. (2017). Emerging preservation technologies in grapes for winemaking. Trends in Food Science & Technology 67: 36-43. https://doi.org/10.1016/j.tifs.2017.06.014

Moreira, N., Mendes, F., Guedes de Pinho, P., Hogg, T. and Vasconcelos, I. (2008). Heavy sulphur compounds, higher alcohols and esters production profile of *Hanseniaspora uvarum* and *Hanseniaspora guilliermondii* grown as pure and mixed cultures in grape must. International Journal of Food Microbiology 124: 231-238. https://doi.org/10.1016/j.ijfoodmicro.2008.03.025.

Naumov, G.I., James, S.A., Naumova, E.S., Louis, E.J. and Roberts, I.N. (2000). Three new species in the *Saccharomyces sensu stricto complex: Saccharomyces cariocanus, Saccharomyces kudriavzevii* and *Saccharomyces mikatae*. International Journal of Systematic and Evolutionary Microbiology 50: 1931-1942. https://doi.org/10.1099/00207713-50-5-1931.

Nisiotou, A.A., Spiropoulos, A.E. and Nychas, G.E. (2007). Yeast community structures and dynamics in healthy and *Botrytis* affected grape must fermentations. Applied and Environmental Microbiology 73(21): 6705-6713. https://doi.org/10.3389/fmicb.2019.01712

Nissen, P. and Arneborg, N. (2003). Characterization of early deaths of non- *Saccharomyces* yeasts in mixed cultures with *Saccharomyces cerevisiae*. Archives of Microbiology 180: 257-263. https://doi.org/10.1007/s00203-003-0585-9

Nocker, A. and Camper, A.K. (2006). Selective removal of DNA from dead cells of mixed bacterial communities by use of ethidium monoazide. Applied and Environmental Microbiology 72:1997-2004.
https://doi.org/10.1128/AEM.72.3.1997-2004.2006

Nocker, A., Cheung, C.Y. and Camper, A.K. (2006). Comparison of propidium monoazide with ethidium monoazide for differentiation of live vs. dead bacteria by selective removal of DNA from dead cells. Journal of Microbiological Methods 67: 310-320.
https://doi.org/10.1016/j.mimet.2006.04.015.

Nogva, H.K., Drømtorp, S.M., Nissen, H. and Rudi, K. (2003). Ethidium monoazide for DNA-based differentiation of viable and dead bacteria by 5'-nuclease PCR. BioTechniques 34: 804-813.
https://doi.org/10.2144/03344rr02

Oliver, J.D. (2005). The viable but nonculturable state in bacteria. The Journal of Microbiology 43: 93-100.
https://pubmed.ncbi.nlm.nih.gov/15765062/

Padilla, B., García-Fernández, D., González, B., Izidoro-Pacheco, I., Esteve-Zarzoso, B., Beltran, G. and Mas, A. (2016). Yeast biodiversity from DOQ priorat uninoculated fermentations. Frontiers in Microbiology 7: 930. https://doi.org/10.3389/fmicb.2016.00930

Plata, C., Millán, C., Mauricio, J.C. and Ortega, J.M. (2003). Formation of ethyl acetate and isoamylacetate by various species of wine yeasts. Food Microbiology 20: 217-224.
https://doi.org/10.1016/S0740-0020(02)00101-6

Poblet, M., Rozès, N., Guillamón, JM. and Mas, A. (2000). Identification of acetic acid bacteria by restriction fragment length polymorphism analysis of a PCR-amplified fragment of the gene coding for 16S rRNA. Letters in Applied Microbiology 31: 63-67.
https://doi.org/10.1046/j.1472-765x.2000.00765.x

Portillo, M.C. and Mas, A. (2016). Analysis of microbial diversity and dynamics during wine fermentation of Grenache grape variety by high-throughput barcoding sequencing. LWT - Food Science and Technology 72: 317-321. https://doi.org/10.1016/j.lwt.2016.05.009

Pretorius, I.S., van der Westhuizen, T.J. and Augustyn, O.P.H. (1999). Yeast biodiversity in vineyards and wineries and its importance to the South African wine industry. South African Journal of Enology and Viticulture 20: 61-70.
https://doi.org/10.21548/20-2-2234

Pretorius, I.S. (2000). Tailoring wine yeast for the new millennium: novel approaches to the ancient art of winemaking. Yeast 16:675-729. https://doi.org/10.1002/1097-0061(20000615)16:8<675::AID-YEA585>3.0.CO;2-B

Prieto, C., Jara, C., Mas, A. and Romero, J. (2007). Application of molecular methods for analysing the distribution and diversity of acetic acid bacteria in Chilean vineyards. International Journal of Food Microbiology 115: 348-355. https://doi.org/10.1016/j.ijfoodmicro.2006.12.017

Querol, A., Barrio, E., Huerta, T., & Ramón, D. (1992). Molecular monitoring of wine fermentations conducted by active dry yeast strains. Applied and environmental microbiology, 58(9), 2948–2953.
https://doi.org/10.1128/AEM.58.9.2948-2953.1992

Querol, A., Fernández-Espinar, M.T., del Olmo, M. and Barrio, E. (2003). Adaptive evolution of wine yeast. International Journal of Food Microbiology 86: 3-10.
https://doi.org/10.1016/s0168-1605(03)00244-7

Rantsiou, K., Urso, R., Iacumin, L., Cantoni, C., Cattaneo, P. and Comi, G. (2005). Culture dependent and independent methods to investigate the microbial ecology of Italian fermented sausages. Applied and Environmental Microbiology 71: 1977-1986.
https://doi.org/10.1128/AEM.71.4.1977-1986.2005

Renault P, Miot-Sertier C, Marullo P, Hernandez-Orte P, Lagarrigue L, Lonvaud-Funel, A. and Bely, M. (2009). Genetic characterization and phenotypic variability in *Torulaspora delbrueckii* species: Potential applications in the wine industry. International Journal of Food Microbiology 134: 201-210.
https://doi.org/10.1016/j.ijfoodmicro.2009.06.008

Renouf, V., Claisse, O. and Lonvaud-Funel, A. (2005). Understanding the microbial ecosystem on the grape berry surface through numeration and identification of yeast and bacteria. Australian Journal of Grape and Wine Research 11: 316-327. https://doi.org/10.1111/j.1755-0238.2005.tb00031.x

Ribéreau-Gayon, P., Dubourdieu, D., Doneche, B. and Lovaud, A. (2000). Handbook of Enology. vol. 1. The Microbiology of Wine and Vinifications. John Wiley & Sons Ltd., Chichester, England.

Ribéreau-Gayon, P., Dubourdieu, D., Doneche, B. and Lovaud, A. (2006). Handbook of Enology vol. 1. The Microbology of wine and Vinifications (2nd ed). John Wiley & Sons Ltd., West Sussex, England. ISBN-13: 978-0-470-01034-1 (HB); ISBN-10: 0-470-01034-7 (HB)

Rodas, A.M., Ferrer, S. and Pardo, I. (2005). Polyphasic study of wine *Lactobacillus strains*: taxonomic implications. International Journal of Systematic Evolutionary Microbiology 55: 197-207.
https://doi.org/10.1099/ijs.0.63249-0

Rodrigues, N., Gonçalves, G., Malfeito-Ferreira, M. and Loureiro, V. (2001). Development and use of a differential

medium to detect yeasts of the genera *Dekkera/ Brettanomyces*. International Journal of Food Microbiology 90: 588-599. https://doi.org/10.1046/j.1365-2672.2001.01275.x

Romano, P., Suzzi, G., Comi, G. and Zironi, R. (1992). Higher alcohol and acetic acid production byapiculate wine yeasts. Journal of Applied Bacteriology 73: 126-130. https://doi.org/10.1111/j.1365-2672.1992.tb01698.x

Romano, P., Capece, A. and Jespersen, L. (2006). Taxonomic and ecological diversity of food and beverage yeasts. In: Yeast in Food Beverages (Ed. A. Querol and G. H. Fleet). Springer, Berlin, Germany, pp. 55-82. https://link.springer.com/chapter/10.1007/978-3-540-28398-0_2

Rudi, K., Naterstad, K., Dromtorp, S.M. and Holo, H. (2005). Detection of viable and dead *Listeria monocytogenes* on goudalike cheeses by real-time PCR. Letters in Applied Microbiology 40: 301-306. https://doi.org/10.1111/j.1472-765X.2005.01672.x

Ruiz, A., Poblet, M., Mas, A. and Guillamon, J.M. (2000). Identification of acetic acid bacteria by RFLP of PCR-amplified 16S rDNA and 16S–23S rDNA intergenic spacer. International Journal of Systematic and Evolutionary Microbiology 50: 1981-1987. https://doi.org/10.1099/00207713-50-6-1981

Sabate, J., Cano, J., Querol, A. and Guillamon, J. M. (1998). Diversity of *Saccharomyces* strains in wine fermentations: analysis for two consecutive years. Letters in Applied Microbiology 26: 452 -455.

Schuller, D., Alves, H., Dequin, S. and Casal, M. (2005). Ecological survey of *Saccharomyces cerevisiae* strains from vineyards in the Vinho Verde region of Portugal. FEMS Microbiology Ecology 51: 167-177. https://doi.org/10.1016/j.femsec.2004.08.003

Schütz, M. and Gafner, J. (1993). Analysis of yeast diversity during spontaneous and induced alcoholic fermentations. Journal of Applied Microbiology 75: 551-558. https://doi.org/10.1111/j.1365-2672.1993.tb01594.x

Setati, M.E., Jacobson, D. and Bauer, F.F. (2015). Sequencebased analysis of the *Vitis vinifera* L. cv Cabernet Sauvignon grape must mycobiome in three south African vineyards employing distinct agronomic systems. Frontiers in Microbiology 6:1358. https://doi.org/10.3389/fmicb.2015.01358

Soden, A., Francis, I.L., Oakey, H. and Henschke, P.A. (2000). Effects of co-fermentation with *Candida stellata* and *Saccharomyces cerevisiae* on the aroma and composition of Chardonnay wine. Australian Journal of Grape and Wine Research 6: 21-30. https://doi.org/10.1111/j.1755-0238.2000.tb00158.x

Sponholz, W. (1993). Wine spoilage by microorganisms. In: Wine Microbiolgy and Biotechnology (Ed. G. H. Fleet). Harwood Academic Publishers, Chur, Switzerland, pp. 395-420.

Sun, S.Y., Gong, H. S., Liu, W.L. and Jin, C.W. (2016). Application and validation of autochthonous *Lactobacillus plantarum* starter cultures for controlled malolactic fermentation and its influence on the aromatic profile of cherry wines. Food Microbiology 55: 16-24. https://doi.org/10.1016/j.fm.2015.11.016

Sunyer-Figueres M, Wang C, Mas A. (2018). Analysis of ribosomal RNA stability in dead cells of wine yeast by quantitative PCR. International Journal of Food Microbiology, 270, 1-4 https://doi.org/10.1016/j.ijfoodmicro.2018.01.020

Tamai, Y., Momma, T., Yoshimoto, H. and Kaneko, Y. (1998). Co-existence of two types of chromosome in the bottom fermenting yeast, *Saccharomyces pastorianus*. Yeast 14: 923-933. https://doi.org/10.1002/(SICI)1097-0061(199807)14:10<923::AID-YEA298>3.0.CO;2-I

Torija, M.J., Rozès, N., Poblet, M., Guillamón, J.M. and Mas, A. (2001). Yeast population dynamics in spontaneous fermentations: Comparison between two different wine-producing areas over a period of three years. Antonie van Leeuwenhoek 79: 345-352. https://doi.org/10.1023/A:1012027718701

Valera, M.J., Federico Laich, F., Sara, S., González, S.S., Torija, M.J., Mateo, E. and Mas, A. (2011). Diversity of acetic acid bacteria present in healthy grapes from the Canary Islands. International Journal of Food Microbiology 151: 105-112. https://doi.org/10.1016/j.ijfoodmicro.2011.08.007

Valera, M.J., Torija, M.J. and Mas, A. (2017). Detrimental Effects of Acetic Acid Bacteria in Foods. Chapter 12. In: Acetic Acid Bacteria: Fundamentals and Food Applications (Ed. I. Y. Sengun). CRC Press, Taylor & Francis Group, Boca Raton, pp. 299-320.

Vegas, C., Mateo, E., González, A., Jara, C., Guillamon, J.M., Poblet, M., Torija, M.J. and Mas, A. (2010). Population dynamics of acetic acid bacteria during traditional wine vinegar production. International Journal of Food Microbiology 138: 130-136. https://doi.org/10.1016/j.ijfoodmicro.2010.01.006.

Vigentini, I., Picozzi, C., Tirelli, A., Giugni, A. and Foschino, R. (2009). Survey on indigenous *Oenococcus oeni* strains isolated from red wines of Valtellina, a cold climate wine - growing Italian area. International Journal of Food Microbiology 136: 123-128.

Wang, C. and Liu, Y. (2013). Dynamic study of yeast species and *Saccharomyces cerevisiae* strains during the spontaneous fermentations of Muscat blanc in Jingyang, China. Food Microbiology 33: 172-177. https://doi.org/10.1016/j.fm.2012.09.014

Wang, C., Mas, A. and Esteve-Zarzoso, B. (2015). Interaction between *Saccharomyces cerevisiae* and *Hanseniaspora uvarum* during alcoholic fermentation. International Journal of Food Microbiology 206: 67-74. https://doi.org/10.1016/j.ijfoodmicro.2015.04.022

Wang, C., Mas, A. and Esteve-Zarzoso, B. (2016). The Interaction between *Saccharomyces cerevisiae* and non-*Saccharomyces* yeast during alcoholic fermentation is species and strain specific. Frontiers in Microbiology 7: 502 https://doi.org/10.3389/fmicb.2016.00502

© 2020 by the author(s). This article is an open-access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).