Patrizia Romano · Maurizio Ciani
Graham H. Fleet *Editors*

Yeasts in the Production of Wine

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In Memory of Graham

The idea of this book originated several years ago from the desire of two friends (Graham Fleet and I) to share a project, focused on the role of yeasts in winemaking. The project was planned to involve colleagues from all over the world, experts in wine yeasts. Our aim was to publish an updated book covering the occurrence and activities of yeasts in wine production, with contributions from an impressive cohort of international authors. I was well aware of Graham's scientific production on wine yeasts. His work has accompanied many students and researchers over the years, always stimulating new and innovative topics. I personally met Graham in Perugia in 1988 at the General Congress of the International Yeast Commission. It was a great emotion for me to finally meet this great scientist known all over the world for his works, in particular concerning yeasts in the wine sector. Since then, a scientific bond based on the common interest on food yeasts has grown between us year after year discussing yeast topics while attending the symposia of the International Commission on Yeasts, of which we were both commissioners.

The project of the book *Yeasts in the Production of Wine* arose from this knowledge and friendship. We were bound by a great enthusiasm and the desire to organize an updated and useful book that would cover all the topics of interest. However, several drawbacks have delayed the work that Graham and I were doing together. The last time we saw each other was in Perugia for the ISSY32, where we discussed the book's progress. Unfortunately, a short time later, Graham suddenly passed, and his painful loss blocked the book. On the one hand, it was difficult and painful to continue this work without him; on the other hand, I felt strongly the feeling of having to complete this book to dedicate it to this great scientist of yeasts. Therefore, I shared this final stage of the book with our colleague and friend, Maurizio Ciani.

For me, he was not only a dear colleague but primarily a benchmark for research on food yeasts. Over the years, our friendship was firmly established, and despite living in such distant countries, we were in touch via email, talking not only about yeasts but also about everyday life. Beyond the great esteem I felt for him, "the scientist", I admired his depth as a generous person and very caring towards others. I have many fond moments and memories of Graham talking about yeasts, and I had the pleasure and honour on numerous occasions of sharing with him many wine tastings while exchanging opinions. He was an educator and a great scientist who shared with all of us his love and passion for yeast and the lifetime he spent in research with great commitment, generosity and joy, trying to understand yeast contributions in foods and beverages.

It was hard to complete the book without him, but thanks to the availability of Maurizio Ciani, who has shared this project, and of all the authors of the various chapters, we were able to complete the book. The greatest satisfaction is to dedicate it to this great researcher and friend who was Graham.

Patrizia Romano

Preface

The main role of yeasts in the bioconversion of grape juice into wine is well established even if other microorganisms may affect the composition of the final product. In the last few decades, research on winemaking process has made more clarity on the complex interactions of many microbial species and the complex ecological and biochemical processes, highlighting the fundamental impact of yeasts on wine production. Researchers across the world have demonstrated the great diversity of yeasts at the species and strain level, which is expressed through different biochemical, physiological and molecular mechanisms, which are the basis of the many roles of yeasts in wine production.

The aim of this book is to collect the new recent developments on the key role of yeast in wine production, evaluating the ecological, genetic and metabolic aspects. The book contains 16 chapters, written by international contributors who are recognized authorities in their field, which cover the most important topics concerning yeasts and their biotechnological applications in wine production. Chapter 1 describes the recent developments and new approaches in yeast ecological distribution from grapes to cellar during the fermentation process and its role in wine production. Chapter 2 gives an exhaustive description of the metabolic impact of yeasts on wine. Chapters 3, 4, 5 and 6 focus a molecular approach on the monitoring and quality assurance of yeasts (Chap. 3), on yeast diversities (Chap. 4), on gene expression during fermentation (Chap. 5) and on synthetic genome engineering in wine yeasts (Chap. 6). Chapter 7 examines the presence and the role of yeasts in a specific environment and fermentation process such as botrytized wines, while Chapter 8 describes the modalities of production of commercial starter cultures in large scale. Chapter 9 describes kinetics and control of alcoholic fermentation, while Chapter 10 is focused on the description of the recent development of strategies for the genetic improvement of wine yeasts. Chapter 11 provides an updated overview on the role of wine yeasts in determining the content of different compounds affecting wine consumer health. Chapter 12 reports the diversity and significance of yeast spoilage species in wine production, analysing the appropriate methodologies for their control. In the next chapters, the occurrence and role of yeasts in specific and peculiar wines are described. Chapter 13 describes the role and influence of yeasts in primary and secondary fermentation during sparkling wine production, while in Chapter 14, the peculiar characteristics and the fundamental role of yeasts associated with fortified wines are discussed. Chapter 15 reports the recent development on fruit wines which are an increasingly widespread alternative to winemaking. Chapter 16, the last chapter, provides an in-depth review on yeasts associated with the production of distilled alcoholic beverages.

In summary, this book provides a comprehensive account on the occurrence, role and biotechnological use of wine yeasts written by a group of expert scientists from key wine production countries and experts in their field. We believe that each chapter contains information which should be valuable to students of winemaking courses, PhD students and researchers who study or work with yeasts. The content of this book can also be useful for wineries and wine yeast companies.

Potenza, Italy Patrizia Romano
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About the Editors

Patrizia Romano graduated in Biological Sciences at the University of Bologna (Italy), where she developed teaching and research activities in the area of agro-food microbiology. Since 1994, she started to work at the University of Basilicata (Potenza, Italy) as Full Professor in Agricultural Microbiology. During her career at the University of Basilicata, she has held various academic positions, as President of the degree course of Food Technology; Vice-Dean of the Agriculture Faculty and successively Vice-Director of the School of Agricultural, Forestry, Food, and Environmental Sciences; Member of the Academic Senate; and actually Vice-Rector for International Education. She is Member of the doctorate boarding in food technology sector, responsible of Erasmus mobility programs with different European countries, and Tutor of research activity of students and researchers from different countries. She is Member of the Italian Academy of Vine and Wine and of International Commission on Yeasts (ICY); Delegate and Expert of Italian Ministry of Agricultural, Food, and Forest Politics for the OIV (International Organisation of Vine and Wine) in the Microbiology Group (Oenology Commission); and Coordinator of the Italian Wine Microbiology Group (GMV). Her research activity is focused on yeast biotechnology, related to different fermentative processes. She is Author and Coauthor of about 230 publications, comprising chapters in books, publications on Italian and international journals, posters, and oral presentations in Italian and international conferences. The main research lines deal with ecology of natural yeasts and their characterization for technological parameters, yeast characterization for secondary compound production related to organoleptic and healthy quality of fermented beverages, and formulation of yeast starters for application at industrial level. She is Co-inventor of two patents on yeasts and Member of an academic spin-off.

Maurizio Ciani is Full Professor of Biotechnology of Microorganisms in the Department of Life and Environmental Sciences at the Polytechnic University of Marche. He graduated in Agricultural Sciences at the University of Perugia, where he was Researcher from 1990 to 2000. In 1993, with a Research Fellow of the

National Research Council (NRC), he spent a period at Viticulture and Enology Department of the University of California, Davis, USA. He is Associate Professor from 2000 at Polytechnic University of Marche and Full Professor from October 2006 in the same university. He is the Department Coordinator for International Mobility at the Faculty of Science and Delegate of the Rector for the orientation of students. He is Member of Italian Academy of Vine and Wine from 2006 and is Expert of Italian Ministry of Agricultural, Food and Forest Politics for the OIV (International Organisation of Vine and Wine) in the Microbiology Group (Oenology Commission). He has been Scientific Responsible of several research projects financially supported by public funds or private institutions and industries. The scientific activity is focused on yeasts: isolation, selection, and characterization of yeasts from various environments. The investigations concerned the new fermentation biotechnology in wine-making (immobilization, multistarter fermentations), the use of nonconventional yeasts in wine and beer production to improve complexity and aroma profile as well as increase/reduce specific compounds, the physiology of wine yeasts (metabolic characterization of yeasts for food and industrial application), and the molecular and biochemical characterization of antimicrobial compounds from yeasts for food and industrial application. He is Author or Coauthor of more than 180 publications including 18 book chapters and a monograph.

Graham H. Fleet had completed his MSc degrees (1969) in Microbiology and Biochemistry from the University of Queensland. He joined the research group of Prof. Herman Phaff at the University of California Davis, where he developed a pioneer project on the biochemistry of yeast cell walls. In 1973, he received his PhD in Microbiology, Food Science, and Technology from the University of California, Davis. After completing his studies in the USA, he went for postdoctoral studies at the Department of Brewery and Biological Sciences, Heriot-Watt University, Edinburgh, UK. In 1975, he returned to Sidney, where he developed a productive academic carrier at the University of New South Wales (UNSW) as Lecturer, Associate Professor (1982), and Professor (1996) of Food Science and Technology, School of Chemical Science and Engineering, until retirement in 2007, when he continued his academic activities at the UNSW as Emeritus Professor. He was a talented Mentor to students from several countries. He left a remarkable legacy in the literature on food science and technology, microbiology, food safety, and other areas, demonstrating his leading role. He was the Editor/Coeditor of 9 books and Author/Coauthor of over 30 book chapters and more than 100 papers in prestigious journals. He was fascinated by the microbial communities associated with natural fermentations of foods. His last book, *Cocoa and Coffee Fermentations* (2015), is focused on this topic, and his book *Wine Microbiology and Biotechnology* (1993) is a known reference. He was Editor of the *International Journal of Food Microbiology* and served at the editorial board of several scientific journals. Since 2010, he served as the Executive Board of the International Committee on Food Microbiology and Hygiene. He was the Chair of the International Commission on Yeasts (ICY, 1996–2000), then ICY Vice-Chair (2000–2004), and later ICY Honorary Member. He was a Member of the Executive Board of the International Union of Microbiological Societies (IUMS) and Chairperson of the IUMS Mycological Division (2002–2008), where he acted as a passionate Representative of yeast researchers. He participated on several ICY meetings, often presenting conferences on yeasts in food and beverages. His last lecture, presented in 2015 during ISSY 32 in Perugia (Italy), was a memorable conference on "yeasts and the fermented food renaissance."

Chapter 1
Yeast Ecology of Wine Production

Maurizio Ciani and Francesca Comitini

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1 Introduction

Wine fermentation is a complex biotechnological process in which yeasts play an essential role. In this context, the ecological distribution of yeasts through the production chain of wine production is a crucial factor the quality of wine. Although

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Saccharomyces cerevisiae is the main microorganism involved during the transformation of grape juice in wine, many other yeasts species occur in grape juice fermentation and may actively take part in the process. Nowadays, selected starter cultures of *S. cerevisiae* are usually added by oenologists to control the fermentative process and to achieve specific desired enological characters (inoculated fermentations). The aim is that to dominate indigenous yeasts belonging to the vineyard environment, winery facilities and cellar equipment. Indeed, it has been clearly demonstrated that the microbial population is a multi-comprehensive consortium that includes filamentous fungi, yeasts and bacteria with different physiological characteristics and different impact on the grape metabolome and final wine quality (Pinto et al. 2015; Verginer et al. 2010). The composition of grape microbiota can be influenced, in complexity and frequency, by various abiotic or biotic factors, including climatic conditions, temperature, UV exposure, rainfall, sunlight and winds, ripeness or variety of grapes and interaction within strains that co-habitat. The study and the monitoring of microbiota of grape barriers is important to recognize the evolution of yeasts and the relationship between the microorganisms, fundamental to predict the progress of fermentative process. The use of conventional and innovative molecular methods allow to analyse the microbial members of consortium from grape berries to wine. Indeed, spontaneous wine fermentation is typically carried out by a complex evolution of microorganisms extensively examined during the years. Now, it is well established that together with *S. cerevisiae*, non-*Saccharomyces* species actively participate during the alcoholic fermentation and their contribution was recently positively revaluated. Non-*Saccharomyces* yeasts, coming from grape berry and winery environment, if well managed, can positively impact on the analytical and sensory characteristics of wines. In this regard, growing interest on the use of controlled mixed fermentation with selected non-*Saccharomyces* and *S. cerevisiae* wine yeasts draw the applied research in oenological field.

2 Yeasts on Grapes

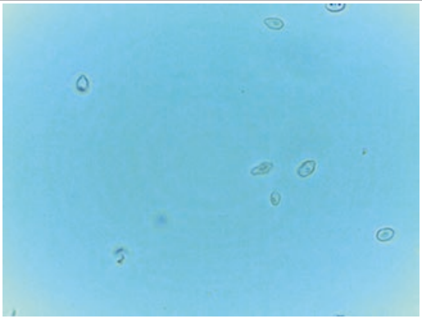
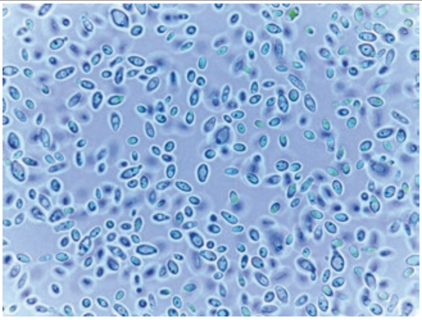
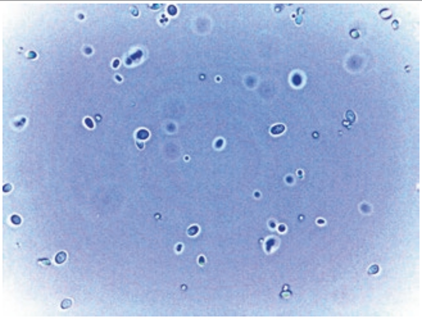
Grapes represent a complex ecological niche where filamentous fungi, yeasts and bacteria cohabit. The microbial community colonizing this ecological niche includes microbial species whose concentration depending on multiple factors; the most important are related to grape ripening and nutrients availability. Actually, the microbial ecology of grape berry is a wide concept including closed relations between the ecosystems and their microbial interactions, microbial vectors and sources of microorganisms. Herman Phaff, the pioneer of yeast ecology, described the concept of ecology as "where microbes live and why they live in one habitat and how yeasts interact with other microorganisms" (Lachance 2003). This comprehensive approach implies that microbial communities may be affected by many other variables in grapes, such as viticultural practices, pedoclimatic factors, diseases and pests that could modify grape integrity. In general, the yeast populations of mature grapes are comprised of between 10^3 and 10^5 cells/g (Fleet et al. 2002), but approximately one log higher values have often been found on damaged berries in presence of higher availability of sugar and nutrients (Barata et al. 2008). Over the last century many researchers have described the occurrence and association of yeasts with grape surface and the results were reviewed by Amerine and Kunkee 1968; Kunkee and Goswell 1977; Kunkee and Bisson 1993. More recently, the yeast ecology of wine grapes was reviewed by Fleet et al. 2002, Barata et al. 2012 and Jolly et al. 2014 evaluating the factors that affect their occurrence and quantitative presence.

2.1 Occurrence and Diversity of Yeasts

The composition, in terms of occurrence and amount, of indigenous microbiota naturally present on grape berry surfaces is crucial during winemaking process, as it can positively or negatively affect the quality of final wine. The presence and fitness of yeasts are essential in alcoholic fermentation, as promoters of transformation of grape sugars into principal products of fermentations: ethanol, carbon dioxide and hundreds of other metabolites responsible for aroma and flavours (Romano et al. 2003; Fleet 2003).

Kurtzman et al. (2011) already several years ago, ascribed overall yeasts potentially associated with grape/wine ecosystem in 15 different yeast genera, such as *Dekkera*/*Brettanomyces*, *Candida*, *Cryptococcus*, *Debaryomyces*, *Hanseniaspora*/*Kloeckera*, *Kluyveromyces*, *Metschnikowia*, *Pichia*, *Rhodotorula*, *Saccharomyces*, *Saccharomycodes*, *Schizosaccharomyces* and *Zygosaccharomyces*. On the other hand, the dynamic yeast taxonomy poses challenge on the nomenclature of wine microbiology (Bisson et al. 2017). The yeast *Hanseniaspora* and its anamorph counterpart *Kloeckera* are the numerically predominant genera present on the surface of grape, with more than 50% of the total yeast population (Fleet and Heard 1993). To a lesser extent, species belonging to *Candida*, *Starmerella*, *Cryptococcus*, *Pichia*, *Metschnikowia* and *Kluyveromyces (Lachancea)* genera are detected (Heard and Fleet 1988; Mills et al. 2002; Rosini et al. 1982). However, the variability may be reduced to few groups of similar physiological characteristics. For instance, the ubiquitous *Candida* spp. and *Pichia* spp. are highly heterogeneous, and new species are likely to be found in each new survey because the accuracy of molecular identifications is constantly increasing. A division of yeast biota of grape berries into three main groups with similar characteristics are proposed: (i) oxidative yeasts as basidiomycetous *Rhodotorula* and *Cryptococcus* along with the yeast-like fungus *Aerobasidium pullulans and some Candida* species; (ii) oxidative-fermentative ascomycetes *Hanseniaspora* spp., *Pichia* spp., and *Metschnikowia* spp. together with some *Candida* species; (iii) strongly fermentative yeasts with higher alcohol producing *Saccharomyces* spp., *Starmerella* spp. *Torulaspora* spp., *Zygosaccharomyces* spp., and *Lachancea* spp. In Table 1.1 are summarized the main yeast species colonizing wine making environment.

Table 1.1 Main yeasts genera found on grape berry surfaces and in winery

Yeast Genera	species	Presence in grapes and characters
Aerobasidium	 *A. pullulans*	Oxidative yeast-like fungus present on the berries surface still before this reach maturity and in the early stages of fermentation.
Dekkera/ Brettanomyces	 *D. bruxellenisis*	Vineries colonization, wine aging; rarely found on grapes; spoilage yeast.
Starmerella	 *S. bombicola (formerly Candida stellata)*	Occurrence on grapes surface at harvest time; low production of volatile acidity;strong fructofilic character;high amounts of glycerol production.

(continued)

Table 1.1 (continued)

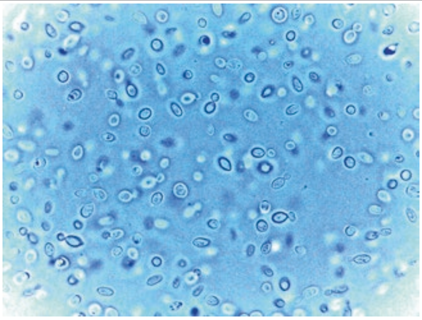
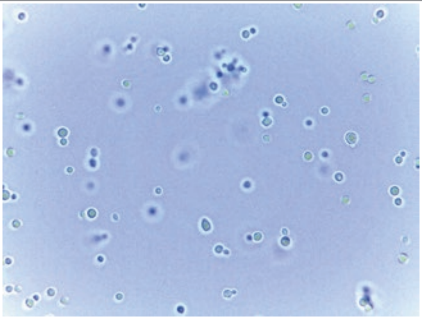
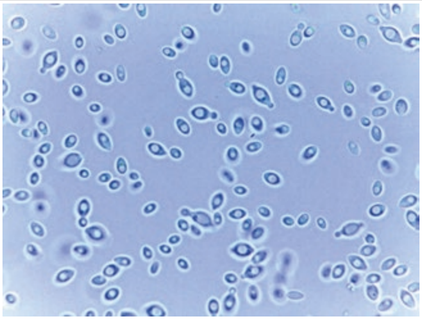
Cryptococcus	 *C. flavescens*	Occurrence on grape berries before and during full ripeness.
Debaryomyces	 *D. hansenii*	Potential presence on grape surface.
Hanseniaspora/ Kloeckera	 *H. uvarum*	Diffuse presence in grape berries surfaces;colonization of grape treated with organic and conventional treatment; occasionally present in cellar; spontaneous fermentation process; generally limited in the first few days of fermentation; weak ethanol tolerance; less efficient fermentation; highest ability for acetate formation.

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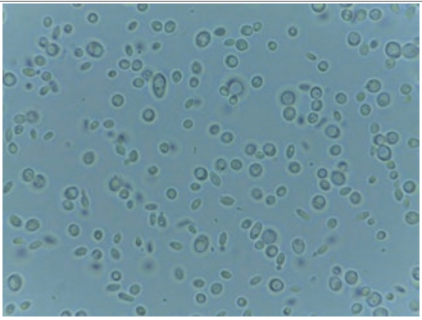
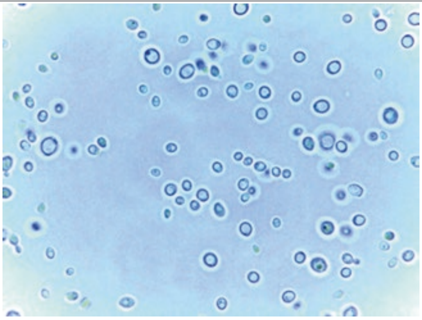
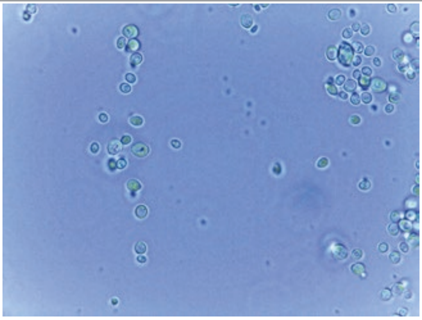
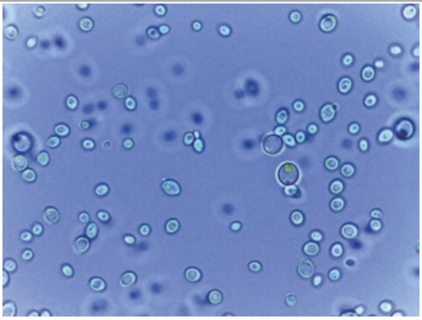
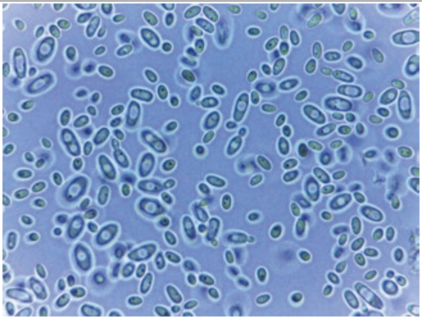
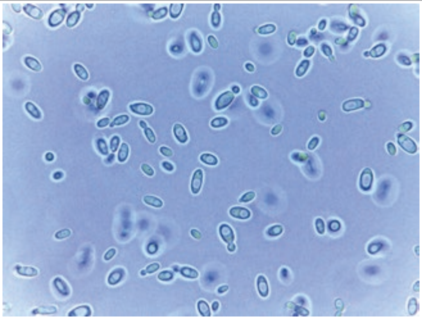
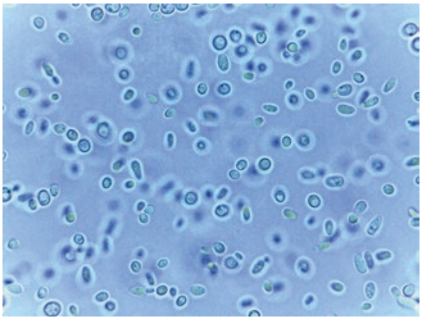
Table 1.1 (continued)

Kluyveromyces	 *K. marxianus*	Generally present in grape berries; found during various stage of fermentation.
Metschnikowia	 *M. pulcherrima*	Widely present in grapes and at harvest time; occurrence during various stage of fermentation (first few days); less efficient fermentation; High ability for acetate and acetate ester formation; antimicrobial activity; positive features to produce polysaccharides; glicosidase activity; ethanol reduction.
	 *M. chrysoperlae*	Present in grape berry; occasionally present in cellar.

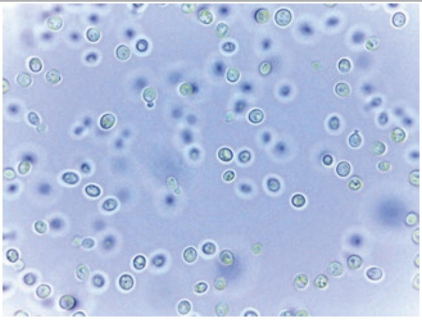
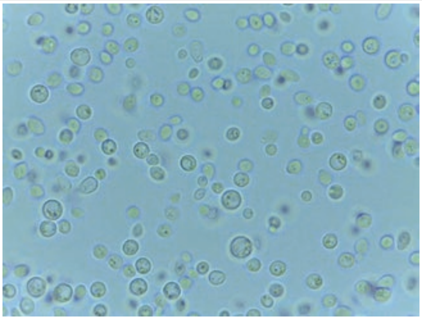
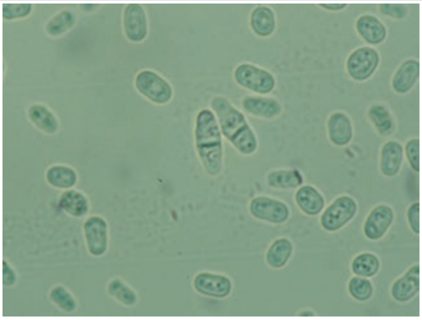
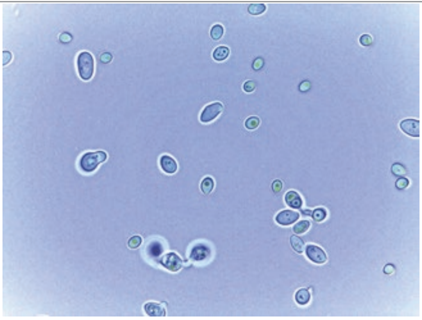
(continued)

Table 1.1 (continued)

	 *M. fructicola*	It possesses divergent copy or rDNA GENE in comparison with other species;unambiguous identification.
Issakentia	 *I. terricola (Pichia terricola)*	Present in grape berry surface and in winery environment; present during various stage of fermentation; potential enzymatic activity.
Candida	 *C. californica*	Present in grape berry surface during various stage of ripening; fructophilic character.
Whickeramomyces	 *W. anomalus (Pichia anomala)*	Less frequent in grape berry; Effect against *Dekkera/Brettanomyces* during fermentation or aging

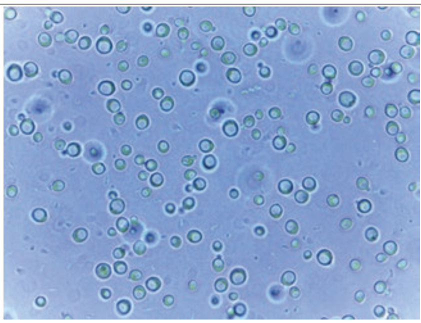
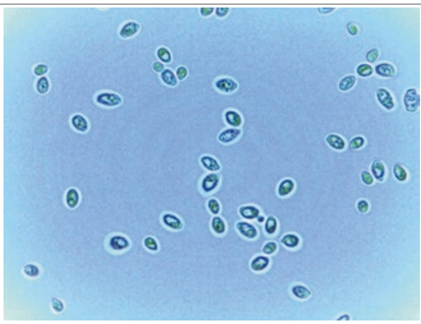
(continued)

Table 1.1 (continued)

Rhodotorula	 *R. mucillaginosa*	Oxidative yeast; colonize grape berries; ubiquitous yeast
Saccharomyces	 *S. cerevisiae*	Rarely present in grape berry; The main fermenting yeast involved in winemaking process; selected starter yeast; used to create new hybrid strains; strong cellar colonization; selective pressure by ethanol and SO_2 in winery is widely present (30-40% of total yeast population)
Schizosaccharomyces	 *S. pombe*	Present in grape/wine ecosystem; malo-alcoholic fermentation; increase in pigment production; High producer of polysaccharides
Zygosaccharomyces	 *Z. bailii*	Strongly fermentative yeast; occasionally present in unripe and overripe grapes; high ethanol tolerance

(continued)

Table 1.1 (continued)

Torulaspora	 *T. delbrueckii*	Strongly fermentative yeast; increase of fruity aroma; high ethanol tolerant; low acetic acid production; high competitiveness with *S. cerevisiae*; frequently isolate in grape surface; impact of aromatic composition of wine; increase of acetate esters, thiols and terpenes and β-phenyl ethanol
Lachancea	 *L. thermotolerans*	Strongly fermentative yeast; most frequently isolated in grape; good competitiveness with *S. cerevisiae* with exception in limited oxygen concentration; large amount of acid lactic together with glycerol and β-phenyl ethanol

Oxidative Yeasts

Relatively to oxidative yeasts, these are present on the surface of the berries still before this reach maturity when there is a high sugar content and can be found in the early stages of fermentation. In the middle and last phase of grape ripeness, the oxidative yeasts decrease in concurrently to the detriment of nutrient availability due to the competition with other yeast species, but they are still widely present at harvest time depending on the agronomical practices (Fleet et al. 2002; Hernández et al. 2018).

Oxidative-Fermenting Yeasts

Hanseniaspora/*Kloeckera* species are the most abundant ascomycetes yeasts colonizing the grape surface of grape berries at harvest time. Regardless of the geographic distribution of winemaking areas, the presence and colonization of the yeasts *Hanseniaspora* / *Kloeckera* on grape surface is everywhere dominant over the other yeast species. Within the apiculate yeasts the species *Hanseniaspora uvarum (Kloeckera apiculata)* are the most frequent but other species such as *Hanseniaspora hosmophila* or *Hanseniaspora guilliermondii* can be found at lower concentration (Giorello et al. 2018). Other ascomycetes widely found at harvest time on grape surfaces are species belonging to *Pichia*, *Candida* and *Metschnikowia* genera. In this regard, several species have been described. Among the species described within *Pichia* genera, *Pichia membranifaciens*, *Pichia fermentans*, *Pichia kluyvery* and *Pichia kudriadzvii* (synonimum *Issatchenkia orientalis*) are the most widely isolated (del Monaco et al. 2014). In the *Candida* genus several fermenting and non-fermenting species were isolated from grapes. The most diffused fermenting species is *Candida stellata* that it was successively reclassified as *Candida zemplinina* and more recently enclosed in clade of *Starmerella* as *Starmerella bacillaris* (Duarte et al. 2012)

Within *Metschnikowia* genera, new species *Metschnikowia viticola* was recently isolated, studied and characterized from a Hungarian vineyard. From a genetic point of view *M. viticola* is well disconnected species within the genus *Metschnikowia*. However, very little is known about the ecological distribution of *M. viticola* and their frequency on grape berries (Peter et al. 2005; Brysch-Herzberg and Seidel 2015). Other many new species have recently been described in the *Metschnikowia* genus, including *Metschnikowia chrysoperlae* (Suh et al. 2004), *Metschnikowia fructicola* (Kurtzman and Droby 2001) and *Metschnikowia andauensis* (Molnar and Prillinger 2005). In these cases, there was a real difficult in the delimitation among new species and the well characterized *Metschnikowia pulcherrima* The experimental results obtained by Sipiczki et al. (2013) explain that the type strains of *M. andauensis* and *M. fructicola* possess divergent copies of the rDNA gene will lead to further investigations of the species concept in the clade. This support the importance of unambiguous yeast identification in any study of the yeast diversity in grape habitat.

Strongly Fermentative Yeasts

Regarding to the fermentative, higher alcohol tolerant yeasts, their colonization is related to the high nutrient availability resulting from grape damage that possess, besides much higher cell counts, wider species diversity than sound grapes (Barata et al. 2012). *S. bacillaris* may be present in higher numbers but its relative proportion also decreases in favour of higher fermentative yeasts such as *Zygosaccharomyces* spp., *Lachancea* spp. and *Torulaspora* spp., which, as mentioned above, may occasionally dominate the overall microbiota.

2.2 Factors Affecting Yeast Community

The composition and complexity of microbiota of grape berries depend on the interactions between individuals. The resulting consortium is generally stable over time and depending on several biotic and abiotic factors (Fig. 1.1). Relative to abiotic factors, the climatic and microclimatic conditions, including the effect of temperature, UV exposure, rainfall, sunlight and winds, can influence microbial populations.

Among biotic factors, microbial vectors, such as bees and wasps, can actively transfer yeasts on the grape surfaces (Francesca et al. 2012; Goddard et al. 2010; Stefanini et al. 2012). The microbial habitat associated with birds represents the

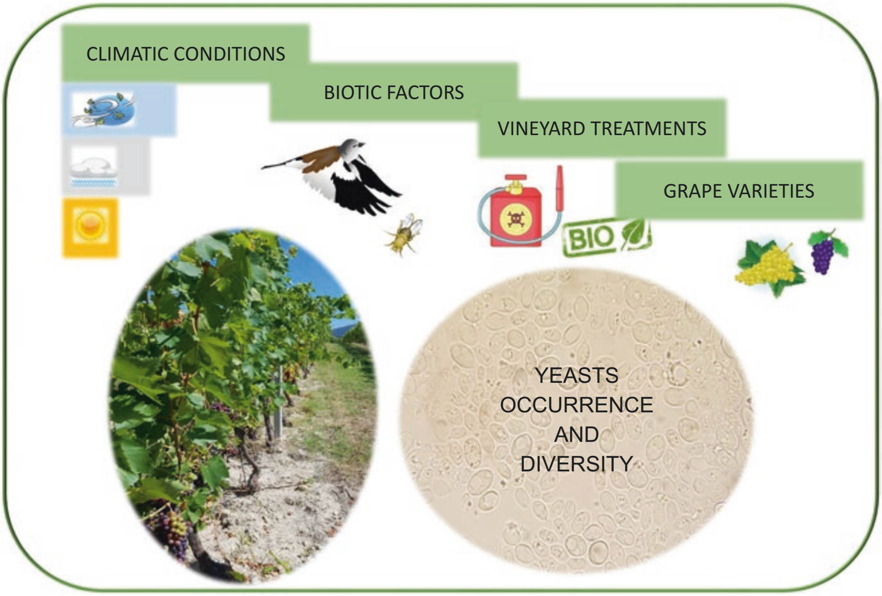


Fig. 1.1 Factors affecting yeast community in vineyard

object of several ecological surveys not only in applied food microbiology. Indeed, associations between wild birds and microorganisms have been studied mainly focusing on bacteria, whereas limited studies on yeasts are available (Cafarchia et al. 2006). The monitoring of bird movements allows the investigation about behavioural and demographical responses to a given environment (Riffell et al. 2006). The migration of birds includes a round trip to the resting areas and a return to the territories of nesting, which occur in autumn and spring respectively. These movements follow the seasonality of food resources. Since birds act as microorganism vectors, the analysis of the microflora they host may be important to evaluate the microbial diversity of the sites visited. From an applicative perspective, yeasts carried out by birds have not been deeply investigated. Nowadays, there is a growing interest of wine producers to perform winemaking employing 'autochthonous' strains which may ensure typical terroir characteristics (Capozzi et al., 2015). At this regard, it was recently reported the dissemination of oenological yeasts by vineyard inhabiting birds, mainly Black birds (*Turdus merula*), although no yeast with technological relevant traits was found in the few migratory birds analysed (Francesca et al. 2012). Those authors evidenced an issue related to the autochthonous status of yeasts, since they may not be indigenous in each environment. Yeasts may be moved at different distances depending on the vector type. Some studies provided evidences that insects such as honey bees disseminate *S. cerevisiae* strains until approximately 10 Km (Goddard et al. 2010), so that the investigation of migratory birds need clarifications for the associated movements of yeasts with the support of technological relevance. During migration, several sites are visited by birds because they represent important stop-over points. For example, during migration from Africa to Europe and vice versa, Lampedusa and Ustica islands are visited in spring when the direction is from sub-Saharan areas to North Europe, while Linosa is visited in autumn during the opposite fly.

It is well known that climate change is partly responsible for the elevated sugar concentration and lower acidity in grape berry and then in must (Godden et al. 2015; Neumann and Matzarakis 2014; Petrie and Sadras 2008; Teslić et al. 2018). This is strictly related to the microbial composition in grape berries. Vine sensitivity to weather properties (Holland and Smit 2014), narrow spatial surfaces suitable for producing high-quality grapes as wine industry raw material, and possibility of perennial plant exploitation (Lereboullet et al. 2014) are indicative of the need for a climate change assessment associated with winemaking. Despite the importance of the global climate change trend, from the vine grower/winemaker perspective, it is more essential to understand regional atmospheric conditions (Orlandini et al. 2009) and local microclimatic environment as well. Generally, increasing average global temperature over the last few decades is more than evident, as is the increasing temperature trend, although is not homogenous in every vine-growing region (Pielke et al. 2002; Van Leeuwen et al. 2013). For example, a significant growing season temperature trend for the majority of northern hemisphere wine-producing regions between 1950 and 1999, with an average increase of 1.26 °C, was demonstrated. However, there was also an insignificant trend in the majority of southern hemisphere wine regions, which emphasizes the necessity to focus study on smaller study areas.

Since climate modifications are vastly complex, examinations of simple temperature and precipitation values are insufficient to explain climate change trends. Therefore, several bioclimatic indices, such as Huglin index (Huglin 1978), Cool night index (Tonietto 1999), Winkler or growing degree day index (Winkler 1962), number of days with maximum temperatures higher than 30 °C (ND > 30 °C) (Ramos et al. 2008), number of days with precipitations <1 mm (Dry spell index, DSI) (Moisselin and Dubuisson 2006), etc. are commonly used in viticulture to provide an improved insight into climate change tendencies. However, the selected bioclimatic indices were mainly based only on-air temperature, as it has the strongest influence on overall growth, productivity, and berry ripening of the grapevine (Jones-Vaid et al. 2012).

Another important parameter influencing the grape microbiota is related to the water intended as rainfall. Indeed, moderate water stress may positively affect berry sugar accumulation during grape-growing season (Coombe et al. 1989), while increasing temperature advances phenological stages and speeds up sugar accumulation in grape berries (Jones-Vaid et al. 2012; Bonnefoy et al. 2013). The association of water stress together with increasing temperature later lead to the production of wines with higher alcohol content and other microbiological, technological, sensorial, and financial implications (Mira de Orduña et al. 2000). As direct consequence, increase of grape sugar content at harvest may cause slow or stuck alcoholic fermentations during hot years as well as alter sensory features due to the ethanol's tendency to increase bitterness perception (Sokolowsky and Fischer 2012), suppress the perception of sourness, and reduce astringency perception.

Concerning the total yeast counts, Combina et al. (2005) found that rainy years increased yeast presence. This climatic condition probably increases the berry volume and permits the release of juice in joint areas, such as the part between the pedicel and the berry, and higher exosmosis leads to nutrients on the grape surface. With careful and sound berry sampling, Čadež et al. (2010) found that colder harvests with higher rainfall lead to increased yeast counts. In contrast, Comitini and Ciani (2006) found ten-fold less total counts in years with high rainfall. In addition, the geographic location, grape variety and vineyard age and size can influence the composition and occurrence of microflora that are present on the surface of grape berries.

Another important aspect is related to vineyard chemical treatments. A lot of studies showed that agronomical practices, such as organic or biodynamic management can modify the microbiota of grape and must (Cordero-Bueso et al. 2011; Milanovic et al. 2013; Pretorius 2000; Mezzasalma et al. 2017). Some authors suggested that the occurrence of specific bacteria in must and wine influences wine characteristics and typicity (Belda et al. 2017a; Liu et al. 2017).

The main vineyard treatment studied is related with the use of pesticide treatments, mainly those against fungi (downy mildew, powdery mildew and grey rot). The studies are either based on analysing grapes after vineyard treatment, which do not exclude the influence of other factors, or from auto-enrichment fermentations which cannot be correctly extrapolated to evaluate the variations on berry microbiota. Conventional pesticides can produce a decrement in the yeast population and diversity in fermenting musts. Ganga and Martínez (2004) detected less diversity of non-*Saccharomyces* species, which was explained using fungicides against *Botritys cinerea*. Differently, there are discordant results on the effect of chemical treatments on *S. cerevisiae* presence on grapes. Ganga and Martínez (2004) did not find reduced *S. cerevisiae* occurrence after fungicide use while other investigations recovered lower numbers of this species (Regueiro et al. 1993; Van der Westhuizen et al. 2000). It is quite evident that the influence of chemical pesticides on microbiota of grape berry is related to other factors, such as climatic conditions or grape variety, which cannot be correctly extrapolated to evaluate the single effect on berry microbiota. About this concern, Ganga and Martínez (2004) detected less diversity of non-*Saccharomyces* species, which was explained using fungicides against *B. cinerea*, while Regueiro et al. (1993) and recovered lower numbers of these species. Milanovic et al. (2013) found that *Candida zemplinina (synonimus S. bacillaris)* and *Hanseniaspora* species colonised surface of grapes treated with both organic and conventional treatment, while *M. pulcherrima* was widely found in conventional samples and only occasionally in organic grapes.

A specific influence of grape varieties on indigenous yeast community of grape berries was found. Clavijo and collegues (2010) carried out an ecological survey of wine yeasts present on grapes growing in two vineyards located in the southern Spain (Serranía de Ronda region). They found that, although *Kluyveromyces (Lachancea) thermotolerans*, *H. guilliermondii*, *H. uvarum and Issatchenkia* *orientalis* (*Pichia kudriavzevii*) are the most frequent species, a specific distribution of strains was found in the three grape varieties studied. The influence of grape varieties on the indigenous yeast community of grape berries was also evaluated by Raspor et al. (2006) The frequency of occurrence of yeast species showed their preferences for certain grape varieties. The white grape variety mostly attracted pigmented *Basidiomycetous* yeasts belonging to the genera *Rhodotorula*, *Sporobolomyces* and *Cryptococcus* that dominated on all sampling locations. Differently, yeast populations isolated from the red grape surfaces belonged both to *Ascomycetous* and *Basidiomycetous* yeasts in the ratio of 1: 1.

In the last 10 years, due to the advances in metagenomics, it has become clearer and clearer that in general, plants host a wide array of bacteria and yeasts most of which are not cultivable and therefore are almost unknown at the taxonomic and metabolic levels. Such microorganisms interact with the plant organs and can influence plant nutrition, development, productivity, and stress responses (Bacon and White 2016).

Another important question regards the influence of grapevine cultivar on the grape microbiota. Recently, it was shown that some epiphytic bacteria were shared by aerial plant portions and the soil (Martins et al. 2013). This finding led them to propose that the physical proximity between soil and the plant might facilitate microbial migration through rain splash, winds, pollinators and other foragers, and parasites.

Moreover, any grapevine cultivar shows peculiar secondary metabolites, and most of these are concentrated in the fruit. Some of these metabolites have antimicrobial properties (Chong et al. 2009; Katalinić et al. 2010) and could influence the composition of grape microbiota both quantitatively and qualitatively. Based on these assumptions, it was hypothesized that each cultivar could have an active and specific role in the interaction with and selection of its microbial community (Mezzasalma et al. 2017).

2.3 Recent Methodologies for Detecting the Presence of Yeasts on the Grape Berry

To know the microbial composition in grape barriers and to further monitor their evolution during wine fermentation understanding the relationship between the microorganisms is of relevant importance in applied studies (Bokulich et al. 2014; Piao et al. 2015; Stefanini and Cavalieri 2018). The use of conventional methods including culture-dependent techniques, allow to analyze culturable fungi, yeasts, acetic acid- and lactic acid-bacteria associated with grape berries and wine. As well as in many other natural habitats, there are several viable but non-culturable wine microorganisms, that could not be studied under conventional laboratory microbial conditions, leaving an incomplete knowledge about the occurrence and dynamics of the microbial community involved in winemaking (Cocolin et al. 2013; Piao et al. 2015). Recent advances