fermentation

MDPI

Review

A Future Place for *Saccharomyces* Mixtures and Hybrids in Wine Making

Helmut König * and Harald Claus

Institute for Molecular Physiology, Johannes Gutenberg-University, Johann-Joachim-Becher-Weg 15, 55128 Mainz, Germany; hclaus@uni-mainz.de

* Correspondence: Helmut.Koenig.Ingelheim@t-online.de

Received: 26 June 2018; Accepted: 15 August 2018; Published: 18 August 2018

Abstract: Each year, winemakers can face sluggish or stuck fermentations during wine making, especially when a spontaneous fermentation is performed, even if strains of the classical wine yeast *Saccharomyces cerevisiae* are applied. Problems are inevitable when low ammonium concentrations (<160 mg L^{-1} grape must) or an excess of fructose compared to glucose are observed during grape must fermentation. *S. cerevisiae* strains cannot use all kinds of amino acids as the sole nitrogen source but usually need free ammonium (optimal concentration: 600 mg L^{-1} grape must). It preferably consumes glucose, leading often to an excess of fructose in the fermenting must, which contains glucose and fructose in an equal ratio at the beginning of fermentation. Yeast hybrids have been isolated from wines several times and different strains are already commercially available. The united properties of the parent strains can provide advantages under sophisticated fermentation conditions. However, the involvement of a hybrid yeast for the rectification of fermentation disorders in spontaneous fermentations has only been described recently in the literature. Recent investigations have provided convincing evidence that fermentation problems can be overcome when must fermentations are successively performed with *Saccharomyces bayanus* strain HL 77 and the triple hybrid *S. cerevisiae* × *Saccharomyces kudriavzevii* × *S. bayanus* strain HL 78. The triple hybrid strain HL 78 uses amino acids as a nitrogen source in the absence of ammonium and it also exhibits a fructophilic character with an enhanced uptake of fructose in comparison to glucose. The application of genetically modified yeast strains is not allowed for starter cultures in wine making, but the usage of yeast mixtures and hybrid strains could be a promising tool for winemakers to solve fermentation problems during spontaneous fermentation or for the creation of novel wine types with desired sensory characteristics under more challenging conditions, especially when the composition of the must components is not optimal because of, e.g., critical climatic or soil conditions.

Keywords: *Saccharomyces*; yeast hybrids; yeast mixtures; spontaneous fermentation; stuck and sluggish fermentation

1. Introduction

In publications about the history of wine making, McGovern [1,2] and Kupfer [3,4] provided convincing indications that cultures of vines and wine making were established between 6000 and 8000 BC in regions between the Black Sea and the Caspian Sea and also along the later Silk Road all the way to China. Successively, viticulture spread via different countries of Asia Minor and northern Africa. It arrived in about 1000 BC in southern European countries such as Italy, France, and Spain. In the end, wine production was well established in more northern and eastern parts of Europe around 1000 AD. Today, the most common vine variety around the earth is *Vitis vinifera* L. subsp. *vinifera*. This variety arose from the Eurasian wild form of *Vitis vinifera* L. subsp. *sylvestris*.

Wine production has a long tradition and has been a part of human culture for thousands of years. Despite this fact, deeper insights into the microbiology and biochemistry of the conversion of the sugars in grape must to ethanol and into other accompanying biochemical reactions were obtained only since the 19th century. In the times before, the art of wine making had been further developed mostly empirically from generation to generation. Fundamental scientific investigations about biochemical transformations during wine making started not before the end of the 18th century and in the course of the 19th century [5–9]. Although, *Saccharomyces cerevisiae* likely played the most important role in wine fermentation from the beginning of viticulture [10], it was not before 1883 that the first pure yeast culture was obtained by Emil Christian Hansen. Originally, these isolates were used for beer production, while around the year 1890, Hermann Mueller-Thurgau also introduced yeast starter cultures to wine making. Only since the 1930s have commercial liquid cultures of yeasts been available as starter cultures for the inoculation of must, which were commonly used after the Second World War.

The variety of microbes growing in fermenting must is limited to three groups of ethanol- and acid-tolerant microorganisms, namely, yeasts, lactic acid bacteria, and acetic acid bacteria [11]. From grapes, must, and wine, more than 100 yeast species belonging to 49 genera have been isolated and characterized [12–15]. The classical wine yeast *S. cerevisiae* and the so-called wild yeasts (non-Saccharomycetes) are involved in the conversion of must into wine. The varieties and succession of yeast species during the fermentation of a certain must sample have a significant impact on the specific sensory profile of the produced wine. Compared to the known wine-related yeasts species, the variety of bacterial species is lower. Around 25 species of wine-related lactic acid bacteria have been obtained in pure culture. They belong to the genera *Lactobacillus*, *Leuconostoc*, *Oenococcus*, *Pediococcus*, and *Weissella* [16]. In addition, 23 acetic acid bacteria have been detected on grapes, in must, and in wine, which belong to the genera *Acetobacter*, *Amayamea*, *Asaia*, *Gluconacetobacter*, *Gluconobacter*, *Komagateibacter*, and *Kozakia* [17]. Molecular biology methods and next-generation sequencing approaches have been applied to examine and quantify the diversity and genetic variations as well as sources and roles of wine-related microorganisms. This knowledge is also helpful for the development of novel starter cultures [18–21].

Up to now, a relatively broad knowledge of the diversity, succession, and physiological and biochemical activities of wine-related microorganisms has been acquired. Mainly, the yeast species *S. cerevisiae* and the two bacterial species *Oenococcus oeni* or *Lactobacillus plantarum* are commercially available and applied for alcoholic and malolactic fermentation, respectively. Despite the deeper microbiological and biochemical knowledge of the backgrounds of wine making, sluggish or stuck fermentations cause significant financial losses for winemakers each year. These unwanted observations stimulate investigations for more improved microbiological strains and novel procedures to circumvent the observed fermentation obstacles.

2. Today's Principal Procedures and Obstacles of Wine Making

In the last decades, the risk of sluggish or stuck fermentations has been significantly reduced by the commercial availability and application of selected strains of the classical wine yeast *S. cerevisiae*, especially when about 10^5 cells/mL are added by winemakers to start controlled fermentation. Today, a great variety of *S. cerevisiae* strains is offered by different companies for the production of wines with different sensory profiles. Because of the high titer of the starter yeast cells, wild yeasts have difficulties developing and fermentation can be carried out relatively reproducibly by starter cultures. While this procedure greatly reduces the risk of fermentation problems, the sensory profiles compared to a spontaneous fermentation are restricted and depend on the starter cultures used.

On the other hand, monitored fermentation is started spontaneously and selected yeast cultures are only added when fermentation problems are observed. Satisfying results can be obtained with optimized yeast strains or yeast mixtures. For this procedure, we isolated and selected yeast strains of *S. cerevisiae* from fermenting must in a certain vineyard in previous years. Harvested cells from grown fermenter cultures were then only added to sluggishly fermenting must in the same vineyard in

order to continue and finish the fermentation. Compared to a solely spontaneous fermentation, by this method, relatively complex wines were also produced which met the special sensory requirements of the winemaker quite well (unpublished special service for certain wineries, provided by the Institute of Microbiology and Wine Research of the Johannes Gutenberg-University in Mainz, Germany).

For millennia, wines have been made by spontaneous fermentation, which is therefore the earliest form of must fermentation. In this case, the yeast strains present in the cellar or on the grapes enter the must and start the fermentation. At the beginning of the fermentation, the classical wine yeast *S. cerevisiae* is present only at a relatively low cell number. The first stage of a spontaneous fermentation is usually dominated by a mixture of some species of so-called wild yeasts. The indigenous wild yeasts, the classic wine yeast *S. cerevisiae*, and the local lactobacilli as well as acetic acid bacteria are involved in the microbial conversion of grape must into wine. Usually, when the ethanol concentration reaches about 4% to 7% (*v*/*v*), *S. cerevisiae* can overgrow the wild yeasts and also most of the bacterial strains in the fermenting must. The corresponding wines are often more complex and are more likely to meet the expectations of a particular terroir. The risk of fermentation problems, however, is obviously increased compared to controlled and monitored fermentations.

Despite the observed increased reliability of must fermentation by adding starter cultures of commercial yeast strains after grape pressing, winemakers, especially of the upper-quality segment, often favor spontaneous fermentation in order to produce more complex wines with a characteristic sensory profile distinctive for a certain winery or terroir. Of course, the sensory profile relies not only on multifactorial environmental and biological features, such as the grape variety and grape quality, the terroir (soil and climate), the conditions in the wine cellar, and the fermentation management, but also, without doubt, to a greater part on the added or indigenous microbiota. In the case of spontaneous fermentation, the bacteria and yeast composition in the fermenting must depends on the microorganisms on the grapes and in the cellar. However, it should be remembered that the risk of fermentation problems in the case of spontaneous fermentation is increased. Some reasons are well known to be responsible for fermentation problems. These include (a) heavily infected grapes, (b) low and fluctuating temperatures, (c) toxic and fungicidal compounds, (d) killer toxins, (e) ratio of glucose to fructose below 1:10, (f) deficiencies of nutrients such as vitamins or trace elements, (g) ammonium concentration below 120 mg/L, (h) pH values below 3.0, and (i) elevated polyphenol concentrations [22–25].

Ordinary attempts to overcome the observed fermentation problems are (a) adjustment of the temperature to 20 °C, (b) addition of yeast nutrients (diammonium hydrogen phosphate), (c) increase of the pH value, and (d) a reinoculation with yeast starter cultures possessing a high ethanol tolerance (e.g. sparkling wine yeasts). However, these measures frequently lead to a change in the initially targeted sensory profile, which is hardly compatible with the conceptions of winemakers in the upper-quality segments and their very special sensory expectations.

3. Suggested Efforts for More Sophisticated Wine Production

3.1. Application of Wild Yeasts and Yeast Mixtures

In general, wild yeasts are suggested to be suitable tools for wine tailoring. They can have an influence on low sulfite formation, reduction of copper content, reduction of ochratoxin A, reduced production of ethyl carbamate, low biogenic amine formation, reducing volatile acidity, alcohol reduction, modulation of acidity, increased glycerol content, modulation of aroma profiles, enhancing varietal aromas, mannoprotein release, and control of spoilage microflora [12,26–28].

When a spontaneous fermentation is started, the wild yeasts (non-Saccharomycetes) dominate in number compared to the classical wine yeast *S. cerevisiae* [12]. The growth of the non-Saccharomycetes is more sensitive to increasing sulfite and ethanol concentrations. Some of them, such as *Torulaspora delbrueckii* and *Lachancea* (*Kluyveromyces*) *thermotolerans*, require higher oxygen concentrations for optimal growth than *S. cerevisiae*. The reason is a different synthesis rate of unsaturated fatty acids

under oxygen-limiting conditions [27,29–32]. Sometimes *S. cerevisiae* grows suboptimally in the presence of wild yeasts which already partly consume nutrients and growth-promoting substances such as sugars, trace elements, or vitamins. Non-Saccharomycetes can produce acetic acid, which is unfavorable for Saccharomycetes. Acetic acid can be sensed at a concentration of 0.6 g/L. The Old World and the New World wine regulations are quite different. Wine making is strictly ruled in the Old World (e.g., France: Appellation d'origine contrôlée (AOC), Italy: Denominazione di origine controllata (DOC), Spain: Denominación de Origen (DO), Portugal: Denominação de Origem Controlada (DOC), Germany: German Wine Law). The regulations are more relaxed and open for innovations in the New Word, but in some cases, more restrictive rules have been established (e.g., Vintner's Quality Alliance, VQA). In the European Economic Community (EEC) (renamed as European Community (EC) after formation of the European Union (EU) in 1993), values are 1.07 g/L for white wine and 1.20 g/L for red wine. The Common Agricultural Policy (CAP) of the EU includes the EU wine regulations. The member states produce about 65% of the global wine. The details of quality classifications are part of the national wine laws. According German law, the upper limits for white and red wines is 1.08 g/L and 1.20 g/L, respectively. For wines of individually selected overripe berries (Trockenbeerenauslese), 2.10 g/L is allowed. In France, wine is of commercial quality according to the appellation d'origine controlée (AOC system) if the acetic acid concentration does not exceed 1.1 g/L. Due to the Australia New Zealand Food Standards Code - Standard 4.5.1—Wine Production Requirements (Australia Only), wine, sparkling wine, and fortified wine must contain no more than 1.5 g/L of volatile acidity, excluding sulfur dioxide, expressed as acetic acid. According to the Code of Federal Regulations (CFR) of the Federal Government of the United States (CFR title 27/4.21), the maximum volatile acidity, calculated as acetic acid and exclusive of sulfur dioxide, is 0.14 gram per 100 mL (20 °C) for natural red wine and 0.12 gram per 100 mL (20 °C) for other grape wines.

The concentration of ethanol is reduced when this alcohol is partly oxidized and converted into the volatile compound acetic acid. Polysaccharide production by wild yeasts can lead to graisse at higher concentrations, while a positive mouth feeling is perceived at a certain concentration. Polysaccharides have an influence on the sensory properties of wines because of their interactions with wine and salivary proteins, tannins, tartrate, and aroma compounds [10]. Wines made with yeast strains that produce inherently higher levels of polysaccharides are naturally softer, have more body, and a better mouthfeel. Regular "batonnage" in this period can further stimulate the release of polysaccharides, which in turn will have a positive influence on the mouthfeel and body of the wine [33–35]. In addition, different fruit and unwanted aromas are caused by the formation of a large variety of esters.

When *L. thermotolerans* and *Candida zemplinina* grow in must, the glycerol content can be increased, which also has an influence on the mouth feeling. *C. zemplinina* can also lower the concentration of the acetic acid produced in must possessing a high sugar content. Members of the genera *Debaryomyces*, *Hansenula*, *Candida*, *Pichia*, and *Kloeckera* can produce aromatic and colored compounds (anthocyanins) from glycoconjugates. An inoculated mixture of *Debaryomyces pseudopolymorphus* and *S. cerevisiae* increased concentrations of terpenols such as citronell, nerol, and geraniol in Chardonnay wines [27]. After addition of *C. zemplinina* and *Pichia kluyveri* to a sample of a Sauvignon Blanc must, the concentration of sulfur-containing compounds, such as 3-mercaptohexan-1-ol (3MH) and 3-mercaptohexan-1-ol acetate (3MHA), was increased. Thioles contribute to the specific aroma of Sauvignon Blanc wines [27]. The yeast *Wickerhamomyces anomalus* can hydrolyze a series of glycosylated aroma precursors [36,37].

Wild yeasts are dominant in the first stage of the must fermentation. Members of the genera *Hanseniaspora*, *Rhodotorula*, *Pichia*, *Candida*, *Metschnikowia*, and *Cryptococcus* were often identified at the beginning of alcoholic fermentation [15,27]. Wild yeasts can be divided into different physiological groups. Species of *Pichia*, *Debaryomyces*, *Rhodotorula*, and *Candida* prefer aerobic growth. *Hanseniaspora uvarum* (perfect form: *Kloeckera apiculata*), *Hanseniaspora guilliermondii* (perfect form: *Kloeckera apiculata* var. *apis*), and *Hanseniaspora occidentalis* (perfect form: *Kloeckera javanica*) have a low

fermentation activity, while strains of *Kluyveromyces marxianus*, *T. delbrueckii*, *Metschnikowia pulcherrima*, and *Zygosaccharomyces bailii* exhibit increased fermentation activities [27,38].

In order to realize certain desirable aromas in wine, fermentation can be started by the addition of individual selected wild yeast species and then continued with a starter culture of the classical wine yeast *S. cerevisiae* in order to complete the fermentation. Wild yeast cultures and also yeast mixtures are already commercially available. For instance, *Torulaspora delbrückii* strains are offered under the designation "Oenoferm wild & pure". Some commercial cultures contain, in addition to *S. cerevisiae* wild yeasts (20–40%), for example, *K. thermotolerans* or *T. delbrueckii*. A mixed culture consisting of *L.* (*K.*) *thermotolerans* (20%), *T. delbrueckii* (20%), and *S. cerevisiae* (60%) is also available. The two known starter cultures Sihaferm PireNature or Level 2 TD are made up of *T. delbrueckii* and *S. cerevisiae*. In this case the must fermentation is started with wild yeast and then continued and completed with *S. cerevisiae*. Viniflora® PRELUDE™ contains a *Torulaspora* starter culture which is added to the must at concentrations of 20 g/hL. The inoculated must is then kept at 7–10 °C for 4–7 days. At an ethanol concentration of 4–6 vol%, a *Saccharomyces* strain is added in the same amount after transfer of the fermenting must to a fermentation tank. The temperature is then increased. Finally, a surplus of malolactic acid can be biologically reduced with the lactic acid bacterium *O. oeni*.

3.2. Application of Hybrid Yeasts for Overcoming Fermentation Problems

During spontaneous fermentation, wine-related microbial species usually grow in succession. In the first stage of the fermentation, the so-called wild yeasts (non-Saccharomycetes) are multiplying. In the harvest years 2011 and 2012, the succession of the microorganisms in the course of the spontaneous fermentation of Riesling must in the winery Heymann-Löwenstein (lower Moselle, Germany) was investigated [39,40]. The wild yeasts in a wine cask without fermentation problems belonged to the genera/species *Candida pararuqosa*, *Saccharomycetes* sp./*Pichia membranifaciens*, *Saccharomycopsis crateagensis*, *Candida boidinii*, *Saccharomycetes* sp., *Aureobasidium* sp., *Metschnikowia* sp., *Metschnikowia chrysoperlae*, *Cryptococcus flavescens*, *C. zemplinina*, *P. kluyveri*, and *H. uvarum*. Interestingly, in some barrels, wild yeast species survived at elevated levels of ethanol. Living cells of *C. boidinii* were found until end of fermentation. The genus *Saccharomyces* contains nine species [41]. Unexpectedly, the fermentation was not initiated by the classical wine yeast *S. cerevisiae* but rather by *Saccharomyces bayanus* [39]. Approximately 4 weeks after an observed stuck fermentation, the alcoholic fermentation was completed by the triple hybrid *S. cerevisiae* × *Saccharomyces kudriavzevii* × *S. bayanus* strain HL78. This hybrid possessed genome sequences of the three mentioned *Saccharomyces* species. The triple hybrid yeast strain HL 78 was not added to the must but grew in the background during the fermentation in the must. Therefore, strain HL 78 must have been already present in low cell numbers after fermentation started. The classical wine yeast *S. cerevisiae* was not able to grow in the investigated must because the temperature in the wine cellar was between 12 and 14 °C and the temperature in the wine cask reached only about 16 °C at the most. The different yeast strains of *S. cerevisiae* require more than 140 mg nitrogen/L for optimal growth [38,39,42]. In the investigations presented here, the available ammonium nitrogen decreased after starting the fermentation from 120 to 40 mg/L in a relatively short time [39]. It is well known that at low ammonium concentrations, the sugar uptake activity also decreases in the case of *S. cerevisiae* [38,39]. In comparison to the classical wine yeast *S. cerevisiae*, *S. bayanus* can also grow better at low temperatures and low available ammonium concentrations. *S. bayanus* strain HL 77 and in particular the triple hybrid strain HL 78 are able to satisfy their nitrogen needs from amino acids or probably proteins, even at low concentrations and without free ammonium. As demonstrated by quantitative proteomics, higher protease activities were detected in the triple hybrid strain HL 78 compared to *S. cerevisiae* [40]. The triple hybrid HL 78 could uptake glucose and especially fructose at lower amino acid concentrations. In addition, the triple hybrid strain exhibits a fructophilic character, which is reflected in a higher uptake rate of radiolabeled fructose compared to glucose [43].

The hybrid *S. cerevisiae* × *S. kudriavzevii* was described by González et al. [44]. The combined characteristics of both parents may be advantageous under certain fermentation conditions. The hybrid is tolerant against high ethanol concentrations and high osmolarity, which is a characteristic feature of *S. cerevisiae*. The tolerance against cool temperatures is a feature of *S. kudriavzevii*. Yeast hybrids have been isolated in Europe by several authors [45–47], although the strains of *S. kudriavzevii* known so far have been isolated from decaying leaves in Japan [48] and from oak bark samples in Portugal [49]. However, the involvement of a hybrid yeast for the elimination of fermentation disorders in spontaneous fermentations has not been mentioned in the literature so far.

4. Discussion

For a few thousand years, must has been fermented spontaneously without the knowledge of the specific physiological and biochemical activities of the involved microorganisms. Therefore, progress in the art of wine making was only gained empirically. In the second half of the last century, spontaneous fermentations were largely successively replaced by the application of selected starter cultures of *S. cerevisiae*, leading to regular use since the 80s of the last century by many winegrowers and cooperatives.

The available improved scientific and practical knowledge led to considerable progress in wine growing and vinification in the last decades. The application of more selected yeasts and methods enables a much more defined control of the fermentation process. The application of molecular biology identification methods and the sequence analysis of nucleic acids have shown that diverse yeast strains occur in the different wine-growing regions which enable the use of region-specific starter cultures after isolation of pure strains [50–53]. Probably occurring fermentation problems during a spontaneous fermentation can thus be remedied by the subsequent addition of terroir-specific yeast strains without the need to accept major changes in the desired flavor profile. Moreover, a partial imitation of spontaneous fermentation is now possible by the use of isolated and selected strains of wild yeasts.

Strategies for the targeted genetic modification of yeasts can in principle be worked out or have already been described [54,55] in order to produce yeast strains with certain desired properties. However, genetically modified yeasts are not authorized for wine making in Europe or Australia. The recognition of safe (GRAS) in the United States applies only for two strains (ML01 and 533EC). Yeast starter cultures can be furthermore improved by selecting certain strains by evolutionary in vitro adaptation or by the production of hybrids. The so-called "evolutionary in vitro adaptation" is performed by a slow change of the culture conditions during several months. A fructophilic yeast was obtained from a normal *S. cerevisiae* isolate by slowly shifting the glucose/fructose ratio towards fructose (Pfeiffer, P.; König, H. unpublished results).

Several yeast hybrid strains are already commercially available. Here, only some examples can be given: strain "Oenoferm® X-treme" is a GMO-free hybrid yeast obtained from the protoplast fusion of two different *S. cerevisiae* strains; strain "Cross Evolution" is a natural cross hybrid between *S. cerevisiae* yeasts; strain NT 202 is a product of the yeast hybridization program; strain S6U is a hybrid of *S. cerevisiae* × *S. bayanus*; and strain VIN7 is an allotriploid interspecific hybrid of a heterozygous diploid complement of *S. cerevisiae* chromosomes and a haploid *S. kudriavzevii* genomic contribution [56].

In the future, the art of wine making includes the simultaneous or sequential use of different strains of *S. cerevisiae* as in the past, but defined mixtures of different species of Saccharomycetes and non-Saccharomycetes will also be used more generally. This will enable quite different wine styles. The future belongs to well-trained creative winemakers who can handle the different protocols with varying compositions of starter cultures to stimulate the microbe orchestra to new sounds.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. McGovern, P.E. *Acient Wine. The Search for the Origins of Viniculture*; Princeton University Press: Princeton, NJ, USA, 2003.
2. McGovern, P.E.; Zhang, J.; Tang, J. Fermented beverages of pre- and proto-historic China. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 261–270. [CrossRef] [PubMed]
3. Kupfer, P. Weinstraße vor der Seidenstraße—Weinkulturen zwischen Georgien und China. In *Kulturgut Rebe und Wein*; König, H., Decker, H., Eds.; Springer Spektrum: Heidelberg, Germany, 2013; pp. 3–17.
4. Kupfer, P. Der älteste Wein der Menschheit in China: Jiahu und die Suche nach den Ursprüngen der eurasischen Weinkultur. In *Wo aber der Wein fehlt, stirbt der Reiz des Lebens*; Decker, H., König, H., Zwickel, W., Eds.; Nünnerich-Asmus Verlag: Mainz, Germany, 2015; pp. 12–25.
5. Gay-Lussac, J.L. Sur l'analyse de l'alcohol et de l'ether sulfurique et sur les produits de la fermentation. *Annales de Chimie et de Physique* **1815**, *95*, 311.
6. Schwann, T. Vorläufige Mitteilung, betreffend Versuche über die Weingährung und Fäulnis. *Annalen der Physik und Chemie* **1837**, *41*, 184–193. [CrossRef]
7. Kützing, F.T. Mikroskopische Untersuchungen über die Hefe und Esssigmutter, nebst mehreren anderen dazu gehörigen vegetabilischen Gebilden. *J. Prakt. Chem.* **1837**, *11*, 385–409. [CrossRef]
8. Cagniard-Latour, C.C. Mémoire sur la fermentation vineuse. *Ann. Chim. Phys.* **1838**, *68*, 206–222.
9. Pasteur, M.L. Animalcules infusoires vivant sans gaz oxygène libre et détermination des fermentation. *Comptes Rendus de l'Academie des Sciences* **1861**, *52*, 344–347.
10. Feldmann, H. *Yeast. Molecular and Cell Biology*; Wiley-Blackwell: Weinheim, Germany, 2010.
11. König, H.; Unden, G.; Fröhlich, J. (Eds.) Biology of Microorganisms on Grapes. In *Must and in Wine*, 2nd ed.; Springer: Heidelberg, Germany, 2017.
12. Bisson, L.F.; Joseph, C.M.L.; Domizio, P. Yeasts. In *Biology of Microorganisms on Grapes, in Must and in Wine*, 2nd ed.; König, H., Unden, G., Fröhlich, J., Eds.; Springer: Heidelberg, Germany, 2017; pp. 65–105.
13. Fugelsang, K.C.; Edwards, C.G. *Wine Microbiology. Practical Applications and Procedures*; Springer: Heidelberg, Germany, 2007.
14. Fleet, G.H.; Heard, G.M. Yeasts—Growth during fermentation. In *Wine Microbiology and Biotechnology*; Fleet, G.H., Ed.; Harwood Academic Publishers: Chur, Switzerland, 1993; pp. 27–54.
15. König, H.; Berkelmann-Löhnertz, B. Maintenance of wine-associated microorganisms. In *Biology of Microorganisms on Grapes, in Must and in Wine*, 2nd ed.; König, H., Unden, G., Fröhlich, J., Eds.; Springer: Heidelberg, Germany, 2017; pp. 549–571.
16. König, H.; Fröhlich, J. Lactic acid bacteria. In *Biology of Microorganisms on Grapes, in Must and in Wine*, 2nd ed.; König, H., Unden, G., Fröhlich, J., Eds.; Springer: Heidelberg, Germany, 2017; pp. 3–41.
17. Guillamón, J.M.; Mas, A. Acetic acid bacteria. In *Biology of Microorganisms on Grapes, in Must and in Wine*, 2nd ed.; König, H., Unden, G., Fröhlich, J., Eds.; Springer: Heidelberg, Germany, 2017; pp. 43–64.
18. Mendes-Ferreira, A.; lí del Olmo, M.; Garcia-Martínez, J.; Pérez-Ortin, J. Functional genomics in wine yeast: DNA arrays and next generation sequencing. In *Biology of Microorganisms on Grapes, in Must and in Wine*, 2nd ed.; König, H., Unden, G., Fröhlich, J., Eds.; Springer: Heidelberg, Germany, 2017; pp. 573–604.
19. Fröhlich, J.; König, H.; Claus, H. Molecular methods for the identification of wine microorganisms and yeast development. In *Biology of Microorganisms on Grapes, in Must and in Wine*, 2nd ed.; König, H., Unden, G., Fröhlich, J., Eds.; Springer: Heidelberg, Germany, 2017; pp. 517–547.
20. Morrison-Whittle, P.; Goddard, M.R. From vineyard to winery: A source map of microbial diversity driving wine fermentation. *Environ. Microbiol.* **2018**, *20*, 75–84. [CrossRef] [PubMed]
21. Borneman, A.R.; Pretorius, I.S.; Chambers, P.J. Comparative genomics: a revolutionary tool for wine yeast strain development. *Curr. Opin. Biotechnol.* **2013**, *24*, 192–199. [CrossRef] [PubMed]
22. Bisson, L.F. Stuck and sluggish fermentations. *Am. J. Enol. Vitic.* **1999**, *50*, 1–13.
23. Bisson, L.F.; Butzke, C.E. Diagnosis and rectification of stuck and sluggish fermentations. *Am. J. Enol. Vitic.* **2000**, *51*, 168–177.
24. Heinisch, J.J.; Rodicio, R. Stress responses in wine yeast. In *Biology of Microorganisms on Grapes, in Must and in Wine*, 2nd ed.; König, H., Unden, G., Fröhlich, J., Eds.; Springer: Heidelberg, Germany, 2017; pp. 377–395.
25. Ribéreau-Gayon, P.; Dubourdieu, D.; Donèche, B.; Lonvaud, A. *Handbook of Enology. The Microbiology of Wine and Vinifications*, 2nd ed.; John Wiley & Sons Ltd.: Chichester, UK, 2006; Volume 1, pp. 79–113.

26. Petruzzi, L.; Capozzi, V.; Berbegal, C.; Corbo, M.R.; Bevilacqua, A.; Spano, G.; Sinigaglia, M. Microbial resources and enological significance: Opportunities and benefits. *Front. Microbiol.* **2017**, *8*, 995. [CrossRef] [PubMed]
27. Jolly, N.P.; Varela, C.; Pretorius, I.S. Not your ordinary yeast: Non-*Saccharomyces* yeasts in wine production uncovered. *FEMS Yeast Res.* **2014**, *14*, 215–237. [CrossRef] [PubMed]
28. Mas, A.; Guillamón, J.M.; Beltran, G. Non-Conventional Yeast in the Wine Industry. *Front. Media* **2016**, *7*, 1494. [CrossRef]
29. Visser, W.; Scheffers, W.A.; Batenburg-van der Vegte, A.H.; van Dijken, J.P. Oxygen requirements of yeasts. *Appl. Environ. Microbiol.* **1990**, *56*, 3785–3792. [PubMed]
30. Hanl, L.; Sommer, P.; Arneborg, N. The effect of decreasing oxygen feed rates on growth and metabolism of *Torulaspora delbrueckii*. *Appl. Microbiol. Biotechnol.* **2005**, *67*, 113–118. [CrossRef] [PubMed]
31. Mauricio, J.C.; Millán, C.; Ortega, J.M. Influence of oxygen on the biosynthesis of cellular fatty acids, sterols and phospholipids during alcoholic fermentation by *Saccharomyces cerevisiae* and *Torulaspora delbrueckii*. *World J. Microbiol. Biotechnol.* **1998**, *14*, 405–410. [CrossRef]
32. Brandam, C.; Lai, Q.P.; Julien-Ortiz, A.; Taillandier, P. Influence of oxygen on alcoholic fermentation by a wine strain of *Torulaspory delbrueckii*: Kinetics and carbon mass balance. *Biosci. Biotechnol. Biochem.* **2013**, *77*, 1848–1853. [CrossRef] [PubMed]
33. Dimopoulou, M.; Lonvaud-Funel, A.; Dols-Lafargue, M. Polysaccharide production by grapes must and wine microorganisms. In *Biology of Microorganisms on Grapes, in Must and in Wine*, 2nd ed.; König, H., Unden, G., Fröhlich, J., Eds.; Springer: Heidelberg, Germany, 2017; pp. 293–314.
34. Sanz, L.M.; Martínez-Castro, I. Carbohydrates. In *Wine Chemistry and Biochemistry*; Moreno-Arribas, M.V., Polo, M.C., Eds.; Springer: Heidelberg, Germany, 2010; pp. 231–248.
35. Santos-Buelga, C.; de Freitas, V. Influence of phenolics on wine organoleptic properties. In *Wine Chemistry and Biochemistry*; Moreno-Arribas, M.V., Polo, M.C., Eds.; Springer: Heidelberg, Germany, 2010; pp. 529–570.
36. Sabel, A.; Claus, H.; Martens, S.; König, H. *Wickerhamomyces anomalus* AS1: A new strain with potential to improve wine aroma. *Ann. Microbiol.* **2014**, *64*, 483–491. [CrossRef]
37. Schwentke, J.; Sabel, A.; Petri, A.; König, H.; Claus, H. The yeast *Wickerhamomyces anomalus* AS1 secretes a multifunctional exo-ß-1,3-glucanase with implications for winemaking. *Yeast* **2014**, *31*, 349–359. [CrossRef] [PubMed]
38. Dittrich, H.H.; Großmann, M. *Mikrobiologie des Weines. 4. aktualisierte Auflage*; Ulmer Verlag: Stuttgart, Germany, 2010.
39. Christ, E.; Kowalczyk, M.; Zuchowska, M.; Claus, H.; Löwenstein, R.; Szopinska-Morawska, A.; Renaut, J.; König, H. An exemplary model study for overcoming stuck fermentation during spontaneous fermentation with the aid of a *Saccharomyces* triple hybrid. *J. Agric. Sci.* **2015**, *7*, 18–34. [CrossRef]
40. Szopinska, A.; Christ, E.; Planchon, S.; König, H.; Evers, D.; Renaut, J. Stuck at work? Quantitative proteomics of environmental wine yeast strains reveals the natural mechanism of overcoming stuck fermentation. *Proteomics* **2016**, *16*, 593–608. [CrossRef] [PubMed]
41. Blättel, V.; Petri, A.; Rabenstein, A.; Kuever, J.; König, H. Differentiation of species of the genus *Saccharomyces* using biomolecular fingerprinting methods. *Appl. Microbiol. Biotechnol.* **2013**, *97*, 4597–4606. [CrossRef] [PubMed]
42. Jiranek, A.; Langridge, P.; Henschke, P.A. Amino acid and ammonium utilization by *Saccharomyces cerevisiae* wine yeasts from a chemically defined medium. *Am. J. Enol. Vitic.* **1995**, *46*, 75–83.
43. Zuchowska, M.; König, H.; Claus, H. Allelic variants of hexose transporter Hxt3p und hexokinase Hxk1p/Hxk2p in strains of *Saccharomyces cerevisiae* und interspecies hybrids. *Yeast* **2015**, *32*, 657–669. [CrossRef] [PubMed]
44. González, S.S.; Barrio, E.; Gafner, J.; Querol, A. Natural hybrids from *Saccharomyces* cerevisiae, Saccharomyces *bayanus* and *Saccharomyces kudriavzevii* in wine fermentations. *FEMS Yeast Res.* **2006**, *6*, 1221–1234. [CrossRef] [PubMed]
45. Bradbury, J.; Richards, K.; Niederer, H.; Lee, S.; Rod Dunbar, P.; Gardner, R. A homozygous diploidsubset of commercial wine yeast strains. *Antonie van Leeuwenhoek* **2006**, *89*, 27–37. [CrossRef] [PubMed]
46. González, S.S.; Barrio, E.; Querol, A. Molecular characterization of new natural hybrids between *Saccharomyces cerevisiae* and *Saccharomyces kudriavzevii* from brewing. *Appl. Environ. Microbiol.* **2008**, *74*, 2314–2320. [CrossRef] [PubMed]

47. Lopes, C.A.; Barrio, E.; Querol, A. Natural hybrids of *S. cerevisiae* × *S. kudriavzevii* share alleles with European wild populations of *Saccharomyces kudriavzevii*. *FEMS Yeast Res.* **2010**, *10*, 412–421. [CrossRef] [PubMed]
48. Naumov, G.I.; James, S.A.; Naumova, E.S.; Louis, E.J.; Roberts, I.N. Three new species in the *Saccharomyces* sensu stricto complex: *Saccharomyces cariocanus*, *Saccharomyces kudriavzevii* and *Saccharomyces mikatae*. *Int. J. System. Evol. Microbiol.* **2000**, *50*, 1931–1942. [CrossRef] [PubMed]
49. Sampaio, J.P.; Gonçalves, P. Natural populations of *Saccharomyces kudriavzevii* in Portugal are associated with oak bark and sympatric with *S. cerevisiae* and *S. paradoxus*. *Appl. Environ. Microbiol.* **2008**, *74*, 2144–2152. [CrossRef] [PubMed]
50. Hirschhäuser, S.; Fröhlich, J.; Gneipel, A.; Schönig, I.; König, H. Fast protocols for the 5S rDNA and ITS-2 based identification of *Oenococcus oeni*. *FEMS Lett.* **2005**, *244*, 165–171. [CrossRef] [PubMed]
51. Sebastian, P.; Herr, P.; Fischer, U.; König, H. Molecular identification of lactic acid bacteria occurring in must and wine. *South Afr. J. Enol. Vitic.* **2011**, *32*, 300–309. [CrossRef]
52. Röder, C.; König, H.; Fröhlich, J. Species-specific identification of *Dekkera/Brettanomyces* yeasts by fluorescently labelled rDNA probes targeting the 26S rRNA. *FEMS Yeast Res.* **2007**, *7*, 1013–1026. [CrossRef] [PubMed]
53. Petri, A.; Pfannebecker, J.; Fröhlich, J.; König, H. Fast identification of wine related lactic acid bacteria by multiplex PCR. *Food Microbiol.* **2013**, *33*, 48–54. [CrossRef] [PubMed]
54. Chambers, P.J.; Pretorius, I.S. Fermenting knowledge: The history of winemaking, science and yeast research. *EMBO Rep.* **2010**, *11*, 914–920. [CrossRef] [PubMed]
55. Pretorius, I.S. Tailoring wine yeast for the new millennium: Novel approaches to the ancient art of wine making. *Yeast* **2000**, *16*, 675–729. [CrossRef]
56. Borneman, A.R.; Desany, B.A.; Riches, D.; Affourtit, J.P.; Forgan, A.H.; Pretorius, I.S.; Egholm, M.; Chambers, P.J. The genome sequence of the wine yeast VIN7 reveals an allotriploid hybrid genome with *Saccharomyces cerevisiae* and *Saccharomyces kudriavzevii* origins. *FEMS Yeast Res.* **2012**, *12*, 88–96. [CrossRef] [PubMed]