fermentation

MDPI

Review

Challenges of the Non-Conventional Yeast *Wickerhamomyces anomalus* in Winemaking

Beatriz Padilla [1], Jose V. Gil [2,3] and Paloma Manzanares [2,*]

1 INCLIVA Health Research Institute, 46010 Valencia, Spain; bpadilla@incliva.es
2 Department of Biotechnology, Instituto de Agroquímica y Tecnología de Alimentos (IATA), Consejo Superior de Investigaciones Científicas (CSIC), Paterna, 46980 Valencia, Spain; giljv@uv.es
3 Departamento de Medicina Preventiva y Salud Pública, Ciencias de la Alimentación, Toxicología y Medicina Legal, Facultad de Farmacia, Universitat de València, Burjassot, 46100 Valencia, Spain
* Correspondence: pmanz@iata.csic.es; Tel.: +34-96 390-0022

Received: 27 July 2018; Accepted: 18 August 2018; Published: 20 August 2018

Abstract: Nowadays it is widely accepted that non-*Saccharomyces* yeasts, which prevail during the early stages of alcoholic fermentation, contribute significantly to the character and quality of the final wine. Among these yeasts, *Wickerhamomyces anomalus* (formerly *Pichia anomala, Hansenula anomala, Candida pelliculosa*) has gained considerable importance for the wine industry since it exhibits interesting and potentially exploitable physiological and metabolic characteristics, although its growth along fermentation can still be seen as an uncontrollable risk. This species is widespread in nature and has been isolated from different environments including grapes and wines. Its use together with *Saccharomyces cerevisiae* in mixed culture fermentations has been proposed to increase wine particular characteristics. Here, we review the ability of *W. anomalus* to produce enzymes and metabolites of oenological relevance and we discuss its potential as a biocontrol agent in winemaking. Finally, biotechnological applications of *W. anomalus* beyond wine fermentation are briefly described.

Keywords: non-*Saccharomyces* yeasts; *Wickerhamomyces anomalus*; *Pichia anomala*; enzymes; glycosidases; acetate esters; biocontrol; mixed starters; wine

1. Introduction

Saccharomyces cerevisiae is the main microorganism involved in the alcoholic fermentation of grape must. Moreover, the use of selected *S. cerevisiae* strains has provided an improvement in the control and homogeneity of fermentations. However, winemaking is a non-sterile process, and many other species of yeasts belonging to various non-*Saccharomyces* genera prevail during the early stages of alcoholic fermentation and contribute significantly to the character and quality of the final wine [1].

In the past, non-*Saccharomyces* yeasts were considered of secondary significance or as undesirable spoilage yeasts. Nowadays, the role of non-*Saccharomyces* has been re-evaluated, and it is widely accepted that selected strains can positively influence the winemaking process [2]. Beyond the contribution of non-*Saccharomyces* yeasts to wine aroma complexity [3], these yeasts can help address some of the modern challenges in winemaking, including the reduction of the ethanol content of wine [4–7] or the control of wine spoilage [8,9].

Ecological studies have shown that species of mainly *Hanseniaspora* (*Kloeckera*), *Candida,* and *Metschnikowia* initiate the fermentation together with species of *Pichia*, *Issatchenkia,* and *Kluyveromyces.* Occasionally, representatives of *Brettanomyces*, *Schizosaccharomyces*, *Torulaspora*, *Rhodotorula*, *Zygosaccharomyces*, and *Cryptococcus* genera are also present. These yeasts decline by mid-fermentation, and then, *S. cerevisiae* becomes predominant and continues the fermentation [10]. Based on the capability of some of these non-*Saccharomyces* yeasts to produce flavor-enhancing enzymes or to modify the concentration

of secondary metabolites, different mixed starters have been designed and proposed as a tool to enhance wine quality [3,11]. Moreover, several species including *Lachancea thermotolerans*, *Metschnikowia pulcherrima*, *Torulaspora delbrueckii*, *Pichia kluyvery*, and *Schizosaccharomyces pombe* are already commercially available.

Wickerhamomyces anomalus, formerly known as *Pichia anomala, Hansenula anomala, Candida pelliculosa* was recently assigned to the genus *Wickerhamomyces* based on phylogenetic analysis of gene sequences, which has caused major changes in the classification of yeasts. [12]. This species has been frequently isolated from grapes and wines. Although traditionally *W. anomalus* is associated with excessive production of ethyl acetate, which represents a serious handicap for their use in winemaking, this species has gained considerable importance for the wine industry since it exhibits interesting and potentially exploitable physiological and metabolic characteristics as summarized in Figure 1. Here, we revisit the contribution of *W. anomalus* in wine production. First, we review the ecology and prevalence of this yeast in winemaking, and we discuss its ability to produce enzymes, killer toxins, and metabolites of enological relevance. Second, we review the design of mixed starters of *W. anomalus* with *S. cerevisiae* to improve wine aroma complexity. Finally, we discuss biotechnological applications of *W. anomalus* beyond wine fermentation. When citing older literature, the original yeast species name will be kept.

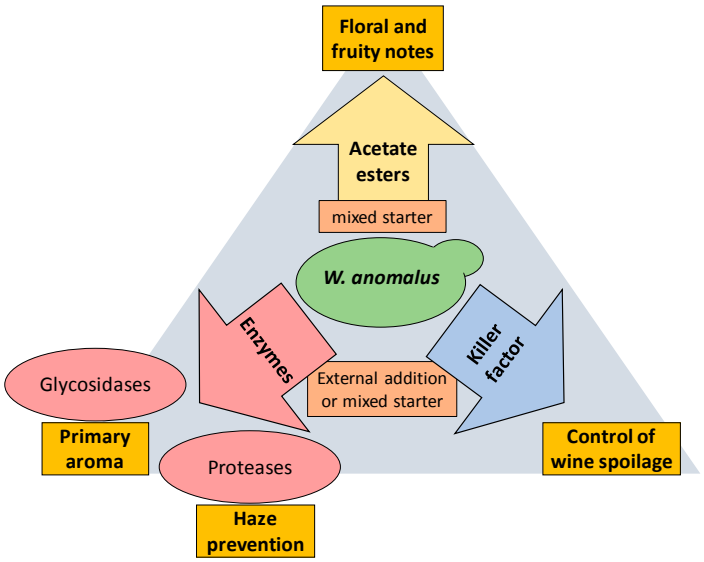

Figure 1. Benefits of *Wickerhamomyces anomalus* in winemaking.

2. *W. anomalus* Is a Ubiquitous Yeast Generally Associated with Winemaking

W. anomalus is a heterothallic, ascomycetous yeast, forming one to four hat-shaped ascospores [13,14]. The placement of *P. anomala* in the genus *Wickerhamomyces* was due to multigene phylogenetic analysis [11]. *W. anomalus* is a widely used name and a proposal to conserve the species name anomala (-us). *W. anomalus* is a biotechnologically relevant yeast species with food, environmental, industrial, and medical applications. Natural habitats of *W. anomalus* are very diverse and include tree exudates, plants and fruit skins, insects, human tissues, and faeces, and also wastewaters and marine environments. The versatility of this species is encouraged by its ability to tolerate extreme environmental conditions like oxidative, salt, and osmotic stress, as well as pH and temperature shocks [15]. Due to these characteristics, this yeast can be a spoilage organism, for instance, in high-sugar food products [16,17] or silage [18]. Its genome sequence is already available, providing the basis to analyse metabolic capabilities, phylogenetic relationships, and biotechnologically important features [19,20]. The main physiological and genetics features of *W. anomalus* are reviewed in Reference [14].

In winemaking, *W. anomalus* is a ubiquitous yeast which has been previously associated with grape, must, wine, and winery facilities. It was shown that *H. anomala* is present during the early stages of red wine fermentation even when the must is inoculated with 10^5 to 10^7 cells of *S. cerevisiae* per mL, thus,

making a significant contribution to fermentation [21]. Different studies described that *W. anomalus* isolated from grape must was able to persist until the end of fermentation [22,23]. Some strains of *W. anomalus* can tolerate up to 12.5% (*v*/*v*) ethanol and are known to produce killer toxins [15,24], allowing this species to compete against other yeasts in the same environment. *W. anomalus* is able to grow abundantly in wine due to its fully aerobic or weakly fermentative metabolism, and it is known for film formation on the surface of bulk wines in unfilled containers and with insufficient sulphite levels to prevent their growth [25].

Grapes are a primary source of non-*Saccharomyces* yeasts including several *Pichia* species [26]. *P. anomala* was found throughout different vineyards over a period of three years in conventional and organic vineyards, representing approximately 20% and 25% of yeast species isolated from musts obtained from Grenache and Shiraz varieties [27]. In a similar study, *W. anomalus* was the second dominant yeast after *Hanseniaspora uvarum* in Cabernet Sauvignon grape must derived from integrated vineyards [28]. However, it was observed that the cell concentration of *W. anomalus* only increased marginally throughout fermentation, suggesting that its growth is severely hampered by the lack of oxygen [28]. This yeast generally shows low growth rates and biomass yields under anaerobic conditions [15]. Yeast isolations from Malvar grape musts pointed out *W. anomalus* as one of the most frequent non-*Saccharomyces* species, and in addition, the yeast was a good producer of extracellular enzymes which may be beneficial in winemaking [29]. Recently, the dynamics of several non-*Saccharomyces* species were evaluated in synthetic must in the presence or absence of *S. cerevisiae* [30]. The study showed that the behaviour of the non-*Saccharomyces* species was differentially influenced by the presence of *S. cerevisiae*. Interestingly, in the absence of *S. cerevisiae*, *W. anomalus* suppressed the rest of non-*Saccharomyces* species suggesting that the yeast can survive in the early stages of the fermentation better than the other yeast species and may utilize the nitrogen released by dead cells. However, in the presence of *S. cerevisiae*, *W. anomalus* specifically declined early in fermentation, suggesting an antagonistic interaction between both yeasts [30]. This interaction has also been proposed in apple cider fermentations [31].

The prevalence of *P. anomala* in cellar equipment has been described in several Spanish wineries, and it was the only species among all detected that it was present in all four wineries evaluated [32]. Previously, it was found that besides *S. cerevisiae*, the most commonly detected species were *P. anomala*, *Pichia membranifaciens*, *Candida* spp. and *Cryptococcus* spp. [33]. Finally, *Pichia* spp. accounted for 83% of non-*Saccharomyces* yeasts present in winery surfaces, such as floor, pumps, and empty tanks, whereas *Hanseniaspora* spp. accounted for the remaining 17% [34].

Ecological studies in different wine regions of the world have also identified other *Pichia* species. Although at lower levels, several species such as *Pichia terricola*, *Pichia kudriavzevii*, and *P. kluyvery* were present in freshly extracted grape musts from Bordeaux region, although they rapidly disappeared from fermenting musts [35]. However, *P. membranifaciens* and *Pichia fermentans* appeared after the starting of the malolactic fermentation, and the former was present in samples of red and white Bordeaux wines examined at 1- and 2-month intervals after fermentation [35]. *P. membranifaciens* was also identified in spontaneous fermentations of musts from La Mancha, Spain [36] and in grape varieties used in India for winemaking [37]. As a minor species, *P. membranifaciens* was described as part of the indigenous population during spontaneous fermentations of wines in Mendoza, Argentina [38]. Yeast diversity studies of grape varieties from vine-growing regions of China identified *P. fermentans* and *Pichia guilliermondii* [39], and the former was also isolated from a Southern Italian autochthonous grape cultivar [40]. *P. kluyveri* and *Pichia farinosa* were found in vineyards and grape musts from four production regions of South Africa, although they were not the predominant species [41]. By contrast, significant amounts of *P. kluyveri* and *P. kudriavzevii* were isolated from grapes varieties of the Strekov winegrowing region in Slovakia, and they were more associated with damaged than with intact berries [42]. Both species are considered as indicators of mould-damaged grapes [26,43]. Recently the species *Pichia galeiformis* was identified for the first time on grape berries by FT-IR spectroscopy [44].

3. *W. anomalus* Is a Good Producer of Relevant Enzymes for Winemaking

W. anomalus strains isolated from enological ecosystems have been reported as an interesting source of different enzymes which could be used in the winemaking industry [45]. Aroma is one of the most appreciable characteristics influencing the overall quality of wine. Non-*Saccharomyces* yeasts may have an impact on both the primary and secondary aroma through the production of enzymes and metabolites, respectively. Strains identified as *W. anomalus* or its former names have been reported to produce glycosidases such as β-D-glucosidase, α-L-arabinofuranosidase, α-L-rhamnosidase, and β-D-xylosidase, which are involved in the release of aroma compounds from grape precursors [3].

Several authors have explored the enzymatic potential of non-*Saccharomyces* isolates with the aim of identifying good producer strains. A study conducted on 20 different yeast species showed that all tested strains of *H. anomala* presented β-glucosidase activity [46]. Results from other screenings concluded that *P. anomala* strains exhibited higher β-glucosidase activity when compared with yeast species belonging to other genera such as *Candida, Dekkera* or *Torulaspora* [47,48].

Besides showing β-D-glucosidase activity, other *P. anomala*/*W. anomalus* strains exhibited α-L-arabinofuranosidase or β-D-xylosidase activity in screenings as well, including more than 300 and 100 wine yeast isolates, respectively [49,50]. Similarly, *P. anomala* produced β-D-xylosidase with activity at pH, temperature, and concentrations of glucose and ethanol usually found during wine fermentation [51]. Interestingly, selected *P. anomala* strains are able to produce several glycosidase activities, for instance one *P. anomala* strain was described as producer of the four glycosidase activities [52], and a *W. anomalus* strain producing β-D-glucosidase, also showed α-L-arabinofuranosidase and β-D-xylosidase activities [24].

Despite the potential of *W. anomalus* to produce glycosidases, the effect of purified *W. anomalus* enzymes on the releasing of wine volatile compounds has been scarcely explored. Terpene production was observed in Muscat-type grape juice and wine treated with β-D-glucosidase from *P. anomala* MDD24 [53,54]. This glucosidase was efficient in releasing desirable aromas particularly during the final stage of alcoholic fermentation due to its tolerance to high concentrations of ethanol. Furthermore, isolates of *W. anomalus* showing β-D-glucosidase activity provoked a moderated overall terpene increase when inoculated to final wines [49]. However, the effectiveness of *W. anomalus* α-L-arabinofuranosidase, α-L-rhamnosidase or β-D-xylosidase for aromatic compounds releasing during winemaking has not been explored yet.

The strain *W. anomalus* AS1 was selected by the capability of its cells to hydrolyze different synthetic and natural glycosides under wine related conditions [24]. Afterwards, the enzyme was purified from the culture supernatant of AS1 and characterized as a multifunctional exo-β-1,3-glucanase active under typical oenological conditions [55]. Thus, the enzyme might have multiple applications in winemaking such as increasing concentrations of sensory and bioactive compounds by splitting glycosylated precursors or to reduce viscosity by hydrolysis of glycan slimes. The role of exo-β-1,3-glucanases in increasing wine aroma through the release of glycosidic precursors has been previously discussed [56].

Besides the contribution to the aromatic profile of wines, other relevant enzymes for winemaking are also produced by *W. anomalus* strains. Degradation of haze forming-proteins by enzymes is an attractive alternative to bentonite fining because it would minimize losses of wine volume and aroma [57]. In fact, wine yeasts secreting proteolytic enzymes are of high biotechnological interest for protein haze prevention because they could be directly added as starter cultures to the grape must. This is the case of *W. anomalus* 227 which secretes the aspartic protease WaAPR1 in white grape juice, suggesting its suitability for reducing grape must protein content [58].

4. *W. anomalus* Is a Good Producer of Acetate Esters

Non-*Saccharomyces* yeast, were traditionally considered as spoilage wine microorganisms due to high ethyl acetate production. In particular, *P. anomala* is a major ethyl acetate producer, and some strains show levels of ethyl acetate higher than 150 mg/L [59], close to the concentration at which this acetate ester can impart spoilage character to wine (150–200 mg/L) [60]. However, *P. anomala* is also a good producer of fruity acetate esters and other volatiles with a positive impact on wine aroma. The ability

of 37 strains of non-*Saccharomyces* yeasts, including seven *P. anomala* strains, to produce the main wine acetate esters; ethyl acetate, isoamyl acetate, and 2-phenylethyl acetate was examined in Reference [59] (Figure 2). Among the genera evaluated, *Pichia* and *Hanseniaspora* stood out as the best producers of acetate esters, although significant differences among strains were found in the production of the three esters, highlighting the convenience of carrying out adequate screenings for selection of the appropriate strains. All the seven *P. anomala* strains included in the study were good isoamyl acetate producers, and interestingly, five of them produced a level of ethyl acetate lower than 200 mg/L. None of them was able to produce 2-phenylethyl acetate [59]. A similar screening studied the main oenological characteristics of 55 non-*Saccharomyces* yeast strains, 14 of them belonging to the *Pichia* genus. Levels of ethyl acetate production of these *Pichia* species ranged between 0.35 and 272 mg/L, whereas the only strain of *P. anomala* evaluated produced around 100 mg/L. This *P. anomala* strain was selected to be included in a mixed starter due to its fermentative characteristics and its ethanol and polysaccharides production [61].

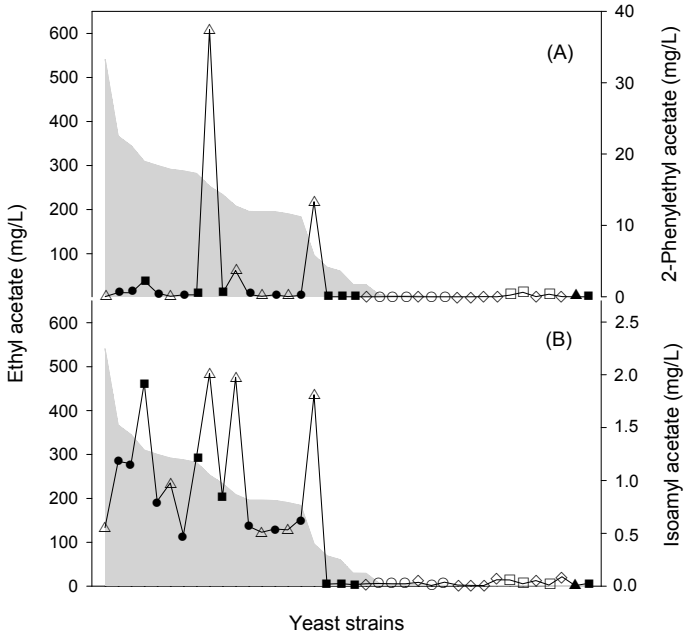

Figure 2. Production of acetate esters by non-*Saccharomyces* yeast strains. (**A**) 2-Phenylethyl acetate (symbols; right axis). (**B**) Isoamyl acetate (symbols; right axis). Ethyl acetate is represented in both panels as an area plot (left axis). Strains: ●*Pichia anomala* (seven different strains), ■ *P. membranifaciens* (seven different strains), ▲ *P. fermentans* (one strain), ◇ *Candida* spp., △ *Hanseniaspora* spp., ○ *Torulaspora* spp., □ *Zygosaccharomyces* spp. Adapted from Reference [59].

5. *W. anomalus* Produces Killer Toxins of Broad Spectrum

After its initial discovery in *S. cerevisiae*, the killer phenotype was described in non-*Saccharomyces* yeasts [62]. Killer toxins represent a biocontrol strategy alternative to the use of chemical preservatives or physical methodologies during the winemaking process [63]. In this context, *W. anomalus* killer proteins have been reported as antimicrobial agents against undesired microorganisms present in different food and beverages [14,64].

In the oenological environment, *W. anomalus* killer toxins are mainly tested against the prevailing wine spoilage microorganism *Dekkera*/*Brettanomyces* [8,65]. Nevertheless, the antimicrobial activity of *W. anomalus* towards other minor yeast species present during the early stages of grape fermentation such as *P. guilliermondii* or *P. membranifaciens* has also been reported [66,67]. Moreover, killer cultures belonging to *P. anomala* showed a broad killer spectrum against regionally relevant spoilage yeast and *Dekkera bruxellensis* collection strains [68]. The killer toxin Pikt produced by the *P. anomala* DBVPG 3003 strain was active on 15 isolates belonging to the genus *Dekkera*/*Brettanomyces*, and its fungicidal

effect in wine was maintained during at least 10 days [69]. Further research revealed that this toxin presented a ubiquitin-like peptide structure with a molecular mass of approximately 8 kDa, and that it selectively interacts with β-1,6 glucans, which are the putative binding sites for Pikt on the cell wall of the sensitive targets [70]. Recently, the killer toxin KTCf20 secreted by the strain *W. anomalus* Cf20 was also suggested to bind to β-1,3 and β-1,6 glucans of the cell wall of sensitive strains. Moreover, the toxin was produced and showed to be stable and highly active at physicochemical conditions suitable for the winemaking process [66]. Finally, the potential use of Pikt from *W. anomalus* D2 as an alternative to sulphur dioxide (SO_2) has been proposed, since differently to SO_2, Pikt produced irreversible damage on sensitive yeasts, ensuring the complete control of spoilage *Brettanomyces* yeasts [71].

Beside the biocontrol effect of *W. anomalus* on non-*Saccharomyces* spoilage yeasts, it has also been reported that some isolates showed killer activity against *S. cerevisiae* strains [66,72]. Thus, the compatibility of selected killer *W. anomalus* strains with the main microbial agents involved in wine production needs to be tested during the selection procedure to avoid technological problems due to sluggish or incomplete alcoholic fermentations.

6. *W. anomalus* in Mixed Starters with *S. cerevisiae*

In recent years, the possibility to improve the fermentation process and the aromatic complexity of wine using selected non-*Saccharomyces* strains in mixed starters with *S. cerevisiae* has been investigated by many authors. This practice is proposed as a way to avoid stuck fermentations, control the ecological balance, achieve unique and distinctive aromatic characteristics, and control some specific oenological aspects, such as acidity, ethanol, or glycerol content [3,61,73–79]. Screening studies are useful to select appropriate non-*Saccharomyces* strains that, based on their quality profiles, could be good candidates to be part of a mixed starter. However, the behaviour of the selected strains could be modified by the presence of *S. cerevisiae* in the mixed starter [61,80]. Moreover, the appropriate modality (sequential or simultaneous) and inoculation time, the proportion of yeasts in the culture, and the potential microorganism interactions should be taken into account [61,81,82].

Different mixed starters containing *P. anomala* and other species of the *Pichia* genus have been proposed to improve wine quality (Table 1). Wines obtained with mixed cultures *P. anomala*/*S. cerevisiae* are characterized by higher concentrations of acetate esters, particularly ethyl acetate and isoamyl acetate [61,79,83–85]. Some of these wines showed levels of ethyl acetate higher than 150 mg/L [61,79], close to the concentration at which this acetate ester can impart spoilage character to wine (150–200 mg/L) [60]. However, wines with the highest concentrations of ethyl acetate were fermented in small volumes (less than 140 mL) where excessive aeration could promote the production of ethyl acetate. In contrast, experimental wines produced in 100 L tanks showed ethyl acetate levels less than 45 mg/L [83,84].

With the aim of reducing the production of ethyl acetate due to *P. anomala* in mixed cultures, the efficacy of a *petite P. anomala* mutant with low respiratory activity was investigated. In mixed cultures with *S. cerevisiae*, the *P. anomala* mutant died quicker and produced lower amounts of ethyl acetate than the wild type. Moreover, wines fermented by mixed cultures with the *petite* mutant strain of *P. anomala* and *S. cerevisiae* presented 100 mg/L of ethyl acetate, half the amount detected using the *P. anomala* wild-type strain, and had a better flavour profile [85]. Increases in acetate esters in wines fermented with *P. anomala* mixed cultures have been correlated with high scores in sensory preference tests, mainly in terms of floral and fruity notes [83,84]. In addition, herbaceous notes were related to higher levels of lineal alcohols in wines fermented with mixed cultures [83].

Table 1. Mixed starters of *Pichia* species and their main impact on wine quality.

Mixed Starter	Impact on Wine	Inoculation	Must	Ref.
P. anomala/*S. cerevisiae*	Isoamyl acetate increase	Co-inoculation	Bobal	[79]
	Isoamyl acetate increase	Co-inoculation	Commercial	[61]
	Acetate ester increase	Co-inoculation	Synthetic	[85]
	Acetate ester increase and alcohol decrease	Sequential	Airén	[84]
	Acetate and ethyl ester increase	Sequential	Mazuela	[83]
P. kudriavzevii/*S. cerevisiae*	Isoamyl acetate increase	Co-inoculation	Cabernet Sauvignon	[86]
P. membranifaciens/*S. cerevisiae*	Isoamyl and 2-phenetyl acetate	Sequential	Muscat	[87]
P. burtonii/*S. cerevisiae*	Ethyl ester increase	Sequential	Synthetic	[88]
P. kluyveri/*S. cerevisiae*	3-Mercaptohexyl acetate increase	Co-inoculation	Sauvignon Blanc	[81]
	3-Sulfanylhexan-1-ol increase	Co-inoculation	Sauvignon Blanc	[89]
	Off-flavor formation	Sequential	Sauvignon Blanc	[90]
P. fermentans/*S. cerevisiae*	Polysaccharide increase	Co-inoculation	Commercial	[91]

Other species of the *Pichia* genus have been included in mixed starter cultures together with *S. cerevisiae*. Some examples are summarized in Table 1. Wines produced with mixed cultures of *P. kudriavzevii* [86] and *P. membranifaciens* [87] presented increases in acetate esters as described for *P. anomala* strains. Wine fermented with a mixed starter of *Pichia burtonii*/*S. cerevisiae* contained higher amounts of ethyl esters [88], whilst co-inoculation of *P. kluyveri* and *S. cerevisiae* increased varietal aromas, mainly 3-mercaptohexyl acetate (3MHA) and 3-sulfanylhexan-1-ol in Sauvignon Blanc wines [81,89]. By contrast, a different *P. kluyvery* isolate did not show a sensorial significant increase in the tropical fruity aromas characterized by 3MHA, and the production of 3-methyl-butanoic acid was associated with an off-putting sour, sweaty, and cheesy aroma that is considered a wine fault [90]. Interestingly, the association of *P. fermentans* with *S. cerevisiae* in mixed cultures produced significant increases in the production of polysaccharides, which improve wine taste and body and exert positive effects on aroma persistence and protein and tartrate stability [91].

7. Applications of *W. anomalus* beyond Wine Fermentation

Different biotechnological applications of *W. anomalus* beyond winemaking are summarized in Table 2. Similar to wine fermentations, the application of non-*Saccharomyces* yeasts in the production of other alcoholic beverages and in bread fermentation is being explored. Besides the use of *Dekkera*/*Brettanomyces* for the production of sour beers, *W. anomalus* stands out as a promising yeast in brewing fermentations mainly due to its diversified enzymatic activities and bioconversion abilities [92]. The fermentation of cider by sequentially mixed cultures of *W. anomalus* and *S. cerevisiae* improved the final quality of cider as a result of a greater variety and amount of esters, higher alcohols, aldehydes, and ketones [31]. *P. anomala* mixed starters have also been proposed to improve the sensorial quality of the sugar cane spirit cachaça since co-inoculation with *S. cerevisiae* led to increases in acetate esters and other volatile compounds associated to good sensory descriptors [93]. Recently a mixed culture of *W. anomalus* with *S. cerevisiae* has been proposed for Chinese Baijiu making due to its positive effects on the end flavor of the beverage [94]. In addition, co-cultures of *S. cerevisiae*, *T. delbrueckii* and *P. anomala* as leavening agents for bread resulted in a higher abundance of volatile organic compounds and in higher sensorial ratings [95]. Finally, the dietary inclusion of *W. anomalus* as single cell protein in aquaculture showed positive effects on rainbow trout gut microbiota abundance and composition [96].

Table 2. Biotechnological applications of *W. anomalus* beyond wine fermentation.

Application	Yeast Strain	Reference
Food and beverage production		
beer	*W. anomalus* [1]	[92]
cider	*W. anomalus* YN6	[31]
cachaça	*P. anomala* UFLA CAF70 and CAF119	[93]
Chinese Baijiu	*W. anomalus* GZ3	[94]
bread	*P. anomala* JK04	[95]
Aquaculture	*W. anomalus* [1]	[96]
Biocontrol		
cereal grain preservation	*P. anomala* J121	[97,98]
antimycotic agent	*P. anomala* C33, C85, Di8, Di28, DBVPG3649	[99]
	P. anomala CMGB88	[72]
Production of fuels and chemicals		
bioethanol	*P. anomala* CBS132101	[100]
	W. anomalus [1]	[101]
ethyl acetate	*W. anomalus* NCYC16	[102]
	W. anomalus DSM 6766	[103]

[1] Strain not specified.

Regarding biocontrol capacity, the positive role of *P. anomala* in grain biopreservation is well established [97]. The yeast improved feed hygiene during storage of moist crimped barley grain by reduction of moulds and *Enterobacteriaceae*. Moreover, *P. anomala* enhanced the nutritional quality of the feed by increasing protein content and reducing the concentration of the antinutritional compound phytate [98]. The killer activity of *P. anomala* is also of interest in biomedical applications due to its activity against potential pathogenic yeast species, which may lead to the development of new antimycotic agents [72,99].

Production of fuels and chemicals is another area of potential application of *W. anomalus*. Bioethanol production exposes yeasts to complex fermentation medium with specific inhibitors and sugar mixtures. *W. anomalus* is able to produce ethanol in multiple biomass hydrolysates with different toxicity levels, is capable of utilizing xylose for growth when supplied with air, and can use nitrate as nitrogen source, making this species a potential ethanol producer using lignocellulosic biomass as a feedstock [100]. Moreover, other studies have identified *W. anomalus* strains that have a comparable ethanol yield to *S. cerevisiae*, although longer fermentation time was needed [101]. In addition to ethanol, *W. anomalus* has the potential to produce the industrially-relevant chemical ethyl acetate from numerous different carbon sources [102]. Ethyl acetate can be used as a microbiologically degradable and environmentally friendly solvent in the manufacture of food, glues, inks, and perfumes, and *W. anomalus* can be an alternative to the chemical processes. Recently, the identification of a novel enzyme Eat1 from *W. anomalus* resulted in high ethyl acetate production when expressed in *S. cerevisiae* and *Escherichia coli*, opening new possibilities for the production of biobased ethyl acetate [103].

8. Final Considerations

Based on the studies reviewed here, the potential positive influence of *W. anomalous* in winemaking seems clear. Indeed, mixed starters with selected *W. anomalus* strains and *S. cerevisiae* can enhance wine aroma, but also control spoilage wine microorganisms. Moreover, *W. anomalus* can exert positive effects in other fermentation processes.

Considering that *W. anomalus* is still seen as a spoilage yeast by winemakers, the commercial application of this yeast seems distant. Since *W. anomalous* is a ubiquitous yeast in the winemaking environment, smart strain screenings will provide appropriate candidates to be included as part of commercial mixed starters. These new strains will allow to exploit positive features of *W. anomalus* while minimizing negative aspects. Undoubtedly, further studies must test the feasibility of *W. anomalus* in different grape musts at industrial or semi-industrial scales, considering the impact of common

oenological practices on the dynamics of this yeast. Finally, interactions among wine yeasts should be considered, taking into account that these interactions seem to be strain-dependent for both non-*Saccharomyces* and *S. cerevisiae* strains.

Author Contributions: B.P., J.V.G., and P.M. contributed to conception and design of the review. B.P. and J.V.G. drafted the manuscript. P.M. supervised and edited the manuscript. All authors commented on the manuscript at all stages.

Funding: This work was funded by grant BIO2015-68790-C2-1-R from the "Ministerio de Economía y Competitividad" (Spain) (MINECO/FEDER Funds). B.P. is the recipient of a Juan de la Cierva Formación post-doctoral research contract (FJCI-2015-23701) from "Ministerio de Economía, Industria y Competitividad" (Spain).

Acknowledgments: Authors thank Sandra Garrigues for critical reading of the manuscript.

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

References

1. Fleet, G.H. Wine yeasts for the future. *FEMS Yeast Res.* **2008**, *8*, 979–995. [CrossRef] [PubMed]
2. Comitini, F.; Capece, A.; Ciani, M.; Romano, P. New insights on the use of wine yeasts. *Curr. Opin. Food Sci.* **2017**, *13*, 44–49. [CrossRef]
3. Padilla, B.; Gil, J.V.; Manzanares, P. Past and future of non-*Saccharomyces* yeasts: From spoilage microorganisms to biotechnological tools for improving wine aroma complexity. *Front. Microbiol.* **2016**, *7*, 411. [CrossRef] [PubMed]
4. Ciani, M.; Morales, P.; Comitini, F.; Tronchoni, J.; Canonico, L.; Curiel, J.A.; Oro, L.; Rodrigues, A.J.; Gonzalez, R. Non-conventional yeast species for lowering ethanol content of wines. *Front. Microbiol.* **2016**, *7*, 642. [CrossRef] [PubMed]
5. Contreras, A.; Hidalgo, C.; Schmidt, S.; Henschke, P.A.; Curtin, C.; Varela, C. The application of non-*Saccharomyces* yeast in fermentations with limited aeration as a strategy for the production of wine with reduced alcohol content. *Int. J. Food Microbiol.* **2015**, *205*, 7–15. [CrossRef] [PubMed]
6. Englezos, V.; Rantsiou, K.; Cravero, F.; Torchio, F.; Ortiz-Julien, A.; Gerbi, V.; Rolle, L.; Cocolin, L. *Starmerella bacillaris* and *Saccharomyces cerevisiae* mixed fermentations to reduce ethanol content in wine. *Appl. Microbiol. Biotechnol.* **2016**, *100*, 5515–5526. [CrossRef] [PubMed]
7. Gonzalez, R.; Quiros, M.; Morales, P. Yeast respiration of sugars by non-*Saccharomyces* yeast species: A promising and barely explored approach to lowering alcohol content of wines. *Trends Food Sci. Technol.* **2013**, *29*, 55–61. [CrossRef]
8. Berbegal, C.; Spano, G.; Fragasso, M.; Grieco, F.; Russo, P.; Capozzi, V. Starter cultures as biocontrol strategy to prevent *Brettanomyces bruxellensis* proliferation in wine. *Appl. Microbiol. Biotechnol.* **2018**, *102*, 569–576. [CrossRef] [PubMed]
9. Oro, L.; Ciani, M.; Comitini, F. Antimicrobial activity of *Metschnikowia pulcherrima* on wine yeasts. *J. Appl. Microbiol.* **2014**, *116*, 1209–1217. [CrossRef] [PubMed]
10. Fleet, G.H.; Heard, G.M. Yeast-growth during winemaking. In *Wine Microbiology and Biotechnology*; Fleet, G.H., Ed.; Harwood Academic Publishers: Chur, Switzerland, 1993; pp. 27–54.
11. 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*, 13. [CrossRef] [PubMed]
12. Kurtzman, C.P. Phylogeny of the ascomycetous yeasts and the renaming of *Pichia anomala* to *Wickerhamomyces anomalus*. *Antonie van Leeuwenhoek* **2011**, *99*, 13–23. [CrossRef] [PubMed]
13. Kurtzman, C.P. *Pichia* e.C. Hansen emend. Kurtzman. In *The Yeasts*, 4th ed.; Kurtzman, C.P., Fell, J.W., Eds.; Elsevier: Amsterdam, The Netherlands, 1998; pp. 273–352.
14. Passoth, V.; Fredlund, E.; Druvefors, U.A.; Schnurer, J. Biotechnology, physiology and genetics of the yeast *Pichia anomala*. *FEMS Yeast Res.* **2006**, *6*, 3–13. [CrossRef] [PubMed]
15. Walker, G.M. *Pichia anomala*: Cell physiology and biotechnology relative to other yeasts. *Antonie van Leeuwenhoek* **2011**, *99*, 25–34. [CrossRef] [PubMed]
16. Lanciotti, R.; Sinigaglia, M.; Gardini, F.; Elisabetta Guerzoni, M. *Hansenula anomala* as spoilage agent of cream-filled cakes. *Microbiol. Res.* **1998**, *153*, 145–148. [CrossRef]
17. Tokuoka, K.; Ishitani, T.; Goto, S.; Komagata, K. Identification of yeasts isolated from high-sugar foods. *J. Gen. Appl. Microbiol.* **1985**, *31*, 411–427. [CrossRef]

18. Jonsson, A.; Pahlow, G. Systematic classification and biochemical characterization of yeasts growing in grass silage inoculated with *Lactobacillus* cultures. *Anim. Res. Dev.* **1984**, *20*, 7–22.
19. Fletcher, E.; Feizi, A.; Kim, S.; Siewers, V.; Nielsen, J. RNA-seq analysis of *Pichia anomala* reveals important mechanisms required for survival at low pH. *Microb. Cell. Fact.* **2015**, *14*, 11. [CrossRef] [PubMed]
20. Schneider, J.; Rupp, O.; Trost, E.; Jaenicke, S.; Passoth, V.; Goesmann, A.; Tauch, A.; Brinkrolf, K. Genome sequence of *Wickerhamomyces anomalus* DSM 6766 reveals genetic basis of biotechnologically important antimicrobial activities. *FEMS Yeast Res.* **2012**, *12*, 382–386. [CrossRef] [PubMed]
21. Heard, G.M.; Fleet, G.H. Growth of natural yeast flora during the fermentation of inoculated wines. *Appl. Environ. Microbiol.* **1985**, *50*, 727–728. [PubMed]
22. Díaz, C.; Molina, A.M.; Nähring, J.; Fischer, R. Characterization and dynamic behavior of wild yeast during spontaneous wine fermentation in steel tanks and amphorae. *BioMed Res. Int.* **2013**, *2013*, 540465. [CrossRef] [PubMed]
23. Renouf, V.; Claisse, O.; Lonvaud-Funel, A. Inventory and monitoring of wine microbial consortia. *Appl. Microbiol. Biotechnol.* **2007**, *75*, 149–164. [CrossRef] [PubMed]
24. Sabel, A.; Martens, S.; Petri, A.; Konig, H.; Claus, H. *Wickerhamomyces anomalus* AS1: A new strain with potential to improve wine aroma. *Ann. Microbiol.* **2014**, *64*, 483–491. [CrossRef]
25. Du Toit, M.; Pretorius, I.S. Microbial spoilage and preservation of wine: Using weapons from nature's own arsenal—A review. *S. Afr. J. Enol. Vitic.* **2000**, *21*, 74–92.
26. Barata, A.; Malfeito-Ferreira, M.; Loureiro, V. The microbial ecology of wine grape berries. *Int. J. Food Microbiol.* **2012**, *153*, 243–259. [CrossRef] [PubMed]
27. Cordero-Bueso, G.; Arroyo, T.; Serrano, A.; Tello, J.; Aporta, I.; Vélez, M.D.; Valero, E. Influence of the farming system and vine variety on yeast communities associated with grape berries. *Int. J. Food Microbiol.* **2011**, *145*, 132–139. [CrossRef] [PubMed]
28. Bagheri, B.; Bauer, F.F.; Setati, M.E. The diversity and dynamics of indigenous yeast communities in grape must from vineyards employing different agronomic practices and their influence on wine fermentation. *S. Afr. J. Enol. Vitic.* **2015**, *36*, 243–251. [CrossRef]
29. Cordero-Bueso, G.; Esteve-Zarzoso, B.; Cabellos, J.M.; Gil-Diaz, M.; Arroyo, T. Biotechnological potential of non-*Saccharomyces* yeasts isolated during spontaneous fermentations of Malvar (*Vitis vinifera* cv. L.). *Eur. Food Res. Technol.* **2013**, *236*, 193–207. [CrossRef]
30. Bagheri, B.; Bauer, F.F.; Setati, M.E. The impact of *Saccharomyces cerevisiae* on a wine yeast consortium in natural and inoculated fermentations. *Front. Microbiol.* **2017**, *8*, 13. [CrossRef] [PubMed]
31. Ye, M.; Yue, T.; Yuan, Y. Effects of sequential mixed cultures of *Wickerhamomyces anomalus* and *Saccharomyces cerevisiae* on apple cider fermentation. *FEMS Yeast Res.* **2014**, *14*, 873–882. [CrossRef] [PubMed]
32. Ocón, E.; Gutiérrez, A.R.; Garijo, P.; López, R.; Santamaría, P. Presence of non-*Saccharomyces* yeasts in cellar equipment and grape juice during harvest time. *Food Microbiol.* **2010**, *27*, 1023–1027. [CrossRef] [PubMed]
33. Martini, A. Origin and domestication of the wine yeast *Saccharomyces cerevisiae*. *J. Wine Res.* **1993**, *4*, 165–176. [CrossRef]
34. Ciani, M.; Mannazzu, I.; Marinangeli, P.; Clementi, F.; Martini, A. Contribution of winery-resident *Saccharomyces cerevisiae* strains to spontaneous grape must fermentation. *Antonie van Leeuwenhoek* **2004**, *85*, 159–164. [CrossRef] [PubMed]
35. Fleet, G.H.; Lafon-Lafourcade, S.; Ribéreau-Gayon, P. Evolution of yeasts and lactic acid bacteria during fermentation and storage of Bordeaux wines. *Appl. Environ. Microbiol.* **1984**, *48*, 1034–1038. [PubMed]
36. Fernández, M.T.; Ubeda, J.F.; Briones, A.I. Comparative study of non-*Saccharomyces* microflora of musts in fermentation by physiological and molecular methods. *FEMS Microbiol. Lett.* **1999**, *173*, 223–229. [CrossRef]
37. Chavan, P.; Mane, S.; Kulkarni, G.; Shaikh, S.; Ghormade, V.; Nerkar, D.P.; Shouche, Y.; Deshpande, M.V. Natural yeast flora of different varieties of grapes used for wine making in India. *Food Microbiol.* **2009**, *26*, 801–808. [CrossRef] [PubMed]
38. Combina, M.; Elia, A.; Mercado, L.; Catania, C.; Ganga, A.; Martinez, C. Dynamics of indigenous yeast populations during spontaneous fermentation of wines from Mendoza, Argentina. *Int. J. Food Microbiol.* **2005**, *99*, 237–243. [CrossRef] [PubMed]
39. Li, S.-S.; Cheng, C.; Li, Z.; Chen, J.-Y.; Yan, B.; Han, B.-Z.; Reeves, M. Yeast species associated with wine grapes in China. *Int. J. Food Microbiol.* **2010**, *138*, 85–90. [CrossRef] [PubMed]

40. Garofalo, C.; Tristezza, M.; Grieco, F.; Spano, G.; Capozzi, V. From grape berries to wine: Population dynamics of cultivable yeasts associated to "Nero di Troia" autochthonous grape cultivar. *World J. Microbiol. Biotechnol.* **2016**, *32*, 10. [CrossRef] [PubMed]
41. Jolly, N.P.; Pretorius, I.S. The effect of non-*Saccharomyces* yeasts on fermentation and wine quality. *S. Afr. J. Enol. Vitic.* **2003**, *24*, 55–62. [CrossRef]
42. Nemcová, K.; Breierová, E.; Vadkertiová, R.; Molnarová, J. The diversity of yeasts associated with grapes and musts of the Strekov winegrowing region, Slovakia. *Folia Microbiol.* **2015**, *60*, 103–109. [CrossRef] [PubMed]
43. Mills, D.A.; Johannsen, E.A.; Cocolin, L. Yeast diversity and persistence in *Botrytis*-affected wine fermentations. *Appl. Environ. Microbiol.* **2002**, *68*, 4884–4893. [CrossRef] [PubMed]
44. Grangeteau, C.; Gerhards, D.; Terrat, S.; Dequiedt, S.; Alexandre, H.; Guilloux-Benatier, M.; von Wallbrunn, C.; Rousseaux, S. FT-IR spectroscopy: A powerful tool for studying the inter- and intraspecific biodiversity of cultivable non-*Saccharomyces* yeasts isolated from grape must. *J. Microbiol. Methods* **2016**, *121*, 50–58. [CrossRef] [PubMed]
45. Madrigal, T.; Maicas, S.; Tolosa, J.J.M. Glucose and ethanol tolerant enzymes produced by *Pichia* (*Wickerhamomyces*) isolates from enological ecosystems. *Am. J. Enol. Vitic.* **2013**, *64*, 126–133. [CrossRef]
46. Rosi, I.; Vinella, M.; Domizio, P. Characterization of β-glucosidase activity in yeasts of oenological origin. *J. Appl. Microbiol.* **1994**, *77*, 519–527. [CrossRef]
47. Charoenchai, C.; Fleet, G.H.; Henschke, P.A.; Todd, B.E.N. Screening of non-*Saccharomyces* wine yeasts for the presence of extracellular hydrolytic enzymes. *Aust. J. Grape Wine Res.* **1997**, *3*, 2–8. [CrossRef]
48. Manzanares, P.; Rojas, V.; Genovés, S.; Vallés, S. A preliminary search for anthocyanin-β-D-glucosidase activity in non-*Saccharomyces* wine yeasts. *Int. J. Food Sci. Tech.* **2000**, *35*, 95–103. [CrossRef]
49. Lopez, M.C.; Mateo, J.J.; Maicas, S. Screening of β-glucosidase and β-xylosidase activities in four non-*Saccharomyces* yeast isolates. *J. Food Sci.* **2015**, *80*, C1696–C1704. [CrossRef] [PubMed]
50. Spagna, G.; Barbagallo, R.N.; Palmeri, R.; Restuccia, C.; Giudici, P. Properties of endogenous β-glucosidase of a *Pichia anomala* strain isolated from Sicilian musts and wines. *Enzym. Microb. Technol.* **2002**, *31*, 1036–1041. [CrossRef]
51. Manzanares, P.; Ramón, D.; Querol, A. Screening of non-*Saccharomyces* wine yeasts for the production of β-D-xylosidase activity. *Int. J. Food Microbiol.* **1999**, *46*, 105–112. [CrossRef]
52. Mateo, J.J.; Peris, L.; Ibañez, C.; Maicas, S. Characterization of glycolytic activities from non-*Saccharomyces* yeasts isolated from Bobal musts. *J. Ind. Microbiol. Biotechnol.* **2011**, *38*, 347–354. [CrossRef] [PubMed]
53. Swangkeaw, J.; Vichitphan, S.; Butzke, C.E.; Vichitphan, K. The characterisation of a novel *Pichia anomala* β-glucosidase with potentially aroma-enhancing capabilities in wine. *Ann. Microbiol.* **2009**, *59*, 335. [CrossRef]
54. Swangkeaw, J.; Vichitphan, S.; Butzke, C.E.; Vichitphan, K. Characterization of β-glucosidases from *Hanseniaspora* sp. and *Pichia anomala* with potentially aroma-enhancing capabilities in juice and wine. *World J. Microbiol. Biotechnol.* **2011**, *27*, 423–430. [CrossRef]
55. Schwentke, J.; Sabel, A.; Petri, A.; Konig, H.; Claus, H. The yeast *Wickerhamomyces anomalus* AS1 secretes a multifunctional exo-β-1,3-glucanase with implications for winemaking. *Yeast* **2014**, *31*, 349–359. [CrossRef] [PubMed]
56. Gil, J.V.; Manzanares, P.; Genovés, S.; Vallés, S.; González-Candelas, L. Over-production of the major exoglucanase of *Saccharomyces cerevisiae* leads to an increase in the aroma of wine. *Int. J. Food Microbiol.* **2005**, *103*, 57–68. [CrossRef] [PubMed]
57. Van Rensburg, P.; Pretorius, I.S. Enzymes in winemaking: Harnessing natural catalysts for efficient biotransformations—A review. *S. Afr. J. Enol. Vitic.* **2000**, *21*, 52–73.
58. Schlander, M.; Distler, U.; Tenzer, S.; Thines, E.; Claus, H. Purification and properties of yeast proteases secreted by *Wickerhamomyces anomalus* 227 and *Metschnikovia pulcherrima* 446 during growth in a white grape juice. *Fermentation* **2017**, *3*, 2. [CrossRef]
59. Viana, F.; Gil, J.V.; Genoves, S.; Valles, S.; Manzanares, P. Rational selection of non-*Saccharomyces* wine yeasts for mixed starters based on ester formation and enological traits. *Food Microbiol.* **2008**, *25*, 778–785. [CrossRef] [PubMed]
60. Lambretchts, M.G.; Pretorius, I.S. Yeast and its importance to wine aroma—A review. *S. Afr. J. Enol. Vitic.* **2000**, *21*, 97–129.

61. Domizio, P.; Romani, C.; Lencioni, L.; Comitini, F.; Gobbi, M.; Mannazzu, I.; Ciani, M. Outlining a future for non-*Saccharomyces* yeasts: Selection of putative spoilage wine strains to be used in association with *Saccharomyces cerevisiae* for grape juice fermentation. *Int. J. Food Microbiol.* **2011**, *147*, 170–180. [CrossRef] [PubMed]
62. Liu, G.L.; Chi, Z.; Wang, G.Y.; Wang, Z.P.; Li, Y.; Chi, Z.M. Yeast killer toxins, molecular mechanisms of their action and their applications. *Crit. Rev. Biotechnol.* **2015**, *35*, 222–234. [CrossRef] [PubMed]
63. Mehlomakulu, N.N.; Setati, M.E.; Divol, B. Non-*Saccharomyces* killer toxins: Possible biocontrol agents against *Brettanomyces* in wine? *S. Afr. J. Enol. Vitic.* **2015**, *36*, 94–104. [CrossRef]
64. Passoth, V.; Olstorpe, M.; Schnürer, J. Past, present and future research directions with *Pichia anomala*. *Antonie van Leeuwenhoek* **2011**, *99*, 121–125. [CrossRef] [PubMed]
65. Ciani, M.; Comitini, F. Non-*Saccharomyces* wine yeasts have a promising role in biotechnological approaches to winemaking. *Ann. Microbiol.* **2011**, *61*, 25–32. [CrossRef]
66. Fernández de Ullivarri, M.; Mendoza, L.M.; Raya, R.R. Characterization of the killer toxin KTCf20 from *Wickerhamomyces anomalus*, a potential biocontrol agent against wine spoilage yeasts. *Biol. Control* **2018**, *121*, 223–228. [CrossRef]
67. Lopes, C.A.; Sáez, J.S.; Sangorrín, M.P. Differential response of *Pichia guilliermondii* spoilage isolates to biological and physico-chemical factors prevailing in Patagonian wine fermentations. *Can. J. Microbiol.* **2009**, *55*, 801–809. [CrossRef] [PubMed]
68. Sangorrín, M.P.; Lopes, C.A.; Jofré, V.; Querol, A.; Caballero, A.C. Spoilage yeasts from Patagonian cellars: Characterization and potential biocontrol based on killer interactions. *World J. Microbiol. Biotechnol.* **2008**, *24*, 945–953. [CrossRef]
69. Comitini, F.; De, J.I.; Pepe, L.; Mannazzu, I.; Ciani, M. *Pichia anomala* and *Kluyveromyces wickerhamii* killer toxins as new tools against *Dekkera/Brettanomyces* spoilage yeasts. *FEMS Microbiol. Lett.* **2004**, *238*, 235–240. [CrossRef] [PubMed]
70. De Ingeniis, J.; Raffaelli, N.; Ciani, M.; Mannazzu, I. *Pichia anomala* DBVPG 3003 secretes a ubiquitin-like protein that has antimicrobial activity. *Appl. Environ. Microbiol.* **2009**, *75*, 1129–1134. [CrossRef] [PubMed]
71. Oro, L.; Ciani, M.; Bizzaro, D.; Comitini, F. Evaluation of damage induced by Kwkt and Pikt zymocins against *Brettanomyces/Dekkera* spoilage yeast, as compared to sulphur dioxide. *J. Appl. Microbiol.* **2016**, *121*, 207–214. [CrossRef] [PubMed]
72. Csutak, O.; Vassu, T.; Corbu, V.; Cirpici, I.; Ionescu, R. Killer activity of *Pichia anomala* CMGB 88. *Biointerface Res. Appl. Chem.* **2017**, *7*, 2085–2089.
73. Andorrà, I.; Berradre, M.; Mas, A.; Esteve-Zarzoso, B.; Guillamón, J.M. Effect of mixed culture fermentations on yeast populations and aroma profile. *LWT-Food Sci. Technol.* **2012**, *49*, 8–13. [CrossRef]
74. Gil, J.V.; Mateo, J.J.; Jiménez, M.; Pastor, A.; Huerta, T. Aroma compounds in wine as influenced by apiculate yeasts. *J. Food Sci.* **1996**, *61*, 1247–1249. [CrossRef]
75. Gobbi, M.; Comitini, F.; Domizio, P.; Romani, C.; Lencioni, L.; Mannazzu, I.; Ciani, M. *Lachancea thermotolerans* and *Saccharomyces cerevisiae* in simultaneous and sequential co-fermentation: A strategy to enhance acidity and improve the overall quality of wine. *Food Microbiol.* **2013**, *33*, 271–281. [CrossRef] [PubMed]
76. Quirós, M.; Rojas, V.; Gonzalez, R.; Morales, P. Selection of non-*Saccharomyces* yeast strains for reducing alcohol levels in wine by sugar respiration. *Int. J. Food Microbiol.* **2014**, *181*, 85–91. [CrossRef] [PubMed]
77. Canonico, L.; Agarbatu, A.; Comitini, F.; Ciani, M. *Torulaspora delbrueckii* in the brewing process: A new approach to enhance bioflavour and to reduce ethanol content. *Food Microbiol.* **2016**, *56*, 45–51. [CrossRef] [PubMed]
78. Contreras, A.; Hidalgo, C.; Henschke, P.A.; Chambers, P.J.; Curtin, C.; Varela, C. Evaluation of non-*Saccharomyces* yeasts for the reduction of alcohol content in wine. *Appl. Environ. Microbiol.* **2011**, *80*, 1670–1678. [CrossRef] [PubMed]
79. Rojas, V.; Gil, J.V.; Piñaga, F.; Manzanares, P. Acetate ester formation in wine by mixed cultures in laboratory fermentations. *Int. J. Food Microbiol.* **2003**, *86*, 181–188. [CrossRef]
80. Bely, M.; Stoeckle, P.; Masneuf-Pomarede, I.; Dubourdieu, D. Impact of mixed *Torulaspora delbrueckii-Saccharomyces cerevisiae* culture on high-sugar fermentation. *Int. J. Food Microbiol.* **2008**, *122*, 312–320. [CrossRef] [PubMed]
81. Anfang, N.; Brajkovich, M.; Goddard, M.R. Co-fermentation with *Pichia kluyveri* increases varietal thiol concentrations in Sauvignon blanc. *Aust. J. Grape Wine Res.* **2009**, *15*, 1–8. [CrossRef]
82. Viana, F.; Gil, J.V.; Valles, S.; Manzanares, P. Increasing the levels of 2-phenylethyl acetate in wine through the use of a mixed culture of *Hanseniaspora osmophila* and *Saccharomyces cerevisiae*. *Int. J. Food Microbiol.* **2009**, *135*, 68–74. [CrossRef] [PubMed]

83. Izquierdo Cañas, P.; García-Romero, E.; Heras Manso, J.; Fernández-González, M. Influence of sequential inoculation of *Wickerhamomyces anomalus* and *Saccharomyces cerevisiae* in the quality of red wines. *Eur. Food Res. Technol.* **2014**, *239*, 279–286. [CrossRef]
84. Izquierdo Cañas, P.M.I.; Garcia, A.T.P.; Romero, E.G. Enhancement of flavour properties in wines using sequential inoculations of non-*Saccharomyces* (*Hansenula* and *Torulaspora*) and *Saccharomyces* yeast starter. *Vitis* **2011**, *50*, 177–182.
85. Kurita, O. Increase of acetate ester-hydrolysing esterase activity in mixed cultures of *Saccharomyces cerevisiae* and *Pichia anomala*. *J. Appl. Microbiol.* **2008**, *104*, 1051–1058. [CrossRef] [PubMed]
86. Luan, Y.; Zhang, B.Q.; Duan, C.Q.; Yan, G.L. Effects of different pre-fermentation cold maceration time on aroma compounds of *Saccharomyces cerevisiae* co-fermentation with *Hanseniaspora opuntiae* or *Pichia kudriavzevii*. *LWT-Food Sci. Technol.* **2018**, *92*, 177–186. [CrossRef]
87. Gobert, A.; Tourdot-Marechal, R.; Morge, C.; Sparrow, C.; Liu, Y.Z.; Quintanilla-Casas, B.; Vichi, S.; Alexandre, H. Non-*Saccharomyces* yeasts nitrogen source preferences: Impact on sequential fermentation and wine volatile compounds profile. *Front. Microbiol.* **2017**, *8*, 2175. [CrossRef] [PubMed]
88. Rollero, S.; Bloem, A.; Ortiz-Julien, A.; Camarasa, C.; Divol, B. Altered fermentation performances, growth, and metabolic footprints reveal competition for nutrients between yeast species inoculated in synthetic grape juice-like medium. *Front. Microbiol.* **2018**, *9*, 196. [CrossRef] [PubMed]
89. Chasseriaud, L.; Coulon, J.; Marullo, P.; Albertin, W.; Bely, M. New oenological practice to promote non-*Saccharomyces* species of interest: Saturating grape juice with carbon dioxide. *Appl. Microbiol. Biotechnol.* **2018**, *102*, 3779–3791. [CrossRef] [PubMed]
90. Beckner Whitener, M.E.; Stanstrup, J.; Panzeri, V.; Carlin, S.; Divol, B.; Du Toit, M.; Vrhovsek, U. Untangling the wine metabolome by combining untargeted SPME–GCxGC-TOF-MS and sensory analysis to profile Sauvignon blanc co-fermented with seven different yeasts. *Metabolomics* **2016**, *12*, 53. [CrossRef]
91. Domizio, P.; Romani, C.; Comitini, F.; Gobi, M.; Lencioni, L.; Mannazzu, I.; Ciani, M. Potential spoilage non-*Saccharomyces* yeasts in mixed cultures with *Saccharomyces cerevisiae*. *Ann. Microbiol.* **2011**, *61*, 137–144. [CrossRef]
92. Basso, R.F.; Alcarde, A.R.; Portugal, C.B. Could non-*Saccharomyces* yeasts contribute on innovative brewing fermentations? *Food Res. Int.* **2016**, *86*, 112–120. [CrossRef]
93. Duarte, W.F.; Amorim, J.C.; Schwan, R.F. The effects of co-culturing non-*Saccharomyces* yeasts with *S. cerevisiae* on the sugar cane spirit (cachaça) fermentation process. *Antonie van Leeuwenhoek* **2013**, *103*, 175–194. [CrossRef] [PubMed]
94. Zha, M.; Sun, B.; Wu, Y.; Yin, S.; Wang, C. Improving flavor metabolism of *Saccharomyces cerevisiae* by mixed culture with *Wickerhamomyces anomalus* for Chinese Baijiu making. *J. Biosci. Bioeng.* **2018**, *126*, 189–195. [CrossRef] [PubMed]
95. Wahyono, A.; Lee, S.B.; Kang, W.W.; Park, H.D. Improving bread quality using co-cultures of *Saccharomyces cerevisiae*, *Torulaspora delbrueckii* JK08, and *Pichia anomala* JK04. *Ital. J. Food Sci.* **2016**, *28*, 298–313.
96. Huyben, D.; Nyman, A.; Vidakovic, A.; Passoth, V.; Moccia, R.; Kiessling, A.; Dicksved, J.; Lundh, T. Effects of dietary inclusion of the yeasts *Saccharomyces cerevisiae* and *Wickerhamomyces anomalus* on gut microbiota of rainbow trout. *Aquaculture* **2017**, *473*, 528–537. [CrossRef]
97. Olstorpe, M.; Passoth, V. *Pichia anomala* in grain biopreservation. *Antonie van Leeuwenhoek* **2011**, *99*, 57–62. [CrossRef] [PubMed]
98. Olstorpe, M.; Borling, J.; Schnürer, J.; Passoth, V. *Pichia anomala* yeast improves feed hygiene during storage of moist crimped barley grain under swedish farm conditions. *Anim. Feed Sci. Technol.* **2010**, *156*, 47–56. [CrossRef]
99. Buzzini, P.; Martini, A. Large-scale screening of selected *Candida maltosa*, *Debaryomyces hansenii* and *Pichia anomala* killer toxin activity against pathogenic yeasts. *Med. Mycol.* **2001**, *39*, 479–482. [CrossRef] [PubMed]
100. Zha, Y.; Hossain, A.H.; Tobola, F.; Sedee, N.; Havekes, M.; Punt, P.J. *Pichia anomala* 29X: A resistant strain for lignocellulosic biomass hydrolysate fermentation. *FEMS Yeast Res.* **2013**, *13*, 609–617. [CrossRef] [PubMed]
101. Ruyters, S.; Mukherjee, V.; Verstrepen, K.J.; Thevelein, J.M.; Willems, K.A.; Lievens, B. Assessing the potential of wild yeasts for bioethanol production. *J. Ind. Microbiol. Biotechnol.* **2015**, *42*, 39–48. [CrossRef] [PubMed]

102. Wu, J.; Elliston, A.; Le Gall, G.; Colquhoun, I.J.; Collins, S.R.A.; Dicks, J.; Roberts, I.N.; Waldron, K.W. Yeast diversity in relation to the production of fuels and chemicals. *Sci. Rep.* **2017**, *7*, 11. [CrossRef] [PubMed]
103. Kruis, A.J.; Levisson, M.; Mars, A.E.; van der Ploeg, M.; Daza, F.G.; Ellena, V.; Kengen, S.W.M.; van der Oost, J.; Weusthuis, R.A. Ethyl acetate production by the elusive alcohol acetyltransferase from yeast. *Metab. Eng.* **2017**, *41*, 92–101. [CrossRef] [PubMed]