Review

Challenges of the Non-Conventional Yeast Wickerhamomyces anomalus in Winemaking

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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 kluyveroy, 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 10⁵ to 10⁷ 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. anomalala 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. anomalala in celler 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. anomalala, 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. kluyveroy 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. kluyveroi 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. kluyveroi and P. kudriavzevii were isolated from grape 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-xilosidas, which are involved in the release of wine compounds from grape precursors [5].

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-xilosidas activity in screenings as well, including more than 300 and 100 wine yeast isolates, respectively [49,50]. Similarly, P. anomala produced β-D-xilosidas 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-xilosidas 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-xilosidas for aromatic compounds releasing during winemaking has not been explored yet.

The strain W. anomalus ASI 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 ASI 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].](image)

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 (SO₂) has been proposed, since differently to SO₂, 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.

<table>
<thead>
<tr>
<th>Mixed Starter</th>
<th>Impact on Wine</th>
<th>Inoculation</th>
<th>Must</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. anomala</em>/<em>S. cerevisiae</em></td>
<td>Isoamyl acetate increase</td>
<td>Co-inoculation</td>
<td>Bobal [79]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ISOamyl acetate increase</td>
<td>Co-inoculation</td>
<td>Commercial [61]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Acetate ester increase</td>
<td>Co-inoculation</td>
<td>Synthetic [85]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Acetate ester increase and alcohol decrease</td>
<td>Sequential</td>
<td>Aïrén [84]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Acetate and ethyl ester increase</td>
<td>Sequential</td>
<td>Mazuela [83]</td>
<td></td>
</tr>
<tr>
<td><em>P. kudriavzevii</em>/<em>S. cerevisiae</em></td>
<td>Isoamyl acetate increase</td>
<td>Co-inoculation</td>
<td>Cabernet Sauvignon [86]</td>
<td></td>
</tr>
<tr>
<td><em>P. membranifaciens</em>/<em>S. cerevisiae</em></td>
<td>Isoamyl and 2-phenetyl acetate</td>
<td>Sequential</td>
<td>Muscat [87]</td>
<td></td>
</tr>
<tr>
<td><em>P. kluveri</em>/<em>S. cerevisiae</em></td>
<td>3-Mercaptohexyl acetate increase</td>
<td>Co-inoculation</td>
<td>Sauvignon Blanc [81]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3-Sulfanylhexan-1-ol increase</td>
<td>Co-inoculation</td>
<td>Sauvignon Blanc [89]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Off-flavor formation</td>
<td>Sequential</td>
<td>Sauvignon Blanc [90]</td>
<td></td>
</tr>
<tr>
<td><em>P. fermentans</em>/<em>S. cerevisiae</em></td>
<td>Polysaccharide increase</td>
<td>Co-inoculation</td>
<td>Commercial [91]</td>
<td></td>
</tr>
</tbody>
</table>

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. kluveri* 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. kluveri* isolate did not show a sensorial significant increase in the tropical fruity aromas characterized by 3MHA, and the production of 3-methyl-butanolic 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.

<table>
<thead>
<tr>
<th>Application</th>
<th>Yeast Strain</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food and beverage production</td>
<td></td>
<td></td>
</tr>
<tr>
<td>beer</td>
<td><em>W. anomalus</em> 1</td>
<td>[92]</td>
</tr>
<tr>
<td>cider</td>
<td><em>W. anomalus</em> YN6</td>
<td>[31]</td>
</tr>
<tr>
<td>cacháça</td>
<td><em>P. anomala</em> UFLA CAF70 and CAF119</td>
<td>[93]</td>
</tr>
<tr>
<td>Chinese Baijiu</td>
<td><em>W. anomalus</em> GZ3</td>
<td>[94]</td>
</tr>
<tr>
<td>bread</td>
<td><em>P. anomala</em> JK04</td>
<td>[95]</td>
</tr>
<tr>
<td>Aquaculture</td>
<td><em>W. anomalus</em> 1</td>
<td>[96]</td>
</tr>
<tr>
<td>Biocontrol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cereal grain preservation</td>
<td><em>P. anomala</em> J121</td>
<td>[97,98]</td>
</tr>
<tr>
<td>antimycotic agent</td>
<td><em>P. anomala</em> C33, C85, D8, D128, DBVPG3649</td>
<td>[99]</td>
</tr>
<tr>
<td></td>
<td><em>P. anomala</em> CMGB88</td>
<td>[72]</td>
</tr>
<tr>
<td>Production of fuels and chemicals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>bioethanol</td>
<td><em>P. anomala</em> CBS132101</td>
<td>[100]</td>
</tr>
<tr>
<td></td>
<td><em>W. anomalus</em> 1</td>
<td>[101]</td>
</tr>
<tr>
<td>ethyl acetate</td>
<td><em>W. anomalus</em> NNCYC16</td>
<td>[102]</td>
</tr>
<tr>
<td></td>
<td><em>W. anomalus</em> DSM 6766</td>
<td>[103]</td>
</tr>
</tbody>
</table>

*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. anomalus* 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. anomalus* 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.

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References

1. Fleet, G.H. Wine yeasts for the future. FEMS Yeast Res. 2008, 8, 979–995. [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]


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]


49. Lopez, M.C.; Mateo, J.J.; Maicas, S. Screening of β-glucosidase and β-xyllosidase activities in four non-Saccharomyces yeast isolates. J. Food Sci. 2015, 80, C1696–C1704. [CrossRef] [PubMed]
58. Schlender, M.; Distler, U.; Tenzer, S.; Thines, E.; Claus, H. Purification and properties of yeast proteases secreted by Wickerhamomyces anomalous 227 and Metschnikowia pulcherrima 446 during growth in a white grape juice. Fermentation 2017, 3, 2. [CrossRef]
Fermentation 2018, 4, 68

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]


64. Anfang, N.; Brajkovich, M.; Goddard, M.R. Co-fermentation with Pichia anomala. Comitini, F.; De, J.I.; Pepe, L.; Mannazzu, I.; Ciani, M. Non-Saccharomyces yeasts: Selection of putative spoilage wine strains to be used in association with Saccharomyces cerevisiae in the brewing process: A new approach to enhance bioflavour and to reduce ethanol content. Food Microbiol. 2013, 33, 271–281. [CrossRef] [PubMed]


84. Izquierdo Cañas, P.M.I.; García, 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.


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]


89. 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] [PubMed]

90. Beckner Whitener, M.E.; Stanstrup, J.; Panzeri, C.B. Could non-Saccharomyces yeasts contribute on innovative brewing fermentations? Food Res. Int. 2016, 86, 112–120. [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]


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