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Bruner, James
Fox, Glen

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Review

Novel Non-*Cerevisiae* *Saccharomyces* Yeast Species Used in Beer and Alcoholic Beverage Fermentations

James Bruner * and Glen Fox *

Food Science and Technology, University of California, Davis, CA 95616, USA

* Correspondence: jrbruner@ucdavis.edu (J.B.); gpfox@ucdavis.edu (G.F.)

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Abstract: A great deal of research in the alcoholic beverage industry was done on non-*Saccharomyces* yeast strains in recent years. The increase in research interest could be attributed to the changing of consumer tastes and the search for new beer sensory experiences, as well as the rise in popularity of mixed-fermentation beers. The search for unique flavors and aromas, such as the higher alcohols and esters, polyfunctional thiols, lactones and furanones, and terpenoids that produce fruity and floral notes led to the use of non-*cerevisiae* *Saccharomyces* species in the fermentation process. Additionally, a desire to invoke new technologies and techniques for making alcoholic beverages also led to the use of new and novel yeast species. Among them, one of the most widely used non-*cerevisiae* strains is *S. pastorianus*, which was used in the production of lager beer for centuries. The goal of this review is to focus on some of the more distinct species, such as those species of *Saccharomyces sensu stricto* yeasts: *S. kudriavzevii*, *S. paradoxus*, *S. mikatae*, *S. uvarum*, and *S. bayanus*. In addition, this review discusses other *Saccharomyces* spp. that were used in alcoholic fermentation. Most importantly, the factors professional brewers might consider when selecting a strain of yeast for fermentation, are reviewed herein. The factors include the metabolism and fermentation potential of carbon sources, attenuation, flavor profile of fermented beverage, flocculation, optimal temperature range of fermentation, and commercial availability of each species. While there is a great deal of research regarding the use of some of these species on a laboratory scale wine fermentation, much work remains for their commercial use and efficacy for the production of beer.

Keywords: yeast; *Saccharomyces*; fermentation; alcohol; beer; wine

1. Introduction

Fermented beverages have played an important and special role over the course of human history due to their economic and cultural importance, perhaps even lending to the beginning of modern civilizations [1,2]. Archaeological evidence places the oldest fermented beverage in the fertile crescent, as far back as 11,000 BCE [3,4], and based on the agricultural evidence of the time and region, that beverage was likely beer. While beer originally could have been an accidental beverage, it progressed into one of the most artfully crafted beverages known to man. No longer thought of as just an art, the science of beer led to several very important landmarks in scientific history (Table 1). As scientific discoveries keep developing, there are some amazing innovations that led to advances in the quality and stability of beer, over the past 40 years [5]. However, minimal advancement was made when considering the raw ingredients used in the brewing process.

Table 1. Significant landmarks of the 150 years from 1760–1910 that came from scientists working at breweries or specifically studying beer and its adjacent ingredients.

Year	Scientist	Employment	Discovery
1762	Michael Combrune	<i>Brewer's Company Middlesex</i>	using a thermometer for analysis [2]
1769	James Baverstock	<i>family brewery</i>	using a hydrometer for analysis [2]
1833	Anselme Payen and Jean-François Perzoz	<i>École Centrale Paris</i>	discovered diastase enzyme and cellulose while working with barley [2]
1843	Karl J.N. Balling	<i>Polytechnic in Prague</i>	invents the balling saccharimeter [6]
1843	James Joule and Lord Kelvin	<i>family brewery</i>	create temperature scale and first law of thermodynamics [7]
1857	Louis Pasteur	<i>University of Lille</i>	microbes are responsible for fermentation [8]
1860	P.E. Marcellin Berthelot	<i>Collège de France</i>	discovered invertase in <i>Saccharomyces</i> [2]
1873	Carl von Linde	<i>Spaten Brewery</i>	invented the refrigeration cycle [2]
1883	Johan Kjeldahl	<i>Carlsberg Brewery</i>	develops method for protein quantification [9]
1888	Emil Christian Hansen	<i>Carlsberg Brewery</i>	first isolation of pure yeast strain [9]
1908	William Sealy Gosset	<i>Guinness Brewery</i>	invents the statistical t-test for students [10]
1909	Søren Sørensen	<i>Carlsberg Brewery</i>	creates pH scale based on H ⁺ ion concentration [11]

On a base level, beer consists of four main ingredients—malt, water, hops, and yeast, and the brewing process could be separated into a hot side and a cold side. In the most basic overview of the brewing process, the hot side begins when malted cereal grains are crushed and combined with warm water so the maltose sugar is hydrolyzed from starch, the liquid is then boiled with hops to add bitterness and flavor; this liquid, called wort, provides the nutrients for yeast. Moving from the hot side to the cold side, wort is subsequently chilled for fermentation (Figure 1a); yeast is added, metabolizing 50 to 80 percent of the sugar and nutrients to fermentation products, leaving behind non metabolized proteins, oligosaccharides, and other compounds [12–14]. The resultant sugar profile of brews can vary, based on the malting and mashing conditions, but typical mashes might contain 60.0% maltose, 20.0% glucose, 10.0% maltotriose, and 5.0% of both sucrose and fructose [15].

Conversely, wine has just two main ingredients, grapes and yeast, and tends to have a much simpler process flow than beer (Figure 1b). Grapes are picked and sorted from the vineyard before being crushed, to release the sugary juice from the interior, for the varying fermentation profiles of red or white wine. When producing white wine the skins and pomace are pressed and filtered from the juice before the addition of yeast for fermentation. While red wine is fermented on the pomace to get the color from the polyphenols within the skins and seeds, before being pressed and filtered for aging. Wine might also have an additional malolactic fermentation to soften the malic acid into lactic acid, but the lactic acid bacteria can produce a buttery diacetyl flavor that is only desirable in certain styles [16]. In wine, the resultant sugar profile can vary, based on that of the grapes used, but the majority (~95.0%) of sugars are already present in monosaccharide form, as equal parts glucose and fructose, which the yeast can ferment without the assistance of enzymes [17,18].

For most of the scientific history of beer, *Saccharomyces cerevisiae* was the yeast used to produce alcohol [19–21] although the first pure culture isolate of brewing yeast was *S. carlsbergensis* (later renamed *S. pastorianus*) [22]. For alcoholic fermentation, the general rule of thumb for the amount of yeast to use, known as the pitching rate, is one million cells per milliliter per percent of sugar in solution [9,12,23]. *S. cerevisiae*, when used at the proper pitching rate, takes the maltose and other sugars produced from the hot side of the brewing process [15], and anaerobically converts the disaccharides into carbon dioxide (CO₂) and ethanol. More than 600 flavor active compounds can also be produced during the alcoholic fermentation process, depending on type of beverage produced (Figure 2) [24–26]. Yeast works via an anaerobic pathway of glycolysis; if oxygen is present it performs respiration and cell reproduction [27].

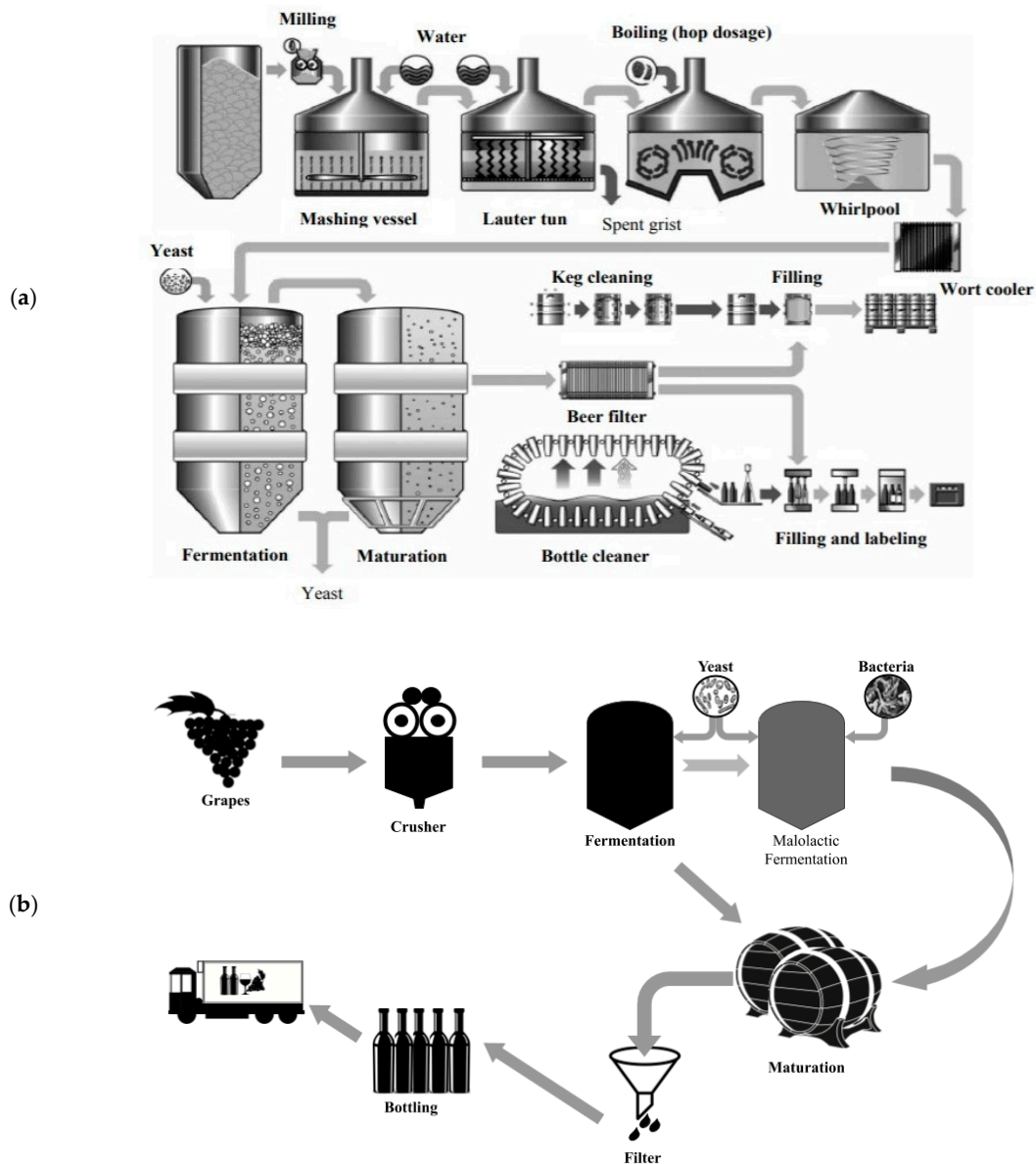


Figure 1. (a) Schematic diagram of the brewing process, as presented in Magalhães et al. 2009 [14]. (b). Schematic diagram of the winemaking process as outlined and described by Water house et al. 2016 [16].

In Stage 1 of alcoholic fermentation, free glucose is assimilated first, followed by the hydrolyzation of maltose or other disaccharides into two glucose, by the enzyme alpha glucosidase (a.k.a maltase, EC 3.2.1.20). Several other enzymatic destabilization and phosphorylation reactions then happen in Stage 1, which turns the substrate into glyceraldehyde-3-phosphate (G3P) and dihydroxyacetone phosphate (DHAP). Stage 2 oxidizes G3P and DHAP, as well as the ADP generated previously, to create ATP as energy for the cell and pyruvate. Stage 3 enzymatically decarboxylates pyruvate to acetaldehyde and CO₂ that leaves the cell, before the alcohol dehydrogenase converts the acetaldehyde to ethanol in Stage 4 (Figure 2). In brewing, yeast is typically reused (repitched) for ten generations or more [9], while in wine, the yeast is generally used far lesser times, due to the prominence of other microorganisms and the higher mortality from more stressful conditions of osmotic pressure and higher ethanol concentrations [28]. In most cases, serial repitching can cause genetic mutation within the cells and the desired flavor profile might no longer be attainable [29–32].

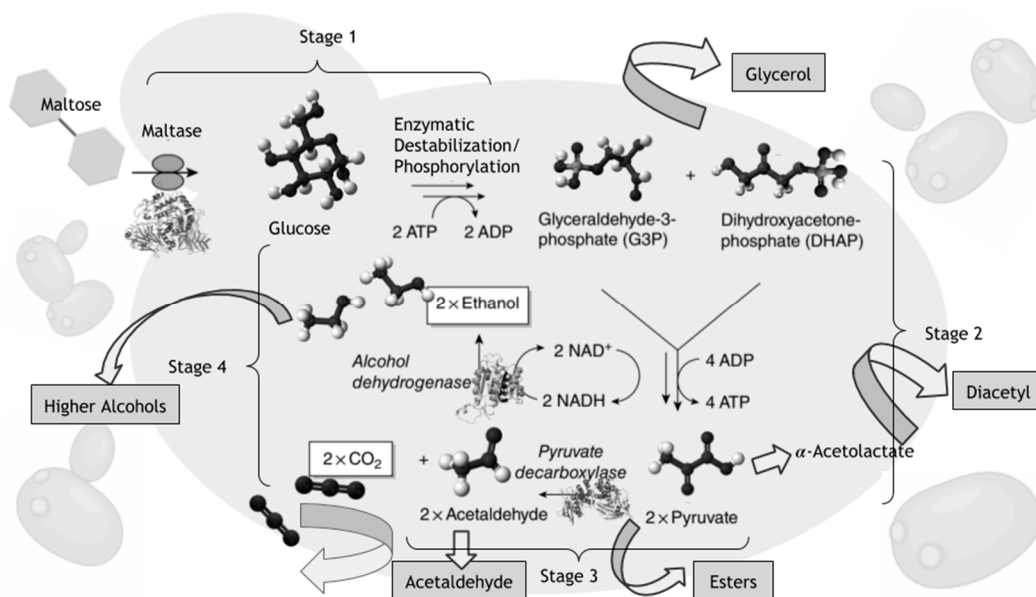


Figure 2. The metabolic role of *Saccharomyces* yeast in the development of flavor for fermented alcoholic beverages. The sole products of yeast fermentation are not just ethanol and CO₂, this schematic representation shows the derivation and synthesis of flavor active compounds from sugar, amino acids, and sulfur metabolism, delineated by the arrows on the diagram. Alcoholic fermentation of beer by *Saccharomyces* is the substrate level phosphorylation anaerobic pathway of glycolysis, which converts maltose sugar into ethanol and carbon dioxide.

Interest in brewing beer with novel yeast strains and applying *Saccharomyces cerevisiae* in new methods outside of traditional beer fermentation [33,34] has increased in recent years, due to the growing consumer tastes of sour and wild mixed-fermentation beers, as well as using some of these novel species for low or no alcohol beer production [35,36]. A great deal of research in the brewing industry was done on non-*Saccharomyces* yeast strains, such as *Brettanomyces*, *Pichia*, *Hanseniaspora*, *Metschnikowia*, and *Torulaspora* [37–39]. Furthermore, the search for unique flavors and aromas, and a desire to invoke new technologies and techniques for making alcoholic beverages led to the use of non-*cerevisiae* *Saccharomyces* spp. in the alcoholic fermentation process [40,41].

While the most widely used non-*cerevisiae* species is *S. pastorianus*, traditionally used in the production of lager beer around the world [42–44], this review focuses on some of the more distinct species. The focus is on five species of *Saccharomyces sensu stricto* (*Sss*) yeasts, *S. kudriavzevii*, *S. paradoxus*, *S. mikatae*, *S. uvarum*, and *S. bayanus*, as well as other novel species not currently in the *Sss*, such as *S. abulensis* and *S. florentinus*. When selecting yeast strains for fermentation, brewers consider its attenuation (the amount of sugar consumed by the yeast), flocculation (the yeast's ability to clump together and fall out of solution), fermentation temperature range, effects on flavor profile, capacity for reuse, and supply chain availability [45]. These facets, as well as a yeast's ability to ferment various carbon sources, morphological characteristics, and genetic hybridization can all assist brewers, when adopting a new strain.

2. *Saccharomyces* Species Diversity

Since Louis Pasteur's groundbreaking and historic report that fermentation was caused by a microorganism instead of a spontaneous mystery [8], the *Saccharomyces* genome was continuously studied, with several distinct species identified [46]. This diversity was termed the *Saccharomyces sensu stricto* (*Sss*) complex and is currently composed of ten genetically distinct species, all of which are capable of metabolizing glucose to produce ethanol (Figure 3). Each of these species was perceptibly delineated from other *Saccharomyces* species, through studies of reproductive isolation and application of the biological species concept [31,47–49]. All *Sss* species were isolated from unique sources in nature,

including tree bark, flowers, fruit, and insects, demonstrating their lineage from wild type to the cultured stock of *Saccharomyces* spp. While all members of the *Sss* were proven to produce energy with fermentation, and many of these species are novel, some were used and studied for their potential use in commercial alcohol production for human consumption. The distribution of *S. cerevisiae* and *S. pastorianus* were long linked to alcoholic beverage production, along with minor mentions of other species in the *Sss* complex. Cultured species, specific to beer production, were shown to have evolved from European wine and Asian sake fermentations [21,50], therefore, its relation to wine production proliferates much of the research.

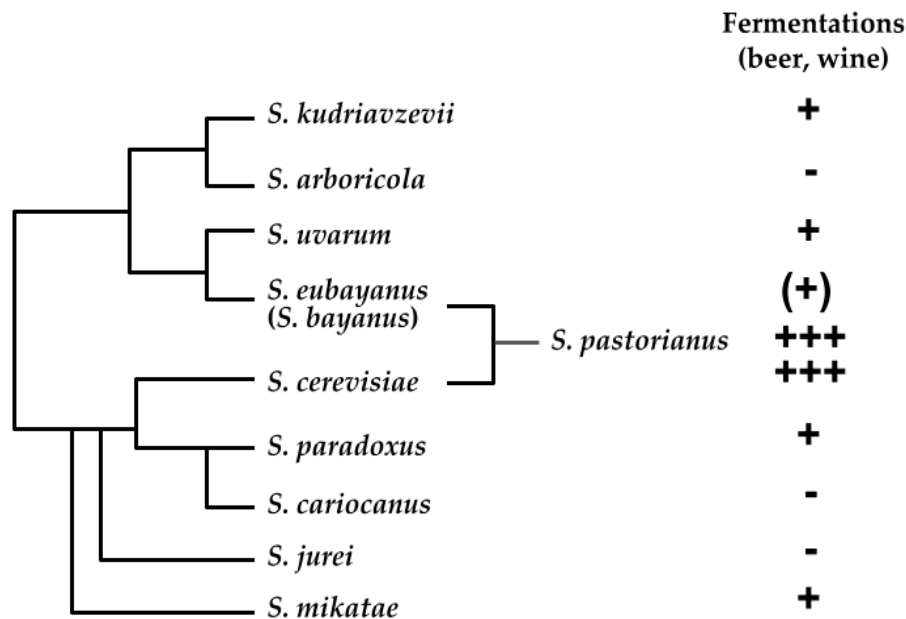


Figure 3. *Saccharomyces* species phylogeny shown; all were effectively isolated from natural sources (i.e., trees, fruit, insects). *Saccharomyces bayanus* is listed in parenthesis to indicate it was derived from multiple hybridization events. *S. pastorianus* is shown as a genetic hybrid of *S. eubayanus* and *S. cerevisiae*. Usage indicated with plus signs (+) for current use in industry, with *S. cerevisiae* and *S. pastorianus* showing the most profound use in the current alcoholic fermentation industry, and negative signs (-) for no known use. *S. cariocanus* is known to be harboring just four translocated chromosomes different than *S. paradoxus*. Figure adapted from Fay 2012 [51].

3. *Saccharomyces kudriavzevii*

S. kudriavzevii was first isolated from a decaying leaf and has since been isolated repeatedly from the bark of oak trees in Portugal and France [52,53]. The yeast is a multipolar budding species, with a size of 5–10 μm, and an oval to slightly elongated shape [47]. It was shown to ferment glucose, sucrose, and maltose, but it did not ferment lactose, melibiose, or starch, which are common characteristics of *Sss* yeast (Table 2). *S. kudriavzevii* is a naturally occurring *S. cerevisiae* hybrid that might constitute 23–100% of the genome for some yeast [54,55], including Belgian trappist ale strains, such as Chimay, Westmalle, and Orval, and was also genetically isolated in draft beer from the United Kingdom, Germany, and New Zealand [56]. This implies that the attenuation, flocculation, and flavor profiles of *S. kudriavzevii* might be similar to that of most Belgian strains. This meant low flocculation, high attenuation, and phenolic off-flavor positive (POF+) [45,57], though there is research in the wine industry that suggests *S. kudriavzevii* ferments slowly and produces less ethanol when used on grape juice [58]. Other research suggests it has high flocculation, as in overnight liquid culture, it grew into spherical 2–3 mm pellets [31].

Table 2. Physiological characteristics that distinguish each species of the *Saccharomyces sensu stricto* complex are discussed. Growth ability scored as positive (+), negative (−), evidence of both positive and negative (−,+), and unknown (u). Ethanol tolerance is defined as being able to grow in the presence of 2.5% v/v EtOH, the low-end strength of standard beer. Attenuation and flocculation scored on a relative basis scale, ranging from low, to moderate, to high. Type strain as defined in MycoBank (mycobank.org), origin, isolation, and commercial availability, as defined in the cited literature.

	<i>S. kudriavzevii</i>	<i>S. paradoxus</i>	<i>S. uvarum</i>	<i>S. mikatae</i>	<i>S. bayanus</i>
<i>Fermentation Of:</i>					
Maltose	+	+	+	+	+
Melibiose	−	−	+	+	−,+
Dextrins (<i>STAI</i>)	−,+	−	−,+	−	−
Ethanol Tolerant	+	+	+	+	+
<i>Characteristics:</i>					
Attenuation	moderate	low-moderate	moderate	moderate	moderate
Flocculation	moderate-high	moderate	high	moderate	moderate
Growth at 10 °C	+	+	+	+	+
Growth at 25 °C	+	+	+	+	+
Growth at 37 °C	−	−	+	−	+
POF	+	u	−	u	+
Region of Origin	Western Europe	Northeastern Europe	Scandinavia	Japan	Europe
Isolated From	Oak tree bark	Oak sap	Fruit/Seeds	Soil/Leaves	Insects/Leaves
Type Strain	NCYC 2889T	DBVPG 6411	DBVPG 6173	NCYC 2888T	CBS 380
Commercial Availability	Anchor Vin7	Anchor Exotics SPH	AWRI 1176 & 1375	AB Biotek/AWRI 2526	Lalvin S6U

It is advised to ferment *S. kudriavzevii* in tandem with a traditional *Saccharomyces* [59] and it was shown to form a triple hybrid complex with *S. cerevisiae* and *S. uvarum*, as it was isolated as such from farmhouse ciders made in France [60,61]. *S. kudriavzevii* is a cryophilic strain in the Sss that prefers fermentation temperatures in the 10–15 °C range [52,62,63], and is currently used to ferment lower temperature pinot noir and lager beer in Europe [54]. The only current commercially available example is Anchor Oenology’s Vin7 strain, developed in Stellenbosch, South Africa, for enhancing thiol aromas in white wine [64,65], but it stands to reason that it can be isolated from previously noted commercial beer examples. Due to its cryophilic tendencies and aromatic potential, *S. kudriavzevii* has potential for use in the production of hoppy lager beers in the brewing industry. Further research remains to be done on this species, considering it is POF+ and it is likely also diastaticus (*STAI*) positive, meaning it could ferment residual maltodextrins. Additionally, minimal commercial production was done with the direct intention of using *S. kudriavzevii*, as most fermentations did not take place with the intention of the use of this species.

4. *Saccharomyces paradoxus*

S. paradoxus is one of the first isolates of the Sss [66], a wild-type strain commonly isolated from the bark of deciduous trees and occasionally from fruit and insects in North America and Eastern Europe [67–69]. Even though genetically *S. cerevisiae* and *S. paradoxus* were proven to be distinct species [70], phylogenetically the two were the closest relatives in the Sss (Figure 3) and were 90% genetically similar [55]. They share the same morphological and phenotypic characteristics, such as being spherical or ellipsoid in shape, with a diameter of 1–5 µm [71]. Previous research indicates mixed results of the fermentative capacity of *S. paradoxus*, but it has the ability to convert glucose into ethanol and a relatively high alcohol tolerance [47,72,73]. It is a positive fermenter for glucose, sucrose, and maltose, but it does not ferment lactose, melibiose, or starch (Table 2). Little evidence exists for the domestication and commercial use of *S. paradoxus* in alcohol fermentation, but it was found to be naturally co-fermenting with *S. cerevisiae* in Eastern European wine fermentations [73,74], as well as with *S. cerevisiae*, *S. bayanus*, *S. cariocanus*, *S. kudriavzevii*, *S. mikatae*, and *S. pastorianus* in indigenous African sorghum beer [75].

In laboratory fermentations, the optimal growth temperature for *S. paradoxus* falls 7 °C lower than *S. cerevisiae*, and is likely cryophilic, due to the climates in which it is found, but *S. paradoxus* is yet to be trialed extensively in a production environment [76,77]. Unfortunately, no information

exists on the attenuation or flocculation characteristics of *S. paradoxus*, nor are there any commercially produced examples of the purely isolated species, but it does seem to have positive sensory attributes in white wine fermentations [73,74]. There is a commercially available hybrid of *S. paradoxus* and *S. cerevisiae* produced by Anchor Oenology, which when used for Syrah and Merlot wine fermentations, shows increased aromas of cherries, strawberries, cocoa, and floral notes, and the wine is described as full-bodied, well-balanced, complex and intense [78]. Much work remains to be done on the versatility of this species for the brewing industry, but it might have potential for unique and novel flavor characteristics if a pure culture from a genetic bank is obtained for further experimentation.

5. *Saccharomyces mikatae*

S. mikatae is a natural genetic hybrid that results from introgression events with *S. cerevisiae* and *S. paradoxus* [55,79], and its current hybrids are described for use in industrial wine fermentation. This hybrid was deliberate, created in a lab with the intent of creating greater complexity in resultant wines, akin to those that are spontaneously fermented, but more easily controlled due to the inclusion of typical *S. cerevisiae* yeast [80,81]. *S. mikatae* was first isolated from decaying leaves and soil in Japan [47,60]. It is ovoid in shape and approximately 5–9 µm in diameter; it reproduces by multipolar budding, and generally appears in pairs or short chains. *S. mikatae* was also shown to form a pellicle after 25 days at 20 °C, similar to *Brettanomyces* and other wild-type yeasts [47]. The inclusion of *S. mikatae* in the Sss means it is capable of alcoholic fermentation and assimilation of glucose, it is also capable of fermenting maltose, sucrose, and melibiose, but not lactose or starch (Table 2). However, *S. mikatae* might have a lower attenuation, due to genetic diversion from *S. cerevisiae*, while still exhibiting similar levels of flocculence [31].

S. mikatae readily creates hybrids with *S. cerevisiae*, and these hybrids were shown to produce higher concentrations of multiple compounds that yield fruity, banana, floral, and sweet perfume aromas in the fermentation of white wine [80,81]. Although no information on beer fermentation with either the type strain or any hybrids exist, the additional amounts of certain volatile compounds in the research by Bellon et al. (2013, 2019) might show signs of this yeast's production of phenolic off flavors. *S. mikatae* grows readily in temperatures from 4–30 °C, with expected slower growth in the range limits and no growth outside the range, making it a cryotolerant fermenter [47,63]. Commercial availability is limited, but yeast manufacturer AB Biotek commenced exploratory production of an *S. mikatae* and *S. cerevisiae* hybrid, AWRI 2526; brewers and winemakers can expect the hybrid as an active dried yeast product that is expected to be available for trials, by the fall of 2020 [81].

6. *Saccharomyces uvarum*

S. uvarum is a fairly well-known member of the Sss, originally believed to be identical to *S. bayanus* and often referred to as *S. bayanus* var. *uvarum*, it was shown to be a genetically distinct *Saccharomyces* species [82–84]. *S. uvarum* is also similar in size and shape to *S. bayanus*, being spherical or ellipsoid in shape, with a diameter of 1–5 µm, and reproducing by multipolar budding. *S. uvarum* was isolated in natural European wine and cider fermentations [85–87], as well as in South American chicha fermentations [88,89], but was first isolated in 1894 and described in 1898 by M.W. Beijerinck, from spontaneous wine fermentation [90]. *S. uvarum* is known to hybridize with *S. cerevisiae*, *S. bayanus*, and *S. pastorianus* [85,91,92], and thus can show signs of being POF+ and possibly might have the *STA1* gene for diastaticus [91,93]. *S. uvarum* showed the capacity to ferment glucose, sucrose, melibiose, and maltose, but it does not ferment lactose. *S. uvarum* is a known bottom-fermenting yeast, meaning it acts similar to a *S. bayanus* or *S. pastorianus* when not in hybrid form, offering cryotolerance [94], moderate attenuation, and high flocculation (Table 2).

Research with wine showed that *S. uvarum* produces comparatively higher amounts of volatile aromatics when fermented cold [95], implying potential use as a lager strain. In Chardonnay winemaking trials, wines were described as showing apricot, cooked orange peel, citrus, lime, honey, and nutty aromas with some tasters, and estery, pineapple, peach, melon, and floral

aromas with others [80,96]. While *S. uvarum* continues to predominate in spontaneous European wine fermentations [86,87,97] and is a known species in some Norwegian kveik hybrid strains [91], its commercial availability is limited to the Australian Wine Research Institute at this time [98]. Cryotolerance and increased aromatic potential means *S. uvarum* could be used in the production of some complex and eccentric lagers in the brewing industry, but currently, it has only been isolated as part of a hybrid culture in the aforementioned kveik beer. Further research remains to be done on the brewing potential of *S. uvarum*, but brewers should be aware of the increased aromatic character that might come from the POF+ genes.

7. *Saccharomyces bayanus*

S. bayanus is a well-studied species in the *Sss* and is often used as a model organism for comparative functional genomics studies of yeast, based on introgression and interspecific hybridization [99]. It is genetically similar to *S. cerevisiae*, but evolved to be a distinct member of the *Saccharomyces sensu stricto* complex [55,100], and is now referred to as *S. bayanus* var. *bayanus*, in order to delineate it from *S. eubayanus* and *S. uvarum* [43,101]. *S. bayanus* was previously thought to be the parent of the lager strain, *S. pastorianus* [43,60,85], but the hybridization event that produced lager brewing yeast is now proven to have occurred between *S. cerevisiae* and *S. eubayanus* [21,50,102,103]. While *S. bayanus* might not be the true hybrid parent of the most used brewing yeast in the world, it still forms natural hybrids with other members of the *Sss* and was identified in these complexes in the fermentation of wine [62,85,104]. While it was first isolated from turbid beer in 1927 [105], *S. bayanus* was also isolated from beer, wine, fruit, and even soda [47]. *S. bayanus* is ellipsoid to elongate in shape, with a diameter of 1–5 µm, and reproduces by multipolar budding. Research showed it to be a positive fermenter for glucose, sucrose, and maltose. Other studies reported *S. bayanus* to show both positive and negative fermentation of melibiose, but it does not ferment lactose or starch (Table 2).

S. bayanus is well-known as a fermenter of beer and cider [43,105,106], but is most commonly used in wine [96,103,107]. It can be purchased from several commercial suppliers, but unfortunately several commercially available strains were genetically identified as *S. cerevisiae*, including the famous Lalvin EC-1118 strain that was originally typed *S. bayanus* [108,109]. *S. bayanus* ferments best in the upper end of the lager strain temperature range of 10 to 21 °C [77,101], is moderately flocculant [31], and has a fairly standard attenuation [110], as expected, given its genetic similarity to *S. pastorianus* lager yeast. The commercially available hybrid of *S. bayanus* and *S. cerevisiae* available from Lallemend, Lalvin S6U, is known to increase the varietal characteristics in white wine, and might produce elevated levels of POF [111,112]. More research needs to be carried out with regards to the flavor profile of beers made with *S. bayanus*, but the research on wine and its history as a potential lager strain means, it is capable of fermenting remarkable lager style beers.

8. Other *Saccharomyces* spp. Used in Alcoholic Fermentation

Several other novel species of *Saccharomyces* used in alcoholic fermentation were determined to be genetically distinct by current research but might not yet be included in the *Saccharomyces sensu stricto* complex. *S. abulensis* is a novel species dubbed the “Santa Maria strain” and was isolated from yeast originating from breweries in Madrid and Sevilla, Spain [92]. *S. florentinus*, formerly known as *S. pyriformis*, is a species of yeast isolated from the scoby of traditionally fermented ginger beer, known as “bees wine,” but is yet to be used in commercial production of beer [113,114]. Three other strains included in the phylogenetic tree of the *Sss* (Figure 3) exist—*S. arboricola*, *S. jurei*, and *S. cariocanus*—but research is limited on their fermentation capacity. *S. arboricola* is a wild-type hybrid of *S. bayanus* and *S. kudriavzevii*, which was isolated from oak and beechwood bark in China [115,116], and is currently being used in sake production [117]. *S. jurei* is closely related to *S. mikatae* and *S. paradoxus* and was isolated from a high altitude tree bark in France; little is known of its fermentative capacity [118]. *S. cariocanus*, isolated from insects in South America [119], is a wild-type hybrid of *S. paradoxus*, which is capable of fermenting sucrose and shows ethanol tolerance [120].

These yeasts are not members of the *Sss*, but is likely to be included, as the complex underwent many changes over the years, in accordance with the system employed in classifying yeast cultures. Very little information exists on these yeasts' ability to ferment beer or their use in a commercial setting, but by contacting genetic banks and yeast culture collections directly, the strain type could be obtained for further experimentation. There also exists multiple variants of *S. cerevisiae*, such as var. *boulardii*, which is known to produce higher levels of polyphenols, and can thus be used in functional probiotic beer [121–126]. Another variant, var. *diastaticus*, can cause over-attenuation [127–129], which was discussed earlier as having the *STA1* gene.

9. Conclusions

The non-*cerevisiae* *Saccharomyces* species discussed in this review, have the ability to ferment glucose and maltose into ethanol, anaerobically. Most are members of the *Saccharomyces sensu stricto* complex, while some are yet to be defined within the species complex classification. *S. kudriavzevii* is available commercially as a hybrid with *S. cerevisiae* and can increase thiol aromatic qualities during fermentation, at lower temperatures, making it ideal for distinctive lagers. *S. paradoxus* is also available commercially as a hybrid with *S. cerevisiae* and showed increased fruity and floral esters in wine, and also lends unique characteristics to African umqombothi beer. Hybrids of *S. mikatae* and *S. cerevisiae* are not yet commercially available, but they were known to produce increased fruity and perfume aromas even when fermented at low temperatures, marking its potential for remarkable lager beer production. Due to debate on the classification and isolation process of *S. uvarum* in genetic research, there is no commercially available version of this species, but it shows potential as a cryotolerant lager yeast, with more character than *S. pastorianus*. After recent research, many commercially available *S. bayanus* strains were reclassified as variants of *S. cerevisiae*, but the true hybrids of *S. bayanus* and *S. cerevisiae* showed increased ethyl esters and spicy notes that could add a complexity to beer production. While much of the research regarding flavor and aroma that is presented in this review might be focused on wine, these species all have potential for novel fermentations and new sensory experiences, if used in beer.

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