M. Hutzler, R. Riedl, J. Koob and F. Jacob

Fermentation and Spoilage Yeasts and their Relevance for the Beverage Industry – A Review

During the production of beverages or in terms of quality control, the question often arises as to which yeast species occur in which beverage as starter cultures or as spoilers? The species name should direct beverage microbiologists and technologists to information that is available about species' specific spoilage and fermentation characteristics. This review provides an overview regarding the yeast flora present in beverage processes in general and provides guidance on how this yeast flora can be identified at the level of genus, species or strain. Additionally, beverage-specific technological and microbiological information about specific yeast species is included. This review also provides an overview of the literature and information on the methods currently available for species identification from the point of view of beverage production, designed to aid microbiologists or technologists in coping with the challenges they face. Descriptions of the yeast flora found in beer, wine, sparkling wine and the mash used for the production of distilled beverages, as well as indigenous fermented beverages are provided below. Yeasts are grouped as either inoculated or spontaneous starter cultures and as direct or indirect spoilage organisms. Spoilage yeasts present in carbonated soft drinks are classified according to their spoilage and fermentative potential in a specific matrix. Detailed information about beverage-relevant characteristics is summarized in alphabetical order according to yeast genus and species name. Fermentation characteristics, fermentation by-products, sources of spoilage, resistance to preservatives and ethanol, osmotolerance, growth conditions and temperatures, characteristics in culture media and other beverage-specific background information are described.

Descriptors: yeast, beverage, beer, wine, soft drink, alcoholic, non-alcoholic, spoilage, fermentation, identification, *Saccharomyces*, *Candida, Debaryomyces, Dekkera, Hanseniaspora, Issatchenkia, Kazachstania, Pichia, Schizosaccharomyces*, *Wickerhamomyces, Torulaspora, Zygosaccharomyces*

1 Introduction

Yeasts can be defined as unicellular fungi of the classes Ascomycetes and Basidomycetes with a vegetative reproduction by budding or fission and their sexual state is not characterized by a fruit body [172]. The word yeast itself derives from the English word for foam that is produced during fermentation. In German the word Hefe = yeast derives from the word "heben" which means to rise or to lift. During the alcoholic fermentation the yeast cells rise to the surface of the liquid. In other languages the word for yeast is also associated with the fermentation process [82]. The origin of the word "yeast" underlines that the historical-cultural importance of yeast is closely connected to yeast cultivation by mankind in various fermentation processes. The most important yeasts within fermentation technologies belong to the genus *Saccharomyces*, which are taxonomically grouped into the *Saccharomyces sensu stricto* complex [131, 170].

The *Saccharomyces sensu stricto* complex consists of *Saccharomyces cerevisiae* which is used for the production of top-fermented beers (ale types), wine distilling mashes, sake and many other alcoholic beverages, *Saccharomyces bayanus* which is used in wine, cider, cidre and apple wine production, *Saccharomyces pastorianus* which is the starter for bottom-fermented beer (lager type) production and apple wine production as well as four additional species which are not used industrially [8, 129, 131]. Within the

Author

Dr.-Ing. Mathias Hutzler, Dipl.-Ing. Jennifer Koob, Dipl.-Ing. Robert Riedl, Dr.-Ing. Fritz Jacob, Research Center Weihenstephan for Brewing and Food Quality, Technische Universität München, Freising, Bavaria, Germany, corresponding author: hutzler@wzw.tum.de

List of abbreviations

B.	Brettanomyces
C.	Candida
Cry.	Cryptococcus
D.	Dekkera
H.	Hanseniaspora
I.	Issatchenkia
K.	Kazachstania
L.	Lachancea
M.	Metschinkowia
N.	Naumovia
P.	Pichia
R.	Rhodotorula
S.	Saccharomyces
S. c.	Saccharomyces cerevisiae
Sch.	Schizosaccharomyces
T.	Torulaspora
W.	Wickerhamomyces
Z.	Zygosaccharomyces

beverage industry spoilage yeasts can lead to uncontrolled fermentations and cause spoilage of products which causes consequently enormous economic loss [48]. These spoilage yeasts spread over approximately 800 known yeast species [11, 157]. Estimations indicate that additional 669000 non-described yeast species exist. [172]. In spite of the great species diversity only a few species are able to grow in beverages and spoil beverages. [123, 157]. The spoilage yeast spectrum and the spoilage yeast susceptibility of a specific beverage depends on the physico-chemical composition of the beverage and its characteristics as a nutrient medium [33, 69, 157, 164]. There are various publications which describe the potential yeast flora and the spoilage yeast flora for specific beverages, pre-stages and groups of beverages as well as raw materials [5, 6, 34, 35, 38, 69, 71, 77, 94, 123, 157, 158]. Up to now there are no scientific publications and estimations about the extent of economic loss that is caused by yeast spoilage of beverages. It is assumed that the economic loss is in dimensions of millions to billions of euros per year in Europe [157]. The reasons therefore are that beverage producers need not obligatorily notify spoilage yeast incidents, consumers do not report product damages caused by yeast spoilage and that only a part of severe product failures (bombages, turbidity, off-flavors) is notified [157]. An identification and indexing of yeast species causing beverage spoilage in a specific beverage enables technologists to contain the beverage specific spoilage yeast flora and the spoilage potential of the occurring spoilage yeasts. Correct species identification of spoilage yeasts enable to improve quality management and product safety systems and to assess raw material quality and product quality. Yeasts are usually identified by phenotypic methods (e.g. fermentation spectra of sugars and nitrogen sources) and by diagnostically established DNA-sequences [46, 86]. DNA-based identification methods are faster and more reliable than phenotypic methods and are already widely spread in science and industry [86]. The identification of beverage relevant spoilage yeasts on species level is in most cases sufficient to conclude the spoilage potential and the technological consequences and countermeasures. For some very specific cases it is necessary to accomplish a differentiation on subspecies, variety or strain level. The differentiation of *Saccharomyces cerevisiae* var. *diastaticus*, the most dangerous spoilage yeast in breweries, is an example for a differentiation on variety level. It has super-attenuating activity, which can be detected using the DNA-sequence encoding for the enzyme glucoamylase [24]. A differentiation of wild or spoilage yeasts on strain level can be used in specific cases, e.g. for strains of *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe* und *Zygosaccharomyces bailii* with non-typical preservative resistances [105, 158]. Strain-level differentiation of industrial used yeasts is of great importance for the production of fermented beverages. The choice of the yeast strain has a great influence on the characteristics and the quality of the final product. Strain characteristics are for example different aroma profiles, temperature optima, fermentation performances, attenuation degrees, flocculation and formation profiles of secondary products of the metabolism such as fermentation by-products [141]. The aim of differentiation methods is to ensure the strain identity of a specific production strain [141]. This review article focuses on identification and differentiation of fermentation and spoilage yeasts and their characteristics for the beverage industry and can be regarded as a useful tool for the quality control in beverage microbiology.

2 Beverage relevant yeasts

2.1 Industrial yeast species

For most fermentation processes in the beverage industry, the three yeast species *Saccharomyces cerevisiae*, *bayanus* and *pastorianus* are used; their position within the *S. sensu stricto* complex is explained in the section on the phylogenetics and systematics of yeast [36, 39, 129]. Top-fermenting brewery yeast strains belong to *S. cerevisiae*, while bottom-fermenting yeast strains are classified as *S. pastorianus* [26, 66, 131]. Pure yeast strains for the brewing of beer are propagated under controlled conditions and used later for fermentation [55, 161]. Additionally, *Dekkera* spp. (anamorph: *Brettanomyces* spp.) are often introduced in later stages of fermentation (secondary) to produce specialty beer styles, such as Berliner Weiße, Belgian beers (Lambic) and English beers (Porter, Ale) [6, 39]. Of these species, the most important is *Dekkera bruxellensis*, which is considered to be an obligate or potential beer spoilage yeast for most beer styles and for other beverages as well [6, 134]. This will be discussed in greater detail below. In wine production, *Saccharomyces* strains are used in starter cultures as well as in methods employing spontaneous fermentation [36, 38]. Many different strains of yeast compete with each other directly during the initial stages of spontaneous fermentation. Generally speaking, *Hanseniaspora uvarum* (anamorph: *Kloeckera apiculata*), *Debaryomyces hansenii, Metschnikowia* spp., *Candida* spp., *Pichia* spp and *Wickerhamomyces* spp. are present, whereby *Hanseniaspora uvarum* usually represents more than 60 % of the total population of cells [6, 36]. The diversity of yeast strains in grape must is dependent on a variety of factors, including geographical location, climate conditions, the type of grape, the degree of damage to the grape skin and the processing conditions [125]. As fermentation progresses, alcohol-tolerant strains of *Saccharomyces* tend to dominate. It has not yet been established if the *Saccharomyces* strains responsible for fermentation originate primarily from the skin of grapes or from the vintner's cellar [36]. A similar relationship is observed with the *Saccharomyces* population in the fermentation of apple wine. *Saccharomyces* yeasts do not naturally colonize the surface of apples; however, they are found in apple presses [8]. In wine production using grapes, the further growth of the original yeast population is also dependent on various stages of processing, including hand sorting, mechanical sorting and fermentation and on the SO_2 concentration [36]. In addition to vigorous *Saccharomyces* strains and other strong fermenters such as *Zygosaccharomyces spp.*, *Zygotorulaspora spp.*, *Kazachstania exigua*, *Lachancea kluyveri*, *Saccharomycodes ludwigii* and *Torulaspora delbrueckii* may be found in spontaneous fermentations [6, 52, 88]. *S. paradoxus* strains isolated in Croatian vineyards are present in greater numbers than *S. cerevisiae* exhibit good oenological properties [132]. The species *S. cerevisiae* and *S. bayanus* are used in controlled wine fermentations. Starter culture combinations consisting of *S. cerevisiae* and various *Candida* spp. were investigated and the resulting wines exhibited sensory characteristics that differed from wines fermented exclusively with pure cultures of *S. cerevisiae* [52, 78, 165]. In western countries, *S. cerevisiae* is utilized in the production of distillery mashes, sparkling wine, apple wine and other fruit wines as a starter culture. In rural areas *Hanseniaspora* spp. are found primarily in spontaneously fermented apple must [38]. In addition to those, *S. bayanus* and *S. pastorianus* are used in apple wine production and often mixtures of these two strains are utilized, although *S. bayanus* generally dominates the earlier stages of fermentation, while *S. pastorianus* tends to be dominant in the later stages [8]. Many *S. bayanus* strains are cryotolerant, meaning that they are capable of stable growth at low fermentation temperatures (8–15 °C). Bottom-fermenting brewery strains of *S. pastorianus*, which possess the ability to grow at low temperatures, are hybrids of *S. cerevisiae* and *S. bayanus* or strains similar to *S. bayanus* in which the mitochondrial DNA stems exclusively from a non-*S. cerevisiae* yeast strain [130]. *S. cerevisiae* and *S. bayanus* hybrids have also been isolated from grape wine and apple wine [106], and cryotolerant *S. cerevisiae* and *S. kudriavzevii* hybrids have been isolated from grape wine, as well [57]. Various chromosomal and mitochondrial genes have been identified from *S. kudriavzevii* [57, 98]. These findings support the theory that the cryotolerant characteristic of yeast which allows them to ferment at low temperatures can be attributed in every case to the genetic material originating from the non-*S. cerevisiae* portion of the DNA [130]. Recent research results concerning the hybrid character of the bottom-fermenting brewing yeast *Saccharomyces pastorianus* are described in section 3. Besides large scale commercially produced alcoholic beverages, there is a broad variety of semi-commercial and artisanal ”indigenous fermented beverages“, in which diverse strains of yeast play a role either as part of starter cultures or in spontaneous fermentation [17, 152]. “Indigenous fermented beverages” refer to fermented beverages made by native people, sometimes referred to as “fermented Ur-beverages“. Examples of such include pulque and chicha from Latin America, sugar cane wine, sake and satho from Asia, and also ajon, busaa, tej, kaffir or sorghum beer from Africa [17, 115, 151, 152]. These beverages contain a great diversity of yeast species; however, *Saccharomyces* species are most frequently associated with them [76, 152]. The identification of the yeast species found in these beverages was taken from older research and therefore was mostly phenotypic [152]. In more recent research, the yeast microflora is more accurately described with the assistance of microbiological methods, such as 18s or 26s rDNA sequencing [23, 76, 91, 166]. The yeast strains that are used as starter cultures, semi-controlled cultures or those used for spontaneous fermentation processes in the production of specific alcoholic beverages are listed in table 1 (see page 36).

2.2 Spoilage Yeasts

2.2.1 Spoilage yeasts in alcoholic beverages

The terms “wild yeast” and spoilage yeast are often used as synonyms, but it is more reasonable to make a distinction between the two. References to wild yeast include yeast strains or species, which are not identical to the yeast strains used in production or in a starter culture [6, 68]. This does not automatically mean that wild yeast act as obligate, potential or indirect spoilage yeasts in alcoholic beverages. Such yeasts can also exist in a latent form in a beverage or in the production area, without causing spoilage [6]. In contrast, the term spoilage yeasts implies the yeasts belonging to this group cause either indirect or direct product spoilage [68]. During the fermentation of alcoholic beverages, spoilage yeasts can compete with the production yeast strains or the “desirable“ yeast flora and negatively affect the course of fermentation [6, 99]. In this way, the presence of a bottom-fermenting brewing yeast strain, which is fermenting along with another bottom-fermenting strain and not identical with the production strain, could be seen as a spoilage yeast in beer production process [6, 65, 66]. Disruptions in fermentation and the creation of undesirable aromas in intermediate products are considered to be indirect product defects and are often irreversible [6]. Indirect spoilage yeasts do not cause damage in finished alcoholic beverages, but are capable of damaging the product in earlier stages of production [6]. Indirect spoilage is frequently observed in the starter culture and during the initial stages of fermentation [6]. If indirect spoilage is detected early enough, measures can be taken to counter it (blending, fine filtration) [6]. Direct product spoilage of a finished alcoholic beverage results from spoilage yeast strains that are able to tolerate and grow in this kind of environment [68]. Indirect spoilage yeasts are generally the primary contaminants, e.g. they are found in the process prior to filling of the product, whereas direct spoilage yeast (potential, obligate) can be primary as well as secondary contaminants, meaning that they can grow in every stage of the process (also after the product has been filled) [154]. With filtered alcoholic beverages, the finished product (from a microbiological perspective) refers to the product once it has been filtered. Only after distillation is complete are distilled beverages considered finished (with the exception of long-term refinement processes, such as the aging of wine or whisky). The point at which unfiltered beverages are considered finished is when the product ceased to undergo microbiological changes, meaning the limit for direct and indirect spoilage has been reached. Yeast strains responsible for direct and indirect spoilage are listed in table 2 (see page 37). The categories beer, wine and mixed beverages made with spirits are relatively new categories. Until recently, only reliable studies involving susceptibility to spoilage yeast existed for beer-based mixed drinks, also called “beer-mix” beverages [21, 69]. Beverage mixtures consist of two parts, an alcoholic beverage and a non-alcoholic beverage. In a beverage mixture, the non-alcoholic beverages vary greatly in their composition and properties (sugar content, type of sugar, aromas, antioxidants, concentrates, fruit extracts, stabilizers, pH, color) as well as their percentage in the beverage mixture [69]. The capabiltity of spoilage yeasts to affect beer-mix beverage negatively is dependent on its ingredients and the properties of the matrix [69]. Spoilage yeasts found in beer and wine can be considered indirect spoilers of beer-mix beverages as they are components of the final beer-mix beverage. In general, there is no indirect spoilage danger to the soft drink component, because the raw materials used to make syrups, concentrates and aromas are first combined with the alcoholic beverages immediately prior to the actual beverage production process (e.g. inline mixing) [143]. Only after mixture completion the medium is conducive to spoilage by yeasts. Direct spoilage yeasts of beer-mix beverages are listed in table 2. Distilled alcoholic beverages used in beverage mixtures are not contaminated by spoilage yeasts due to their high alcohol content. The spoilage yeast flora of beer or wine made from apples or grapes is well-researched [6, 34, 35, 52, 99]. At present, there are no studies available about which strains of spoilage yeasts are found in fruit wine; however, it can be assumed that these species are comparable to those found in wine made with grapes or apples. This also applies to indigenous fermented beverages; information regarding indirect and direct spoilage yeasts can be found in table 2. Because the yeast strains in many studies of indigenous beverages (refer to 2.1) must first

Table 1 **Yeast species in starter cultures and in spontaneously fermenting communities of microbes, both of which are used in the production of alcoholic beverages [5, 7, 19, 23, 24, 26, 41, 56, 57, 65, 66, 74]**

Type of beverage	Yeast species in starter cultures	Yeast species in spontaneous fermenting communities
Beer		
beer (bottom-fermented)	*S. pastorianus* (bottom-fermenter)	–
beer (top-fermented)	*S. cerevisiae*	–
beer (top-fermented, specialty beers)	*S. cerevisiae, D. bruxellensis*	–
Wine		
grape wine	*S. bayanus, S. cerevisiae*	*S. bayanus, S. cerevisiae, S paradoxus, C.* spp., *Debaryomyces hansenii, P. fermentans, Hanseniaspora uvarum, I. orientalis, M. pulcherrima, Kregervanrija fluxuum, Saccharomycodes ludwigii, T delbrueckii, Zygotorulaspora* spp., *K. exigua, L. kluyveri, W. anomalus, Z.* spp.
apple wine/cider	*S. cerevisiae, S. bayanus, S. pastorianus*	*C. oleophila, C. sake, C. stellata, C. tropicalis, H. uvarum, H. osmophila, H. valbyensis, L. cidri, Kluyveromyces marxianus, Kregervanrija delftensis, M. pulcherrima, Lindnera misumaiensis, P. guilliermondi, P. nakasei*
wines made with other fruits	*S. cerevisiae*	–
wine containing CO_2 (sparkling wine, prosecco, champagne)	*S. cerevisiae*	–
Distilled alcoholic beverages		
Whisky	*S. cerevisiae, S. cerevisiae var. diastaticus*	–
cognac, armagnac, brandy	see wine made with grapes	see grape wine
Rum	*S. cerevisiae, S. bayanus, Sch. pombe*	–
Indigenous fermented beverages		
kaffir/sorghum beer (millet beer)	*S. cerevisiae*	–
pulque (fermented agave juice)	*S. cerevisiae*	*S. cerevisiae, Hanseniaspora uvarum*
sake (alcoholic rice beverage)	*S. cerevisiae* var. *sake, C. sake*	–
palm wine	-	*S. cerevisiae, Sch. pombe, P. membranifaciens, P. fermentans, C. tropicalis*
honey wine (tej)	-	*S. cerevisiae*
chicha (alcoholic corn beverage)	-	*S. cerevisiae, S. pastorianus, Kregervanrija fluxuum*
busaa (corn, millet beverage)	-	*S. cerevisiae, I. orientalis*
sugar cane wine	-	**S. cerevisiae, Saccharomycopsis fibuligera*
*takju/yakju (alcoholic rice or wheat beverage)	-	**S. cerevisiae, C. inconspicua, C. sake), P. triangularis, W. anomalus*
*sweet-sour alcoholic rice and manioc beverages based on ragi, loogpang, bubod starter cultures	-	**S. cerevisiae, C. intermedia, C. parapsilosis, C. solani, Cryptococcus humicola, Filobasidium capsuligenum, Kodamaea ohmeri, Kregervanrija fluxuum, P. membranifaciens, P. guilliermondii, Saccharomycopsis fibuligera, Saccharomycopsis malanga, W. canadensis, W. anomalus, W. subpelliculosa*

*semi-controlled fermentations, based on naturally cultivated mixed starter cultures

be identified in order to ensure a controlled process with defined starter cultures, research of the spoilage yeast flora has long been of secondary importance.

2.2.2 Spoilage yeasts in non-alcoholic beverages

A limited number of yeast species can be found in alcoholic beverages that offer specific environmental conditions. However, many yeast species cannot tolerate these conditions [158]. A large number of yeast strains from different genera and species have been isolated from non-alcoholic beverages [3, 128, 137–140, 147, 157, 181]. Many of the isolated yeast strains have no effect on the beverage, but simply "came into contact with the beverage by chance" and are not capable of growth or die within a short time under the existing conditions. The ability to separate the true spoilage yeasts from the background flora is important [158]. For this situation, yeast strains from non-alcoholic beverages can be divided into three different main categories according to Davenport [30, 158]. table 3 (see page 38) lists yeast strains classified into three categories. Category I consists of spoilage yeast species which have the potential to spoil the beverage if one cell per package is present and capable of growth [30]. Yeast strains from category II can cause spoilage if there are errors in production or if the beverage is susceptible to microbial contamination [30]. They are present only in small numbers if high standards of hygiene are maintained. Category III contains microbes present under unhygienic conditions, which are considered to be indicator microbes

Table 2 Direct and indirect spoilage yeasts associated with different types of beverages [5, 19, 20, 31, 37, 42, 46]

Type of beverage	Indirect spoilage yeasts	Direct spoilage yeasts
Beer and beer based beverages		
Beer	*S.* spp. *(bayanus, cerevisiae, pastorianus), C.* spp. *(inconspicua, intermedia, rugosa, sake, stellata, tropicalis), Debaryomyces hansenii, H. uvarum, I. orientalis, K. exigua, Kluyveromyces marxianus* (esp. in "Sauergut" for biological acidification), *Kregervanrija fluxuum, P.* spp. *(fermentans, jadinii, membranifaciens), R. glutinis, Saccharomycopsis fibuligera, Saccharomycodes ludwigii, W. anomalus*	*S.* spp. *(bayanus, pastorianus, cerevisiae, cerevisiae var. diastaticus) B.* spp. *(custersianus, nanus), D.* spp. *(anomala, bruxellensis)*
beer-mix beverages	see beer (direct and indirect spoilage yeasts)	*S.* spp. *(bayanus, cerevisiae, cerevisiae var. diastaticus, paradoxus, pastorianus), B. naardenensis, D* spp. *(anomala, bruxellensis), K. exigua, L. kluyveri, N. castelli, Sch. pombe*
Wine		
grape wine	*S.* spp. *(bayanus, cerevisiae, pastorianus), C.* spp. *(boidinii, glabrata, inconspicua, norvegica, parapsilosis, rugosa, sake, stellata, tropicalis, zeylanoides), Cryptococcus laurentii, Debaryomyces* spp. *(etchellsii, hansenii), H.* spp. *(guilliermondii, uvarum), I. orientalis, Torulaspora delbrueckii, K. exigua, Kluyveromyces marxianus Kregervanrija fluxuum, L. kluyveri, M. pulcherrima, P.* spp. *(guilliermondii, jadinii, membranifaciens, farinosa, fermentans), R.* spp. *(glutinis, mucilaginosa), Saccharomycopsis fibuligera, Sch. pombe, T. delbrueckii, W.* spp. *(anomalus, subpelliculosa*	*S.* spp. *(cerevisiae), D.* spp. *(anomala, bruxellensis), Saccharomycodes ludwigii, Zygosaccharomyces* spp. *(bailii, rouxii)*
apple wine/cider	*S.* spp., *C.* spp. *(anglica, boidinii, cidri, norvegica, pomicola), Hanseniaspora* spp. *(uvarum, osmophila, valbyensis), M. pulcherrima, P.* spp. *(guilliermondi), Saccharomycodes ludwigii*	*S.* spp. *(bayanus, cerevisiae), D.* spp. *(anomala, bruxellensis), Z. bailii*
beverages made with wine	see wine made from grapes (indirect and direct spoilage yeast)	no references exist
Distilled alcoholic beverages		
Whisky	see beer	–
cognac, armagnac, brandy	see wine	–
beverages made with spirits (alcopops)	–	no references exist
Indigenous fermented beverages		
alcoholic beverages produced with uncontrolled or partially controlled yeast flora	yeast strains that are different from the commercial or proprietary strains and negatively affect fermentation and product quality	yeast strains that are capable of growth in the final product, causing spoilage

but normally do not cause any spoilage. Yeast species from other habitats that are not typically found in production areas for non-alcoholic beverages can be classified in the additional category IV. For example, *Kluyveromyces lactis* is typically found in dairies. If this yeast is found in a production area for non-alcoholic beverages, this is an indication that there was contact with dairy products or equipment [158]. *Back* divided the yeast strains encountered in the non-alcoholic beverage industry according to their fermentative potential: specifically, into the categories of strong-fermenters, those capable of fermentation, weak-fermenters and respiratory yeasts (incapable of fermentation) [5, 7]. Table 3 shows the yeast strains grouped into categories I-III according to their fermentative potential.

2.2.3 Beverage relevant spoilage yeasts – an overview

Evidence of the spoilage of beverages through the action of yeasts appears in many forms. The spoilage effects that may occur in both non-alcoholic and alcoholic beverages are gas production, turbidity, sedimentation and off aromas [100]. Formation of a film on the surface of the beverage has been observed only with alcoholic beverages [100].

Deak and *Beuchat* describe the percentage of the 96 species of spoilage yeasts that occur most frequently in fruits, non-alcoholic beverages, beer and wine [35]. In subsections 2.2.1 and 2.2.2, yeast strains capable of causing direct or indirect spoilage of various beverages are reviewed. A combination of these data with the percentage of distribution by Deak and Beuchat allows a preliminary classification of the spoilage potential of a particular yeast strain as well as the probability for it causing spoilage in a given beverage. In section 5, general information about the most important beverage-spoiling yeast species is included. The yeast species are arranged and described in alphabetical order. The description also includes additional information about their occurrence along with a description of the growth and metabolic properties relevant for beverages. Particular emphasis is given to aspects such as the resistance to preservatives, osmotic tolerance and temperature resistance. Morphology descriptions and metabolic

Table 3 Yeast strains from non-alcoholic beverages (soft drinks) and their production surroundings grouped according to their potential for spoilage as well as fermentative potential (adapted from [4, 6, 8, 20, 58, 69])

Fermentative potential	**Category I: spoilage yeast species**	**Category II: potential spoilage yeast species/ hygiene indicator flora**	**Category III: hygiene indicator flora**
strong fermenters	*S. cerevisiae* *Z. bailii*	*S. bayanus* *Zygotorulaspora florentinus*	
average fermenters	*K. exigua* *Sch. Pombe* *Z. rouxii* *Z. bisporus* *Z. lentus*	*I. orientalis* *L. fermentati* *L. kluyveri* *T. delbrueckii* *T. microellipsoides*	
weak fermenters	*B. naardenensis* *D. anomala* *D. bruxellensis*	*C. boidinii* *C. davenportii* *C. etchellsii* *C. intermedia* *C. parapsilosis* *C. stellata* *Debaryomyces hansenii* *H. uvarum* *L. thermotolerans* *Lodderomyces elongisporus* *P. fermentans* *P. minuta* *P. guilliermondii* *W. anomalus*	*C. lactis-condensi* *C. sake* *C. solani* *C. tropicalis* *C. lusitaniae* *D. etchellsii*
non-fermenters (not capable of fermentation)		*C. inconspicua* *P. membranifaciens*	*(Aureobasidium pullulans)* *Cryptococcus albidus* *Cryptococcus laurentii* *Rhodotorula glutinis* *Rhodotorula mucilaginosa*

spectra (e.g. for sugar, nitrogen sources) are only briefly covered, as reference works and data banks from authors such as *Barnett* et al., *Boekhout* et al., *Kurtzman* and *Fell* and *Robert* et al. already exist [11, 20, 87, 133]. Section 5 is meant to serve as a reference source, in which an overview of the yeast species is provided with regard to beverage microbiology. Section 5 includes descriptions of yeast species that are of greater importance or those for which DNA-based rapid analysis methods (e.g. Real-Time PCR) have already been developed.

3 Phylogenetics and Classification of beverage relevant yeasts

Yeasts are generally identified on the basis of their phenotype or according to genetic sequencing [20]. Many methods based on molecular biology have been developed and are considered to be an alternative to physiological methods [46]. The majority of these methods utilize the ribosomal DNA as target sequence. The ribosomal genes (5S, 5.8S, 18S and 26S) of the species *Saccharomyces cerevisiae* and most of the other yeast species are arranged in tandem repeat units which repeat in a head to tail manner in the genome of chromosome XII approximately 100–200 times [12, 46, 112]. The term “tandem-repeats” should not be confused with the term “short-tandem-repeats” (microsatellite DNA fragments). The encoded DNA fragments (5S, 5.8S, 18S and 26S) are separated from the non-coding fragments, the ITS (internal transcribed spacer) and the IGS regions (intergenic spacer) [53,112]. The ribosomal genes as well as the ITS and IGS regions are well-established tools for identifying species and bringing phylogenetic relationships to light [89]. The ITS and IGS regions exhibit a high level of interspecific variation [45]. At the same time, the intraspecific polymorphisms in the ITS regions are not very pronounced, which makes these regions more suitable for the identification of closely related species than 18S or 26S rDNA fragments [43,79,108]. Partial DNA fragments of the IGS regions can exhibit intraspecific variation, e.g. variation in the DNA sequences of individual strains within a species [53, 67, 111, 112]. The two most frequently referenced DNA regions for identifying yeast at the species level are the 18S genes and the D1/D2 domains, which are located at the 5‘ end of the 26S genes [72, 89]. These sequences can be found in the public Internet databases of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/), the European Bioinformatics Institute (http://www.ebi.ac.uk/embl/) and the DNA Data Bank of Japan (http://www.ddbj.nig.ac.jp/). Especially for the D1/D2 domains of the 26s gene, a great number of sequences is available and may be used for comparison with an unknown sequence. Species identity is given at a sequence identity of 99 % identical nucleotides [46, 89]. Additional genes and DNA fragments such as histone promoter sequences as well as actin-1, beta-tubuline, cytochrome oxidase II, RNA polymerase II, pyruvate decarboxylase, EF1-α, YCL008c, URA3, MET10, LRE1, GRC3, SED1, STE2, BUL2 genes are discussed, which can aid in the identification of relationships among yeast species [13, 26, 29, 90, 103, 104, 129]. These fragments are amplified using PCR and then sequenced or determined using gel electrophoresis or real-time methods. The genes LRE1 and GRC3, among others, provide a means for investigating the phylogenetic relationships between the species *S. pastorianus*, including bottom-fermenting brewery yeast species and *S. bayanus* [65, 129]. Other species of the *S. sensu stricto* complex may also be identified using these genes [68]. Furthermore, it has also been reported that mitochondrial DNA can be utilized for the differentiation of brewing yeast and species of the *S. sensu stricto* complex [25, 46, 90, 130, 135, 164]. Differentiation of the members of the *Saccharomyces sensu stricto* complex was performed using phylogenetic multiple gene analysis [90]. *Saccharomyces* species that could not be identified as *Saccharomyces sensu stricto* species by multiple gene analysis have subsequently been determined to be members of other genera and have been classified as such in one of 14 recommended genera [85, 87, 90]. The *Saccharomyces sensu lato* complex, including former *Saccharomyces* species only remotely related to *S. cerevisiae*, is no longer in existence [85, 90, 116, 131]. The species formerly counted among the *S. sensu lato* complex are now distributed among various genera, for example *Kazachstania*, *Naumovia* and *Lachancea* [85, 88, 116]. Therefore, the typical beverage spoilage yeast species *Saccharomyces exiguus* is now classified under *Kazachstania exigua* [133]. The *S. sensu stricto* complex originally consisted of the four *Saccharomyces* strains *bayanus, cerevisiae, paradoxus* and *pastorianus*, which since the year 2000, includes three additional *Saccharomyces* species, *S. cariocanus, S. kudriavzevii* and *S. mikatae*, bringing it to a total of seven strains [117, 131, 170]. Recently *S. arboricolus* sp. nov. and *S. eubayanus* sp. nov. were described and proposed as eighth and ninth species within the *S. sensu stricto* complex [95, 174]. *S. arbolicolus* was isolated from oak trees in china [174]. *Libkind et al.* report that the draft genome sequence of *Saccharomyces eubayanus* is 99,5 % identical to the non-*Saccharomyces cerevisiae* portion of the *Saccharomyces pastorianus* genome sequence [95]. Consequentely Libkind et al. postulate that *S. eubayanus* sp. nov. is the missing wild genetic stock of bottom-fermenting *S. pastorianus* brewing strains [95]. The review article of *Rainieri et al.* focuses on *S. sensu stricto* species and the relationship between non-hybrid species and hybrid groups [131]. It has been the topic of discussion, that the *S. bayanus* species actually includes two groups. The one group is made up of yeast strains which were formerly considered to be similar to *S. uvarum*, which was formerly classified as an independent species. The other group consists of pure *S. bayanus* strains. A current hypothesis postulates that the *S. bayanus*-type CBS 380 strain is a hybrid, that is a genetic mixture of *S. uvarum* and *S. bayanus* [26, 80, 83, 160]. It is very probable that use of this strain as a reference strain has led to misinterpretations and confusion [131]. According to the hypothesis detailed above, the *S. bayanus*-type strain shares the same *S. bayanus* parent strain with the bottom-fermenting brewing yeast, which is grouped under *S. pastorianus* and was formerly named *S. carlsbergensis* [26, 80, 83, 160]. *S. pastorianus* can also be divided into two groups: *S. pastorianus*-type strains and strains, which were represented by the bottom-fermenting yeast formerly classified as *S. carlsbergensis* [180]. *S. pastorianus*-type strains from different microorganism collections appear to differ from one another. The strain CBS 1538 exhibits a hybrid character comparable to *S. carlsbergensis*, whereas the NRRL-Y 1551 strain is not a hybrid [80]. It is probable that incorrect interpretations and conclusions resulted through the use of these strain-types as reference strains in identification and characterization studies [131].

4 Yeast Identification and Differentiation

4.1 Definition and impact of identification and differentiation

Identification can be defined as the assignment of an unknown microorganism to a specific class or species within an existing classification system [126]. Identification at the species level by means of rDNA sequencing is now the current reference method and considered to be state of the art [46, 86, 155]. Generally, differentiation is the distinction – independent of the taxonomic level – between two organisms. Identification and differentiation based on genetic information can be performed on different levels of expression: at the genome level, the protein level, the morphology/cell component level and the behavior level [60]. In the area of beverage microbiology, differentiation of species, subspecies, strains and other groups with practical relevance is performed [6, 31, 46, 155]. The differentiation of different strains within a single species (= strain differentiation) is extremely important, especially for yeasts used commercially, whereby the terms strain characterization or typing are often used synonymously [46, 141, 142]. With spoilage organisms, the identification at the species or strain level is an important analytical tool in localizing the source of infection – as part of a routine quality control program – within a process [155].

4.2 State of the art identification and differentiation methods – an overview

Different methods used for identification and differentiation are listed and arranged by technique in table 4 (see page 40). Additionally, the individual methods, the degree of differentiation and the corresponding publications regarding yeasts relevant for beverages are given.

Table 4 shows how the molecular biology methods and PCR-based methods have grown in importance in recent years, evidenced by the numerous publications regarding the identification and differentiation of yeast strains relevant for beverage production. Recently *Ivey* and *Phister* published a review of molecular techniques for the detection and identification of microorganisms in wine [70].

Table 4 Overview of the methods for identification and differentiation used for yeast (adapted from [29, 68])

Methods	Degree of differentiation	Previously researched yeast group, genus, species/type/matrix, reference source
Physiological, morphological methods		
Standard methods	genus, species	various yeast strains/foods, beverages [8, 40, 58]
Miniature commercial systems (e.g. API 20C AUX, Rapid IDYeast Plus,	genus, species	*C.* spp., *Claviaspora lusitanae, I. orientalis, H.* spp., *M.* spp., *P.* spp., *R.* spp., *S.* spp., *Saccharomycopsis cratagenesis., T.* spp., *W.* spp./orange juice [3]
Chemotaxonomic methods		
Total fatty acids analysis (FAME = determination of fatty acid methyl ester compounds)	species	*B. naardenensis, Debaryomyces hansenii, D.* spp., *I. orientalis, Kluyveromyces marxianus, P.* spp., *S.* spp., *Z. rouxii, W.* spp./fruit concentrates [61] *C.* spp., *K.* unispora, *S. cerevisiae, T. delbrueckii, W. anomalus*/brewery isolates [70]
Protein fingerprinting (e.g. 2D protein map)	species, strain	*S. cerevisiae, S. pastorianus, Sch.* sp, *Z. rouxii*/ beer, beer yeasts, wine yeasts [1] *S. cerevisiae, S. pastorianus*/beer yeasts [38]
Mass spectrometry methods (e.g. MALDI-TOF MS, Py-MS, DIMS, GC-TOF MS)	species, strain	various yeast strains (depending on data bank)/ foods, beverages [64] *S. cerevisiae, S. pastorianus*/beer yeast [53, 71]
Fourier-transform infrared spectroscopy (FT-IR)	species, strain	*S. cerevisiae, S. pastorianus*/beer yeast [71] various yeast strains/ foods, beverages [76]
Immunological methods		
Technique based on monoclonal antibodies (e.g. ELISA)	species, strain	*B.* spp., *D.* spp./wine [39] *S. cerevisiae, S. pastorianus, Sch.* sp, *Z. rouxii*/ beer, beer yeasts, wine yeasts[1]
Molecular genetics methods		
Sequencing	species	*S. cerevisiae*/sorghum beer [75] *C.* spp., *Claviaspora* spp., *Geotrichum* spp., *H.* spp., *I.* spp., *M.* spp., *P.* spp., *R.* spp., *S. cerevisiae, S. bayanus, Saccharomycopsis* spp., *T.* spp., *W.* spp./orange juice [3] *Bulleromyces* spp., *C.* spp., *Clavispora* spp., *Cryptococcus* spp., *Filobasidium* spp., *Galactomyces* spp., *H.* spp., *I.* spp., *P.* spp., *R.* spp., *S.* spp., *Sporobolomyces* spp., *Trichosporon* spp., *W.* spp., *Williopsis* spp./malting process [43] *C.* spp., *S.* spp., *P.* spp., *W.* spp./beer [70]
Karyotyping	species	*C. guilliermondi, C. pulcherima, H. uvarum, S. cerevisiae*/grape wine [60] *S. bayanus*/wine [22,49,50] *S. cerevisiae*/ sherry [28] *S. cerevisiae*/ cachaca [35] *S. cerevisiae, S. bayanus*/ cider [51] *S. cerevisiae*/wine [47] *S. cerevisiae*/must [2]
RFLP mt DNA	species	*S. cerevisiae*/ grapes [18] *S. cerevisiae, C. stellata, H. uvarum, M. pulcherrima, T. delbrueckii*/wine [55] *S. cerevisiae*/sherry [27, 28] *S. cerevisiae*/must [13] *S. cerevisiae*/must, wine [30] *S. cerevisiae*/wine [9, 34, 45, 47, 72, 73] *C. guilliermondi, ,C. pulcherima, Kloeckera apiculata, S. cerevisiae*/grapes, wine [60]
Fluorescence/ Chemoluminescence in-situ Hybridization (FisH/ CisH)	genus, species	*B. custersianus, B. naardenensis, B. nanus, D. anomala, D. bruxellensis*/wine [59] *C. stellata, H. uvarum, H. guillermondii, Kluyveromyces marxianus, Kluyveromyces thermotolerans, P. membranifaciens, S. cerevisiae, T. delbrueckii, W. anomalus*/must, wine [77] *D. bruxellensis*/wine [67]
Polymerase Chain Reaction (PCR) -based methods	genus, species, strain	

...continued next page

PCR-RFLP of the 5.8s ITS rDNA region	species	*S. cerevisiae*/sorghum beer [75] *C.* spp., *D. anomala, H.* spp., *I. terricola, Kloeckera apiculata, Kluyveromyces thermotolerans, L. kluyveri, M. pulcherrima, R. glutinis, S.* spp., *Sch.* spp., *T. delbrueckii Z.* spp./wine [9, 28, 32, 55, 60, 73] *C.* spp., *Claviaspora* spp., *Geotrichum* spp., *H.* spp., *I.* spp., *M.* spp., *P.* spp., *R.* spp., *S. cerevisiae, S. bayanus, Saccharomycopsis* spp., *T.* spp., *W.* spp./orange juice [3] *C.* spp., *Claviaspora* sp, *H.* spp., *P.* spp., *R.* spp., *Trichosporon* spp., *W.* spp./oranges, orange juice [44] *D.* spp., *Debaryomyces* spp., *H.* spp., *M.* spp., *P.* spp., *S.* spp., *Saccharomycodes* spp., *W.* spp./cider [48]
PCR-DGGE, PCR TGGE	species, strain	*Aureobasidium pullulans, H.* spp., *M.* spp./grapes [54] *C.* spp., *H.* spp., *Kloeckera apiculata, Kluyveromyces* spp., *P.* spp., *M.* spp., *S. cerevisiae, W.* spp./wine [17]
Real-Time PCR	species, subspecies	*D. bruxellensis*/wine [21, 52] *C. tropicalis, K. exigua, P. membranifaciens, D. anomala, D. bruxellensis, S. cerevisiae, S. cerevisiae var. diastaticus., S.pastorianus, Saccharomycodes ludwigii, W. anomalus*/beer [11,12] spoilage yeast for beverages and foods (group identification, screening)/juice [10,15] beverage relevant yeasts (group identification, screening), *S. cerevisiae* var. *diastaticus*/beverages [25]
RAPD-PCR	strain	*S. cerevisiae*/cachaca [35] *Sch. pombe*/cachaca [33] *S. cerevisiae, S. pastorianus*/beer yeasts [62]
Microsatellite PCR	strain	*S. cerevisiae, Kloeckera apiculata*/wine [14] *S. cerevisiae*/wine [36] *S. cerevisiae, S. pastorianus*/beer yeasts [62]
AFLP-PCR	strain	*S. cerevisiae, S. pastorianus*/beer yeasts [63]
δ-Sequence PCR	strain	*S. cerevisiae, S. pastorianus*/beer yeasts [62] *S. cerevisiae*/wine [16,22,45,55] *S. cerevisiae*/must [13]

One focus of this review is the detection and identification of wine related yeasts.

5 Beverage relevant information about important beverage relevant yeast species and related species in alphabetic order

5.1 *Brettanomyces/Dekkera* spp. (*B. custersianus, B. naardenensis, B. nanus, D. anomala, D. bruxellensis*)

Dekkera is an ascospore-forming genus and is also the teleomorphic form of the genus *Brettanomyces* [158]. This genus included five species, of these *D. bruxellensis* and *D. anomala* are typical spoilage organisms of alcoholic and non-alcoholic beverages [3, 6, 11, 99]. Both species are found in mixed starter cultures used in the production of Belgian lambic/gueuze beers and Berliner Weiße and are responsible for the vinegary aroma typical of these beers [39, 169, 171]. *B. claussenii* is classified as *D. anomala*, while the species *B. intermedius*, lambicus, schanderlii are classified as *D. bruxellensis* [52, 133]. Along with *S. cerevisiae* and *Z. bailii*, *D. anomala* is the yeast species that has been most frequently identified as responsible for spoilage in non-alcoholic beverages [35]. Spoilage caused by *D. naardenensis* is normally restricted to non-alcoholic beverages, whereby its capacity to grow in beer-mix beverages has also been observed [35, 69, 84]. In 1961, *van der Walt* characterized the species *B. custersianus*, which was isolated from the African Bantu beer [168]. The strains of this species, which are in the CBS strain collection, were isolated exclusively from Bantu beer (CBS T4805, 4806, 5207, 5208) and olives (CBS 8347) [133]. *Vanderhaegen et al.* reported *B. custersianus* being isolated from Belgian top-fermented beers [169]. The genus *Eeniella* was established with a single species, *Eeniella nana*, in 1981. Because the phylogenetic relationships were not absolutely clear, it was recommended that this genus be allowed to remain classified as such [179]. The current classification for this species is *B. nanus* (CBS T1945, 1955, 1956) [133]. The three strains that are found in the CBS yeast collection were isolated from beer samples from filled bottles [133]. As yet, *B. custersianus*, naardenensis and nanus have not been isolated from wine production surroundings [62]. *Brettanomyces/Dekkera* species can cause characteristic changes in the general properties of beverages such as sediment, turbidity, off-aromas (e.g. mousy, rodent cage litter, clove, spice, medicinal, wet wool, cedar, horse, farmyard and sewage notes), acetic acid, ethyl acetate, volatile fatty acids and volatile phenolic compounds [27, 49, 52, 148]. Volatile phenolic compounds such as 4-vinyl phenol and 4-vinyl guaiacol are formed from hydroxycinnamic acids like coumaric and ferulic acids via an enzymatic decarboxylation reaction [27, 52]. Additionally, *D. bruxellensis* and *D. anomala* are also capable of reducing/oxidizing 4-vinylphenol and 4-vinyl guaiacol to form 4 ethylphenol and 4-ethylguaiacol [27]. Caffeic and sinapic acids also may serve as reactants for two similar reaction steps; however, they are converted in smaller quantities [40, 61]. Although *B. custersianus*, naardenensis and nanus are capable of growth in the presence of hydroxycinnamic acid, their capacity to take up these substances and convert them to the corresponding vinyl or ethyl compounds is very low with the exception of sinapic acid [61]. The sensory impression associated with 4 ethylphenol is described as "smoky, medicinal" and that of 4-vinyl guaiacol as "clove" or "spice-like" [52]. In addition to *Brettanomyces/Dekkera* strains, which cause a broad spectrum of undesirable sensory problems, there are also strains that are considered to be neutral sensorially as they do not cause any undesirable aromas [52]. In theory, *Brettanomyces/Dekkera* yeasts can reduce/oxidize vinyl phenols created by other microorganisms to ethyl phenols. It has been confirmed that *D. bruxellensis* can use 4-vinyl phenol as a precursor to produce 4-ethyl phenol in the absence of coumaric acid [37]. It has also been reported that *D. bruxellensis* strains can tolerate sulfur dioxide concentrations between 10-50 mg/l [9]. All five *Brettanomyces/Dekkera* species can produce two of the three substances responsible for the "mousy" off-aroma, namely 2 ethyltetrahydropyridine and 2-acetylhydropyridine [148]. It is suspected that *Brettanomyces/Dekkera* yeasts enter the wine production process through recovered wine, poor hygiene in tanks, hoses and during general operations or through vinegar flies also known as fruit flies [52]. *Brettanomyces/Dekkera* yeasts have also been found in air samples collected in various wineries [28] and are most frequently found in the areas of wineries where wooden barrels are present. *Brettanomyces/Dekkera* yeasts can utilize the disaccharide cellobiose, which is formed during barrel production from bending the staves [19, 52]. *D. bruxellensis* is the dominant wine spoilage species, producing the highest concentrations of undesirable volatile substances while also causing spoilage in beer, beer-mix beverages and non-alcoholic beverages [6, 10, 69, 158]. The differences between the *Brettanomyces/Dekkera* species are listed in table 5 with respect to their selected growth characteristics and capacity to produce acetic acid. All *Brettanomyces/Dekkera* species produce acetic acid and are capable of growth in ethanol with the exception of certain *B. nanus* strains. Due to their different maximum growth temperatures, distinguishing one species from the next is possible. *D. bruxellensis* and *D. anomala* can be differentiated from one another with relative certainty on the basis of their utilization of lactose [52]. Most of the *D. anomala* strains can assimilate lactose as opposed to *D. bruxellensis* [52]. All *Brettanomyces/Dekkera* species are sensitive to alkaline pH conditions and cannot grow at a pH higher than 8.0 [35].

5.2 *Candida* sp. (*C. glabrata, C. intermedia, C. parapsilosis, C. sake, C. tropicalis*)

Species belonging to the genus *Candida* are suspected to be pathogens or opportunistic pathogens. In this relationship, *C. albicans* is the species that is most frequently responsible for clinical/medical yeast infections [56]. Other *Candida* species such as *C. dubliniensis, C. famata, C. kefyr, C. krusei, C. glabrata, C. parapsilosis, C. tropicalis* and *C. guilliermondi* are being isolated with increasing frequency from clinical samples and are suspected as the causative agents for instances of candidosis [81, 102]. *C. parapsilosis* is currently the third leading cause of pathogenic *Candida* blood infections in North America and second in Europe and the rest of the world (excluding Africa) [97, 121]. *C. tropicalis* is second as the leading cause of blood infections in Latin America and fourth place in the rest of the world (excluding Africa); *C. glabrata* is the third most frequent in the world overall (excluding Africa) [121]. As Tables 2 and 3 illustrate, *C. glabrata, C. parapsilosis* und *C. tropicalis* play an important role not only in spontaneous fermentations, but also as beverage-spoiling microorganisms. The suspicion or suggestion that beverage-spoiling *Candida* species can be (opportunistic) pathogens is not well-documented in microbiological tests performed on beverages and as a consequence is not given much attention in the relevant literature. *C. parapsilosis* is an anamorphic species of spoilage yeast and does not form spores. It has been suggested that *Lodderomyces elongisporus* is its teleomorphic form [158]. However, it was confirmed through comparison of ribosomal 18s DNA that they are two different species [74]. Their similarities can lead to incorrect identification of isolates from spoiled beverages. *C. parapsilosis* is a yeast species that acts in an opportunistic manner and, although frequently isolated, only causes spoilage in soft drinks from time to time [158]. *C. parapsilosis* can ferment glucose and several strains are capable of fermenting galactose, maltose, sucrose, trehalose and melezitose [11]. This yeast species also exhibits lipolytic activity [75, 123]. *C. parapsilosis* has been isolated from tea-beer, water, sour pickles, soft drinks and fruit juices [11]. *C. parapsilosis* is very closely related to the pathogenic yeast species *C. tropicalis* and *C. albicans*, both of which are the most common yeast isolates associated with human infections [1, 18, 158, 178]. *C. tropicalis* has also been isolated from soft drinks, fruit juices and filling lines [128, 158]. *C. parapsilosis* and *C. tropicalis* are capable of growth at temperatures above 37 °C. *C. sake* is a yeast species characterized by multilateral budding, with the formation of pseudohyphae, which creates whitish, cream-colored colonies; it also multiplies asexually. *C. sake* can ferment glucose and galactose; some strains are able to ferment maltose, sucrose and trehalose. *C. sake* has been found in sake and beer, on brewery equipment and grapes, as well as in juice, sauerkraut, water and non-alcoholic beverages [11]. Starter cultures are used in the production of aguardente, a Brazilian alcoholic beverage

Table 5 **Summary of typical growth characteristics of the five species belonging to the genera *Brettanomyces* and *Dekkera* [58]**

Brettanomyces/Dekkera species	Production of acetic acid	Growth in ethanol	Growth in the presence of cycloheximide [%]		Growth at various temperatures [°C]				
			0.01	0.1	25	30	35	37	40
Brettanomyces custersianus	+	+	r	r	+	+	+	+	–
Brettanomyces naardenensis	+/s	+	+	+	+	+/–	–	–	–
Brettanomyces nanus	+	+/r/–	+	r/–	+	–	–	–	–
Dekkera anomala	+	+	+	+/r/–	+	+	+/–	+/–	–
Dekkera bruxellensis	+	+	+	+/r	+	+	+	+	+/–

\+ = production or growth, s = slight, r = retarded growth, – = no growth +/r/– = strains with growth/strains with retarded growth/strains without growth distilled from a spontaneously fermented sugar cane mash. The mash consists of fermenting sugar cane juice or a mixture of sugar cane juice with ground corn, a fermenting fruit or rice preparation and contains *C. sake* and *S. cerevisiae* as the primary yeast strains [114]. These strains of *C. sake* possess the unusual quality of killer activity targeting other yeast strains also originally found in the starter culture [114]. *C. glabrata* ferments glucose; several strains can ferment trehalose. *C. glabrata* has been isolated from insect larva feces, mammals, birds, baker's yeast and fruit juice [11]. *C. intermedia* can utilize glucose, galactose, melezitose and sucrose, while several strains can ferment maltose, trehalose, cellobiose and raffinose. *C. intermedia* has been isolated from earth samples, humans, salt water, grapes and beer [11].

5.3 *Debaryomyces hansenii* (anamorph *C. famata*)

Debaryomyces hansenii is the teleomorphic form of *C. famata* [35, 51] and possesses a high osmotolerance [75, 123]. The maximum a_w-values for glucose, fructose, sucrose and NaCl are 0.84, 0.86, 0.81 and 0.84, respectively. The maximum growth temperature for *Debaryomyces hansenii* is 32–37 °C [35]. *Debaryomyces hansenii* suppresses mold growth on apples, citrus fruits and cereal grains [35]. *Debaryomyces hansenii* strains can also possess pectolytic activity [35]. The lag phase of *Debaryomyces hansenii* in strawberry juice at 15 °Brix and a pH of 4.0 at a temperature of 30 °C is 3 h with a generation time of 0.8 h [35]. *Debaryomyces hansenii* has been found in fruit juices, wine and sorghum beer [35]. In fruit juices containing peach, apricot and pear juice, *Debaryomyces hansenii* is one of the most frequently isolated yeast strains [35] and is found on apples, grapes, citrus fruits, tropical fruits, barley and corn. It has already been isolated from the raw materials used in the production of non-alcoholic beverages, such as from syrups, molasses and raw sugar [35]. *Debaryomyces hansenii* is one of the primary spoilage yeasts for foods preserved with acid, for example, salad dressings and mayonnaise [35]. Contaminations found in baker's yeast, milk and meat products by *Debaryomyces hansenii* have also been observed [35]. According to Deak and Beuchat, *Debaryomyces hansenii* is the yeast species which is most frequently found in foods after *S. cerevisiae* and is the fourth most common species in fruits and beverages [35]. The genome of *Debaryomyces hansenii* has been sequenced and compared with the genomes of *S. cerevisiae* and other yeasts of the Hemiascomycetes, in order to examine the evolutionary development of the yeast genome [2, 22].

5.4 *Hanseniaspora uvarum* (anamorph *Kloeckera apiculata*)

The yeast species *Hanseniaspora uvarum* belongs to category "average fermentation or capable of fermentation" [75, 123]. *H. uvarum* is the teleomorphic form of *Kloeckera apiculata* and is described in many publications along with *Metschinkowia* sp. as the dominant yeast species on ripe grapes [35, 47]. *H. uvarum* often makes up more than 60 % of the total yeast population present on grapes [36]. There have only been a few instances in which *H. uvarum* has not been found on ripe grapes [47].

H. uvarum has been isolated from apples, citrus fruits, cherries, plums, corn, cocoa, steeped barley, fruit juices, soft drinks, must, beer, wine and cider [35]. The other *Hanseniaspora* species (*H. guilliermondii, H. osmophila, H. valbyensis*) generally prefer the same type of habitats; however, they do occur in different distributions [35]. After inoculation of intact fruit with *H. uvarum*, pectolytic activity was observed [35]. As shown in tables 2 and 3 , *H. uvarum* is a potential spoilage yeast for non-alcoholic beverages and an indirect spoilage yeast for alcoholic beverages; this is due to their sensitivity to alcohol [36, 158]. So-called apiculate yeasts such as *H. uvarum* are bypassed by the development of *S. cerevisiae* during wine fermentations at an ethanol concentration of approximately 3–5 % v/v [36]. The alcohol tolerance of *H. uvarum* is dependent on the fermentation temperature and the particular strain [35]. In research conducted by *Gao* and *Fleet*, a *H. uvarum* strain was shown to tolerate an alcohol concentration of 9.0 % v/v at 10, 15 and 20 °C, but only 5.0 % v/v at 30 °C [54]. By comparison, another strain only tolerated a maximum of 5.0 % v/v at 10, 15 and 30 °C, at 20 °C up to 7 % ethanol by volume [54]. *Heard, Fleet, Holloway et al.* were able to show that wild yeasts such as *H. uvarum* are capable of surviving the entire wine fermentation process [63, 64]. *Hanseniaspora* sp. play an important role in the production of volatile aroma-active wine components [64]. The lag phase of *H. uvarum* in strawberry juice at 15° Brix and a pH of 4.0 at a temperature of 30 °C is 3 h with a generation time of 0.9 h [34]. *H. uvarum* has been isolated in indigenous beer from Ghana, known as pito, with an ethanol content of 1.5–3.5 % v/v [118]. A unique characteristic of this beverage is that it is frequently inoculated by means of a woven belt, a so-called "inoculation belt" [118]. Bilinski et al. tested 400 yeast strains from 31 genera for antibacterial activity. Only the two species *Kluyveromyces marxianus* and *H. uvarum* were shown to be effective against *Lactobacillus plantarum* and *Bacillus cereus*. No effects were observed on Gram-negative bacteria [16]. *Hanseniaspora uvarum* belongs to the ten most frequently occurring spoilage yeast strains for fruits and beverages; other types of foods are rarely infected with this species [35].

5.5 *Issatchenkia orientalis* (anamorph *Candida krusei*)

Issatchenkia orientalis is the teleomorphic form of *Candida krusei*, is resistant to preservatives and forms a filamentous covering on the surfaces of liquids [75, 123]. The prominent surface film can contain gas bubbles, as this yeast species is capable of fermentation [158]. *I. orientalis* is resistant to sorbic, benzoic and acetic acids as well as ethanol and SO_2, but to a lesser extent than *Z. bailii* [158, 175, 177]. Furthermore, it possesses a moderate osmotolerance for sugar and salt as well as an unusually high resistance to pasteurization temperatures and strongly acidic environments [42, 122, 158]. *I. orientalis* is very widely distributed and has been found in different habitats, for example, in fresh water, on fruits, in fruit juices, cereals, sorghum beer, in Kombucha tea starters, fermented beverages and soft drinks [58, 107, 119, 133, 146, 153, 159]. A possible entry point for *I. orientalis* into non-alcoholic beverages in production facilities is via the fruit fly *Drosophila* sp. with which it is associated [158].

5.6 *Kazachstania exigua* (anamorph *Candida holmii*)

Kazachstania exigua is the teleomorphic form of *Candida (Torulopsis) holmii*, with which it shares 99.9 % homologous 18S rDNA.

Evidence of beverage spoilage is reminiscent of *S. cerevisiae*, meaning that it shows signs of fermentation and strong growth [123]. *K. exigua* is resistant to moderate concentrations of preservatives [75, 123] and is linked to the spoilage of fruit juices, carbonated non-alcoholic beverages, non-carbonated beverages containing fruit juice and beer [133]. This yeast species is only moderately osmotolerant with regard to salt and sugar and also exhibits resistance to sorbic, benzoic and acetic acids [14, 122]. Through 26S rDNA D1/D2 sequencing, it has been established that the resistance properties among the different strains vary greatly. Several strains exhibit resistance to 500 ppm of sorbic acid as well as 800 ppm of benzoic acid at a pH of 4.0 [158]. In contrast to *S. cerevisiae*, *K. exigua* cannot grow at temperatures above 37 °C, has a low ethanol tolerance and a high tolerance of acidic media [11, 122, 123]. As was described in section 2 *K. exigua* was previously a member of the genus *Saccharomyces* and the *Saccharomyces sensu lato* complex. Molecular biology identification systems are available for *Kazachstania exigua*, such as real-time PCR identification system [24, 65].

5.7 *Lachancea kluyveri*

Lachancea kluyveri is capable of fermentation and is found in fruit juices, fruit juice concentrates, soft drinks and wine. It possesses less potential for spoilage than *S. cerevisiae* [7, 35]. The pectolytic activity of *L. kluyveri* has been demonstrated in connection with the steeping of olives [35]. In addition, *L. kluyveri* is a part of the natural starter flora of tesguino, an indigenous fermented beverage made of corn from South America [152]. *L. kluyveri* belongs to the *S. sensu lato* complex and serves in genetic comparison studies with *Saccharomyces* species [22, 85, 88, 116].

5.8 *Pichia fermentans* (anamorph *Candida lambica*)

Pichia fermentans is a typical beverage-spoiling yeast species, which has been found in apple juice, beer, soft drinks, must and wine [35]. Additionally, it has been isolated from fruit and cocoa fermentations and is a typical spoilage yeast of cheese and processed meat (e.g. ground meat) [35, 50, 51, 136].

P. fermentans has a maximum growth temperature between 35 and 39 °C and is the teleomorphic form of *C. lambica* [35, 167]. Carrot extract inhibits the growth of *P. fermentans*. It is probable that nonpolar components such as free fatty acids, for example, are responsible for this effect [35].

5.9 *Pichia membranifaciens* (anamorph *Candida valida*)

Pichia membranifaciens and its anamorphic form *Candida valida* are widespread and can be found on fruits, corn and in fruit juices, sorghum beer, conventional beer, palm wine, wine from grapes and molasses [4, 77, 110, 113, 119, 120]. *P. membranifaciens* is resistant to preservatives and creates a filamentous covering on the surfaces of liquids [75, 123]. Most types of filamentous yeasts grow under aerobic conditions extremely rapidly [11]. As a yeast incapable of fermentation, *P. membranifaciens* does not pose a strong threat as a spoilage microorganism in carbonated beverages. In non-carbonated beverages with a greater surface at the liquid/gas interface, a small amount of growth on the surface is possible [7]. *P. membranifaciens* is capable of spoiling beverages due to its ability to grow at low temperatures [59]. The maximum growth temperature lies between 32 °C and 37 °C [173]. This yeast species is vulnerable to dry heat, but is more heat resistant than other yeast strains such as *S. cerevisiae*, for example [15, 144]. *P. membranifaciens* is resistant to salt, SO_2 as well as to sorbic, benzoic and acetic acids [44, 124, 158]. Insects such as fruit flies can serve as vectors for *P. membranifaciens* contamination in beverage production processes [158].

5.10 *Pichia guilliermondii* (anamorph *Candida guilliermondii*)

Pichia guilliermondii is a typical beverage-spoiling yeast species, which is found in fruit juices, soft drinks, cider, sorghum beer and wine [35]. *P. guilliermondii* also occurs in acid-preserved, slightly salted foods, in processed meats, in seafood and in baked goods [35]. *P. guilliermondii* suppresses mold growth on apples, citrus fruits and cereal grain kernels [35].

5.11 *Saccharomyces sensu stricto* sp. (*bayanus, cariocanus, cerevisiae, kudriavzevii, mikatae, paradoxus, pastorianus*)

In section 2, reference was made to the discussions surrounding the taxonomy of the *Saccharomyces* group of yeasts in the last 20 years, and the changes which have occurred over the years as well as the nine species currently part of the *S. sensu stricto* complex [88, 90, 131]. *S. bayanus, S. cerevisiae* and *S. pastorianus* are the main spoilage yeast strains responsible for problems encountered in soft drinks, whereas *S. cerevisiae* is by far the most common spoilage microorganism in non-carbonated and carbonated non-alcoholic beverages [7, 158]. To date, *S. cariocanus* has only been isolated from fruit flies (*Drosophila* sp.) and pulque, a fermented agave beverage from Mexico [133]. *S. mikatae* and *S. kudriavzevii* have only been isolated from environmental samples, such as partially decomposed leaves [133]. Of the three yeast species *S. cariocanus, S. mikatae* and *S. kudriavzevii*, which were the latest additions to the *S. sensu stricto* complex, none of the strains was isolated from food or beverages with the exception of the *S. cariocanus* CBS 5313 strain, which was isolated from pulque [133, 158]. The *S. paradoxus* available in yeast collections are mostly from environmental samples and were isolated from oak exudate, oak cell juice, tree bark and soil samples [96, 133]. Strains more relevant to beverages were isolated from the surfaces of grape skins, from spoiled mayonnaise and from fruit flies [96, 132, 133]. To date, there are no *S. paradoxus* strains available from yeast collections that have originated in spoiled beverages [133]. *S. paradoxus* and *S. cerevisiae* are very closely related and often sympatric [101]. *Maclean et al.* showed that interspecies hybrids brought about through the fusion of ascospores from both species occurs less frequently than one would expect based on the statistical information. This would be the first confirmation of a reproductive, prezygotic isolation of two very closely related yeast strains [101]. *S. cerevisiae* is a domesticated organism and is widespread due to its intensive cultivation in the various industries where it is utilized for fermentation (refer to 2.1) [158]. It stands to reason that *S. cerevisiae* has been isolated from diverse beverages and raw materials. Of all yeast strains, it has been the species most commonly found in products with low a_w and pH values [11, 35]. In facilities producing beer and non-alcoholic beverages, the brewery yeast strains are the most frequent yeasts responsible for the spoilage of non-alcoholic beverages [109]. Other potential sources of infection for non-alcoholic beverages can usually be traced to poorly cleaned bottles and insects serving as vectors for contamination [92,109]. *Back* and *Anthes* determined that 65 % of the samples tested from spoiled non-alcoholic beverages were contaminated with *S. cerevisiae* (top-fermenting brewery yeast) and *S. pastorianus* (bottom-fermenting brewery yeast) [3]. Facilities where only non-alcoholic beverages are produced and filled have significantly lower values for the number of yeast cells able to multiply, as opposed to facilities where beer and carbonated soft drinks come into contact with the same filling equipment [109]. Brewery yeast exhibits a very short adaptation time for growth in a carbonated drink medium, on average, around 5 days at temperatures of 6–20 °C. For a massive infection with bottom-fermenting yeast *S. pastorianus* consisting of 4000 cells per ml, the adaptation time is reduced to 2 days at 20 °C. For carbonated, non-alcoholic beverages containing fruit juice, the generation time of the brewing yeast after adaptation can be assumed to be around 8 hours [109]. At a starting concentration of 10 cells/ml (brewery yeast), after 5 days, the critical cell count of 100,000 cells/ml would be reached, as is evidenced by noticeable fermented off-flavors and turbidity in clear beverages [109]. Interestingly, research on the spoilage flora in the non-alcoholic beverage industry in Iraq showed that *S. cerevisiae* and *S. pastorianus* were not present as spoilage yeasts [139]. This could be explained by the absence of breweries in this region [158]. Haploid *S. cerevisiae* strains under optimal growth conditions have a generation time of 75 minutes, for diploid/aneuploid/polyploid strains it is 120 minutes [158]. Bottles explosions and the formation of ethanol is characteristic for *S. cerevisiae* spoilage [158]. *S. cerevisiae* utilizes the sugars that are typically found in non-alcoholic beverages, that is glucose, fructose and sucrose. It is capable of growth in most of these beverages. A typical fruity-fermented aroma is present, caused by the pungent aromas of fermentation by-products such as diacetyl, 2,3-pentandione, acetaldehyde, ester compounds and higher alcohols [7]. Through the formation of ethanol as a product of fermentation, the danger exists that the concentration of ethanol will exceed 0.5 % by volume, meaning that the beverage is not allowed to be sold [7]. The presence of *S. cerevisiae* ascospores increases the heat resistance of the microorganisms greatly [127]. *S. cerevisiae* is moderately osmotolerant in the presence of sugar and salt, and its vitamin requirement varies from strain to strain [11, 30, 123]. *S. cerevisiae* is a strong fermenter and some strains are resistant to preservatives [75, 123]. While most strains exhibit some resistance to sorbic and benzoic acids, certain atypical strains possess a significant resistance to preservatives and therefore a higher potential to cause spoilage [122]. The pectolytic activity of several *S. cerevisiae* strains can lead to the clearing of cloudy non-alcoholic beverages containing fruit accompanied by the formation of sediment. The S. cerevisiae strains, which occur in non-alcoholic beverages as contaminants, differ from the brewery yeast strains in their extremely high tolerance to acidic conditions, their low sensitivity to acidic disinfection agents and in part, their reduced ability to utilize maltose [7]. Top-fermenting brewery yeast, also belonging to the *S. cerevisiae* species, which come into contact with non-alcoholic and beer-mix beverages, can also act as a spoilage microorganism in these beverages [7, 69]. *S. pastorianus* strains that are present in non-alcoholic beverages as spoilage yeast, generally exhibit the same characteristics as bottom-fermenting brewery yeasts, but are often more acid-tolerant [7]. TIMKE et al. were able to prove that S. cerevisiae strains isolated from the filling areas in breweries did not show a tendency to form biofilms [163]. The same *S. cerevisiae* strains investigated in this study exhibited a high potential for beer spoilage [163].

5.12 *Schizosaccharomyces pombe*

Sch. pombe is only rarely found in soft drinks. This can most likely be attributed to the irregular occurrence of this yeast species [158]. *Sch. pombe* is a strong-fermenter, resistant to preservatives and is osmotolerant [30, 75, 123]. *Sch. pombe* has been isolated from African beers, brewing yeast, apples, apple juice, grapes, grape juice, wines, palm wines, sugar cane molasses (for rum production), in the arak production process, from cachaça mash, juice concentrates and soft drinks [11, 133, 145, 158]. *Sch. pombe* exhibits a greater osmotolerance for sugars as opposed to salt. As with *S. cerevisiae*, *Sch. pombe* is positive for the Crabtree effect and requires B vitamins and adenine for growth [11, 35]. *Sch. pombe* is resistant to the preservatives sulfite as well as to benzoic and acetic acids [123, 176, 177]. Its resistance to sorbic acid is not as strong [158]. In comparison to other yeast strains, *Sch. pombe* is very heat resistant and exhibits strong growth at 37 °C [123]. *Sch.* species are sensitive to alkaline conditions and cannot grow at a pH above 8.0 [35]. With an average generation time of 4 hours, *Sch. pombe* growth is relatively slow. This can result, in turn, in the delayed spoilage of beverages. Despite its slow growth, *Sch. pombe* is a strong fermenter and is capable of creating undesirable H_2S odors from sulfite. If *Sch. pombe* is involved in the spoilage of soft drinks, the source of contamination is frequently sugar syrups and fruit juice concentrates [158].

5.13 *Torulaspora delbrueckii* (anamorph *Candida colliculosa*)

Torulaspora delbrueckii occurs as a spoilage organism in non-alcoholic beverages; however, it is not a strong-fermenter like *S. cerevisiae* [7]. According to *James* and *Stratford*, this yeast species occurs commonly in soft drink manufacturing plants and is resistant to moderate preservative concentrations [76]. It is a close relative of *T. microellipsoides* and is osmotolerant [75]. The maximum a_w-values for a *T. delbrueckii* strain isolated from sweet bean paste, were measured for glucose, fructose, sucrose and NaCl at 0.86, 0.89, 0.87 and 0.90 [35]. *T. delbrueckii* has been isolated from apples, grapefruit, grapes, tropical fruits, as well as from raw sugar, sugar molasses, fruit juice concentrates, soft drinks, must, beer and wine [4, 35]. *T. delbrueckii* is often found on the raw materials and equipment used for the production of non-alcoholic beverages; however, it is only rarely detected in finished, filled products [35]. T. delbrueckii is also found as a spoilage yeast in bakery and milk products [35]. Lysine agar may be used to detect *T. delbrueckii*, whereas Lin's Wild Yeast Agar cannot [35]. Both types of nutrient media are used in breweries.

T. delbrueckii grows on CHROMagar *Candida*, a nutrient medium utilized in the medical field. *T. delbrueckii* produces colonies yellow in color with grey edges on this medium [32]. According to Deak and Beuchat, *T. delbrueckii* is the third most frequent yeast species observed in fruits and beverages [35]. *T. delbrueckii* is also part of the natural starter culture flora used in the production of the indigenous fermented beverage called pulque in Mexico [118].

5.14 *Torulaspora microellipsoides* (earlier known as *Zygosaccharomyces microellipsoides*)

Torulaspora microellipsoides is found in non-alcoholic beverages and is less harmful than *S. cerevisiae* [7]. According to James and Stratford, this yeast species commonly occurs in soft drink manufacturing plants and is resistant to moderate preservative concentrations [75]. The preservative concentrations currently used in the industry are sufficient to prevent product spoilage by this yeast species. If the concentrations of such preservatives were to be reduced, this species would pose a serious threat as a beverage spoiler [75].

5.15 *Wickerhamomyces anomalus* (earlier known as *Pichia anomala*, anamorph *Candida pelliculosa*)

Wickerhamomyces anomalus is widespread in the beverage industry. It is commonly found in fruit juices, fruit juice concentrate, purees, fruit pulp, fruit preparations and is a spoilage yeast in various processing steps in the production of alcoholic beverages. It is primarily responsible for intense solvent-like odors (ethyl acetate, amyl acetate) [7]. In carbonated beverages, weak-fermenting yeast strains are either incapable of growth or exhibit slight growth. In non-carbonated beverages (juices, nectars, fruit juice beverages) strong growth occurs, which can result in severe flavor and aroma defects [7]. *W. anomalus* can grow on 50 % glucose agar and, in some cases, also on 60 % glucose agar; therefore it is considered to be osmotolerant. This strain can utilize various alcohols, organic acids and starch [7]. The maximum growth temperature for *W. anomalus* is between 35 and 37 °C [35]. The lag phase of *W. anomalus* in strawberry juice with 15 °Brix and a pH of 4.0 at a temperature of 30 °C is 3 h with a generation time of 1.3 h. In malt extract bouillon with a pH of 3.5 at 30 °C, the generation time of *W. anomalus* is 2.0 h [35]. *W. anomalus* is the dominant yeast strain in the colonization of biofilms on the steel surfaces of equipment in beer filling lines [156]. *Timke et al.* were also successful in isolating *W. anomalus* strains from the filling areas of breweries and confirmed its capacity for the creation of biofilms [163]. *W. anomalus* suppresses mold growth on apples, citrus fruits and cereal grains [35]. *Laitila et al.* confirmed this finding by investigating the inhibition effects of *W. anomalus*, isolated from malt, against Fusarium species, which are suspected of causing gushing in beer [93]. Of the 12 yeast strains isolated, the *W. anomalus* strain (VTT C-04565) showed the highest antagonistic activity against field and storage flora [93].

5.16 *Zygosaccharomyces bailii*

Zygosaccharomyces bailii as well as *Z. bisporus*, *Z. lentus, Z. rouxii* and *Sch. pombe* all belong to the group of osmophilic yeasts that can grow under conditions in which the a_w-value is less than 0.85 or equivalent to that of a glucose solution with a concentration of 60 % by mass [11, 158]. *Z. bailii* is even capable of growth at an a_w-value of 0.8 at 25 °C [123]. In the production of non-alcoholic beverages, syrup bases with a high concentration of sugar are frequently used which are susceptible to infection by the osmotolerant yeasts described above [158]. *Z. baillii* is the most frequent contaminant and the most dangerous yeast in fruit concentrates. Most often, this species is detected in citrus concentrates or in concentrated apple juice (up to 73 °Brix) [7]. The spoilage potential of *Z. bailii* is enhanced by the fact that this species is a strong fermenter and is resistant to preservatives [75, 123]. This species exhibits a particularly strong resistance to slightly acidic preservatives, such as acetic, benzoic, propionic and sorbic acids. Some *Z. bailii* cells can develop into so-called “super cells” which are capable of growth in media containing double the average concentrations of sorbic acid (compared to the resistance limit of normal *Z. bailii* cells) [150]. Highly resistant *Z. bailii* strains are often isolated and possess the capability for growth in non-alcoholic beverages at concentrations above the allowable limits for preservatives in Europe (300 mg/l) [75]. *Z. bailii* possesses the ability to adapt to preservatives present at low concentrations, which then allow it to soon after develop the ability to grow in the presence of higher concentrations [75]. The following characteristics further increase the spoilage potential of *Z. bailii*: a) strong ability for glucose fermentation; b) beginning with a contamination of even one individual cell per package, the subsequent growth can result in product spoilage; c) despite significant pressure levels and the presence of preservatives, *Z. bailii* is still able to ferment glucose; d) fructose is utilized preferentially to glucose, thereby increasing the rate of growth if fructose composes more than 1 % of the ingredients in the product [41, 75].

5.17 *Zygosaccharomyces rouxii* (anamorph *Candida mogii*)

Similar to *Z. bailii, Z. rouxii* is a strong fermenter and is resistant to preservatives [75]. Furthermore, *Z. rouxii* is recognized as the most osmotolerant of all yeast strains. Only the mold *Xeromyces bisporus* exhibits a higher level of osmotolerance [123]. *Z. rouxii* is capable of growth at an a_w-value of 0.62 in fructose and at an a_w-value of 0.65 in sucrose/glycerin [162]. As a result, *Z. rouxii* is responsible for the spoilage of products containing high concentrations of sugar such as raw sugar, sugar syrups and juice concentrates [11, 162]. Its resistance to preservatives is not as strong as *Z. bailii*. Within the genus *Zygosaccharomyces*, after *Z. bailii, Z. rouxii* is the yeast species most responsible for spoilage [123].

5.18 *Zygosaccharomyces spp.* (*Z. bisporus, Z. lentus, Z. mellis*)

Comparisons of the rDNA have shown that *Z. bisporus* is very closely related to *Z. bailii* [73]. It is logical that this species shares many of the characteristics of *Z. bailii*, making it difficult to definitely identify it based on physiological methods [75]. *Z. bisporus* is also tolerant with regard to osmotic pressure differences and is resistant to preservatives. It has been determined that *Z. bisporus* possesses a slightly higher tolerance to osmotic pressure than *Z. bailii* but is less resistant to preservatives [75,162]. Although *Z. bisporus* is very similar to *Z. bailii*, this species is much less common and is not considered as much of a danger as *Z. bailii* [11]. *Z. lentus* was characterized in 1999 by *Steels et al.* [149]. It differs from *Z. bailii* and *Z. bisporus* primarily in its slow growth under aerobic conditions and inhibited growth in the presence of 1 % acetic acid. *Z. lentus* can also grow in the presence of weakly acidic preservatives and dimethyl dicarbonate (DMDC, Velcorin, E242) and is also osmotolerant [149]. Dimethyl dicarbonate is a commonly used microbial control agent found in cold-filled beverages. In contrast to *Z. bailii* and *Z. bisporus, Z. lentus* is capable of growth at low temperatures (e.g. 4 °C). This characteristic is the reason that *Z. lentus* will need to be regarded as a potential threat to cooled beverages in the future [149]. *Z. mellis* is often mistaken for *Z. rouxii* and is a known spoilage yeast in honey. In terms of spoilage in beverages, this species does not play an important role [75].

5.19 *Zygotorulaspora florentinus* (formerly *Zygosaccharomyces florentinus*)

Zygotorulaspora florentinus is capable of fermentation, grows in fruit juices and soft drinks; however, it occurs less frequently than *S. cerevisiae*. It grows more slowly than *S. cerevisiae* and it generally does not affect beverages in an extreme manner. This yeast species exhibits an extremely high tolerance for cycloheximide (actidione) [7].

6 References

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