Mgr inż. Krystian KLIMCZAK
Dr inż. Monika CIOCH-SKONECZNY
Department of Fermentation Technology and Microbiology, Faculty of Food Technology
University of Agriculture in Krakow, Poland
Katedra Technologii Fermentacji i Mikrobiologii, Wydział Technologii Żywności
Uniwersytet Rolniczy w Krakowie, Polska

UNCONVENTIONAL YEASTS IN THE BREWING INDUSTRY®

Niekonwencjonalne drożdże w piwowarstwie®

Key words: beer, yeast, non-*Saccharomyces*.

Nowadays, brewers are looking for new ways to impart original and desirable sensory qualities to their products. Hundreds of different types of malts, raw materials, spices and hop varieties are available at their disposal. Nevertheless, virtually all of the currently used brewing yeast strains belong to two closely related species: S. cerevisiae and S. pastorianus. The possibility of using yeasts from outside the Saccharomyces genus to obtain interesting sensory characteristics and to lower alcohol content has been studied extensively in the wine industry. More recently, this issue has also gained attention in the brewing industry. However, a major obstacle to the use of these yeasts is the fact that they are often characterized by insufficient ethanol production, as well as low levels of resistance to this compound. For this reason, research on the use of non-Saccharomyces yeasts focuses on selecting strains that exhibit desirable properties, or they are often used in mixed fermentations with Saccharomyces yeast.

The article presents the current state of knowledge on selected non-Saccharomyces yeasts: Brettanomyces spp., Torulaspora delbrueckii, Lachancea thermotolerans, and Metschnikowia pulcherrima, and describes their possible applications in the brewing industry.

Słowa kluczowe: piwo, drożdże, drożdże nie-*Saccharomyces*.

W dzisiejszych czasach piwowarzy poszukują sposobów na nadanie oryginalnych i pożądanych cech sensorycznych swoim produktom. Do ich dyspozycji dostępne są setki różnych rodzajów słodów, surowców niesłodowanych, przypraw oraz odmian chmielu. Mimo to, praktycznie wszystkie z aktualnie używanych szczepów drożdży piwowarskich należą do dwóch blisko spokrewnionych gatunków: S. cerevisiae i S. pastorianus. Możliwość zastosowania drożdży spoza rodzaju Saccharomyces do uzyskiwania interesujących cech sensorycznych oraz obniżania zawartości alkoholu jest od lat badana w przemyśle winiarskim. Od niedawna, to zagadnienie zainteresowało również przemysł piwowarski. Dużą przeszkodą przy zastosowaniu tych drożdży jest jednak fakt, że często ich szczepy charakteryzują się niedostateczną produkcją alkoholu, jak i niskim poziomem odporności na ten związek. Z tego powodu, badania dotyczące zastosowania drożdży spoza rodzaju Saccharomyces skupiają się na wytypowaniu szczepów wykazujących pożądane właściwości lub stosowane są tzw. fermentacje mieszane, głównie z drożdżami Saccharomyces.

Artykuł przedstawia aktualny stan wiedzy na temat wybranych drożdży nie-Saccharomyces: Brettanomyces spp., Torulaspora delbrueckii, Lachancea thermotolerans i Metschnikowia pulcherrima oraz opisuje ich możliwe zastosowanie w przemyśle piwowarskim.

INTRODUCTION

Recent decades have resulted in a multidirectional development of the brewing industry, both in the domestic and global markets. The market, which previously sought to consolidate under the banner of multinational corporations, that focused mainly on bottom-fermented beers, has undergone a breakthrough, which we now refer to as the beer revolution. This phenomenon resulted in the emergence of many new beer styles, as well as the evolution of existing ones. As a result, brewers both at home and in breweries, are looking today for newer and newer ways to achieve beers with unique sensory qualities that can meet consumer expectations. It should be noted that brewers already have a whole range of options available for this purpose. The huge variety of different malts, as well as many adjuncts, the possibility of using, among others, fruits, more than 260 varieties of hops, and spices such as hot peppers, tea, coffee, as well as many others, make it possible for an ingenious brewer to obtain a product with unprecedented characteristics.

However, one of the most important components of beer – yeast – remains virtually unchanged. Nearly all of the currently available strains of brewer's yeast belong to two closely related species: *Saccharomyces cerevisiae* and *S. pastorianus*. Individual strains often differ significantly in the

Corresponding author - Adres do korespondencji: Monika Cioch – Skoneczny, Katedra Technologii Fermentacji i Mikrobiologii, Wydział Technologii Żywności, Uniwersytet Rolniczy w Krakowie, ul. Balicka 122, 30-149 Kraków; e-mail: monika.cioch@urk.edu.pl

degree of sugar utilization, production of volatile compounds, as well as enzymatic activities, among other things. However, there is no denying that a huge biodiversity of other yeast species is left untapped. Yeast, as the organisms conducting the alcoholic fermentation process, exerts a decisive influence on the quality characteristics of the finished product. Currently available studies indicate that individual strains of non-*Saccharomyces* yeast may for example be characterized by a more intense ability to release glycosidically bound volatile compounds. In conjunction with a different profile of volatile compounds synthesis, they may allow to obtain unique beers. However, a factor that eliminates a significant portion of non -*Saccharomyces* yeasts is their low alcohol resistance, slow fermentation rates, and inability to utilize sugars found in the wort. One way to solve this problem is to conduct mixed fermentations, by inoculating the wort simultaneously with the yeast strain of the interest, and the *S. cerevisiae* strain that will complete the fermentation. However, this solution has its own problems, because, among other things, it greatly complicates the process of inoculating the yeast, and performing subsequent fermentations using recovered yeast slurry [24].

The aim of this article is to present the current state of knowledge and determine the usefulness in the brewing industry of selected yeast species from outside the *Saccharomyces* genus.

BRETTANOMYCES SPP.

Microorganisms of this genus were first isolated from beer in the United Kingdom in 1903 by Hjelte Claussen. The sensory characteristics of beers obtained using those yeast strains were typical for a significant portion of beers produced in the Britain at the time, as reflected in the genus name *Brettanomyces*, meaning "British Brewing Fungus". In the literature, species from this genus are often referred to using the name of their anamorphic form, *Dekkera*. They are characterized by a high degree of adaption to conduct a fermentation under harsh environmental conditions and show high survival rates. For this reason, they can often be a part of the undesirable microflora in the beer, wine, or biofuel industries. Yeast of this genus are also a part of the native microflora of spontaneously fermented lambic and gueuze beers, where they impart distinctive sensory characteristics. The wort used in production of those beers contains significant amounts of dextrins, which can be broken down by these microorganisms. Hence, *Brettanomyces* yeast species are detected in significant quantities during the aging of these beers. The species detected in the highest quantities at the ageing stage are *B. bruxellensis*, *B. anomalus* and *B. custersianus*. The currently available literature regarding the use of these yeast in the brewing industry focuses mainly on the first two species [4, 12, 43].

Yeast from *Brettanomyces* spp., similarly to *S. cerevisiae* exhibit the Crabtree effect, whereby aerobic respiration is repressed in the presence of fermentable sugars. Literature sources reports that *B. bruxellensis* and *B. anomalus* strains can metabolize most of mono-, di-,tri-saccharides, as well as dextrins. The ability to use dextrins, which is rare among brewer's yeasts, is attributed to intracellular and extracellular α-glucosidase activity. These enzymes allow these organisms to utilize polysaccharides, from maltotetraoses to maltoheptaoses, which typically constitute the residual sugar content of beer, when fermented with *S. cerevisiae*. Thus, they can be used to produce highly attenuated beers with higher alcohol content [22, 30].

Literature data indicates that they can produce up to 15% of ethanol. Unlike *S. cerevisiae*, they produce little or no glycerol, an ingredient that is quantitatively the third fermentation product of *S. cerevisiae*, after CO_2 and ethanol. The rate of fermentation itself is also slower than in *S. cerevisiae*. When oxygen is present in the environment, they can produce significant levels of acetic acid, but the degree of this compound production depends strongly on the strain. Their high resistance to unfavorable environmental conditions is combined with significant resistance to low pH levels. Depending on the literature, they can survive in pH equal to 3, or even 2.3. They can grow at moderate temperatures of 19-35°C. This information is confirmed by the study of Tyrawa et al. [48], where conducting wort fermentations at 28°C allowed the tested *B. bruxellensis* strains to achieve full attenuation. Fermentations conducted at 15°C were characterized by a slower process rate, and a lower degree of attenuation. At that temperature, it was the control strain *S. cerevisiae* US-05 that achieved fuller attenuation. The results indicate that fermentations which use *Brettanomyces* strains should be carried out at higher temperatures, but the exact range is strongly dependent on the strain applied [12, 21, 30, 38, 48].

Beers obtained with *Brettanomyces* yeast have distinctive sensory characteristics. This is due in part to their ability to produce a wide range of phenolic compounds (POF+). In the brewing community, the aromas of these compounds are typically associated with beers made with this yeast. Among these compounds, 4-vinylguaiacol – 4VG (an aroma described as clove-like), and 4-vinylphenol – 4VP (phenolic, medicinal) are most often listed, and are considered as most important. They have a low sensory threshold of 0.2–0.4 mg/L, and are formed by decarboxylation of hydroxycinnamic acids, 4-VG from ferulic acid and 4-VP from p-coumaric acid, respectively. Since these compounds are extracted from the malt during the mashing process, it is not possible to remove the precursors of these reactions. They are highly undesirable in most beer styles, except for a few, such as hefeweizen or lambic. The literature reports that all strains of *B. bruxellensis* and *B. anomalus* exhibit, to varying degrees, the enzymatic activities required to produce these compounds. The only strain known so far that does not produce these off-flavors was found by Colomer et al. [45]. Tyrawa et al. [48] report that the final content of these compounds in fermented beverages is probably not dependent on fermentation temperature. Although lower fermentation temperatures limit the production of these compounds, by the time fermentation is complete, the levels of volatile phenols in beers fermented at lower temperatures equalize to those found in beers fermented at higher temperatures [12, 28, 31, 45, 47, 48].

The literature sources often attribute significant activity of the β-glucosidase enzyme to species of *Brettanomyces* yeast. During hopping, odorless precursors of volatile compounds pass from the hops into the wort. These precursors can be cleaved by the action of this enzyme, releasing a volatile compound. Lafontaine et al. [27] report that the largest fraction of glycosides in hops are bound linalool (floral, coriander aroma) and α-terpineol (lilac, pine). For this reason, there has been considerable interest in recent years in β-glucosidase activities among brewer's yeast. Daenen et al. [15] report, that species of *Saccharomyces* genus currently used in brewing have a low capacity to degrade such compounds. The results presented by Colomer et al. [45] add to the knowledge of these enzymatic activities among *Brettanomyces* spp. The authors report, that this trait is strongly strain-dependent among *B. bruxellensis*, and some strains may not exhibit such activities at all. Interestingly, in this study, most of the *B. bruxellensis* strains isolated from beers had reduced enzymatic activity, compared to those isolated, for example, from wines. The strains of other species assessed in the study – *B. anomalus*, *B. custersianus*, *B. naardenesis* – showed high enzymatic activity. However, it was a single strain of *B. bruxellensis* that showed the highest ability to release glycosidically bound volatile compounds. Interestingly, the authors report that among *B. bruxellensis* strains tested, two of them had a very high ability to biotransform geraniol to β-citroneol. Levels of this compound in post-fermentation beers exceeded its sensory threshold and were comparable to those found in dry-hopped beers. Concurrently, these were the strains with the lowest levels of β-glucosidase activity. Daenen et al. [15] also did not detect uniformly high enzymatic activity in *Brettanomyces* spp., except for one strain of *B. custersii*. That strain showed significantly higher activity than any of *Brettanomyces* spp., *S. cerevisiae* and *S. pastorianus* strains tested. Recent scientific reports somewhat confirm the literature data. Indeed, yeasts of *Brettanomyces* spp. can exhibit very high levels of β-glucosidase activity, but high expression of this enzyme is relatively uncommon among individual strains [15, 26, 27, 42, 44].

Unfortunately, the current state of knowledge does not allow to present a complete characterization of volatile compound synthesis profile of these yeast. Crauwels et al. [14] report that yeast of this genus can synthesize significant amounts of acetic acid esters, with low acetates content which may be due to theirs high esterase activity. These assumptions are consistent with the results obtained by Tyrawa et al. [48]. In that study, most *Brettanomyces* strains produced higher amounts of ethyl decanoate, ethyl capronate and ethyl caprylate than *S. cerevisiae*. The latter two compounds are found in significant amounts in lambic and gueuze beers and are suspected to be responsible for the fruity and vinous character of these beers. They are also typical for this yeast. Most of these beers were also characterized by a diacetyl content above the perceptible threshold. Yeasts from the *Brettanomyces* genus impart distinctive sensory characteristics to beverages made with them, largely due to their volatile phenol content. As a result, they have been commercially available for years, to be used for the production of beers such as lambic, gueuze, or wild beers. Beers made with these yeasts can also be found on the market [11, 14, 48].

TORULASPORA DELBRUECKII

Torulaspora delbrueckii yeast has found use in the wine industry, where in 2003 those yeasts became the first commercially available non-*Saccharomyces* wine culture. They are often used in mixed fermentations, where they impart fruity aromas to wines. Studies report that in wine fermentations, this yeast allows to obtain a clean profile of volatile compounds, as well as low volatile acidity levels. There are reports that they may have been domesticated by humans as early as 4000 years ago. Over the years, the species has undergone many changes in classification. In earlier literature sources, it was classified as *Saccharomyces rosei* or *S. delbrueckii* [5, 33].

The literature reports, that yeast strains within this species are characterized by high variability in their ability to assimilate specific carbon sources. In a study by Michel et al. [32] of the 10 strains tested, all of them metabolized glucose, fructose, and sucrose, while maltose and maltotriose were consumed by only one strain. Similar results were obtained by van Breda et al. [5]. In a study by Canonico et al. [7], 8 of the 28 strains examined metabolized maltose. Equally diverse is their fermentation rate capacity, where some authors report that they may have a higher fermentation rate than *S. cerevisiae*, or much slower one, as reported by Canonico et al. [7]. In that study, pure cultures failed to achieve full attenuation, and produced about 50% less ethanol during fermentation than the control *S. cerevisiae* strain. In a study by Michel et al. [33], most strains grew well in a 5% ethanol environment, while a concentration of 10% was lethal to them. In this study, the only maltose-fermenting strain produced 4% of alcohol from 12°P wort. In contrast, Drosou et al. [17] investigated the utilization of sugars using different media that contained only one of each tested sugar. They found, that assessed *T. delbrueckii* strains produced alcohol levels not dissimilar to *S. cerevisiae* control strains, even in a medium that contained only maltose. The strains evaluated by van Breda et al. [5] in wine fermentations produced 8–13% of alcohol. In these studies, *T. delbrueckii* strains also produced higher amounts of glycerol than *S. cerevisiae*. The reports of these authors clearly indicate that the fermentation capacities of *T. delbrueckii* strains are highly strain dependent. In a study by Silva-Sousa et al. [46] the examined strains developed best at temperatures in the range of 25–30°C, but some strains showed good growth at 15°C. A temperature of 37°C inhibited the growth of most strains. Literature sources also report that, unlike yeast of the *Brettanomyces* genus, *T. delbrueckii* strains do not show production of phenolic aromas [5, 7, 17, 20, 46].

Despite many problems with the variability of the fermentation characteristics of *T. delbrueckii* strains, yeasts of this species are of interest to researchers because of their ability to impart interesting sensory characteristics to wines and beers. Canonico et al. [7] report that beers made with *T. delbrueckii* were characterized by fruity and citrus notes, and fullness of flavor. The beers contained lower concentrations of secondary metabolites formed during the fermentation process, compared to *S. cerevisiae*, with the exception of acetaldehyde. In a follow-up to this study, Canonico et al. [8] report, that mixed fermentations with *S. cerevisiae* allow to obtain higher levels of higher alcohols, ethyl acetate and isoamyl acetate. Similar results were obtained by Michel et al. [33], where flavors in beers were described as honey and pear, and also, depending on the strain, additionally plum or citrus. In a study by Einfalt [18] beers made with the addition of sorghum, fermented with *T. delbrueckii* were preferred to those made with *Metschnikowia pulcherrima*, mainly due to a sweeter flavor that may have resulted from the lower attenuation levels found. The presence of β-glucosidase activity is an ambiguous issue. Despite the failure to detect such activities among the 5 strains evaluated by Escribano et al. [19], other authors such as Azzolini et al. [2] and Maturano et al. [29], have found it to be higher than in *S. cerevisiae*. An interesting feature of this yeast is its high ability to biotransform terpenoids derived from hops. King et al. [26] report, that *T. delbrueckii* showed the ability to produce geraniol from linalool, which was then converted to α-terpineol. There are also reports that they can synthesize some of the terpenes *de novo*. These activities may be responsible for the characteristic aroma of beers obtained using those yeast. The relatively high proportion of maltose-fermenting strains, the interesting sensory characteristics of products made with their application, as well as the significant production of ethanol and resistance to that compound make this yeast species an interesting candidate for beer production. Unfortunately, there are no commercially available strains of *T. delbrueckii* aimed for brewing applications [2, 7, 8, 18, 19, 26, 29].

LACHANCEA THERMOTOLERANS

Lachancea thermotolerans, formerly known as *Kluyveromyces thermotolerans* has attracted the interest of researchers in the wine industry for its ability to significantly lower the pH of wine, resulting in a pleasant acidity, thanks to the production of significant amounts of lactic acid. Production of this compound takes place at the beginning of the fermentation process, and due to the consumption of sugars in the process, its synthesis comes at the expense of ethanol. Therefore, when using strains characterized by a significant production of this compound, the resulting beers and wines are characterized by lower ethanol content. The production of lactic acid, as well as the interesting sensory characteristics impaired by this yeast, make them the subject of research in the fermentation-oriented industries. As of today, one of *L. thermotolerans* strains is commercially available. It's aimed to produce sour beers without the use of lactic acid bacteria, and it shows a high level of attenuation due to its ability to utilize maltose [34].

Literature data report that this yeast utilize glucose and sucrose. The ability to utilize maltose is a variable trait within the species. In a study by Domizio et al. [16] 3 of the evaluated strains utilized maltose to a similar extent as *S. cerevisiae*, but none of them utilized maltotriose. The strain tested by Callejo et al. [6] fermented maltose to a low degree, but again, did not utilize maltotriose. Nevertheless, in a study by Domizio et al. [16], beer obtained with *L. thermotolerans* was characterized by only 6–12% lower alcohol content, than one prepared with *S. cerevisiae*. A similar relationship was observed by Zdaniewicz et al. [50], where beers fermented with *L. thermotolerans* strain with low lactic acid production phenotype were characterized by lower alcohol levels (4.25–4.3%), and higher residual sugar content than those made with the *S. cerevisiae* control strain (5.22–5.37%). These results may confirm reports that they have a lower fermentation capacity than *S. cerevisiae*. *L. thermotolerans* strains show tolerance to up to 5–9% ethanol in the environment. Morata et al. [34] report, that species of this yeast has a similar temperature requirement to *S. cerevisiae*. Literature data indicate that *L. thermotolerans* exhibit higher levels of glycerol synthesis than *S. cerevisiae*, which is confirmed by studies by many authors, such as Domizio et al. [16], Zdaniewicz et al. [50] and Hranilovic et al. [25]. As mentioned, a unique feature of *L. thermotolerans* is the production of significant amounts of lactic acid. In sour beers made with lactic acid bacteria, the content of this compound is usually in the range of 3–6 g/L. In wine fermentations conducted by Hranilovic et al. [25], the strains produced 1.8 (±0.2) – 12.0 (±0.2) g/L of this compound. In the case of brewing, levels of lactic acid in beverages obtained by Domizio et al. [16], Zdaniewicz et al. [50], and Canonico et al. [9] were much lower, 0.1–0.25, 0.01 (±0.00) – 0.06 (±0.07) and 1.83 (±0.07) g/L, respectively. The authors' results indicate that the strains show significant differences in the production of this compound. So far, the strains of *L. thermotolerans* that have been examined did not show production of phenolic off-flavors [6, 9, 10, 16, 25, 34, 50].

In a study by Domizio et al. [16], aromas of the beers obtained with *L. thermotolerans* were described as fruity, floral, clove, melon, strawberry and sour. Unfortunately, the authors did not examine whether the aroma described as clove was due to the presence of volatile phenols. Similar fruity and spicy aromas, in the case of wines, were reported by Morata et al. [34]. Beers obtained by Zdaniewicz et al. [50] with *L. thermotolerans*, compared to *S. cerevisiae*, were characterized by a lower content of all analyzed esters except ethyl 2-methyloctanoate (higher content) and ethyl acetate (similar). The beers also differed significantly in the profile of higher alcohols. In contrast, in a study by Canonico et al. [9], beers obtained with *L. thermotolerans*, were characterized by a higher content of ethyl acetate. The aroma of beers produced with these yeasts may also be partly attributed to their enzymatic activities. Among the strains tested by Escribano et al. [19], 6% of *L. thermotolerans* strains exhibited β-glucosidase activity. Comitini et al. [13] also reported the detection of such activities among 2 of the 5 strains tested. In a study by Zdaniewicz et al. [50], the examined strain reduced the limonene, perillene, nerol and humulene present in the wort, and the previously absent citronellol, β-damascenone and nerolidol appeared in the beer. In addition, the concentrations of other terpenes were higher than in the beer produced with *S. cerevisiae* yeast. These activities, that result in the transformation of terpenes may be partly responsible for the aroma of beverages obtained with these yeast [13, 19, 50].

The interesting sensory characteristics of beers produced with this yeast species, combined with the possibility of using some of the strains to produce sour beers, or those with reduced alcohol content, mean that *L. thermotolerans* strains are currently being studied for their application in the brewing industry.

METSCHNIKOWIA PULCHERRIMA

The yeast from the *Metschnikowia pulcherrima* species were first isolated in 1884, by Ilya Metchnikoff. They were initially classified as *Monospora bicuspidata*, but their classification has changed over the years. A characteristic feature of these microorganisms is the production of red dye, called pulcherrimin, which permeates into the substrate during their growth. Due to their broad antagonistic activity against molds, among others, they are used in natural plant protection products. At the same time, they do not produce mycotoxins or allergens, so they are not harmful to human health. These yeasts are often detected at the initial stages of wine fermentation, due to the fact that they constitute the native microbiota of fruits. Strains from this genus are characterized by the production of a wide range of extracellular enzymes, such as pectinases, proteases, β-glucanases, β-glucosidases, cellulases, cellobiases and amylases. Because of these activities, they are the subject of research, mainly in the wine industry, where they are used mainly in mixed fermentations. As of today, strains of *M. pulcherrima* are the least studied organisms among all those mentioned, especially in the case of brewing [36].

Literature data indicate that this yeast can consume glucose, sucrose, fructose, galactose and maltose as a carbon source. Abeln et al. [1] confirm the assimilation of maltose and maltotriose. In contrast, in fermentation trials conducted by Postigo et al. [37] and Rodríguez Madrera et al. [39], *M. pulcherrima* did not utilize these two sugars. In the literature data, it can be found that they have low fermentation capacity. In the case of wine fermentation, they produce about 4% of alcohol. However, in a few studies by other authors, higher values occur, as in study by Sadoudi et al. [41], where the *M. pulcherrima* strain allowed to produce a wine with 10.89 (±0.32) % of alcohol. So far, studies on their use in brewing are as inconclusive as those from winemaking. In studies by Postigo et al. [37] and Rodríguez Madrera et al. [39], the alcohol concentrations in beers after fermentation were 0.62 (±0.03) and 0.60 (±0.00)%, respectively. In those studies, they also had lower glycerol production than the control *S. cerevisiae* strain, despite reports that they can increase glycerol concentrations in wines. Different results were obtained by Einfalt [18], where fermenting wort with addition of sorghum, the *M. pulcherrima* strain allowed to produce a beer with 33.7 (±0.5) g/L ethanol, compared to *S. cerevisiae* 37.9 (±0.5) g/L. So far, this is the one of few available examples of such a high alcohol content in beverages obtained with this yeast. The fact that these yeasts are detected only at the initial stages of must fermentations is explained in the literature by their low resistance to the presence of ethanol in the environment. However, in a study by Barbosa et al. [3], 62 of the 65 assessed strains showed growth at alcohol concentration of 6%, while 4 strains expressed growth at a concentration of 9%. An important feature of these yeasts is the expression of aerobic metabolism. With the right degree of oxygenation, they can utilize more than 40% of the available sugars in respiratory processes, effectively reducing the amount of alcohol in the finished product. For this reason, they are being extensively studied in wine fermentations, as they could allow to obtain wines with lower alcohol content. In a study by González-Arenzan et al. [23], half of the studied strains had activities that condition the production of phenolic aromas. Similar results were obtained by Escribano et al. [1, 3, 18, 19, 23, 37, 39, 41].

As mentioned, the species *M. pulcherrima* has attracted the attention of researchers due to their broad spectrum of enzymatic activities. In a study by Escribano et al. [19], 63% of the strains showed β-glucosidase activity. A similarly high proportion is presented by Bedrinaña et al. [35] – 73% and Barbosa et al. [3] – 62 of 64 tested strains were found to possess this trait. Another group of compounds that in recent years have gathered the attention of researchers are the precursors of volatile sulfur compounds, found in both beer and wine. It is suspected that due to the β-lyase activity, they can be released into volatile forms, often characterized by a pleasant, fruity aroma and a very low sensory threshold. In a study by Barbosa et al. [3], all of the tested strains showed this activity, and 17 of them did so to a very high degree. In a study by Zott et al. [51], *M. pulcherrima* strains allowed the release of higher amounts of 3SH in wines, than the control *S. cerevisiae* strain. Einfalt [18] reports, that beers obtained with *M. pulcherrima* were characterized, compared to *S. cerevisiae*, by a lower content of 3-methylbutanolol, 2-phenylethyl alcohol, propanol, 2-methylbutanol, isoamyl acetate and 2-phenylethyl acetate, and a higher level of isobutanol. A similar, significant isobutanol content was observed by Postigo et al. [37], in sequential fermentations with *S. cerevisiae*, while the concentration of isoamyl acetate was higher than in the control sample. In this study, in sequential fermentations, beers obtained with *M. pulcherrima* were preferred over those obtained with *Hanseniaspora valbyensis*, *H. guilliermondii*, *Zygosaccharomyces bailii*, *Torulaspora delbrueckii* and *Wickerhamomyces anomalus*. In addition, in literature, there are many reports from the wine industry on the positive effects on the sensory characteristics when used in mixed fermentations with *S. cerevisiae*, such as those published by, among others, Varela et al. [49], Ruiz et al. [40] or Sadoudi et al. [41]. These reports indicate that, as in the case of winemaking, *M. pulcherrima* yeast may be of particular interest for use in mixed fermentations. Additionally, thanks to the ability to oxidize sugars they may find application in the production of alcohol-reduced beers [3, 19, 35, 37, 39–41, 49, 51].

CONCLUSIONS

In recent years, a number of researchers have undertaken a search for new brewing and wine yeast, among the non-*Saccharomyces* species. Although few of the existing microorganisms have been studied thoroughly so far, reports of their properties suggest that within the next few years, some of them may find application in the production of fermented beverages. The sensory qualities that are possible to obtain in beers thanks to their use already made some of the strains commercially available, as in case of *Brettanomyces* spp. and *Lachancea thermotolerans*. The relatively high fermentation capacity of *T. delbrueckii* and the broad spectrum of enzymatic activities of *M. pulcherrima* species make them an interesting candidate for the use in the brewing industry.

PODSUMOWANIE

W ostatnich latach wielu badaczy podjęło się szukania nowych drożdży piwowarskich i winiarskich wśród gatunków spoza rodzaju *Saccharomyces*. Mimo że, jak dotąd przebadano niewiele spośród istniejących mikroorganizmów, doniesienia o ich właściwościach pozwalają sądzić, że w ciągu następnych lat część z nich może znaleźć zastosowanie w produkcji napojów fermentowanych. Możliwe do uzyskania z ich użyciem cechy sensoryczne sprawiają, że już dziś w przypadku piwowarstwa dostępne komercyjnie są szczepy *Brettanomyces* spp. oraz *L. thermotolerans*. Stosunkowo wysoka zdolność fermentacyjna *T. delbrueckii* oraz szerokie spektrum aktywności enzymatycznych *M. pulcherrima* sprawiają, że mogą one również znaleźć zastosowanie w przemyśle piwowarskim.

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