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Torulaspora delbrueckii: Towards Innovating in the Legendary Baking and Brewing Industries

Ángel De La Cruz Pech-Canul, David Ortega,
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Abstract

Baking and brewing are among the oldest bioprocesses refined by human societies. Both fermentative processes have successfully used domesticated strains of *Saccharomyces cerevisiae* in their process as the biocatalyst throughout their evolution. However, the dominance of *S. cerevisiae* has limited the capability for diversification of many organoleptic properties of the final product, such as aroma and flavours. The use of non-*Saccharomyces* yeasts can be an enormous source of opportunities for innovation in both fermentative processes. *Torulaspora delbrueckii* is a ubiquitous yeast species, and numerous strains have been isolated from many different bioprocesses. The strains of *T. delbrueckii*, once considered microbial contamination, have recently shown several advantages over *S. cerevisiae* strains, including higher ethanol tolerance; better capabilities to consume wort sugars; higher resistance to hop/pH/osmotic stress; and freeze-thaw resistance, among others. This chapter aims to present a comprehensive review of frontier research on *T. delbrueckii* regarding its potential and prospects for the baking and brewing industries.

Keywords: alcoholic beverages, beer production, baking industry, brewing industry, *Torulaspora delbrueckii*

1. Introduction

Bread and beer are among the oldest foods in the human history. The consumption of both of these fermented products has been rooted as a basic human food, and at the present time, these are still among the most consumed foods around the world. The ubiquity of their production allowed a diversification and development of refined, artisan techniques, which currently comprises innumerable recipes [1, 2]. All the recipes include essentially the same basic ingredients such as cereals, yeast, and water. However, the organoleptic properties (aroma, flavours, etc.) of the final product differ greatly between recipes.

Since early times, both cereals and water were identified as fundamental ingredients for the preparation of beer or bread. Despite the fact that these ingredients have been recognised as essentials for centuries, the experimental approaches developed by Pasteur during the mid-nineteenth century revealed the existence of a third element much more essential to the fermentation process: yeast. The fermentation performed by yeast is undoubtedly the oldest and the largest biotechnology

application. There are many types of yeast strains used for fermented foods commonly known as commercial strains: baker's yeast in bread production and brewer's yeast in beer fermentation. After centuries of selection, due to the refinement of the fermentation processes, today it is easy to find a wide variety of dedicated yeast strains that are suitable for different types or styles for either beer or bread.

The yeast strains responsible of these fermented foods are able to ferment sugars present in the flour or in the wort (starch, glucose, fructose, sucrose, and maltose, among others), which is concomitant with the production of other molecules such as CO₂, the main causes of dough leavening and the natural carbonation of beer. The type of yeast strain used in the fermentation process also greatly influences the properties displayed in the final product such as the texture of the dough and the flavour, and ideally, certain yeasts can also add some nutritional values to the final product [3].

The capabilities of the yeast strains to grow fast (fast propagation) and to produce a valuable product are still currently exploited by the industrial field. Depending on the final product, the yeast strains also possess other advantages, such as high tolerance to stresses caused by high sugar concentration or the drying/freezing of dough present in baker's yeast [2]. On the other hand, brewer's yeast which is similar to wine's yeast offers particular characteristics to the fermentation process such as an optimal flocculation, high ethanol tolerance, and rapid growth at high osmolarity [4].

Since the beginning, the food industry has mainly used *Saccharomyces* or closely related strains, which could be a result of the ubiquity of this genus. As consequence, scientific research efforts have been focused mainly on *Saccharomyces cerevisiae*, a yeast that nowadays is still the most commonly used microorganism in baking, brewing, and other fermented foods [5, 6]. Furthermore, *S. cerevisiae* has also been historically used as a model organism for the research on fundamental aspects of eukaryotic cell biology. The popularity of *S. cerevisiae* in the food industry has led to the standardisation of the organoleptic properties of the final product, which limits the capability to improve or to create new properties for the final product.

The use of non-*Saccharomyces cerevisiae* yeasts, also known as “nonconventional” or “non-commercial” yeast, offers new alternatives for the development of products with improved properties, such as biomass yield, distinct flavour complexity, aroma profile, and other advantages. Among nonconventional yeasts, *Torulaspora delbrueckii* (formerly *Saccharomyces rosei* or *S. rosei*) has been considered of oenological interest for decades.

At the present time, *T. delbrueckii* is starting to be recognised as a model organism of study because most of its biological features, like its sugar metabolism, differ greatly from the more prevalent *S. cerevisiae* [7]. These differences provide many advantages of biotechnological importance in several fermentative processes; for example, it is quite interesting how *T. delbrueckii* can adapt its physiological state in order to endure the harsh conditions present during the frozen dough preservation [8]. On the other hand, the use of *T. delbrueckii* in the brewing industry has had a positive effect on the aroma and taste of the final alcoholic beverage [9–11]. The industrial brewing process has appreciated several features of this yeast strain that also complement its suitability for the fermented food preparation processes, such as its higher ability to retain cell viability under stress conditions, high ethanol tolerance, an increased osmotic resistance, and the advantageous capability to yield a final product with lower levels of unpleasant acetaldehyde, acetoin, acetate, and ethyl acetate [12]. Fermentation with *T. delbrueckii* also provides an excellent example of improvement in the industrial process with the consequent creation of new value-added products. In the present chapter, we review the current and potential applications of *T. delbrueckii* strains in the bread and brewing industries discussing their physiological perspective.

2. The genetics of *Torulaspora delbrueckii*: an overview

Torulaspora delbrueckii has unique abilities when compared to other yeasts; the use of this microorganism has many advantages yet to be potentially exploited in the biotech industry. The progress in understanding its physicochemical and physiological mechanisms of action has been hampered by the lack of genetic and molecular tools specific for this yeast. The development of tools for metabolic engineering of a specific microorganism is essential for the improvement of industrial processes. Once the microorganism of interest has been isolated and identified, one of the first steps for the development of the genetic tools is to determine its exact genome sequence. With the advent of modern, state-of-the-art equipment and facilities dedicated to unravel the DNA sequence from diverse biological samples; nowadays, the sequencing of small genomes (<50 MB) has become even cheaper and more reliable than just a decade ago.

A pioneering work presented in 2002 described the isolation of genes from *T. delbrueckii* and their subsequent heterotrophic expression using *Saccharomyces cerevisiae* as a host [13, 14]. From this work, the first yeast genomic library of *T. delbrueckii* PYCC532 was derived. In 2011, a draft genome sequence of *T. delbrueckii* CBS 1146 was published as part of a molecular evolution study [15]. The CBS 1146 strain has been preserved since 1970 under laboratory conditions and thus raises the question regarding the potential differences when compared to a fresh strain isolated from an active alcoholic fermentation. A complementary study that addressed this question was published recently in 2015 [16], where a set of differential genes were identified. The most recent sequencing projects have had much better coverage than preliminary studies. Most recently, other *T. delbrueckii* genomes have been published (Table 1). For example, the first complete genome map of *T. delbrueckii* COFT1 has

Strain	Source	GenBank accession	Genome assembly level	Reference
CBS 1146 (NRRL Y-866)	Unknown. Isolated by the Wallenstein Lab., No. 129, 119,077. Deposited by the NRRL	GCA_000243375.1	Chromosome	[15]
COFT1	Isolated from the spontaneous wine fermentation at the Yalumba Wine Company (Angaston, Australia)	CP027647.1 to CP027655.1	Whole-genome map	[17]
NRRL Y-50541	Isolated from the mezcal-fermenting process at Oaxaca, Mexico	CP011778 to CP011785	Chromosome	[16, 18]
SRCM101298	Isolated from fermented food by Sung Ho Cho, Microbial Institute for Fermentation Industry, South Korea	GCA_002214845.1	Contig	[19]

Table 1.
Genomes sequencing projects developed for *Torulaspora delbrueckii*.

been recently assembled, having been isolated from a spontaneous wine fermentation from Australia. Its genome consists of eight chromosomes and one mitochondrial chromosome. Altogether, these published data sets are proving to be useful as they guide the design of experimental approaches to study enzymes involved in the biosynthesis and/or the catabolism of biotechnological molecules of interest.

The discovery of the circular plasmid pTD1 from *T. delbrueckii* CBS 1090 provided an excellent platform for the development of modern genetic tools. The circular pTD1 (4.8 kbp) is an unusual finding in the diverse yeast genera that normally possess linear plasmids [20]. The plasmid pTD1 belongs to the 2- μ m family, and it has been shown that the members of this plasmid family keep a high-copy number with minimal impact on the host. Recently, a model was suggested for the 2- μ m protein complexes required for plasmid partitioning and transcriptional regulation [21]. pTD1 also contains an AT-hook motifs (residues 222–234) which are small DNA-binding modules with a preference for binding AT-rich DNA [22] and have been frequently identified in a large number of proteins, such as transcription and chromatin remodelling factors, such as the yeast centromere-binding protein and Mif2 DNA replication origin recognition factor Orc2, respectively [21, 23]. Two decades after the discovery of pTD1, its importance has been recently brought to light when a modified version for the TD recombinase (variant TD1–40) was employed to the development and analysis of two Flp-like site-specific recombination systems. The evolved variants of the TD recombinase, unlike the wild-type enzyme, were suitable for genome engineering in *Escherichia coli* and mammalian cells [24]. The authors also suggest that its application could be potentially extended to plant and insect cells.

The allele-coupled exchange (ACE) and the allelic exchange (AE) technologies are becoming more common for the manipulation of microorganisms for biotech purposes. For example, researchers lead by Professor Nigel Minton at the Synthetic Biology Research Centre (SBRC) (University of Nottingham, UK) have successfully dedicated their efforts to develop the ACE and the AE technologies in diverse *Clostridia* strains and other Gram-positive bacteria [25, 26]. The genetic manipulation of *T. delbrueckii* was initially achieved using the genetic tools developed originally for *S. cerevisiae*. The improved gene disruption method for *T. delbrueckii* was performed using the classic PCR-based disruption cassettes, one of the most commonly used strategies for gene targeting in *S. cerevisiae* and other organisms [12]. Eventually, ACE was adapted for baker's yeast *T. delbrueckii* IGC5323 and was one of the goals for the development of genetic tools towards the metabolic engineering of *T. delbrueckii* [27].

At the present time, many attempts have been made to expand the number of selection markers available for the construction of new molecular tools for *T. delbrueckii*; however, the pursuit of selection markers remains unsuccessful [12]. A successful auxotrophic selection marker was the creation of a screening system under glucose as sole carbon source. The deletion of LGT1 gene, identified as coding for a hexose transporter, was successfully conducted, despite the fact that the deletion did not impaired glucose uptake ability [28]. According to previous works, it is likely that the genome of *T. delbrueckii* contains other hexose transporters [29]. Other approaches are still being developed towards genome editing, and trends indicate that eventually the modern CRISPR-CAS9 technique could be adapted for *T. delbrueckii*, just as it was recently fully adapted for *S. cerevisiae* [30].

3. From the lab to the kitchen: efforts towards the incorporation of scientific research into the baking and brewing industries

Results derived from scientific research have positively influenced many aspects of human wellbeing, such as food production. Many food industries are currently

interested in innovation through the usage of *T. delbrueckii* strains in their fermentative processes. Consequently, the brewing and baking industries are starting to use nonconventional yeast strains in their processes.

Effective biomass production from molasses is a crucial aspect to consider in the selection of baker's yeast for the industrial process. Baker's yeast is able to propagate and to create biomass from sugarcane molasses, which contains mainly glucose, fructose, and sucrose as well as trisaccharide raffinose [31, 32]. On the other hand, invertase activity is crucial for the hydrolysis of disaccharide into free glucose and fructose monomers, required for yeast growing on molasses.

Yeast strains with high invertase activity exhibit a low production of CO₂ in the sweet dough. This last observation can be explained by the rapid entry of free sugars into the cells causing a massive increase in the osmotic pressure that affects the cellular homeostasis which eventually slows glycolysis [33–35].

Moreover, both trehalose and glycogen are known as the major store of glucose into *Saccharomyces cerevisiae* cells. A rapid increase of the levels of these sugars is an early metabolic response during conditions of oxidative, heat, or salt/osmotic stresses. It has been proposed that these sugars have several physiological functions. For example, the accumulation of these sugars has a function as glycolytic safety valves to escape “substrate-accelerated death”: a phenomenon that follows when the starved yeast is exposed to the substrate, which limits their growth [36–39].

Oppositely, *Torulaspora delbrueckii* strains under hyperosmotic/frozen stresses into sweet dough have shown the ability to adapt promptly to high-osmotic-pressure environments which correlates in part with a low-invertase activity, as well as a slow rate of trehalose mobilisation, displaying a higher accumulation of trehalose than *S. cerevisiae* [8, 27]. Moreover, the intracellular trehalose accumulation, induced by desiccation of *T. delbrueckii*, protects the yeast cell and presents a lower oxidative stress, which correlates with a higher fermentative capacity, when compared to other non-conventional yeasts [40]. The trehalose content may possibly be correlated to their types of trehalase. Strain D2–4 was found to harbour two types of trehalase activities, which have different optimum reaction pH at 4.3 and 6.7. On the other hand, the freeze-sensitive mutant strain 60B3 bears additional activity at pH 5.7, which adds a third type of trehalase activity in that sensitive mutant. The change of trehalose content during the growth of the freeze-sensitive 60B3 strain was directly correlated with the change of the trehalase activity at pH 5.7. Despite the fact that higher trehalose levels are always correlated with higher stress resistance, experimental results suggest that other factors are involved in the maintenance of stress resistance [41–43].

As mentioned above, the metabolism of *T. delbrueckii* and *S. cerevisiae* differs greatly, such as their regulatory mechanisms in the adaptive response to salt stress [44]. The gene ENA1, encoding a Na⁺-ATPase, is a key determinant in the *Saccharomyces cerevisiae* tolerance when it is exposed to salt stress as high concentrations of extracellular toxic monovalent cations (i.e. Na⁺ and Li⁺) [45]. The regulation of ENA1 required for cell survival is mainly under the control of three diverse signaling pathways, (1) high-osmolarity glycerol (HOG), (2) cell wall integrity (CWI), and (3) calcineurin/Crz1p pathways [46–48]. Glycerol production is essential for *S. cerevisiae* to deal with osmotic stresses. Hog1 phosphorylation occurs as a response to osmotic stress, and the phosphorylated Hog1 interacts with transcription factors to modulate its gene expression patterns during stress responses [49, 50].

In order to understand the high-osmolarity glycerol (HOG) pathway in *T. delbrueckii*, the Hog1 homologue in *T. delbrueckii* (*TdHog1*) was characterised as well as its mutant versions. The expression of GPD1 was not strongly affected in the *TdHog1*-deleted mutant. Also, *S. cerevisiae* and *T. delbrueckii* have divergent regulatory mechanisms that control glycerol accumulation under a moderate osmotic stress condition [51]. As a consequence of glycerol accumulation, in anaerobiosis,

glycerol production of *T. delbrueckii* represents around 6% of the total carbon flux, a value that is comparable to *S. cerevisiae* under the same conditions. However, acetic acid levels are increased which allows for reduction of NAD⁺ generated during glycerol formation in *S. cerevisiae* as a side-effect of the overexpression of GPD1 and/or GPD2 in the presence of high-glycerol concentration. A significant relationship between glycerol and acetic acid production was not found in *Torulaspora delbrueckii* [52, 53]. When an excess of acetic acid is produced in alcoholic beverages such as a beer or wine, it provides a characteristic vinegar-like flavour or sour taste [54, 55]. A higher percentage of acetic acid also results in decreased CO₂ production [9, 56–58].

Saccharomyces cerevisiae exhibits an adaptive response to cell wall stress mediated by cell wall integrity MAPK signalling factor Slt2, which once activated triggers Rlm1 phosphorylation [59]. Additionally, the *slt2Δ* mutant allowed for the discovery of a novel regulatory mechanism associated with PKA inhibition [60, 61]. During the study of *hog1* mutant from *S. cerevisiae*, it has been reported that Slt2p is rapidly dephosphorylated (1 min). On the other hand, a Tdhog1 mutant from *T. delbrueckii* showed a significant reduction of the phospho-Slt2p signal, and it was delayed around 30–60 min [51].

The induction of the ENA1 gene is mediated by calcineurin which plays a role in the dephosphorylation of Crz1. Two Crz1-binding regions have been identified into the ENA1 promoter; the antagonistic regulation of ENA1 is via protein kinase A (PKA) that is involved in the Crz1 phosphorylation [62]. Additionally, phosphatases Ppz1 and Ppz2, individually or both, influenced the expression of ENA1. For example, a higher increase of the ENA1 expression in Ppz1 mutants was observed which was correlated to an increase halotolerance. Interestingly, Ppz1 showed to be dependent of an intact calcineurin/Crz1-signalling pathway to keep its ENA1 promoter activity [63]. During the analysis of TdCRZ1, the homologue to CRZ1 in *Torulaspora delbrueckii*, the authors tried to enhance the salt tolerance of *S. cerevisiae* HS13. So, the TdCrz1p was linked to the freeze tolerance capability, a very important feature with great potential for industrial applications [44].

4. Yeast cocultures: *Torulaspora delbrueckii*—combining to enhance the product quality

At the present time, commercial strains of *Torulaspora delbrueckii* are used in the bread and brewing industries and are summarised in **Table 2**. The success of *T. delbrueckii* relies mainly on the superior flavour that its fermentation can provide to the final product and also in its incredible ability to withstand stress conditions described previously. These properties will lead to improve the product quality and enhance the flavour complexity. However, there are still some challenges to overcome to employ a pure culture of *T. delbrueckii* in industrial fermentations.

Name	Supplier	Industry		Reference
		Bakery	Brewery	
Biodiva™TD291	Lallemand		✓	[64]
ZYMAFLORE® ALPHA ^{TD n. sacch}	Laffort		✓	[10]
H299–18	Nikka Uisukii KK	✓		[65]
NS8422 (FERM P-12709)	Nikka Whiskey KK	✓		[66]

Table 2.
A brief list of *Torulaspora delbrueckii* commercially available.

For this reason, these yeast strains are usually employed in co-fermentations with *Saccharomyces cerevisiae* and/or other yeast strains [9, 10]. For example, in mixed fermentations, it plays an important role to enhance its organoleptic properties such as flavour, aroma, and a final product with low-alcohol content [9].

The current key market players for global yeast market are listed below:

- AB Mauri
- Biospringer
- Chr. Hansen Holding A/S
- Lesaffre
- AB Vista
- Alltech
- Angel Yeast Co., Ltd.
- Biorigin—Art in Natural Ingredients
- DSM N.V.
- ICC
- Kerry Group
- Lallemand Inc.
- Leiber GmbH
- Minn-Dak Yeast Company
- Ohly
- Oriental Yeast Co., Ltd.
- Pacific Ethanol, Inc.
- Pakmaya
- Suboneyo Chem. Pharmaceuticals Pvt. Lim.
- Synergy Flavours

The flavour profiles of co-fermentations in brewing showed significant differences, revealing a species-dependent relationship. Analysis of the main volatile compounds on the beers produced by *T. delbrueckii* and *S. cerevisiae* in the pure cultures and their simultaneous co-fermentation at different ratios was performed. *T. delbrueckii* was able to increase the levels of higher alcohols, where β -phenyl ethanol was excluded. These results are opposite to the data obtained in winemaking, where lower levels of higher alcohols are detected. β -phenyl ethanol and ethyl

butyrate levels were lowered when a major presence of *T. delbrueckii* was present in the inoculum ratio [9–11]. A yeast population change has been correlated with the inoculum size ratio and the type of cell-to-cell interactions that occur during mixed culture fermentation. The faster decay of *T. delbrueckii* during cocultures with *S. cerevisiae* is notable below a 2:1 ratio. The last observation could be related to multiple factors, for example, due to the *T. delbrueckii*'s killer activity: a kind of immune system that secretes protein toxins, which become lethal to other yeast species [67, 68].

Sequential inoculation of *T. delbrueckii* with *S. cerevisiae* has shown higher levels of desired aroma compounds compared to simultaneous inoculation, exemplified by the enhanced production of 4-vinyl guaiacol (clove-like aroma) [69].

Unlike the production of beer and wine, co-fermentation with *T. delbrueckii* during dough preparation has been barely explored; only simultaneous co-fermentations have been performed. *T. delbrueckii* JK08 and *P. anomala* JK04 exhibited a superior flavour and mouthfeel, respectively, as well as an improvement in the colour, when compared to *S. cerevisiae*. Even when these strains did not fulfil the exact criteria of commercial baker's yeasts [70], the use of these three yeasts together has been proven to enhance the bread quality [70].

5. Conclusion

The history of fermented foods has long accompanied the evolution of the human civilisation. While the ingredients may have changed, their selection has also been shaped to meet new consumer demands. In fact, over centuries, man has refined the art of fermented food by pure empirical observation, for example, through the selection of the best cereals with better performing characteristics or through the domestication of yeast strains. Despite the fact that the fermented food industry has mastered the domestication of *Saccharomyces cerevisiae*, the use of nonconventional yeast strains has proved to be advantageous for the fermented food industry.

Once considered a microbial contamination, *Torulaspora delbrueckii* has been shown to have a positive influence on several organoleptic properties for the final products of the brewing and bread industries. For example, when this nonconventional yeast is used in the brewing industry, the aromatic profiles and flavours of the beer are enhanced; and some strains have the peculiarity to produce naturally less-ethanol content while retaining all its improved properties. Additionally, many of its other biological features were conveniently suitable for the industrial processes, such as its robustness potential to resist frozen/thaw stress. These advantages will be useful for biotechnological exploitation that will lead to innovation of new products or even to process improvement for the food industry. As usual in new trends in science, to date, the information regarding *T. delbrueckii* genetics and its metabolic pathways remain scarce. Fortunately, the scientific community is starting to focus their efforts to deeply understand the nature of this splendid genus. Continued scientific research will gradually lead to the accumulation of more data that will impact positively the fermented food industry.

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Conflict of interest

The authors declare that they no conflicts of interest.

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