Non-Conventional Yeasts as Alternatives in Modern Baking for Improved Performance and Aroma Enhancement

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Abstract: *Saccharomyces cerevisiae* remains the baker's yeast of choice in the baking industry. However, its ability to ferment cereal flour sugars and accumulate CO_2 as a principal role of yeast in baking is not as unique as previously thought decades ago. The widely conserved fermentative lifestyle among the *Saccharomycotina* has increased our interest in the search for non-conventional yeast strains to either augment conventional baker's yeast or develop robust strains to cater for the now diverse consumer-driven markets. A decade of research on alternative baker's yeasts has shown that non-conventional yeasts are increasingly becoming important due to their wide carbon fermentation ranges, their novel aromatic flavour generation, and their robust stress tolerance. This review presents the credentials of non-conventional yeasts as attractive yeasts for modern baking. The evolution of the fermentative trait and tolerance to baking-associated stresses as two important attributes of baker's yeast are discussed besides their contribution to aroma enhancement. The review further discusses the approaches to obtain new strains suitable for baking applications.

Keywords: alternative baker's yeast; non-conventional yeasts; stress tolerance; aroma profile; carbon metabolism

1. Background

Saccharomyces cerevisiae, the "conventional" baker's yeast, is the most decorated industrial workhorse of all times [1–6]. Due to its monopoly in the baking sector, beverages and, recently, in the pharmaceutical industry coupled with its long list of credentials of being the pioneer in a number of areas, it is deemed indispensable [4,5]. The principal role of this yeast in baking is the fermentative degradation of simple sugars found in flour dough, generating CO_2 required for leavening of dough during fermentation and rising of the dough during baking [6–10]. This yeast also exhibit a secondary role of a baker's yeast, associated with formation of desirable secondary products of fermentation such as higher alcohols, aromatic esters, fatty acids, and other organic compounds which are responsible for aromatic and sensorial properties of bread [11–14]. In addition to the fermentative role, a baker's yeast of preference should exhibit additional phenotypes such as ability to tolerate baking-associated stresses and ability to assimilate or ferment other sugars found in flour dough [15–18]. Yeasts encounter numerous stresses during preparation of biomass and fermentation such as osmotic stress, thermal stress, salinity stress, oxidative stress, air-drying stress, freezing and thawing stress, ethanol stress, and others [16–19]. However, the conventional baker's yeast is sensitive to many of these baking-associated stresses [9,17], has a streamlined carbon substrate utilisation base [20,21], and has poor aromatic profiles [22] as its major drawbacks. Given these three major drawbacks, it still baffles consumers and the scientific community why the conventional baker's yeast has

remain as the baker's yeast of choice. Its long historical intimacy with humankind could have earned itself its GRAS (Generally Regarded As Safe) status (FDA, USA) and its QPS (Qualified Presumption of Safety) status (European Food Safety Authority, EFSA) rather than its outright suitability. New research into the existence of other yeasts, also known as non-conventional yeasts, suggests that there are many other yeasts with the same credentials that qualifies them as alternative baker's yeast [6,7,10,23,24]. The increasing interest in diversity of bread and farinaceous products and the demand for natural baking ingredients in the modern markets, driven by diverse consumer preferences, is prompting the review of potential non-conventional yeasts as alternatives to the conventional baker's yeast, *S. cerevisiae*. By 2025, the global yeast market size is estimated to reach about USD 6 billion, which is an annual growth rate of about 10% from the year ending 2020 [25]. The increasing demand of novel bakery products further exacerbates the need for alternative baker's yeast.

Against this background, this review is aimed at giving an extensive overview of the potential of non-conventional yeasts for use as alternative baker's yeast. The shortfalls as well as challenges encountered by the conventional baker's yeast are discussed. In addition, the baking credentials such as the fermentative ability and its evolution among the *Saccharomycotina*, as the primary role of a baker's yeast, is briefly discussed. Key stress tolerance traits in non-conventional yeasts as advantageous traits and strategies to improve the desirable traits towards development of robust non-conventional baker's yeasts are summed up, especially regarding fermentation ability and aroma improvement.

## 2. Non-Conventional Yeasts as Leavening Agents: Gaps, Limitations and Challenges

The credentials behind the primary and secondary roles of a baker's yeast are not exclusive to the conventional baker's yeast *S. cerevisiae* [6,10,24,26,27]. The traits are widespread among non-conventional yeasts, a circumscription of "other yeasts" [28] comprising of 11 genera of non-*Saccharomyces* yeasts (Figure 1). Yeasts from these genera have been extensively studied and are known to have evolved alcoholic fermentation traits (Figure 2) [6,10,26,29]. During alcoholic fermentation, CO_2 is also accumulated as a by-product of decarboxylation of pyruvate [30,31], a trait desirable in baking. Extensive characterisation of fermentative physiology of several non-conventional yeasts exhibiting the ability to ferment irrespective of the presence of O_2, also known as Crabtree effect, conducted by Hagman and his co-workers [32] suggests that there is a huge dough leavening potential among non-conventional yeasts as shown in Figure 1. It then baffles the consumer and scientific community as to why the conventional baker's yeast continues to monopolise the baking industry. Elsewhere, other researchers have even isolated several non-conventional yeasts from sourdough to further suggest the immense potential of non-conventional baker's as alternative baker's yeasts [33–35]. Other than the fermentative capacity as the primary role, there is a requirement for baker's yeast with aromatic diversity to replace synthetic aromas in baked farinaceous products [36]. The poor aromatic profile exhibited by the conventional baker's yeast [22] is a major metabolic drawback in modern consumer lifestyles. In addition, the use of chemical leavening agents has become common but the increase in chemophobic individuals and production of off-flavours are punitive [37]. The former circumscribes a new target group for the search of alternative baker's yeasts with natural aromatic flavours. Other than the two attributes discussed above, conventional baker's yeast has been extensively described as characterised by a poor performance under baking-associated stressful environments [8,15]. The conventional baker's yeast fermentative performance is heavily impaired by the presence of high sugar doughs, which exert a high osmotic pressure [38,39].
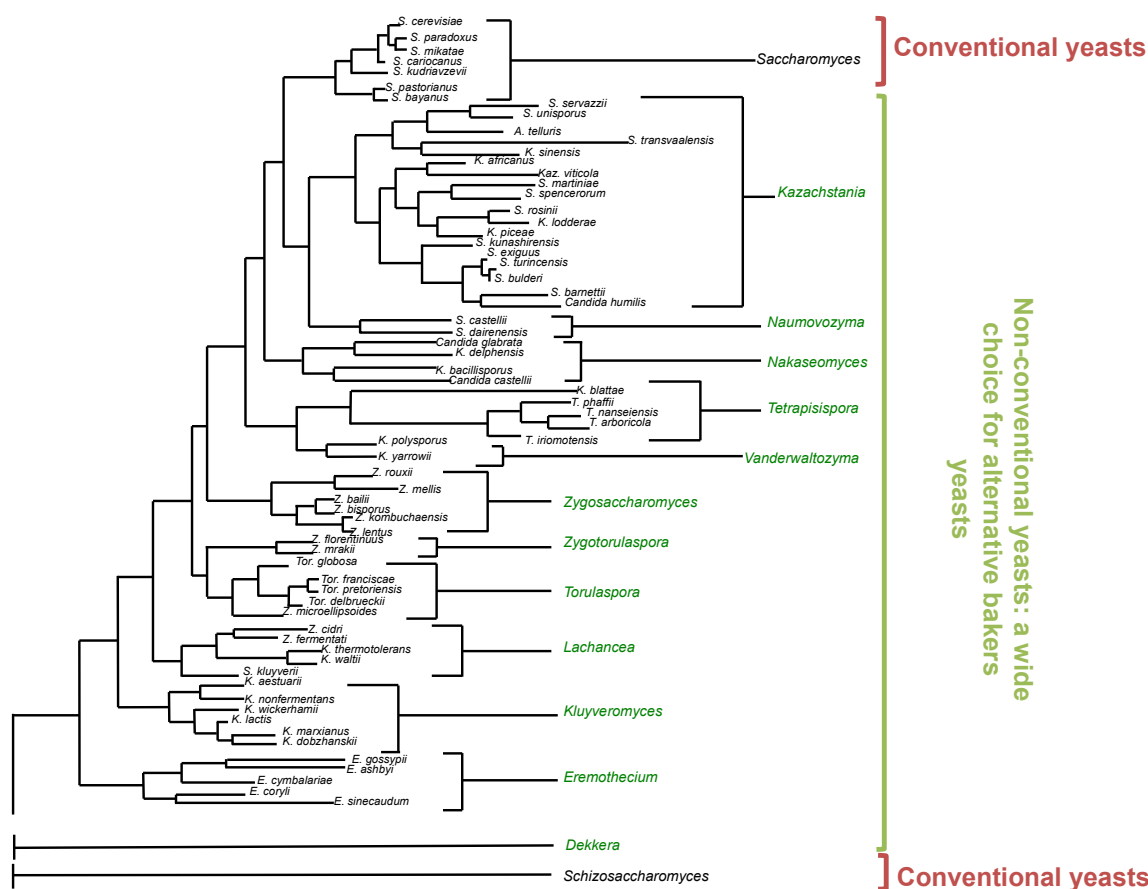
Figure 1. Phylogenetic position of non-conventional yeasts. This figure was adapted from [6]. The figure shows 12 clades resolved by a concatenation of gene sequences reported [40]. Non-conventional yeasts and their attributes of importance as alternative baker's yeasts are highlighted in green, whereas the conventional yeasts are highlighted in red.

Despite its monopoly of the baking industry, the conventional baker's yeast has a streamlined carbon substrate utilisation range [20,21]. Native strains of *S. cerevisiae* cannot utilise carbon sources found in flour such as starch and xylose [21,41–44]. For bread with reduced amounts of starch, costly gelatinisation and enzymatic liquefaction would be required. In addition, other farinaceous products often include use of milk for emulsification purposes. A major concern is the inability of the native conventional yeast to ferment lactose due to the absence of amylase or glucoamyase activity (lacks the β-galactosidase) [21,45,46]. Since there are increasing incidences of individuals in the human population who become lactose intolerant with age [47], an inability to ferment lactose by the conventional baker's yeast is a major drawback for the quality of the bakery products. Such inabilities heavily impact on the carbohydrate content and, subsequently, the bread quality as many sugars remain in the final product. There has been an increasing trend to utilise inexpensive carbon sources to reduce the costs of modern biomass production. An inability to utilise lactose in abundant and inexpensive cheese whey waste streams is a major cause for concern. It is also noteworthy that some *S. cerevisiae* strains lack the α-galactosidase enzyme and therefore cannot utilise melibiose [48], a disaccharide accumulated after the breakdown of raffinose when molasses are used for biomass production [21]. This inability reduces the production efficiency and hence affecting cost-effective productivity of the biomass production process. Furthermore, poor tolerance to metabolic stress due to the presence of accumulated ethanol, organic acid production, and reactive oxygen species, characteristic of conventional baker's yeast, is a major challenge [49]. Extensive research suggests that most of these major challenges evident in the usage of the conventional baker's yeast can be ameliorated by use of non-conventional yeasts whose desirable attributes as baker's yeast have come to light [6].

However, there are two challenges, on the other hand with exploitation of non-conventional yeasts as baker's yeast. One major obstacle, probably due to a poor history of domestication, information on their safety designated as GRAS or QPS status is lacking. However, a majority of non-conventional yeasts are increasingly being isolated from traditionally and spontaneous fermented foods, which bears testimony of their silver lining [50,51]. In addition, with the advent of advanced technologies in standard toxicology and clinical trials, the safety of non-conventional yeasts can be conclusively established rather than just based on substantial historical safe use evident in the use of *S. cerevisiae* as the conventional baker's yeast. A second obstacle is the limited availability of information required to establish if these non-conventional yeasts are virulent. Information on many of these non-conventional yeasts is, however, becoming readily available due to the advent of omic technologies. The number of available genomes of non-conventional yeasts, both privately and publicly owned database, has increased three-fold in recent years [52–54]. The knowledge of genomic architecture for understanding molecular events, metabolic pathways, and their associated regulatory mechanisms is indispensable for developing tools and other processes important for their exploitation in the baking industry [55,56]. The combination of genomic and physiological data is increasingly becoming instrumental to allow inferences of genomic features and metabolic pathways with a potential in baking applications [57]. Such information was trivial for the conventional baker's yeast for many decades, but the power of comparative genomics on non-conventional yeasts could also allow us today to study molecular mechanisms and undesirable genes that could be responsible for virulence or emergence thereof. Recent advances in genome editing technologies, such as CRISPR-Cas9 [58] which can be used to modify large spectrum of genomes, could also allow specific targeting of genes and pathways with potential in baking to allow expansion of desirable baking traits among non-conventional yeasts. Such gaps, limitations, and challenges pertinent to the search for non-conventional yeasts are discussed in the next sections.
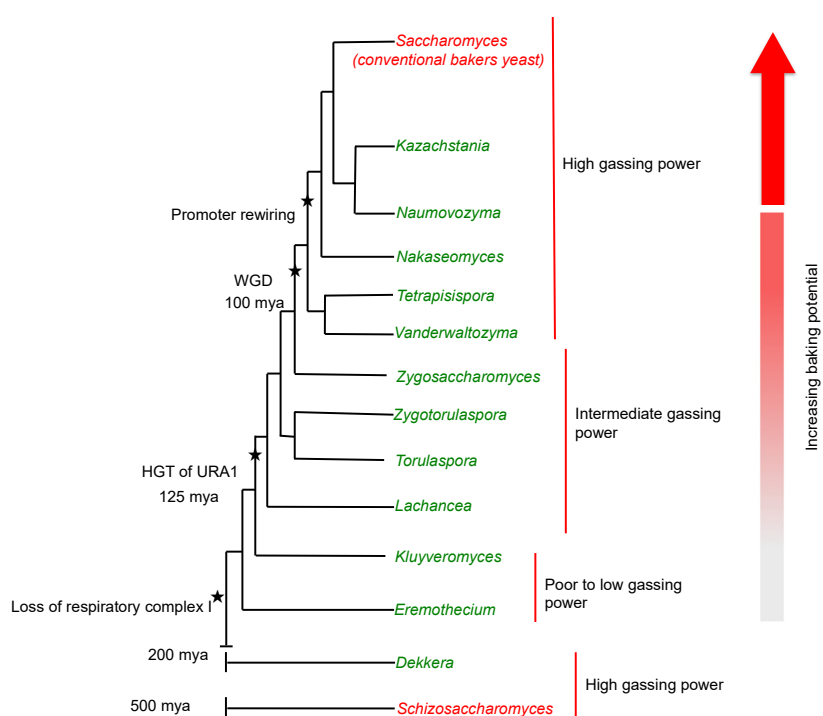
Figure 2. Alternative yeasts and molecular events behind their respirofermentative lifestyle. The figure is redrawn from [6]. Promoter rewiring and loss of rapid growth elements [59], WGD [60], the horizontal gene transfer of *URA1* [61] and the loss of respiratory complex I [62] as molecular mechanisms responsible for fermentative metabolism are shown. Specific phylogenetic positioning and ability to ferment under aerobic conditions (Crabtree positivity) reflects the work by [32]. Green colour shows non-conventional/alternative baker's yeasts.

3. Fermentation of Multiple Simple Sugars Found in Flour Dough: A Key Baking Yeast Trait Is Conserved in Non-Conventional Yeasts

Wheat flour contains a wide range of fermentable monosaccharides and disaccharides, with glucose, galactose, and fructose as monosaccharides and sucrose and maltose as disaccharides [63]. The baking potential of baker's yeast is directly linked to the ability to ferment these sugars [64]. The ability of yeasts to assimilate even a small amount of a variety of all available sugars could enhance the leavening productivity as well as its economic potential. Utilisation of sugars occurs in this respective order: glucose, sucrose, galactose, fructose and, lastly, maltose in *S. cerevisiae*. The first four carbon sources are utilised within an hour of dough fermentation [65,66]. The conventional baker's yeast utilises maltose at the very end of dough fermentation as the most predominant sugar in dough without sucrose added and, thus, the leavening ability is closely linked to the fermentation of maltose [67,68]. Fermentation of simple sugars is well conserved in the *Saccharomycotina* and in the distant relatives that separated 200–500 mya, the *Dekkera/Brettanomyces* and *Schizosaccharomyces pombe* lineages (Figure 2). The fermentative capacity, which directly translates to the gassing power of the baker's yeast, although well pronounced in the *Saccharomyces* genera of which the conventional baker's yeast is found, is not unique [26,32,69]. Many non-conventional yeasts that separated from *Saccharomyces–Lachancea Kluyveromyces–Eremothecium* lineages about 125–150 mya exhibit the alcoholic fermentation lifestyle [26,32]. This trait, extensively described as the Crabtree effect, suggests that there are multitudes of non-conventional yeasts with potential as alternative baker's yeasts. Extensive comparative genomics work suggests that all non-conventional yeasts harbouring the *URA1* gene and that underwent whole genome duplication (WGD) as well as promoter rewiring have the potential to be used as baker's yeast (Figure 2). The baking potential increases with decreasing phylogenetic distance to the conventional baker's yeast (Figure 2).

4. Assimilation of Multiple Complex Sugars in Flour Dough: A Desirable Trait in Baking Yeasts Is Patchily Distributed in Non-Conventional Yeasts

In addition to simple fermentable sugars, non-fermentable oligosaccharides such as glucofructans and trisaccharides such as starch, raffinose and glucose and fructose are also found in wheat flour [70]. Starch is the principal carbohydrate in plant seeds such as wheat and rye, often used for making flour [71]. However, native strains of the conventional baker's yeast cannot break down starch [21]. The addition of fungal amylases to wheat flour for degradation of starch, increasing the amounts of maltose in the dough [72], is common among bakers [73,74]. Starch-degrading traits among non-conventional yeasts such as *Sacharomycopsis fibigulera*, *Debaryomyces castelli*, *Cryptococci* spp., *Candida famata*, *Aureobasidium pullulans*, and *Clavispora lusitanie* has been reported [75]. Other sugars that could be important in influencing the outcome of the bread, for example in whole wheat bread where the outer layer of kennel (bran) is also used, are cellulose and hemicellulose. Again, these sugars cannot be utilised or fermented by *S. cerevisiae* [18]. Cellulases and hemicellulases are exogenously added during baking to release simple sugars [76]. The addition of enzymes during baking increases the cost of production of bread. "One step forward, many steps backwards", the conventional baker's yeast strains again cannot naturally utilise pentose sugars released upon degradation of hemicellulose such as xylose, arabinose, and mannose [77]. By contrast, fermentation of these carbon sources has been reported in non-conventional yeasts such as *Pichia stipitis*, *Pichia kudriavzevii*, *Schfferomyces shehatae*, *Candida tropicalis*, and others [78]. A number of approaches to enhance fermentation by the introduction of specific baking traits into the conventional baker's yeast via pathway bioengineering have been reported [79]. However, consumers' negative perception and acceptance remains the major obstacle in the use of genetically modified strains in the baking industry. Use of yeasts with such desirable genetic traits to reduce the final producer price of bread and farinaceous products remain a priority.

5. Robust Stress Tolerance of Non-Conventional Yeasts: A Key Trait Sought for in Alternative Baking Yeasts

The baker's yeast encounters a number of environmental and metabolic stressors during preparation of biomass and during dough fermentation [15]. Osmotic stress, thermal stress, salinity stress, oxidative stress, air-drying stress, freezing and thawing stress, and ethanol stress, among others, are typical examples. These stresses potentially damage cellular components, which subsequently leads to a reduction in fermentation performance. Non-conventional yeasts, as previously reported, possess beneficial robust stress tolerance when compared to *S. cerevisiae* [6,80]. *S. cerevisiae* is characterised by poor resistance to many of these baking-associated stresses [9,81]. Process optimisations such as using chemical stress protectants as ingredients and formulations to improve the quality of bread are well documented [82]. Several desirable traits of non-conventional yeasts have been documented suggesting extreme stress tolerance phenotypes such as ethanol tolerance, osmotolerance, thermotolerance, halotolerance, and freezing and thawing tolerance, among others (Table 1). These non-conventional yeasts are increasingly becoming attractive alternatives for baking as well as other different industrial bioprocesses as their extremophilic stress tolerance traits improve the fermentation performance and subsequently improve the techno-economics in the baking industry. Tolerance under different stressors among non-conventional yeasts is discussed in the next sections highlighting current literature.

Table 1. Overview of non-conventional yeasts and their relevant traits for baking.

Trait	Yeast Species
Gassing power	*Wickerhamomyces anomalus* [80,83] *Wickerhamomyces subpellicullosus* [10] *Torulospora delbrueckii* [83,84] *Torulospora pretoriensis* [85] *Kluyveromyces marxianus* [66,86] *Picha kudriavzevii* [83,87,88] *Kazachstania gamospora* [10] *Kazakstania humilis* [83] *Kazachstania exigua* [89] *Brettanomyces (=Dekkera) bruxellensis* [69,80,90] *Kluyveromyces marxianus* [91]
Thermotolerance	*Wickerhamomyces anomalus* [80] *Metschnikowia pulcherima* [92] *Wickerhamomyces subpellicullosus* [10] *Kluyveromyces marxianus* [86,92,93] *Pichia kudriavzevii* [88] *Torulospora delbrueckii* [49,94] *Pichia kudriavzevii* [87,92,95] *Lachancea thermotolerans (Hino* et al. 1987) [96] *Candida thermophila* [95]
Osmotolerance	*Wickerhamomyces anomalus* [97] *Wickerhamomyces subpellicullosus* [10] *Torulospora delbrueckii* [84,92] *Metschnikowia pulcherima* [92] *Zygosaccharomyces spp* [92] *Pichia kudriavzevii* [98] *Kazachstania gamospora* [99] *Brettanomyces (=Dekkera) bruxellensis* [100] *Kluyveromyces marxianus* [101]

Table 1. *Cont.*

Trait	Yeast Species
Halotolerance	*Wickerhamomyces anomalus* [102] *Wickerhamomyces subpellicullosus* [10] *Torulospora delbrueckii* [87] *Pichia kudriavzevii* [87] *Brettanomyces (=Dekkera) bruxellensis* [100] *Kluyveromyces marxianus* [101] *Debaryomyces hanseni* [103]
Ethanol tolerance	*Wickerhamomyces anomalus* [92] *Lachancea thermotolerans* [92] *Saccharomycoides ludwigii* [92] *Wickerhamomyces subpellicullosus* *Zygosaccharomyces rouxii* [92] *Torulospora delbrueckii* [104] *Pichia kudriavzevii* [87,92] *Hanseniaspora valbyensis* [92] *Brettanomyces (=Dekkera) bruxellensis* [90] *Kluyveromyces marxianus* [105]
Freezing and thawing stress tolerance	*Torulospora delbrueckii* [84,106] *Zygosaccharomyces rouxii* [106] *Saccharomyces rosei* [106] *Kluyveromyces thermotolerans* [106]
Wide range of sugar utilisation	*Kluyveromyces marxianus* [80,107] *Pichia kudriavzevii* [108]
Aroma complexity	*Wickerhamomyces anomalus* [109] *Saccharomycopsis fibigulera* [109] *Wickerhamomyces subpellicullosus* [10] *Torulospora delbrueckii* [110] *Pichia kudriavzevii* [109] *Kazachstania gamospora* [10] *Kazachstania humilis* [109] *Kazachstania exigua* [89] *Kazachstania zonata* [111] *Brettanomyces (=Dekkera) bruxellensis* [112] *Kluyveromyces marxianus* [7]

5.1. Osmotolerance

Bread can be made as lean or sweet dough, which depends on the concentration of added sucrose in flour. Lean dough is characterised by no sugar or very little amounts of added sucrose, whereas sweet dough contains approximately 25–40% sucrose [18]. High sugar concentrations exert an unwarranted osmotic stress to the baking yeast, hindering its optimal fermentative capacity [18,113]. The conventional baker's yeast's poor fermentative capacity in sweet doughs has been extensively characterised [8,15]. Osmotic stress leads to rapid cell dehydration and a reduction in gassing power [114]. Fermentation of sweet dough increases the relative concentrations of reactive oxygen species (ROS), further reducing the fermentative capacity of the baking strain [115]. The presence of osmotolerant strains among non-conventional yeasts that can survive such concentrations has been reported in literature [92]. Recent research highlights that not all non-conventional yeasts possess this attribute [75] but increasing number of non-conventional yeasts are continuously being explored. Table 1 shows a few osmotolerant non-conventional yeasts.

5.2. Thermotolerance

Thermotolerance of yeasts is an important attribute of interest in the baking industry. Despite the fact that dough preparation and fermentation are carried out at mesophilic temperatures, baker's yeast may encounter very low or very high temperatures during baking

and storage [15]. Downstream processing for preparation of dried baker's yeast biomass involves air-drying, where hot air temperature can easily increase to over 37 °C [18]. The increase in temperatures during preparation and storage of biomass may affect many factors such as misfolding of proteins, change in vacuolar pH, and malfunction of mitochondria [18,116]. In addition, during fermentation of the dough, elevated temperatures could become another obstacle. There is a reduction in fermentation efficiency of *S. cerevisiae* strains grown at high temperatures. An increased fluidity and permeability of membranes increase the sensitivity of yeast cells to organic acids, which then impairs fermentation [117]. In addition, high temperatures denature and inactivate enzymes, leading to an imbalance of metabolic activities together with those specific for fermentation of dough. Thermotolerance is a rare trait among non-conventional yeasts. The trait is required for biomass production process but not exclusively for fermentation during baking. Such a trait is desirable in biomass production to reduce costs associated with cooling after sterilisation of growth media as well as to reduce contamination [118]. Yeast strains usually tolerate only a narrow mesophilic temperature range and, so far, only a few species such as *Kluyveromyces marxianus* and *Ogataea polymorpha* have been found to present fermentation capability at temperatures above 40 °C [119]. Other species with thermotolerance ability have been reported (Table 1).

5.3. Freezing and Thawing Stress Tolerance

Freezing and thawing stress tolerance is another desirable characteristic of a baker's yeast. The practice of storing frozen dough is very common among bakeries since historical times. This follows the need to reduce the costs of labour associated with starting the dough every time it is needed. In modern bakeries, frozen doughs are indispensable for providing a constant supply of oven-ready frozen doughs to the consumers [18,120]. However, freezing and thawing conditions are detrimental to the conventional baker's yeast [121]. Freezing and thawing physically damages cellular components, which leads to reduction in fermentation capacity [18,120,122]. Studies have shown that freeze-injured yeasts have an impaired leavening capacity, leading to poor bread quality [122]. ROS are also generated during freezing and thawing, which further exerts oxidative stress on the fermentative yeasts [121]. Attempts to develop freeze-tolerant strains of the conventional baker's yeast using mutation procedures and recombinant DNA technologies have, however, yielded strains with poor flavour profile [122]. Yeast trains that retain their fermentative and flavouring abilities after freezing and thawing are highly desirable. A few examples of non-conventional yeasts with freezing and thawing stress tolerance are presented (Table 1).

5.4. Ethanol Tolerance

One of the most important attributes of the industrial workhorse, *S. cerevisiae,* in baking as well as in other processes, such as beer brewing and wine making, is its ability to ferment sugars and accumulate ethanol and CO_2. However, the accumulation of ethanol negatively impacts the fermentative performance of the yeast. Ethanol is toxic as it denatures enzymes required for alcoholic fermentation such as hexokinases and dehydrogenases [123] and alters cellular lipid and unsaturated fatty acid concentrations, subsequently impairing growth and fermentation [124]. Ethanol tolerance is a desirable and beneficial trait in non-conventional yeasts with potential as baker's yeast. Many studies have reported yeasts with such an ability (Table 1).

5.5. Oxidative Stress Tolerance

During production of biomass and during fermentation of dough, oxidative stress is a major concern in the baking industry. ROS generated during normal aerobic cellular metabolism are known to exert oxidative stress. ROS inactivate proteins, damage nucleic acids, denature proteins, and damage mitochondrial membranes [18,125–129]. Serial repitching of fresh dough with previously fermented dough is a common practice among traditional bakeries. However, due to the deterioration of the baker's yeast due to the pres-

ence of ROS, this practice is not ideal because of increased cellular ageing and replicative lifespan after every pitch cycle [130,131]. Thus, non-conventional yeasts with oxidative stress protection mechanisms are attractive. Oxidative stress can also be exerted by the presence of other stressors such as freezing and thawing and air-drying, as discussed in the respective sections above. Yeasts with such desirable attributes among non-conventional yeasts are tabled (Table 1).

6. Aromatic Diversity of Non-Conventional Yeasts: A Key Trait Sought for in Baking Yeasts

The aroma profile constitutes an important quality parameter of bread affected not only by the baking ingredients but also by the secondary metabolites produced by yeasts during fermentation, such as esters, aldehydes, and ketones [9,132,133]. Aroma can also be derived from the Maillard reaction between reducing sugars and amino acids [134] as well as from lipid oxidation [135]. Nevertheless, microbial metabolic pathways are considered the main source of aromas in bread and other food products [133].

Yeast species principally contribute to the leavening of flour dough and aroma of bread [136]. However, *S. cerevisiae* produces a limited diversity of aromas, whereas certain non-conventional yeasts recently tested in baking have shown a huge potential in this sense, especially those present or isolated from sourdoughs [7,10,22,136,137]. In sourdoughs, generally, there is a single predominant yeast species, but the diversity among different doughs is known to be relatively high. Around 30 different species have been isolated. The genera *Saccharomyces* (*S. cerevisiae*), *Kazachstania* (*K. humilis*, *K. exigua*), *Wickerhamomyces* (*W. anomalus*), *Pichia* (*P. kudriavzevii*), and *Torulaspora* (*T. delbrueckii*) are the most isolated and geographically widespread in Asia, Europe, as well as in Africa and Australia in certain cases [136]. Studies detailing the use of non-conventional yeasts in bakery are still limited, but certain authors have made several attempts in this regard either using non-conventional yeasts as pure cultures or in combination with lactic acid bacteria [7,10,137,138]. Aslankoohi et al. [7] evaluated the ability of eight non-conventional yeasts as alternatives for bread fermentation as well as two non-baker's *Saccharomyces* species, all of them isolated from food. Among the evaluated yeasts, *T. delbrueckii* and *S. bayanus* presented adequate dough fermentation ability and novel flavour profiles, as presented by trained sensory panellists. The obtained bread employing *T. delbrueckii* was described as having a more complex, nutty, forest-like flavour reminiscent of some bread types resulted from spontaneous fermentation, whereas the bread prepared using *S. bayanus* exhibited a more aromatic profile, characterised by fruity notes, when compared to the control. GC–MS analyses showed that the bread produced by *T. delbrueckii* and *S. bayanus* presented several volatiles in higher concentrations than the control, some of them being already described as relevant aromas in bread crumb, such as 1-heptanol, 2-phenyl ethanol, benzaldehyde, heptanal, ethyl octanoate and phenylacetaldehyde [132,133,139,140]. *T. delbrueckii* bread contained more aldehydes and ketones (carbonyl compounds) than the control, suggesting that these compounds were reduced to their corresponding acids, alcohols, and esters at a lower rate. In addition, other aroma compounds produced by these non-conventional yeasts were not present in control bread. This indicated that non-conventional strains produce different concentrations of certain aroma compounds and they can also be responsible for whole new set of aroma compounds that are not synthesised by the conventional baker's yeast [7]. Zhou et al. [10] tested seven non-conventional yeasts isolated from environment and food for their baking potential. These authors found that when the dough was leavened with the alternative baker's yeasts, the buttery, nutty, and fruity aromas were significantly more pronounced than in the control bread prepared using conventional baker's yeast. In particular, bread produced by *Kazachstania gamospora* and *Wickerhamomyces subpelliculosus* displayed more complex aroma profiles and obtained better overall results in sensory analyses. In addition, these strains presented higher stress tolerance to sugar and salt [10].

Studies involving combinations of non-conventional yeasts and lactic acid bacteria have also been carried out as mentioned above. Plessas et al. [138] tested diverse starter cul-

tures for sourdough bread making using different combinations: (i) the non-conventional yeast *Kluyveromyces marxianus* as pure starter culture; (ii) *K. marxianus* in mixed culture with the lactic acid bacteria *Lactobacillus delbrueckii ssp. bulgaricus*; (iii) *K. marxianus* in mixed culture with the lactic acid bacteria *Lb. helveticus*. Fermentation employing natural sourdough microflora was used as a control. The aroma of the resulting bread after employing mixed cultures was more complex as evidenced by the higher number of aroma compounds identified. GC–MS analyses showed the presence of relevant volatiles to bread quality in these cases, such as 2-nonen-1-ol, 3-nonen-1-ol, benzyl alcohol, and furfural as well as a variety of esters. In addition, bread produced by mixed cultures reached the highest overall quality scores in sensory evaluation [138]. Another study tested *Meyerozyma guilliermondii* and *P. kudriavzevii*, together with *Lb. sanfranciscensis* isolated from Chinese liquor Daqu. These microorganisms were used as mixed starter cultures on sourdough bread making. The outcome was an improved consumer acceptance and flavour complexity due to a higher production of esters, aldehydes, and other aroma compounds [137].

7. Improvement of Non-Conventional Yeasts for Baking

Improvement of production strains is important to increase the product yields and productivity required for economic viability. There are multiple strategies to develop yeast strains towards specific industrial characteristics [79,141]. Current methods can be either through non-genetic modification (non-GM) or genetic modification (GM) strategies.

8. Non-GM Strategies

When the developed strains are for use in the food production sectors, consumer acceptance is of utmost importance. Genetic improvement of food grade yeasts is controversial [142]. Non–genetic modification techniques used to improve strains are therefore attractive. The isolation of yeasts from their natural environment or screening from collection centres is one way to explore the wealthy biodiversity of yeast. Another strategy is to artificially develop such yeasts using classical and emerging methods with potential to alter productivity of the strains. However, this method's major drawback is the limit or absence of natural yeasts with such desirable characteristics. Yeasts are known to be only adapted for survival and reproduction in their niches, which are completely different from the highly stressful production environments, such as those encountered during baking, brewing, or many other industrial processes [79]. Such native phenotypic traits are therefore often not easily transferred to baking applications. Thus, maximising phenotypic traits through artificial strain development strategies is attractive. Evolutionary engineering is one of the simplest yet very attractive and powerful ways that exploit the natural biodiversity by selecting robust strains for specific industrial processes. This technique, also known as adaptive laboratory evolution, exploits the plasticity of microbial genomes. When a selective pressure is applied, it confers a specific selective advantage to evolving yeasts, leading to derived mutants presenting an industrially relevant trait as set by the investigator [79,141]. The selective advantage of derived strains is often responsible for an increased growth rate and decreased death rate as well as increased retention in culture [143]. This technique has been shown to be important for improvements of wild type and yeast strains engineered towards stress tolerance, ability to consume new substrates, and increased product formation rates [79,141]. This strategy is highly advantageous in certain situations over rational metabolic engineering. For example, not much genetic background information for the trait of interest is required before evolution [144]. In addition, this method is considered a non-genetic modification strategy and, hence, the evolved strains developed using this technique will have no challenges with consumer acceptance [145–147]. Furthermore, limited laboratory equipment is required, serial transfer in simple shake flasks is an inexpensive approach to develop strains.

9. GM Strategies

The commercial application of genetically modified yeasts is attractive as alternative baker's yeast. Randez-Gil et al. recommended the use of recombinant DNA technology to develop strains with improved baking attributes [8]. Although consumer acceptance is the major drawback of such a development strategy, technological advancements demonstrating clear, safe, and beneficial use of microorganisms in foods may be appreciated sooner than anticipated [148]. Many countries have approved the use of genetically modified strains but there has been a lag on the entrance of those foods on the market [148].

10. Conclusions and Future of Non-Conventional Yeasts in Modern Baking

Increasing number of studies highlight the potential of non-conventional yeasts to develop new bakery products with novel aromas. Some of these yeasts present several attributes interesting for bakery industry such as osmotolerance, halotolerance, or thermotolerance, among others, in addition to the ability of synthesise a huge range of aroma compounds that can result in higher aroma complexity. These species constitute an untapped source for product diversification and innovation. However, further studies are needed to adjust the different manufacturing processes as well as to demonstrate the QPS or GRAS status of most of them such that they can be freely used in the food industry.

References

1. Donalies, U.E.; Nguyen, H.T.; Stahl, U.; Nevoigt, E. Improvement of *Saccharomyces* yeast strains used in brewing, wine making and baking. *Adv. Biochem. Eng. Biotechnol.* **2008**, *111*, 67–98. [CrossRef] [PubMed]
2. Johnson, E.A.; Echavarri-Erasun, C. Yeast biotechnology. In *The Yeasts*; Elsevier: Amsterdam, The Netherlands, 2011; pp. 21–44.
3. Kang, A.; Lee, T.S. Converting sugars to biofuels: Ethanol and beyond. *Bioengineering* **2015**, *2*, 184. [CrossRef]
4. Krogerus, K.; Magalhães, F.; Vidgren, V.; Gibson, B. Novel brewing yeast hybrids: Creation and application. *Appl. Microbiol. Biotechnol.* **2017**, *101*, 65–78. [CrossRef] [PubMed]
5. Parapouli, M.; Vasileiadis, A.; Afendra, A.-S.; Hatziloukas, E. *Saccharomyces cerevisiae* and its industrial applications. *AIMS Microbiol.* **2020**, *6*, 1. [CrossRef]
6. Zhou, N. Carbon Metabolism in Non-Conventional Yeasts: Biodiversity, Origins of Aerobic Fermentation and Industrial Applications. Ph.D. Thesis, Lund University, Lund, Sweden, 2015.
7. Aslankoohi, E.; Herrera-Malaver, B.; Rezaei, M.N.; Steensels, J.; Courtin, C.M.; Verstrepen, K.J. Non-Conventional Yeast Strains Increase the Aroma Complexity of Bread. *PLoS ONE* **2016**, *11*, e0165126. [CrossRef]
8. Randez-Gil, F.; Sanz, P.; Prieto, J.A. Engineering baker's yeast: Room for improvement. *Trends Biotechnol.* **1999**, *17*, 237–244. [CrossRef]
9. Struyf, N.; Van der Maelen, E.; Hemdane, S.; Verspreet, J.; Verstrepen, K.J.; Courtin, C.M. Bread dough and baker's yeast: An uplifting synergy. *Compr. Rev. Food Sci. Food Saf.* **2017**, *16*, 850–867. [CrossRef] [PubMed]
10. Zhou, N.; Schifferdecker, A.J.; Gamero, A.; Compagno, C.; Boekhout, T.; Piškur, J.; Knecht, W. *Kazachstania gamospora* and *Wickerhamomyces subpelliculosus*: Two alternative baker's yeasts in the modern bakery. *Int. J. Food Microbiol.* **2017**, *250*, 45–48. [CrossRef] [PubMed]
11. Ali, A.; Shehzad, A.; Khan, M.R.; Shabbir, M.A.; Amjid, M. Yeast, its types and role in fermentation during bread making process—A review. *Pak. J. Food Sci.* **2012**, *22*, 170–178.

12. Gamero, A.; Ingoglia, C.; De Jong, C. Microbread: Use of a micro-scale screening breadbaking platform for high-throughput screening of new ingredients and formulations in baked goods. In Proceedings of the 10th Wartburg Symposium on Current Topics in Flavor Chemistry & Biology, Eisenach, Germany, 16–19 April 2013; pp. 359–362.
13. Maicas, S. The Role of Yeasts in Fermentation Processes. *Microorganisms* **2020**, *8*, 1142. [CrossRef] [PubMed]
14. Xu, D.; Yin, Y.; Ali, B.; Zhang, Y.; Guo, L.; Xu, X. Isolation of yeast strains from Chinese liquor Daqu and its use in the wheat sourdough bread making. *Food Biosci.* **2019**, *31*, 100443. [CrossRef]
15. Attfield, P.V. Stress tolerance: The key to effective strains of industrial baker's yeast. *Nat. Biotechnol.* **1997**, *15*, 1351–1357. [CrossRef]
16. Randez-Gil, F.; Córcoles-Sáez, I.; Prieto, J.A. Genetic and phenotypic characteristics of baker's yeast: Relevance to baking. *Annu. Rev. Food Sci. Technol.* **2013**, *4*, 191–214. [CrossRef]
17. Takagi, H. Construction of baker's yeast strains with enhanced tolerance to baking-associated stresses. In *Biotechnology of Yeasts and Filamentous Fungi*; Springer: Berlin/Heidelberg, Germany, 2017; pp. 63–85.
18. Takagi, H.; Shima, J. Stress Tolerance of Baker's Yeast During Bread-Making Processes. In *Stress Biology of Yeasts and Fungi: Applications for Industrial Brewing and Fermentation*; Takagi, H., Kitagaki, H., Eds.; Springer: Japan, Tokyo, 2015; pp. 23–42.
19. Lahue, C.; Madden, A.; Dunn, R.; Smukowski Heil, C. History and Domestication of *Saccharomyces cerevisiae* in Bread Baking. *Front. Genet.* **2020**, *11*. [CrossRef] [PubMed]
20. Flores, C.L.; Rodriguez, C.; Petit, T.; Gancedo, C. Carbohydrate and energy-yielding metabolism in non-conventional yeasts. *FEMS Microbiol. Rev.* **2000**, *24*, 507–529. [CrossRef]
21. Ostergaard, S.; Olsson, L.; Nielsen, J. Metabolic Engineering of *Saccharomyces cerevisiae*. *Microbiol. Mol. Biol. Rev.* **2000**, *64*, 34–50. [CrossRef]
22. Chiva, R.; Celador-Lera, L.; Uña, J.A.; Jiménez-López, A.; Espinosa-Alcantud, M.; Mateos-Horganero, E.; Vega, S.; Santos, M.Á.; Velázquez, E.; Tamame, M.J.M. Yeast Biodiversity in Fermented Doughs and Raw Cereal Matrices and the Study of Technological Traits of Selected Strains Isolated in Spain. *Microorganisms* **2021**, *9*, 47. [CrossRef]
23. Steensels, J.; Verstrepen, K.J. Taming wild yeast: Potential of conventional and nonconventional yeasts in industrial fermentations. *Annu. Rev. Microbiol.* **2014**, *68*, 61–80. [CrossRef] [PubMed]
24. Gamero, A.; Quintilla, R.; Groenewald, M.; Alkema, W.; Boekhout, T.; Hazelwood, L. High-throughput screening of a large collection of non-conventional yeasts reveals their potential for aroma formation in food fermentation. *Food Microbiol.* **2016**, *60*, 147–159. [CrossRef] [PubMed]
25. Yeasts Markets. Available online: http://www.marketsandmarkets.com/Market-Reports/yeast-industry-268.html (accessed on 1 June 2021).
26. Dashko, S.; Zhou, N.; Compagno, C.; Piskur, J. Why, when, and how did yeast evolve alcoholic fermentation? *FEMS Yeast Res.* **2014**, *14*, 826–832. [CrossRef] [PubMed]
27. Gamero Lluna, A.; de Jong, C. Novel yeasts, novel flavours. *New Food Mag.* **2013**, *16*, 26–28.
28. Spencer, J.; Ragout de Spencer, A.; Laluce, C. Non-conventional yeasts. *Appl. Microbiol. Biotechnol.* **2002**, *58*, 147–156. [CrossRef]
29. Piskur, J.; Rozpedowska, E.; Polakova, S.; Merico, A.; Compagno, C. How did Saccharomyces evolve to become a good brewer? *Trends Genet.* **2006**, *22*, 183–186. [CrossRef]
30. De Deken, R.H. The Crabtree effect: A regulatory system in yeast. *J. Gen. Microbiol.* **1966**, *44*, 149–156. [CrossRef]
31. Pronk, J.T.; Steensma, H.Y.; van Dijken, J.P. Pyruvate metabolism in *Saccharomyces cerevisiae*. *Yeast* **1996**, *12*, 1607–1633. [CrossRef]
32. Hagman, A.; Sall, T.; Compagno, C.; Piskur, J. Yeast "make-accumulate-consume" life strategy evolved as a multi-step process that predates the whole genome duplication. *PLoS ONE* **2013**, *8*, e68734. [CrossRef] [PubMed]
33. Martinez-Anaya, M.; Pitarch, B.; Bayarri, P.; De Barber, B.C. Microflora of the sourdoughs of wheat flour bread. X. Interactions between yeasts and lactic acid bacteria in wheat doughs and their effects on bread quality. *Cereal Chem.* **1990**, *67*, 85–91.
34. Meroth, C.B.; Hammes, W.P.; Hertel, C. Identification and Population Dynamics of Yeasts in Sourdough Fermentation Processes by PCR-Denaturing Gradient Gel Electrophoresis. *Appl. Environ. Microbiol.* **2003**, *69*, 7453–7461. [CrossRef]
35. Meroth, C.B.; Walter, J.; Hertel, C.; Brandt, M.J.; Hammes, W.P. Monitoring the bacterial population dynamics in sourdough fermentation processes by using PCR-denaturing gradient gel electrophoresis. *Appl. Environ. Microbiol.* **2003**, *69*, 475–482. [CrossRef]
36. Lee, M.E.; DeLoache, W.C.; Cervantes, B.; Dueber, J.E. A highly characterized yeast toolkit for modular, multipart assembly. *ACS Synth. Biol.* **2015**, *4*, 975–986. [CrossRef] [PubMed]
37. Heitmann, M.; Zannini, E.; Arendt, E. Impact of *Saccharomyces cerevisiae* metabolites produced during fermentation on bread quality parameters: A review. *Crit. Rev. Food Sci. Nutr.* **2018**, *58*, 1152–1164. [CrossRef] [PubMed]
38. Takagi, H.; Iwamoto, F.; Nakamori, S. Isolation of freeze-tolerant laboratory strains of *Saccharomyces cerevisiae* from proline-analogue-resistant mutants. *Appl. Microbiol. Biotechnol.* **1997**, *47*, 405–411. [CrossRef]
39. Verstrepen, K.; Chambers, P.; Pretorius, I. The Development of Superior Yeast Strains for the Food and Beverage Industries: Challenges, Opportunities and Potential Benefits. In *Yeasts in Food and Beverages*; Querol, A., Fleet, G., Eds.; Springer: Berlin/Heidelberg, Germany, 2006; pp. 399–444.
40. Kurtzman, C.; Fell, J.W.; Boekhout, T. *The Yeasts: A Taxonomic Study*; Elsevier: Amsterdam, The Netherlands, 2011.
41. Barnett, J.A. The utilization of sugars by yeasts. *Adv. Carbohydr. Chem. Biochem.* **1976**, *32*, 125–234. [PubMed]
42. Jeffries, T.W. Engineering yeasts for xylose metabolism. *Curr. Opin. Biotechnol.* **2006**, *17*, 320–326. [CrossRef] [PubMed]

43. Jeffries, T.W.; Jin, Y.S. Ethanol and thermotolerance in the bioconversion of xylose by yeasts. *Adv. Appl. Microbiol.* **2000**, *47*, 221–268.

44. Jeffries, T.W. Conversion of xylose to ethanol under aerobic conditions by *Candida tropicalis*. *Biotechnol. Lett.* **1981**, *3*, 213–218. [CrossRef]

45. Compagno, C.; Porro, D.; Smeraldi, C.; Ranzi, B.M. Fermentation of whey and starch by transformed *Saccharomyces cerevisiae* cells. *Appl. Microbiol. Biotechnol.* **1995**, *43*, 822–825. [CrossRef]

46. Sreekrishna, K.; Dickson, R.C. Construction of strains of *Saccharomyces cerevisiae* that grow on lactose. *Proc. Natl. Acad. Sci. USA* **1985**, *82*, 7909–7913. [CrossRef]

47. Scrimshaw, N.S.; Murray, E.B. The acceptability of milk and milk products in populations with a high prevalence of lactose intolerance. *Am. J. Clin. Nutr.* **1988**, *48*, 1142–1159. [CrossRef]

48. Naumov, G.I.; Naumova, E.S.; Turakainen, H.; Korhola, M. Identification of the alpha-galactosidase MEL genes in some populations of *Saccharomyces cerevisiae*: A new gene MEL11. *Genet. Res.* **1996**, *67*, 101–108. [CrossRef]

49. Hernandez-Lopez, M.J.; Prieto, J.A.; Randez-Gil, F. Osmotolerance and leavening ability in sweet and frozen sweet dough. Comparative analysis between *Torulaspora delbrueckii* and *Saccharomyces cerevisiae* baker's yeast strains. *Antonie Van Leeuwenhoek* **2003**, *84*, 125–134. [CrossRef]

50. Johansen, P.G.; Owusu-Kwarteng, J.; Parkouda, C.; Padonou, S.W.; Jespersen, L. Occurrence and Importance of Yeasts in Indigenous Fermented Food and Beverages Produced in Sub-Saharan Africa. *Front. Microbiol.* **2019**, *10*. [CrossRef]

51. Motlhanka, K.; Zhou, N.; Lebani, K. Microbial and Chemical Diversity of Traditional Non-Cereal Based Alcoholic Beverages of Sub-Saharan Africa. *Beverages* **2018**, *4*, 36. [CrossRef]

52. Hittinger, C.T.; Rokas, A.; Bai, F.-Y.; Boekhout, T.; Goncalves, P.; Jeffries, T.W.; Kominek, J.; Lachance, M.-A.; Libkind, D.; Rosa, C.A.; et al. Genomics and the making of yeast biodiversity. *Curr. Opin. Genet. Dev.* **2015**, *35*, 100–109. [CrossRef] [PubMed]

53. Kurtzman, C.P.; Fell, J.W.; Boekhout, T. (Eds.) Chapter 1—Definition, Classification and Nomenclature of the Yeasts. In *The Yeasts*, 5th ed.; Elsevier: London, UK, 2011; pp. 3–5.

54. Shen, X.-X.; Opulente, D.A.; Kominek, J.; Zhou, X.; Steenwyk, J.L.; Buh, K.V.; Haase, M.A.; Wisecaver, J.H.; Wang, M.; Doering, D.T.; et al. Tempo and mode of genome evolution in the budding yeast subphylum. *Cell* **2018**, *175*, 1533–1545.e20. [CrossRef] [PubMed]

55. Bellora, N.; Moliné, M.; David-Palma, M.; Coelho, M.A.; Hittinger, C.T.; Sampaio, J.P.; Gonçalves, P.; Libkind, D. Comparative genomics provides new insights into the diversity, physiology, and sexuality of the only industrially exploited tremellomycete: *Phaffia rhodozyma*. *BMC Genom.* **2016**, *17*, 901. [CrossRef] [PubMed]

56. Libkind, D.; Peris, D.; Cubillos, F.A.; Steenwyk, J.L.; Opulente, D.A.; Langdon, Q.K.; Rokas, A.; Hittinger, C.T. Into the wild: New yeast genomes from natural environments and new tools for their analysis. *FEMS Yeast Res.* **2020**, *20*. [CrossRef] [PubMed]

57. Riley, R.; Haridas, S.; Wolfe, K.H.; Lopes, M.R.; Hittinger, C.T.; Göker, M.; Salamov, A.A.; Wisecaver, J.H.; Long, T.M.; Calvey, C.H.; et al. Comparative genomics of biotechnologically important yeasts. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 9882–9887. [CrossRef] [PubMed]

58. Donohoue, P.D.; Barrangou, R.; May, A.P. Advances in industrial biotechnology using CRISPR-Cas systems. *Trends Biotechnol.* **2018**, *36*, 134–146. [CrossRef] [PubMed]

59. Ihmels, J.; Bergmann, S.; Gerami-Nejad, M.; Yanai, I.; McClellan, M.; Berman, J.; Barkai, N. Rewiring of the yeast transcriptional network through the evolution of motif usage. *Science* **2005**, *309*, 938–940. [CrossRef]

60. Wolfe, K.H.; Shields, D.C. Molecular evidence for an ancient duplication of the entire yeast genome. *Nature* **1997**, *387*, 708–713. [CrossRef]

61. Gojkovic, Z.; Knecht, W.; Zameitat, E.; Warneboldt, J.; Coutelis, J.B.; Pynyaha, Y.; Neuveglise, C.; Moller, K.; Loffler, M.; Piskur, J. Horizontal gene transfer promoted evolution of the ability to propagate under anaerobic conditions in yeasts. *Mol. Genet. Genom.* **2004**, *271*, 387–393. [CrossRef]

62. Dujon, B. Yeast evolutionary genomics. *Nat. Rev. Genet.* **2010**, *11*, 512–524. [CrossRef] [PubMed]

63. Lineback, D.R.; Rasper, V.F. Wheat: Chemistry and Technology. In *Wheat Carbohydrates*; Pomeranz, Y., Ed.; American Association of Cereal Chemists: St. Paul, MN, USA, 1988; Volume I, pp. 277–372.

64. Oura, H.; Suomalainen, H.; Viskari, R. Breadmaking. In *Economic Microbiology. Fermented Foods*; Rose, A.H., Ed.; Academic Press: London, UK, 1982; Volume 7.

65. Bell, P.J.L.; Higgins, V.J.; Attfield, P.V. Comparison of fermentative capacities of industrial baking and wild-type yeasts of the species *Saccharomyces cerevisiae* in different sugar media. *Lett. Appl. Microbiol.* **2001**, *32*, 224–229. [CrossRef] [PubMed]

66. Struyf, N.; Laurent, J.; Verspreet, J.; Verstrepen, K.J.; Courtin, C.M. *Saccharomyces cerevisiae* and *Kluyveromyces marxianus* Cocultures Allow Reduction of Fermentable Oligo-, Di-, and Monosaccharides and Polyols Levels in Whole Wheat Bread. *J. Agric. Food Chem.* **2017**, *65*, 8704–8713. [CrossRef] [PubMed]

67. Beudeker, R.J. Developments in bakers' yeast production. *Yeast Biotechnol. Biocatal.* **1990**, 103–146.

68. Oda, Y.; Ouchi, K. Principal-component analysis of the characteristics desirable in baker's yeasts. *Appl. Environ. Microbiol.* **1989**, *55*, 1495–1499. [CrossRef]

69. Rozpedowska, E.; Hellborg, L.; Ishchuk, O.P.; Orhan, F.; Galafassi, S.; Merico, A.; Woolfit, M.; Compagno, C.; Piskur, J. Parallel evolution of the make-accumulate-consume strategy in *Saccharomyces* and *Dekkera* yeasts. *Nat. Commun.* **2011**, *2*, 302. [CrossRef]

70. Sahlström, S.; Park, W.; Shelton, D.R. Factors influencing yeast fermentation and the effect of LMW sugars and yeast fermentation on hearth bread quality. *Cereal Chem.* **2004**, *81*, 328–335. [CrossRef]

71. D'Appolonia, B.; Rayas-Duarte, P. Wheat carbohydrates: Structure and functionality. In *Wheat*; Springer: Berlin/Heidelberg, Germany, 1994; pp. 107–127.

72. Randez-Gil, F.; Sanz, P. Construction of industrial baker's yeast strains able to assimilate maltose under catabolite repression conditions. *Appl. Microbiol. Biotechnol.* **1994**, *42*, 581–586. [CrossRef]

73. Cauvain, S.P.; Young, L.S. *Technology of Breadmaking*; Springer: Berlin/Heidelberg, Germany, 2007.

74. Hebeda, R. *Baked Goods Freshness: Technology, Evaluation, and Inhibition of Staling*; CRC Press: Boca Raton, FL, USA, 1996; Volume 75.

75. Kręgiel, D.; Pawlikowska, E.; Antolak, H. *Non-Conventional Yeasts in Fermentation Processes: Potentialities and Limitations*; IntechOpen: London, UK, 2017. [CrossRef]

76. Limayem, A.; Ricke, S.C. Lignocellulosic biomass for bioethanol production: Current perspectives, potential issues and future prospects. *Prog. Energy Combust. Sci.* **2012**, *38*, 449–467. [CrossRef]

77. Tabañag, I.D.F.; Tsai, S.-L. Hemicellulose Degradation and Utilization by a Synthetic *Saccharomyces cerevisiae* Consortium. *bioRxiv* **2018**. [CrossRef]

78. Nandal, P.; Sharma, S.; Arora, A. Bioprospecting non-conventional yeasts for ethanol production from rice straw hydrolysate and their inhibitor tolerance. *Renew. Energy* **2020**, *147*, 1694–1703. [CrossRef]

79. Steensels, J.; Snoek, T.; Meersman, E.; Nicolino, M.P.; Voordeckers, K.; Verstrepen, K.J. Improving industrial yeast strains: Exploiting natural and artificial diversity. *FEMS Microbiol. Rev.* **2014**, *38*, 947–995. [CrossRef]

80. Radecka, D.; Mukherjee, V.; Mateo, R.Q.; Stojiljkovic, M.; Foulquie-Moreno, M.R.; Thevelein, J.M. Looking beyond Saccharomyces: The potential of non-conventional yeast species for desirable traits in bioethanol fermentation. *FEMS Yeast Res.* **2015**, *15*. [CrossRef]

81. Hernández-López, J.; Vargas-Albores, F. A microplate technique to quantify nutrients (NO_2^-, NO_3^-, NH_4^+ and PO_4^{3-}) in seawater. *Aquac. Res.* **2003**, *34*, 1201–1204. [CrossRef]

82. Shima, J.; Takagi, H. Stress-tolerance of baker's-yeast (*Saccharomyces cerevisiae*) cells: Stress-protective molecules and genes involved in stress tolerance. *Biotechnol. Appl. Biochem.* **2009**, *53*, 155–164. [CrossRef] [PubMed]

83. De Vuyst, L.; Van Kerrebroeck, S.; Leroy, F. Chapter Two—Microbial Ecology and Process Technology of Sourdough Fermentation. In *Advances in Applied Microbiology*; Sariaslani, S., Gadd, G.M., Eds.; Academic Press: Cambridge, MA, USA, 2017; Volume 100, pp. 49–160.

84. Alves-Araújo, C.; Almeida, M.J.; Sousa, M.J.; Leão, C. Freeze tolerance of the yeast *Torulaspora delbrueckii*: Cellular and biochemical basis. *FEMS Microbiol. Lett.* **2004**, *240*, 7–14. [CrossRef]

85. Oda, Y.; Tonomura, K. Selection of a Novel Baking Strain from the Torulaspora Yeasts. *Biosci. Biotechnol. Biochem.* **1993**, *57*, 1320–1322. [CrossRef]

86. Lane, M.M.; Morrissey, J.P. *Kluyveromyces marxianus*: A yeast emerging from its sister's shadow. *Fungal Biol. Rev.* **2010**, *24*, 17–26. [CrossRef]

87. Chamnipa, N.; Thanonkeo, S.; Klanrit, P.; Thanonkeo, P. The potential of the newly isolated thermotolerant yeast *Pichia kudriavzevii* RZ8-1 for high-temperature ethanol production. *Braz. J. Microbiol.* **2018**, *49*, 378–391. [CrossRef]

88. Yuangsaard, N.; Yongmanitchai, W.; Yamada, M.; Limtong, S. Selection and characterization of a newly isolated thermotolerant *Pichia kudriavzevii* strain for ethanol production at high temperature from cassava starch hydrolysate. *Antonie van Leeuwenhoek* **2013**, *103*, 577–588. [CrossRef] [PubMed]

89. Vaudano, E.; Eleonora, B.; Petrozziello, M. Exploring the possibility of using Kazachstania exigua (ex. Saccharomyces exiguus) in wine production. In *Industrial, Medical and Environmental Applications of Microorganisms: Current Status and Trends*; Wageningen Academic Publishers: Wageningen, The Netherlands, 2014; pp. 304–309.

90. Galafassi, S.; Merico, A.; Pizza, F.; Hellborg, L.; Molinari, F.; Piškur, J.; Compagno, C. *Dekkera/Brettanomyces* yeasts for ethanol production from renewable sources under oxygen-limited and low-pH conditions. *J. Ind. Microbiol. Biotechnol.* **2011**, *38*, 1079–1088. [CrossRef]

91. Caballero, R.; Olguín, P.; Cruz-Guerrero, A.; Gallardo, F.; García-Garibay, M.; Gómez-Ruiz, L. Evaluation of *Kluyveromyces marxianus* as baker's yeast. *Food Res. Int.* **1995**, *28*, 37–41. [CrossRef]

92. Mukherjee, V.; Radecka, D.; Aerts, G.; Verstrepen, K.J.; Lievens, B.; Thevelein, J.M. Phenotypic landscape of non-conventional yeast species for different stress tolerance traits desirable in bioethanol fermentation. *Biotechnol. Biofuels* **2017**, *10*, 216. [CrossRef] [PubMed]

93. Limtong, S.; Sringiew, C.; Yongmanitchai, W. Production of fuel ethanol at high temperature from sugar cane juice by a newly isolated *Kluyveromyces marxianus*. *Bioresour. Technol.* **2007**, *98*, 3367–3374. [CrossRef]

94. Albertin, W.; Chasseriaud, L.; Comte, G.; Panfili, A.; Delcamp, A.; Salin, F.; Marullo, P.; Bely, M. Winemaking and bioprocesses strongly shaped the genetic diversity of the ubiquitous yeast *Torulaspora delbrueckii*. *PLoS ONE* **2014**, *9*, e94246. [CrossRef] [PubMed]

95. Isono, N.; Hayakawa, H.; Usami, A.; Mishima, T.; Hisamatsu, M. A comparative study of ethanol production by *Issatchenkia orientalis* strains under stress conditions. *J. Biosci. Bioeng.* **2012**, *113*, 76–78. [CrossRef] [PubMed]

96. Zhou, N.; Ishchuk, O.P.; Knecht, W.; Compagno, C.; Piškur, J. Acquisition of thermotolerance in *Lachancea thermotolerans* using a bacterial selection pressure. *J. Ind. Microbiol. Biotechnol.* **2017**, *46*, 133–145. [CrossRef] [PubMed]

97. Daniel, H.-M.; Moons, M.-C.; Huret, S.; Vrancken, G.; De Vuyst, L. *Wickerhamomyces anomalus* in the sourdough microbial ecosystem. *Antonie van Leeuwenhoek* **2011**, *99*, 63–73. [CrossRef] [PubMed]
98. Wang, H.; Hu, Z.; Long, F.; Niu, C.; Yuan, Y.; Yue, T. Characterization of osmotolerant yeasts and yeast-like molds from apple orchards and apple juice processing plants in China and investigation of their spoilage potential. *J. Food Sci.* **2015**, *80*, M1850–M1860. [CrossRef] [PubMed]
99. Korcari, D.; Ricci, G.; Capusoni, C.; Fortina, M.G. Physiological performance of *Kazachstania unispora* in sourdough environments. *World J. Microbiol. Biotechnol.* **2021**, *37*, 1–8. [CrossRef]
100. Curtin, C.D.; Pretorius, I.S. Genomic insights into the evolution of industrial yeast species *Brettanomyces bruxellensis*. *FEMS Yeast Res.* **2014**, *14*, 997–1005.
101. Saini, P.; Beniwal, A.; Kokkiligadda, A.; Vij, S. Evolutionary adaptation of *Kluyveromyces marxianus* strain for efficient conversion of whey lactose to bioethanol. *Process Biochem.* **2017**, *62*, 69–79. [CrossRef]
102. Sehnem, N.; Machado, Â.; Matte, C.; de Morais, M., Jr.; Ayub, M. Second-generation ethanol production by *Wickerhamomyces anomalus* strain adapted to furfural, 5-hydroxymethylfurfural (HMF), and high osmotic pressure. *An. Acad. Bras. Ciênc.* **2020**, *92*. [CrossRef]
103. Prista, C.; Michán, C.; Miranda, I.M.; Ramos, J. The halotolerant *Debaryomyces hansenii*, the Cinderella of non-conventional yeasts. *Yeast* **2016**, *33*, 523–533. [CrossRef] [PubMed]
104. Ciani, M.; Maccarelli, F. Oenological properties of non-Saccharomyces yeasts associated with wine-making. *World J. Microbiol. Biotechnol.* **1997**, *14*, 199–203. [CrossRef]
105. Mo, W.; Wang, M.; Zhan, R.; Yu, Y.; He, Y.; Lu, H. *Kluyveromyces marxianus* developing ethanol tolerance during adaptive evolution with significant improvements of multiple pathways. *Biotechnol. Biofuels* **2019**, *12*, 1–15. [CrossRef]
106. Hahn, Y.-S.; Kawai, H. Isolation and Characterization of Freeze-tolerant Yeasts from Nature Available for the Frozen-dough Method. *Agric. Biol. Chem.* **1990**, *54*, 829–831. [CrossRef]
107. Nurcholis, M.; Lertwattanasakul, N.; Rodrussamee, N.; Kosaka, T.; Murata, M.; Yamada, M. Integration of comprehensive data and biotechnological tools for industrial applications of *Kluyveromyces marxianus*. *Appl. Microbiol. Biotechnol.* **2020**, *104*, 475–488. [CrossRef] [PubMed]
108. Rahmadhani, N.; Astuti, R.; Meryandini, A. Substrate utilization of ethanologenic yeasts co-cultivation of *Pichia kudriavzevii* and *Saccharomyces cerevisiae*. In *IOP Conference Series: Earth and Environmental Science*; IOP Publishing: Bristol, UK, 2020; p. 012072.
109. Liu, T.; Li, Y.; Sadiq, F.A.; Yang, H.; Gu, J.; Yuan, L.; Lee, Y.K.; He, G. Predominant yeasts in Chinese traditional sourdough and their influence on aroma formation in Chinese steamed bread. *Food Chem.* **2018**, *242*, 404–411. [CrossRef] [PubMed]
110. Pacheco, A.; Santos, J.; Chaves, S.; Almeida, J.; Sousa, M. The emerging role of the *Torulaspora delbrueckii* in bread and wine production: Using genetic manipulation to study molecular basis of physiological responses. In *Structure and Function of Food Engineering*; IntechOpen: London, UK, 2012.
111. Gutiérrez, A.; Boekhout, T.; Gojkovic, Z.; Katz, M. Evaluation of non-Saccharomyces yeasts in the fermentation of wine, beer and cider for the development of new beverages. *J. Inst. Brew.* **2018**, *124*, 389–402. [CrossRef]
112. Joseph, C.L.; Albino, E.; Bisson, L.F. Creation and use of a *Brettanomyces* aroma wheel. *Catal. Discov. Pract.* **2017**, *1*, 12–20. [CrossRef]
113. Verstrepen, K.J.; Iserentant, D.; Malcorps, P.; Derdelinckx, G.; Van Dijck, P.; Winderickx, J.; Pretorius, I.S.; Thevelein, J.M.; Delvaux, F.R. Glucose and sucrose: Hazardous fast-food for industrial yeast? *Trends Biotechnol.* **2004**, *22*, 531–537. [CrossRef] [PubMed]
114. Blomberg, A.; Adler, L. Physiology of osmotolerance in fungi. *Adv. Microb. Physiol.* **1992**, *33*, 145–212.
115. Landolfo, S.; Politi, H.; Angelozzi, D.; Mannazzu, I. ROS accumulation and oxidative damage to cell structures in *Saccharomyces cerevisiae* wine strains during fermentation of high-sugar-containing medium. *Biochim. Biophys. Acta BBA Gen. Subj.* **2008**, *1780*, 892–898. [CrossRef]
116. Nakamura, T.; Lipton, S.A. Cell death: Protein misfolding and neurodegenerative diseases. *Apoptosis* **2009**, *14*, 455–468. [CrossRef]
117. Henderson, C.M.; Zeno, W.F.; Lerno, L.A.; Longo, M.L.; Block, D.E. Fermentation temperature modulates phosphatidylethanolamine and phosphatidylinositol levels in the cell membrane of *Saccharomyces cerevisiae*. *Appl. Environ. Microbiol.* **2013**, *79*, 5345–5356. [CrossRef]
118. Nwaefuna, A.E.; Rumbold, K.; Boekhout, T.; Zhou, N. Bioethanolic yeasts from dung beetles: Tapping the potential of extremophilic yeasts for improvement of lignocellulolytic feedstock fermentation. *Biotechnol. Biofuels* **2021**, *14*, 86. [CrossRef]
119. Lehnen, M.; Ebert, B.E.; Blank, L.M. Elevated temperatures do not trigger a conserved metabolic network response among thermotolerant yeasts. *BMC Microbiol.* **2019**, *19*, 100. [CrossRef]
120. Hsu, K.; Rc, H.; Pa, S. Frozen Dough. I: Factors Affecting Stability of Yeasted Doughs. *Cereal Chem.* **1979**, *56*, 419–424.
121. Park, J.-I.; Grant, C.M.; Attfield, P.V.; Dawes, I.W. The freeze-thaw stress response of the yeast *Saccharomyces cerevisiae* is growth phase specific and is controlled by nutritional state via the RAS-cyclic AMP signal transduction pathway. *Appl. Environ. Microbiol.* **1997**, *63*, 3818–3824. [CrossRef]
122. Shima, J.; Hino, A.; Yamada-Iyo, C.; Suzuki, Y.; Nakajima, R.; Watanabe, H.; Mori, K.; Takano, H. Stress tolerance in doughs of *Saccharomyces cerevisiae* trehalase mutants derived from commercial Baker's yeast. *Appl. Environ. Microbiol.* **1999**, *65*, 2841–2846. [CrossRef]
123. Manzo-Avalos, S.; Saavedra-Molina, A. Cellular and mitochondrial effects of alcohol consumption. *Int. J. Environ. Res. Public Health* **2010**, *7*, 4281. [CrossRef]

124. Stanley, D.; Bandara, A.; Fraser, S.; Chambers, P.J.; Stanley, G.A. The ethanol stress response and ethanol tolerance of *Saccharomyces cerevisiae*. *J. Appl. Microbiol.* **2010**, *109*, 13–24. [CrossRef]

125. Cabiscol, E.; Piulats, E.; Echave, P.; Herrero, E.; Ros, J. Oxidative stress promotes specific protein damage in *Saccharomyces cerevisiae*. *J. Biol. Chem.* **2000**, *275*, 27393–27398. [CrossRef]

126. Gibson, B.R.; Lawrence, S.J.; Leclaire, J.P.; Powell, C.D.; Smart, K.A. Yeast responses to stresses associated with industrial brewery handling. *FEMS Microbiol. Rev.* **2007**, *31*, 535–569. [CrossRef]

127. Gibson, B.R.; Lawrence, S.J.; Smith, J.M.; Shelton, N.; Smith, J.M.; Smart, K.A. Oxygen as toxin: Oxidative stress and brewing yeast physiology. *Cerevisia* **2006**, *31*, 25.

128. Girotti, A.W. Lipid hydroperoxide generation, turnover, and effector action in biological systems. *J. Lipid Res.* **1998**, *39*, 1529–1542. [CrossRef]

129. Salmon, T.B.; Evert, B.A.; Song, B.; Doetsch, P.W. Biological consequences of oxidative stress-induced DNA damage in *Saccharomyces cerevisiae*. *Nucleic Acids Res.* **2004**, *32*, 3712–3723. [CrossRef]

130. Barker, M.G.; Brimage, L.J.; Smart, K.A. Effect of Cu,Zn superoxide dismutase disruption mutation on replicative senescence in *Saccharomyces cerevisiae*. *FEMS Microbiol. Lett.* **1999**, *177*, 199–204. [CrossRef] [PubMed]

131. Halliwell, B.; Gutteridge, J.M. *Free Radicals in Biology and Medicine*; Oxford University Press: New York, NY, USA, 2015.

132. Schieberle, P.; Grosch, W. Potent odorants of the wheat bread crumb Differences to the crust and effect of a longer dough fermentation. *Z. Lebensm. Unters. Forsch.* **1991**, *192*, 130–135. [CrossRef]

133. Birch, A.N.; Petersen, M.A.; Hansen, Å.S. The aroma profile of wheat bread crumb influenced by yeast concentration and fermentation temperature. *LWT Food Sci. Technol.* **2013**, *50*, 480–488. [CrossRef]

134. Purlis, E. Browning development in bakery products—A review. *J. Food Eng.* **2010**, *99*, 239–249. [CrossRef]

135. Frasse, P.; Lambert, S.; Levesque, C.; Melcion, D.; Richard-Molard, D.; Chiron, H. The influence of fermentation on volatile compounds in French bread crumb. *Food Sci. Technol.* **1992**, *25*, 66–70.

136. Carbonetto, B.; Ramsayer, J.; Nidelet, T.; Legrand, J.; Sicard, D. Bakery yeasts, a new model for studies in ecology and evolution. *Yeast* **2018**, *35*, 591–603. [CrossRef]

137. Xu, D.; Zhang, Y.; Tang, K.; Hu, Y.; Xu, X.; Gänzle, M.G. Effect of mixed cultures of yeast and lactobacilli on the quality of wheat sourdough bread. *Front. Microbiol.* **2019**, *10*, 2113. [CrossRef] [PubMed]

138. Plessas, S.; Fisher, A.; Koureta, K.; Psarianos, C.; Nigam, P.; Koutinas, A.A. Application of *Kluyveromyces marxianus*, *Lactobacillus delbrueckii* ssp. *bulgaricus* and *L. helveticus* for sourdough bread making. *Food Chem.* **2008**, *106*, 985–990. [CrossRef]

139. Poinot, P.; Grua-Priol, J.; Arvisenet, G.; Rannou, C.; Semenou, M.; Le Bail, A.; Prost, C. Optimisation of HS-SPME to study representativeness of partially baked bread odorant extracts. *Food Res. Int.* **2007**, *40*, 1170–1184. [CrossRef]

140. Birch, A.N.; Petersen, M.A.; Hansen, Å.S. Aroma of wheat bread crumb. *Cereal Chem.* **2014**, *91*, 105–114. [CrossRef]

141. Swamy, K.B.S.; Zhou, N. Experimental evolution: Its principles and applications in developing stress-tolerant yeasts. *Appl. Microbiol. Biotechnol.* **2019**, *103*, 2067–2077. [CrossRef] [PubMed]

142. Da Silva, N.A.; Srikrishnan, S. Introduction and expression of genes for metabolic engineering applications in *Saccharomyces cerevisiae*. *FEMS Yeast Res.* **2012**, *12*, 197–214. [CrossRef] [PubMed]

143. Stevenson, B.S.; Schmidt, T.M. Life history implications of rRNA gene copy number in *Escherichia coli*. *Appl. Environ. Microbiol.* **2004**, *70*, 6670–6677. [CrossRef] [PubMed]

144. Jeffries, T.; Jin, Y.-S. Metabolic engineering for improved fermentation of pentoses by yeasts. *Appl. Microbiol. Biotechnol.* **2004**, *63*, 495–509. [CrossRef] [PubMed]

145. Deckers, M.; Deforce, D.; Fraiture, M.-A.; Roosens, N.H. Genetically Modified Micro-Organisms for Industrial Food Enzyme Production: An Overview. *Foods* **2020**, *9*, 326. [CrossRef] [PubMed]

146. Zhou, N.; Katz, M.; Knecht, W.; Compagno, C.; Piškur, J. Genome dynamics and evolution in yeasts: A long-term yeast-bacteria competition experiment. *PLoS ONE* **2017**, *13*, e0194911. [CrossRef] [PubMed]

147. Zhou, N.; Bottagisi, S.; Katz, M.; Schacherer, J.; Friedrich, A.; Gojkovic, Z.; Swamy, K.B.S.; Knecht, W.; Compagno, C.; Piškur, J. Yeast-bacteria competition induced new metabolic traits through large-scale genomic rearrangements in *Lachancea kluyveri*. *FEMS Yeast Res.* **2017**. [CrossRef] [PubMed]

148. Harlander, S.; Roller, S.; Harlander, S.K. *Genetic Modification in the Food Industry: A Strategy for Food Quality Improvement*; Springer Science & Business Media: Berlin/Heidelberg, Germany, 1998.