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REVIEW ARTICLE

Strategies to Improve *Saccharomyces cerevisiae*: Technological Advancements and Evolutionary Engineering

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Abstract Bakery industries are thriving to augment the diverse properties of *Saccharomyces cerevisiae* to increase its flavor, texture and nutritional parameters to attract the more consumers. The improved technologies adopted for quality improvement of baker's yeast are attracting the attention of industry and it is playing a pivotal role in redesigning the quality parameters. Modern yeast strain improvement tactics revolve around the use of several advanced technologies such as evolutionary engineering, systems biology, metabolic engineering, genome editing. The review mainly deals with the technologies for improving *S. cerevisiae*, with the objective of broadening the range of its industrial applications.

Keywords *Saccharomyces cerevisiae* · Baker's yeast · Metabolic engineering · Systems biology · Dough leavening · Strain improvement

Introduction

Baking is a very old process, which uses extended dry heat for cooking [1]. Generally, yeast, *Saccharomyces cerevisiae* has been used for the baking process commonly known as baker's yeast. It is a unicellular microorganism classified under fungi (Ascomycota and Fungi imperfecti). Baker's yeast is used as a primary leavening agent for bread production and other products such as bread, cookies, crackers, cakes, pretzels, pastries, pies, tarts, quiches and more, collectively known as baking goods [2, 3]. It uses the sugar present in dough for its metabolic processes and generates carbon dioxide (CO_2) and ethanol, which causes the dough leavening during fermentation and oven rises [4]. Besides these, some other metabolites like organic acids, glycerol and aroma compounds are also produced [5]. These metabolites play significant role in fine quality, texture and rheological properties of bread. Although, yeast have been engaged in bakery industries since long times, the performance is limited due to various industrial constraints and requirements [6]. At industrial scale, baker's yeast gets exposed to several multiple and fluctuating environmental stresses, which ultimately diminish the product yield and also negatively affect the quality of bakery products [7]. Additionally, these environmental constraints largely affect cellular metabolism and viability.

Therefore, in order to overcome these constraints, it is very essential to improve the conventional baker's yeast using advanced techniques such as systems biology, bioinformatics tools, metabolic engineering, genome editing using CRISPR-Cas, etc. [8–11], along with the conventional approach like evolutionary engineering and genetic engineering. A schematic diagram of various technologies used for improvement of baker's yeast is described in Fig. 1. The approaches used for obtaining genetically engineered host depends on the target to be customized and on a phylogentically distant source of molecular determinants [12]. Many research reports have been published for improvement of baker's yeast for stress tolerance, enhancing fermentation and leavening abilities and improving aroma of bakery products [13]. It has been noticed that most of the commercial strains of baker's yeast are polyploids in nature with the lacking any mating type

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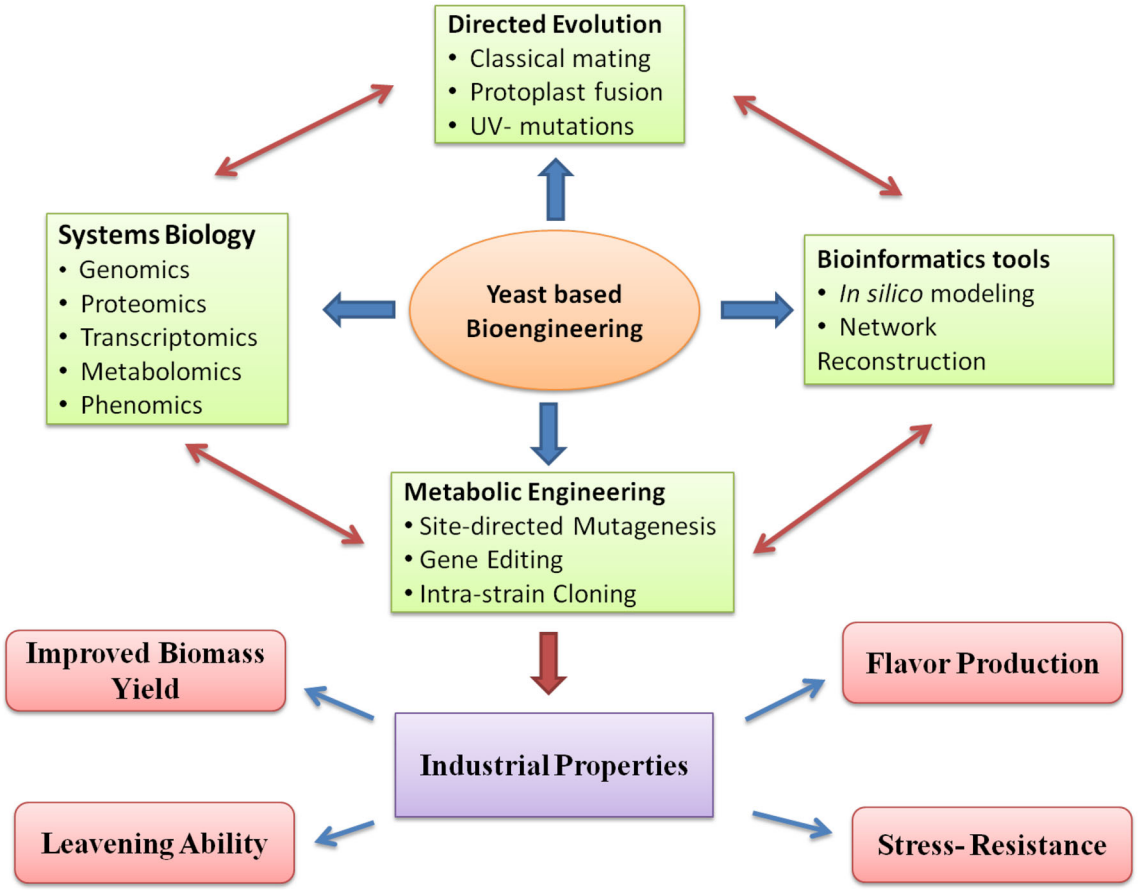

Fig. 1 Combinatorial approaches and modern techniques towards baker's yeast quality improvement

behavior, a low level of sporulation or very poor spore viability. The advances in genetic engineering and protoplast fusion technologies to the yeast genetics have solved the problems at certain levels [14, 15].

Considering the importance of baker's yeast, in this review, we highlighted the major bakery industries manufacturing the baker's yeast and its various products worldwide. Further, we describe the baker's yeast strain improvement for increased flavor induction, various industrial stress tolerances, and enhanced leavening ability. Moreover, we have illustrated different advance technologies such as systems biology, metabolic engineering, gene editing used for strain microbes improvement along with conventional techniques like recombinant DNA technologies, evolutionary engineering that can play very important role in further desired trait improvement.

Bakery Industry

Baker's products have been used as a vital component of the balanced diet for many centuries. Today, the baker's industry is one of the largest sectors of the food industry worldwide, which creates thousands of employments and generates billions of dollars in revenue. As the data available for the year 2016, the U.K. bread and morning goods are worth almost £4 billion. While in India, estimated turnover of the bakery products is US $ 1.3 billion and is also one of the largest manufacturing sectors. This constant growth has been driven by demands of consumer for appropriate and premium baked goods which are fresh, shelf-stable, nutritious and conveniently packaged. A large numbers of yeast manufacturing industries as described in Table 1 are situated around the globe. These industries are involved in production of different types of baker's yeast and other bakery products.

Improvement of Baker's Yeast and Flavor Induction

Initially, the wild strain of baker's yeast was not very effective for the production of bakery products. Thus, several industrial initiative to improve conventional baker's yeast strains for (1) pleasant flavor, (2) increased resistance to various stresses like high/low temperature,

Table 1 Major baker's yeast manufacturing industries in different countries

Industries	Country	Products	URL
Anchor Yeast	South Africa	Cream yeast, Instant yeast, Compressed yeast	http://www.anchor.co.za/
Lesaffre	France	Instant dry yeast, Compressed yeast, Liquid yeast, Crumbled yeast, Frozen semi-dry yeast (FSDY), Active dry yeast	http://www.lesaffre.com/
Red Star®	Wisconsin, United States	Active dry yeast, Quick rise™ yeast, Platinum® yeast, Cake (Fresh) yeast	http://redstaryeast.com/
AngelYeast Co., Ltd	China	Dry yeast, Semi-dry yeast and Fresh yeast	http://en.angelyeast.com/index.html
Goodrich	India	Compressed yeast	http://goodrichworld.org/index.html
Lallemand Inc.	Canadian	Baking products and Yeast	http://www.lallemand.com/our-business/baking/
Abmauri	India	Liquid yeast, Fresh yeast, Active dry and Instant dry yeast	http://www.abmauri.in/index.php/yeast-manufacturers-in-bangalore
Fleischmann's Yeast	United States And Canada	Rapid rise® yeast, Active dry yeast, Pizza crust yeast, Bread machine yeast, Fresh active yeast, Simply homemade®, Baking mixes	http://www.breadworld.com/
Zanae	Greece	Liquid yeast, Baker yeast	http://zanae.gr/en/product/liquid-yeast
Oriental Yeast Co., Ltd.	Japan	Bakers' yeast, Dough improver and Other baking products	http://www.oyc.co.jp/en/business/food.html
Zeus Iba	Italy	Baker's yeast	http://www.zeusiba.com/

pH, osmotic pressure, oxidation, high sugar and salt tolerances, (3) increased fermentation, and (4) good leavening ability.

The pleasing and appealing flavor of yeast raised products gives them universal acceptance and public preference. The characteristics flavor of yeast raised products arises from yeast fermentation and subsequent reactions of the by-products with other dough compounds during baking [16]. Fermentation by yeast produced large number of volatile compounds that provide different aroma to bakery products. Any single component cannot be responsible for the bread aroma while they act in synergistically [17]. Moreover, it is not necessary that specific component present during dough can induce specific flavor in the bread. Generally it is assumed that many secondary metabolites like aldehydes, ketoses, organic acids, higher alcohols, esters and various enzymes involved in the biochemical reactions as listed in Table 2 during dough fermentation are responsible for bearing of characteristics flavor [18–21]. As we know that, vanilla is most pleasing and a high demanding flavor produced from vanillin compound among the population. So to meet the demands, various chemical methods were developed for the synthesis of vanillin. But vanillin synthesized by chemical route limits its usability [22]. Thus biotechnological approaches have been developed for vanillin production such as biocatalytic transformation of lignin biopolymer to vanillin catalyzed by genetically modified *Rhodococcus jostii* [23]. Hansen et al. [17], has also been de novo synthesized the vanillin in the presence of glucose by metabolically engineered yeast. However, new non-conventional yeast strains *Torulaspora delbrueckii* and *S. bayanus* have been identified which provides satisfactory dough fermentation with an interesting flavor profile to the bread [24].

Stress Tolerance

In most of the bakery process, yeast strain is exposed to the various environmental conditions such as temperatures, pressure, pH, water content, osmotic, oxidation and various chemical compounds [30, 31]. These harsh conditions cause drastic damage to cellular organelles and membranes, which ultimately leads to growth inhibition or cell death [32]. So, it is necessary to acquire or induces different stress-adaptation cellular mechanisms such as stress protein induction, favorable changes in membrane structure, up and down-regulation of corresponding gene expression to survive under these stresses [33, 34]. Stress tolerance properties can significantly enhance the growth of yeast strain during fermentation conditions which ultimately increase the product yield. Many studies have been conducted for improvement of wild strain of baker's yeast in order to reduce these stresses [32, 35–37]. Recently, *AtTIL* gene encoding temperature-induced lipocalins, TIL protein from *Arabidopsis thaliana* was expressed in *S. cerevisiae* using genetic engineering approach. The recombinant strain conferred a high tolerance to the

Table 2 Flavors bearing organic compounds and enzymes during dough formation

Organic compounds	Flavoring agents	References
Organic acids	Butyric, succinic, propionic, n-butyric, iso-butyric, iso-valeric, heptanoic, pelargonic, palmitic, crotonic, itaonic, levulinic, benzylic, valeric, caprylic, lauric, myristic, vanillin,	[16, 25, 26]
Alcohols	Ethanol, n-propanol, 2-propanol, n-butanol, isobutanol, amyl alcohol, isoamyl alcohol, 2-3 butanediol, β-phenylethanol, benzylalcohol, 4-alyl-guayacol, furfuryl alcohol, n-pentanol, n-hexanol, n-octanol	[26, 27]
Aldehydes and Ketones	Formaldehyde, acetaldehyde, isovaleraldehyde, n-valeraldehyde, 2-methyl butanol, n-hexaldehyde, acetone, propionaldehyde, iso-butyraldehyde, methyl-ethyl ketone, 2-butanone, diacetyl, acetoin, dodecanal, 2-furaldehyde, 3-furaldehyde	[26, 27]
Esters and furan derivatives	Ethyl formate, ethyl acetate, ethyl pyruvate, ethyl levullnate, ethyl caprinate, ethyl laurate, ethyl myristinate, ethyl pentadecanoate, ethyl palmitate, glycol-di-acetate, amyl benzoate, furan, 2-methyl-furan, 2-acety-furan, 2-phenyl-furan, 2-propyl-furan	[26, 28]
Enzymes	α-Amylase, β-amylase, aminopeptidases, carboxypeptidase, xylanases, lipoxygenases, invertase, lipases, glucose oxidase, sulfhydryl oxidase, hemicellulases, pentosanases	[6, 28, 29]

stresses i.e. oxidative agents, heat shock, freezing and exposure to organic acids [38]. Similarly in another research, the recombinant strain of *S. cerevisiae* has been created by over expression of *MAL62* gene. This gene is responsible for trehalose accumulation in the cytoplasm which improve the cell for freezing tolerance [36]. Furthermore, some of the recent examples are also described in Table 3.

Fermentation and Leavening Ability

The higher fermentation and leavening ability of *S. cerevisiae* is essential for the manufacturing of various bakery goods. As we discussed above, during baking process, baker's yeast exposed to several environmental stresses which significantly lowers the cell survival and leavening ability [39, 40]. Several other key factors such as salt, carbohydrate sources, dough textures are also significantly affected the fermentation and leavening abilities [41]. In yeast, Glycogen is the main source of carbon and energy reserve. It is involved in many metabolic processes. Regulation of glycogen metabolism directly influences the fermentation ability [42]. Recently, *S. cerevisiae* was engineered for increasing the fermentation performance by inserting the promoter of a gluconeogenic gene, *PCK1*, into the upstream side of *RIM 15* gene to achieve its repression in glucose rich medium. *RIM 15* gene encode a protein kinase and down regulation of this gene increases the fermentation ability [43]. Many studies have been conducted in order to improve the leavening ability of bakers' yeast. In a study, the leavening property of *S. cerevisiae* has been enhanced by reducing the sucrase activity in sweet dough. The yeast strain of varying sucrose activity were constructed by *SUC2* gene deletion which encodes for sucrase enzyme [44]. Along with these, some other studies are enlisted in Table 3.

Techniques Used for Strain Improvement

Evolutionary Engineering

Evolutionary engineering is most widely used and very cost-effective technique for improvement of strains that includes mutagenesis and recombination of genes, pathways or sometimes whole cells followed by screening of improved strains developing desired phenotype [45]. This can be natural or induced by providing specific conditions to the cells. In this approach first cells are exposed to specific stress conditions which induce the mutation (addition, subtraction and substitution) in the genome. These mutations may be at a single site or at multiple sites. Treated cells are then screened for particular phenotype. This approach requires multiple generation cycles of random genetic changes and selections which finally give needful phenotype [46]. In this connection, several techniques of strain improvement like (1) classical mating, (2) protoplast fusion i.e. hybridization, (3) UV-induced mutations, and (4) stress-induced mutation are frequently used [14, 15, 47]. Many recent examples have demonstrated the satisfactory performance of this approach for strain improvement [45, 48, 49]. Moreover, this conventional approach has significantly improved *S. cerevisiae* for stress tolerance to various environmental conditions. Recently, *S. cerevisiae* has been engineered for improving ethanol tolerance using this approach. The strain was cultivated in the presence of gradually increasing ethanol concentration and selected mutants showed tolerance up to 12% (v/v) of ethanol. Along with this, the improved strain has also been shown the diplodization of haploid cells under ethanol stress [31]. Some of the recent examples are listed in Table 3. Although, this approach has been widely exploited for strain improvement, but it has several limitations such as lack of suitable screening methods for most of the traits, less chances of getting desired phenotype,

Table 3 Genetically improved baker's yeast with the role in bakery industries

Strain with improvement	Method of improvement	Role in bakery industry	References
Strain improvement for flavor induction			
S. cerevisiae for production of trans-cinnamic acid derivatives (cinnamaldehyde, cinnamyl alcohol, and hydrocinnamyl alcohol)	Metabolic engineering of *S. cerevisiae* by heterologous over expression of the genes encoding phenylalanine ammonia lyase 2 from *Arabidopsis thaliana* (*AtPAL2*), aryl carboxylic acid reductase (*acar*) from *Nocardia* sp., and phosphopantetheinyl transferase (*entD*) from *E. coli*	Production of high-value aromatic compounds	[66]
S. cerevisiae S288c for biosynthesis of 2-Furfurylthiol	Two genes *STR3* and *CYS3* from *S. cerevisiae* were cloned and over expressed in the reference strain *S. cerevisiae* S288c	2-Furfurylthiol is an important aroma compound with characteristic sesame flavor	[67]
S. cerevisiae for increased isobutanol production	Over-expression of ILV2, ILV3, ILV5, and BAT2 genes involved in valine metabolism	Enhanced production of vanillin, natural flavoring agent	[68]
Baker's yeast, showing higher vanillin production	In silico stoichiometries modeling	The fivefold increase in free vanillin production provides vanillin flavor	[16]
S. cerevisiae mutants for vanillin production	Pathway engineering by the introduction of four genes using Recombinant DNA Technology	Enhanced production of vanillin, natural flavoring agent	[17]
Strain improvement for stress tolerance			
S. cerevisiae strains with increased freeze-tolerance	Harbor the *TDH3p-PDE2* genes which are heterozygous and homozygous respectively by Intra-strain self-cloning procedure	Exhibits freeze tolerance	[69]
S. cerevisiae for resistance to proline analogue azetidine-2-carboxylate	Conventional mutagenesis	More tolerant to freezing-stress	[35]
S. cerevisiae with freezing tolerance and leavening ability	Deletion of *NTH1* in combination of *MAL62* gene over-expression	Enhanced anti-freezing and leavening ability during dough fermentation	[36]
Baker's yeast strains with the anti-freeze ability	Simultaneous deletion trehalase-encoded *NTH1* gene and proline oxidase-encoded *PUT1* gene	Enhanced anti-freezing ability	[70]
S. cerevisiae mutant by redox engineering	Glutamate dehydrogenase genes expression	High freeze-tolerance ability	[71]
Baker's yeast, showing higher trehalose accumulation	*NTH1* gene deletion	Displays a higher viability of yeast cells after freezing	[72]
Baker's yeast cells resistant to many types of baking-associated stress	Alteration of *POG1* gene by breeding method	Increased fermentation ability in bread dough after freeze–thaw stress	[37]
Strain improvement for higher fermentation and leavening ability			
Baker's yeast AY77 deficient in γ-aminobutyric acid (GABA) assimilation	Amber mutation in *DAL81* gene	Prevent reduction of GABA in dough fermentation	[73]
S. cerevisiae for leavening ability	*MAL61* and/or *MAL62* gene over-expression	Improved leavening ability of yeast during dough fermentation	[74]
S. cerevisiae having a high Maltose metabolism	SNF1 gene over-expression	Improved maltose metabolism and leavening ability of yeast during dough fermentation	[75]
S. cerevisiae with high storage carbohydrate metabolism	Over-expressing the *GSY2* gene and deleting NTH1 gene	High fermentation and metabolic capacity	[42]
S. cerevisiae for Bread leavening ability	Over-expression of SNR84 with PGM2 deletion	Enhanced leavening ability of baker's yeast	[40]
Baker's yeast for maltose metabolism	Disruption of *MIG1* and or *TUP1* and/or *SSN6* genes	Higher maltose metabolism resulted higher leavening ability during dough fermentation	[39]
S. cerevisiae mutant with altering of *POG1* gene expression	Genetic engineering approach	Higher fermentation abilities	[37]

randomness, hit and trial method, slow, labor intensive and require multiple generation cycles for a particular trait. Moreover, it is very difficult to determine which genetic modification is responsible for the improvement. Additionally, it is not possible to transfer the improved characteristics to other strains [31].

Systems Biology

The rapid growth of high throughput systems biology approaches in diversified fields, including strain improvement made this approach very important. It provides complete information of all cellular responses in different conditions which helps in determination of major changes at cellular levels or community levels and their interactions under specified conditions [10, 50]. The 'omics' techniques, i.e. genomics, transcriptomics, proteomics, metabolomics and phenomics are used alone or in combinations for the complete study of systems biology [51, 52]. The huge amount of data generated using systems biology tools is compiled, processed using several computational tools which provides valuable information about the systems. Further, complete genome of *S. cerevisiae* has already been sequenced and stored in the yeast genome database (YGD) developed by Stanford University, Stanford. In addition to this, complete information on all metabolic pathways of *S. cerevisiae* is present in YGD. Another database, Yeast metabolome Database (YMDB 2.0) having complete information of yeast metabolome are also developed [53]. The behavior of yeast varies according to their ambient and systems biology approach provides complete information about deferential behavior of yeast in varied environments. This data provide preliminary information for designing of strategies for further improvement. Recently, proteomics data has been analysis of two baker's yeast strains exposed to near freezing point (4 °C). From this data, it has been deduced that glycolytic protein expression up regulated, increased intracellular accumulation of glycerol and trials to prevent severe cold and freeze injury. At 4 °C, total of 16 hyper-expressed proteins were identified which takes part in energy-metabolism, translation and redox homeostasis [54]. In another research study, comparative transcriptomic and metabolic analysis was performed during fermentation of four different types of *S. cerevisiae* strains for rational identification of new targets for improving aroma production. The advance technologies (HPLC, GC-MS and microarrays) was used for comparative analysis [55].

Metabolic Engineering

Metabolic engineering is a powerful approach which has been successfully used to improve the various microbial strains for production of metabolites which cannot be formed naturally [56]. Moreover, this approach provides the ability to microorganisms to survive under stress conditions. This technique directly modifies the metabolic fluxes [57]. Generally, metabolic engineering involves manipulation of cellular pathways by altering the enzymatic, transport and regulatory functions of the cell by using recombinant DNA technology [58]. Basically, for metabolic engineering, it is necessary to have complete knowledge of metabolic pathways and thanks to the next generation sequencing, which provides complete information of genome sequences of baker's yeast [59]. Along with this, many bioinformatics tools and models have been developed which decode these genome information and provide full information about all metabolic pathways. This information is further used for construction of new metabolic networks, which will be executed in real by various molecular techniques such as genetic engineering and genome engineering, etc. A number of reviews and research work have been published for successful improvement of strains using metabolic engineering [60–62]. Recently, the metabolic pathways of *S. cerevisiae* have been engineered for the production of ethanol and its isomers which are used for biofuel production [76]. In this approach, a combination of valine synthesis and degradation and complete re-localization of cytosolic and mitochondrial pathways has been done [63]. Generally, recombinant DNA approach is used for metabolic engineering, but recently the emergence of gene editing or genome engineering tools using CRISPR/Cas9 made the metabolic engineering more uncomplicated [8, 9, 64, 65].

Conclusions and Perspectives

Bakery products have been used as an essential part of a balanced diet for many centuries. A wide variety of morning fast foods are found on the supermarket shelves. There is vast competition in the baking industries for the above-said bakery products in terms of premium quality, increased shelf-life, good flavor, high nutritional contents and pleasing appearance with cosmetic touch [2]. Yeast plays an important role in dough maturation due to production of CO_2 during fermentation. Along with this, different types of metabolites such as organic acids, alcohols, aldehydes, ketones, esters and enzymes responsible for flavor induction. The textures of bread are also dependent upon all these metabolites. There are lots of industrial stress, such as temperature, pH, salt, sugar, freezing and thawing, lower growth of yeast, less leavening ability, increase the CO_2 production, etc., which limits the use of native yeast strain for the production of bakery products and that ultimately negatively affect the overall yield [5]. So, in order to overcome these limitations, several approaches have been exploited for further strain improvement [35, 66, 67, 69, 73]. Many conventional techniques including genetic engineering and evolutionary engineering have been used successfully worldwide, but still there is some limitation with conventional yeast strain, i.e. *S. cerevisiae*. Moreover, most of the traits of industrial

importance like stress tolerance and increase dough raising power have been polygenic in nature with multi-step regulations at many sites, difficult to be dealt with recombinant DNA technology and evolutionary engineering. Unfortunately, even after putting much effort towards the production of genetically modified microorganisms, these are still not acceptable for human consumption. Besides this, the rheology and leavening properties of dough and bread characteristics are not yet fully tested for the newly created genetically modified strains derived from Baker's yeast. The emergence of some advanced technologies such as systems biology, genome engineering, metabolic engineering, bioinformatics tools and computational biology provide better understanding and analysis of intracellular metabolic networks and metabolites produced under varying conditions. Moreover, the future perspective might be in the field of isolation and screening of non-conventional yeast strains that can grow in high stress conditions, produce different flavors and cost-effective.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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