**MAL62 overexpression and NTH1 deletion enhance the freezing tolerance and fermentation capacity of the baker’s yeast in lean dough**

Xi Sun¹,², Cui-Ying Zhang³,⁴, Ming-Yue Wu⁵, Zhi-Hua Fan¹,², Shan-Na Liu¹,², Wen-Bi Zhu¹ and Dong-Guang Xiao³,⁴*

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**Abstract**

**Background:** Trehalose is related to several types of stress responses, especially freezing response in baker’s yeast (*Saccharomyces cerevisiae*). It is desirable to manipulate trehalose-related genes to create yeast strains that better tolerate freezing-thaw stress with improved fermentation capacity, which are in high demand in the baking industry.

**Results:** The strain overexpressing MAL62 gene showed increased trehalose content and cell viability after prefer-mention-freezing and long-term frozen. Deletion of NTH1 in combination of MAL62 overexpression further strengthens freezing tolerance and improves the leavening ability after freezing-thaw stress.

**Conclusions:** The mutants of the industrial baker’s yeast with enhanced freezing tolerance and leavening ability in lean dough were developed by genetic engineering. These strains had excellent potential industrial applications.

**Keywords:** Baker’s yeast, MAL62, NTH1, Freezing tolerance, Cell viability, Leavening ability

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**Background**

Frozen dough technology has been used in bakery industry to provide consumers with high-quality fresh bakery and convenience. However, cellular macromolecules, including proteins, nucleic acids and lipids of the yeast used in frozen dough, could be seriously damaged under the freezing and the subsequent thawing treatments, leading to inhibition of cell growth, cell viability and the leavening ability [1].

A great body of knowledge is already available regarding the molecular responses of the baker’s yeast (*Saccharomyces cerevisiae*) to frozen dough-associated stresses [2]. Among other molecules, trehalose has been highlighted due to its main function as a protective molecule in stress response [3]. This effect is achieved either by protecting membrane integrity through the union with phospholipids [4], or by preserving the native conformation of proteins and preventing aggregation of partially denatured proteins [5].

When yeast cells suffer from freezing stress, they accumulate large amounts of trehalose [6]. The accumulation is mainly induced by the classical the UDPG-dependent trehalose synthesis pathway, or referred as system I. It contains a trehalose-6-phosphate synthase encoded by TPS1 [7], a trehalose-6-phosphate phosphatase encoded by TPS2 [8] and a trehalose-synthesis protein complex encoded by TSL1 [9]. In addition, an alternative trehalose synthesis pathway, called ADPG-dependent trehalose synthesis pathway or the system II, has been proposed [10, 11]. It is specifically linked to maltose utilization.

Maltose metabolism in yeast depends on at least one of the five unlinked MAL loci (*MAL1* through *MAL4* and *MAL6*). A typical MAL locus consists of a *MALx1* (*MALxT*) gene (where *x* is the locus), encoding maltose permease, a *MALx2* (*MALxS*) gene, coding for...
alpha-glucosidase (maltase), and a MALx3 (MALxR) gene, encoding a positive regulatory protein [9]. It is reported that the expression of any one of the MAL loci in MAL-constitutive strains could elicit a maltose-induced trehalose accumulation [11]. Studies have shown that maltose and trehalose seem to share a common regulating mechanism [17, 18]. The maltose permease has been considered the rate-limiting enzyme in the MAL genes induction and maltose metabolism [4, 6, 12]. Hence, attempts to increase the trehalose content by system II had been concentrated on the modification of maltose permease or the entire MAL gene cluster [19–21]. However, recent studies showed that the alpha-glucosidase (maltase) is more important than maltose permease in maltose metabolism and leavening ability of baker’s yeast in lean dough [22–24]. In addition, the system II might be dependent of the system I, due to the fact that the system II is completely prevented when TPS1, a key gene in system I, is deleted [12].

Trehalose degradation could also be induced under certain stress [13, 14]. The best characterized trehalase is the neutral trehalase encoded by the NTH1 gene, which is induced by stress, such as heat. Nth1p is involved in thermos-tolerance and hydrolyzes intracellular trehalose into glucose [15, 16]. Deletion of NTH1 results in accumulation of trehalose, and heat sensitivity.

To better understand the role of trehalose in freezing tolerance of baker’s yeast in lean dough, and its possible mechanism, we investigated the effects of overexpression of MAL62, the gene encoding an alpha glucosidase, and deletion of NTH1 gene, on trehalose accumulation and on the freezing tolerance and leavening ability of baker’s yeast in lean dough.

**Methods**

**Strains, plasmids and growth conditions**

The genetic properties of all *S. cerevisiae* strains and plasmids used in the present study are summarized in Table 1. The BY14a was selected as a high leavening capacity haploid from 32 clones derived from the diploid BY14 strain, which has been maintained at the Tianjin Key Laboratory of Industrial Microbiology, Tianjin University of Science and Technology.

Recombinant DNA was amplified in *Escherichia coli* DH5α. Transformants were grown in Luria–Bertani medium (10 g/L tryptone, 5 g/L yeast extract, and 10 g/L NaCl) with 100 mg/L ampicillin. The plasmid was obtained using Plasmid Mini Kit II (D6945, Omega, USA).

The yeast strain was grown at 30°C in 3% yeast extract peptone dextrose (YPEPD) medium (10 g/L yeast extract, 20 g/L peptone, and 20 g/L dextrose). Approximately 800 mg/L of G418 was added to the YEPD plates for selecting Geneticin (G418)-resistant transformants. After cultivation in YEPD for 24 h, 20 mL of the cell culture was inoculated into 200 mL of cane molasses medium (5 g/L yeast extract, 0.5 g/L (NH₄)₂SO₄, and 12° Brix cane molasses) at the initial OD₆₀₀ = 0.4 and cultivated for 24 h at 30°C with 180 rpm rotary shaking to the final OD₆₀₀ = 1.8. Cells were harvested through centrifugation (4°C, 1500 × g, 5 min) and were washed twice with sterile water at 4°C for the succeeding fermentation experiments. To investigate the degradation of trehalose during prefermentation and the freezing tolerance, a modified low sugar model liquid dough (LSMLD) medium was used [17]. The modified medium contains 2.5 g/L (NH₄)₂SO₄, 5 g/L urea, 16 g/L KH₂PO₄, 5 g/L Na₃HPO₄, 0.6 g/L MgSO₄, 22.5 mg/L nicotinic acid, 5 mg/L Ca-pantothenate, 2.5 mg/L thiamine, 1.25 g/L pyridoxine, 1 mg/L riboflavin, and 0.5 mg/L folic acid and carbon sources (33.25 g/L maltose with 5 g/L glucose).

**Plasmid construction and yeast transformation**

Genomic yeast DNA was prepared from the industrial baker’s yeast BY14a using a yeast DNA kit (D3370-01, Omega, Norcross, GA, USA). Table 2 shows the PCR primers used in this study.

Plasmid Yep-PMK (Yep-PGK1-MAL62-KanMX), an episomal plasmid with MAL62 under the control of the constitutive yeast phosphoglycerate Kinase gene (PGK1) promoter (PGK1p) and terminator (PGK1T), was constructed as follows: a KpnI/BamHI/KanMX fragment, which was the dominant selection marker during yeast conversion, was amplified through PCR using pUG6 as template with Kan-U and Kan-D primers, and was cloned to the Yep352 vector to construct the empty plasmid Yep-K (Yep-KanMX). A XhoI fragment of MAL62 amplified with MAL62-U and MAL62-D primers from the genomes of the parental strain BY14a was inserted into the PGK1 fragment of pPGK1 vector and resulted in plasmid pPGK. Then, the BamHI fragment of PGK (the entire PGK1 and the inserted MAL62) amplified with PGK-U and PGK-D from pPGK was cloned to Yep-K to produce the final plasmid Yep-PMK.

Baker’s yeast transformation was achieved through lithium acetate/PEG method [18]. The deletion cassette of NA-loxP-KanMX-loxP-NB was amplified with N-S and N-X and transformed into the industrial baker’s yeast BY14a. The fragment was integrated into the chromosome at the NTH1 locus of BY14a by homologous recombination to construct the NTH1 deletion strain. The selection of NTH1 deletion strain was performed using the YEPD medium supplemented with 800 mg/L geneticin (G418). After selection, recombinant strains were verified with the primers N-S, K-S and N-X, K-X. Cre recombinase was expressed and KanMX was excised after introducing the plasmid pSH-Zeocin into
the NTH1 deletion strain, thus resulting in B-NTH1. The respective transformation plasmids Yep-K, Yep-PMK were then transformed to select the G418-resistant strains BY14a+K, B-NTH1+K, B+MAL62 and B-NTH1 + MAL62. BY14a+K and B-NTH1 + K were BY14a and B-NTH1 carrying the vector Yep-K, respectively, used as a blank control to demonstrate any possible effect of the empty vector. The transformants were then verified by PCR using the primers Kan-U and Kan-D.

### Table 1 Characteristics of strains and plasmids used in the present study

| Strains or plasmids | Relevant characteristic                                                                 | Reference or source                                                                 |
|---------------------|----------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|
| Strains             |                                                                                       |                                       |
| Escherichia coli    | Ø80 lacZD1M1 S carpU169 recA1 endA1 hsdR17 supE44 thi-1                                  | Yeast Collection Center of the Tianjin Key Laboratory of Industrial Microbiology     |
| DH5α                |                                                                                       |                                       |
| BY14                | MA790a Industrial baker’s yeast                                                        | Yeast Collection Center of the Tianjin Key Laboratory of Industrial Microbiology     |
| BY14a               | MA790, haploid derived from BY14 strain                                                | This study                                                                           |
| B-NTH1              | MA790, ΔNTH1::loxP                                                                    | This study                                                                           |
| B-NTH1 + K          | MA790, ΔNTH1::loxP, Yep-K                                                              | This study                                                                           |
| BY14a + K           | MA790, Yep-K                                                                          | This study                                                                           |
| B + MAL62           | MA790, Yep-PMK                                                                        | This study                                                                           |
| B-NTH1 + MAL62      | MA790, ΔNTH1::loxP, Yep-PMK                                                           | This study                                                                           |
| Plasmids            |                                                                                       |                                       |
| pUG6                | E. coli/S. cerevisiae shuttle vector, containing Amp*, loxP-kanMX-loxP disruption cassette | [41]                                                                                 |
| Yep352              | URA3*, Amp' or control vector                                                         | Invitrogen, Carlsbad, Ca, USA                                                       |
| Yep-K               | KanMX ARS URA3*, Amp' or control vector                                                | This study                                                                           |
| pPGK1               | bla LEU2 PGK1::PGK1                                                                   | This study                                                                           |
| pPGKM               | bla LEU2 PGK1::MAL62-PGK1                                                              | This study                                                                           |
| pUC-ABK             | NA-loxP-KanMX-loxP-NB                                                                  | Yeast Collection Center of the Tianjin Key Laboratory of Industrial Microbiology     |
| Yep-PMK             | bla LEU2 PGK1::MAL62-PGK1::KanMX                                                      | This study                                                                           |
| pSH-Zeocin          | Zet', Cre expression vector                                                           | Yeast Collection Center of the Tianjin Key Laboratory of Industrial Microbiology     |

*BY14a was selected as high leavening capacity haploid from 32 clones derived from BY14 strain (data not shown)*

### Table 2 Primers used in the present study (restriction sites are underlined)

| Primer name | Sequence 5'-3' |
|-------------|----------------|
| Kan-U       | CCGGGTACCAGCTGAGCTTCCGCTGACGC          |
| Kan-D       | CCGCGGTACCGCCATGATGTCGAAGCTGCTG          |
| MAL62-U     | CCGCTCGAGATAGCTTCTCTAGCTCCATGCTC          |
| MAL62-D     | CCGCTCGAGATGCTTCTCTAGCTGCAGCTGCTG          |
| PGK-U       | CCGGGATCCAGCTGAGCTTCCGCTGACGC          |
| PGK-D       | CCGGGATCCAGCTGAGCTTCCGCTGACGC          |
| N-S         | ATCATCATCTGATACGCTACCT                      |
| K-S         | CCGGGATCCAGCTGAGCTTCCGCTGACGC          |
| N-X         | TACAGCGTAAAGTCCTGATGCTGCTGCTG          |
| K-X         | TACAGCGTAAAGTCCTGATGCTGCTG          |

**Assay of the intracellular trehalose content**

Fresh yeast cells were dried overnight at 85 °C to calculate the cell dry weight (CDW). Trehalose was extracted from 0.1 g of fresh yeast cells (previously washed with distilled water twice) with 4 mL of 0.5 mol/L cold trichloroacetic acid and the extract was employed for measuring the trehalose content as described previously [19, 20]. Experiments were conducted three times.

**Determination of neutral trehalase activity**

The activities of neutral trehalase in crude extracts were measured as described previously [21]. The liberated glucose was analyzed by HPLC employing an Aminex HPX-87H column with 5 mmol/L H₂SO₄ as the mobile phase at a flow rate of 0.6 mL/min at 65 °C. One unit of trehalase activity was defined as the amount of trehalase producing 1.0 μmol glucose per min under assay conditions. The specific trehalase activity was expressed as the units per gram CDW. Experiments were conducted three times.

**Determination of Tps1 (trehalose-6-phosphate synthase) activity**

Tps1 activity was measured as described previously [22]. The trehalose-6-phosphate formed during the reaction...
was quantitatively determined using the Anthrone method [19]. One unit of Tps1 activity was defined as the amount of Tps1 producing 1.0 μmol 6-phosphate-trehalose per min under assay conditions. The specific Tps1 activity was expressed as the units per gram CDW. Experiments were conducted three times.

**Determination of alpha-glucosidase activity**

Crude extracts were prepared using the Salema-Oom method to determine enzyme activities [23]. Alpha-glucosidase were determined following the Houghton-Larsen method [24]. Standard errors were less than 10 %.

**Determination of the cell viability of baker’s yeast after freezing and thaw**

For the freeze–thaw stress, yeast cells were harvested from the cane molasses medium and inoculated into the LSMLD medium at 30 °C for 25 min. One milliliter of cell culture was shifted to −20 °C and at 5 min intervals for different prefermentation time periods. After freezing for 1–3 week, the frozen suspensions were thawed at 30 °C for 30 min then diluted and plated on YEPD plates for 2 days. Cell viability was determined by the percentage of the number of colonies after stressing relative to the number of colonies before stress. Three independent experiments were performed.

**Determination of leavening ability**

The leavening ability of yeast cells was assayed by measuring the CO₂ production in lean dough. Lean dough was composed of 280 g of standard flour, 150 mL of water, 4 g of salt, and 9 g of fresh yeast. The dough was evenly and rapidly stirred for 5 min at 30 ± 0.2 °C then divided into pieces (50 g each) and placed in a fermentograph box 171 (Type JM451, Sweden). CO₂ production was recorded at 30 °C for 120 min. Experiments were conducted three times.

To assay the leavening ability after freeze–thaw, the mixed dough was stored at −20 °C. After freezing for 1 week, the frozen dough was thawed at 30 °C for 30 min, and the CO₂ production was assayed for 120 min at 30 °C. Experiments were conducted at least thrice.

**Statistical analysis**

Data were expressed as mean ± SD and were accompanied by the number of experiments independently performed. Differences among all the strains were analyzed using ANOVA. P < 0.05 were considered statistically significant. The differences between the transformants and the parental strain were confirmed by Student’s t test. Differences at P < 0.05 were considered statistically significant.

**Results**

**Overexpression of MAL62 enhances the Tps1 activity and intracellular trehalose content of baker’s yeast**

Previous studies have reported that the MAL gene has a positive effect on the activity of Tps1, a trehalose-6-phosphate synthase that synthesizes trehalose under stress conditions [12]. We first tested if the Tps1 activity is affected by MAL62 overexpression. As shown in Table 3, overexpression of MAL62 (in both B + MAL62 and B-NTH1 + MAL62) significantly increased the Tps1 activity (P < 0.05). The alpha-glucosidase activities of these two strains were also increased significantly (Table 3). These results suggest that overexpression of MAL62 induces trehalose production.

To further confirm this, we measured and compared the trehalose levels in different strains. We found that all six strains (BY14a, B-NTH1, B + MAL62, B-NTH1 + MAL62, BY14a + K and B-NTH1 + K) had similar growth curves. Cells entered exponential phase 3 h after inoculation, and stationary phase 10 h after inoculation (data not shown). Our results showed in strains overexpressing MAL62 (B + MAL62 and

### Table 3  Alpha-glucosidase activities, Tps1 activities and the neutral trehalase activities of strains

|                | Alpha-glucosidase activity⁴ (μmol/mg/min) | Tps1 activity⁵ (U/g CDW) | Neutral trehalase activity⁶ (U/g CDW) |
|----------------|------------------------------------------|--------------------------|---------------------------------------|
| BY14a          | 2.46 ± 0.25                              | 0.80 ± 0.07              | 12.28 ± 0.88                          |
| BY14a + K      | 2.45 ± 0.22                              | 0.83 ± 0.10              | 12.26 ± 0.81                          |
| B-NTH1         | 2.45 ± 0.21                              | 0.82 ± 0.07              | 8.68 ± 0.74*                          |
| B-NTH1 + K     | 2.45 ± 0.23                              | 0.81 ± 0.09              | 8.39 ± 0.53*                          |
| B + MAL62      | 4.43 ± 0.37**                            | 1.06 ± 0.10*             | 12.36 ± 0.93                          |
| B-NTH1 + MAL62 | 3.66 ± 0.32**                            | 1.01 ± 0.11*             | 8.31 ± 0.61*                          |

Values shown represent at least three independent experiments (data are mean ± SD). Significant difference of the transformants (BY14a + K, B-NTH1,B-NTH1 + K, B + MAL62, B-NTH1 + MAL62) from the parental strain was confirmed by Student’s t-test (**P< 0.01, *P < 0.05, n = 3)

⁴ Alpha-glucosidase activities and Tps1 activities were calculated from the cells grown in cane molasses medium

⁵ Neutral trehalase activities were calculated from the cells prefermentation in LSMLD medium
B-NTH1 + MAL62), trehalose started to accumulate in late exponential stage at a rate of 21.9 mg/h/g CDW. In contrast, in strains having no MAL62 overexpression (BY14a, B-NTH1, BY14a + K and B-NTH1 + K), trehalose accumulation started only in stationary phase and at a lower rate (19.1 mg/h/g CDW) (Fig. 1).

**MAL62 overexpression does not affect the rate of trehalose degradation**

To examine if MAL62 overexpression or NTH1 is involved in trehalose degradation, we compared the neutral trehalase activity and the degradation rate of intracellular trehalose among the six strains. As shown in Table 3, the B + MAL62 strain had a similar neutral trehalase activity compared to its control (BY14a and BY14a + K), suggesting that overexpression of MAL62 did not affect the trehalose degradation. This is further confirmed by direct measurement of the intracellular trehalose content (Fig. 2), which showed a similar degradation rate among B + MAL62, BY14a and BY14a + K. In addition, both the neutral trehalase activity and the rate of trehalose degradation were significantly lower in all NTH1 deletion strains (B-NTH1, B-NTH1 + K and B-NTH1 + MAL62) (Table 3, Fig. 2), regardless whether MAL62 was overexpressed or not. These results suggest that NTH1, but not MAL62, is important for trehalose degradation.

**High trehalose content increases viability of yeast cells after freezing**

Although a number of reports have shown that the degradation of trehalose during prefermentation is necessary [25], the residual intracellular trehalose is still considered to be important to freezing tolerance of yeast [26, 27]. Hereby, we assessed the cell viability of the six strains to investigate the effect of MAL62 overexpression and/or NTH1 deletion on the freezing tolerance of yeasts after prefermentation and 7 d freezing.

As shown in Fig. 3, the cell viability of strains with MAL62 overexpression (B + MAL62 and B-NTH1 + MAL62) was significantly higher than the other strains before prepermentation (time = 0 min). Cell viability of all strains decreased as prefermentation time increased. 25 min after prefermentation, the cell viability of the strain with both MAL62 overexpression...
and NTH1 deletion (B-NTH1 + MAL62) was significantly higher than other strains (ANOVA, P < 0.05). The cell viability of strains with either MAL62 overexpression or NTH1 deletion remained in the middle, while the control strains (BY14a and BY14a + K) had the lowest viability, dropping from about 80 % to about 40 %. The cell viability is in agreement with the trehalose content (Fig. 2), which showed that 25 min after prefermentation, the B-NTH1 + MAL62 had the highest trehalose content (95 mg/g CDW) and the controls (BY14a and BY14a + K) had the lowest (about 55 mg/g CDW). These results suggest that the residual trehalose content has a positive correlation with the viability of yeast cells after prefermentation and freezing [28].

Overexpression of MAL62 or deletion of NTH1 confers long-term freezing tolerance of baker’s yeast

In order to access the long-term freezing tolerance of the NTH1-deletion and/or the MAL62-overexpression strains, we examined the trehalose content before freezing and the cell viability 21d after freezing (Fig. 4). As shown in Fig. 4, both the trehalose content and the cell viability were significantly higher in strains with MAL62 overexpression (B + MAL62 and B-NTH1 + MAL62) (ANOVA, P < 0.05). Compared with the control (BY14a and BY14a + K), deletion of NTH1 alone (B-NTH1 and B-NTH1 + K) also induced a higher trehalose content and higher cell viability, which is in agreement with previous studies [26, 29].

Overexpression of MAL62 and deletion of NTH1 enhance the fermentation characteristics of baker’s yeast exposed to freezing-thaw stress

Leavening ability is an important fermentation characteristic of baker’s yeast used in frozen dough. We next explored the possible effects of MAL62 overexpression and NTH1 deletion on the leavening ability after freezing and thaw by measuring the CO₂ production. Our results showed that freezing-thaw caused a reduction of CO₂ production in all strains (comparing Fig. 5a with 5b). However, either before or after freezing-thaw, overexpression of MAL62 (B + MAL62 and B-NTH1 + MAL62) significantly enhanced the CO₂ production (ANOVA, P < 0.05). NTH1 deletion alone had no effect on CO₂ production before freezing-thaw (Fig. 5a) but enhanced the CO₂ production after freezing-thaw (Fig. 5b). Interestingly, MAL62 overexpression and NTH1 deletion (B-NTH1 + MAL62) had a lower CO₂ production than MAL62 overexpression alone (B + MAL62) before freezing-thaw, but the CO₂ production was higher after the freezing-thaw, suggesting that MAL62 overexpression and NTH1 deletion provide the best enhancement on leavening ability upon freezing-thaw stress.

Discussion

Biological macromolecules and membranes are liable to denaturation under freezing conditions [30]. Freezing also causes the formation of intracellular ice crystals, which are harmful to cells. It has been suggested that trehalose could act as a stabilizer of cellular membranes and proteins under freezing stress [28]. Previous studies have reported that the modification of the whole MAL gene cluster is necessary to elicit trehalose synthesis [31, 32]. In this study, we demonstrated that the single-gene-overexpression of MAL62 in industrial baker’s yeast is capable of increasing trehalose accumulation and cell viability under freezing stress. Trehalose formation in MAL62 overexpressing strains (B + MAL62 and B-NTH1 + MAL62) was earlier and faster than the controls (Fig. 1), suggesting the positive effects on the intracellular trehalose content and freezing tolerance (Figs. 2, 4). Moreover, although MAL62 overexpression had little effect on protecting trehalose against degradation during prefermentation, the cell viability assay showed that the MAL62 overexpression could protect cells against freezing stress after prefermentation. This is in line with a previous report [33], showing that the trehalose accumulation before the induction of stress was more important for stress tolerance.

One explanation is that MAL62 overexpression enhances the activity of Tps1. This hypothesis relies on the fact that maltose constitutive genes could partially relieve Tps1 from the catabolite repression [34], and the alpha-glucosidase (coded by gene MAL62) is the rate-limiting factor in maltose metabolism [35]. Our result is consistent with this hypothesis, since Tps1 activity could increase when the alpha-glucosidase activity was enhanced by MAL62 overexpression (Table 3). Another explanation is that the existence of
adenosine-diphosphoglucose (ADPG)-dependent trehalose synthase, which requires ADPG instead of UDPG as donor of glucose units for trehalose synthesis [36]. Since the expression of ADPG-pyrophosphorylase gene and MAL genes shared the common regulation, any of the MAL gene products either by means of control at the transcription level, or by acting directly on enzyme activity could regulate the activity of the ADPG-pyrophosphorylase activity [11]. Hence, overexpression of MAL62 alone could increase intracellular trehalose content and bring about further enhancements in freezing tolerance.

The fermentation characteristics of baker's yeast as a strong correlation with the tolerance in stress conditions [37]. After exposure to freeze–thaw stress, response to the environmental change involved in rapid accumulation of relevant protectants and rapid production of enzymes related to stress-protective effect [7, 19]. In this work, we found that the freezing tolerance and the fermentation characteristics of the double mutant (B-NTH1 + MAL62) were significantly enhanced than that of either single mutant (B-NTH1 or B + MAL62) after the freezing-thaw stress (Figs. 4, 5b). In addition, we found that NHT1 deletion (B-NTH1, B-NTH1 + K and B-NTH1 + MAL62) induced a low neutral trehalase (Table 3), which caused a lower level of trehalose degradation. High activity of trehalose synthase (+MAL62) [38] and low activity of neutral trehalase (−NTH1) increase the intracellular trehalose level [26], which explains why the double mutant (B-NTH1 + MAL62) provides the best freezing tolerance and fermentation characteristics [39].

In summary, our study showed that MAL62 overexpression and NTH1 deletion in baker’s yeast significantly enhanced the freezing tolerance and fermentation characteristics, which is in high demand in the frozen dough baking industry [40]. On the basis of our findings, it is also possible to lengthen the storage period of frozen dough through overexpression of the MAL62 with NTH1 deletion. It provides valuable insights for breeding novel stress-tolerant and fast-fermented baker’s yeast strains that are useful for baking industry.

Conclusion

The results of this study show that overexpression of MAL62 was an effective way of increasing trehalose content and cell viability after prefreezer-freezing and long-term frozen. Deletion of NTH1 in combination of MAL62 overexpression could further strengthen freezing tolerance and improve the leavening ability after freezing-thaw stress. Furthermore, the single-gene-overexpression of MAL62 in industrial baker’s yeast is capable of increasing trehalose accumulation, therefore, promoting cell viability and the leavening ability of baker’s yeast in lean dough under freezing stress. Hence, such baker’s yeast has excellent commercial and industrial applications.

Authors’ contributions

XS carried out the experiments and drafted the manuscript. MYW and SNL participated in the plasmid and strain construction. ZHF and WBZ assisted in fermentation experiments. CYZ and DGX conceived the study and reviewed the final manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.
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