Deletion of \textit{NTH1} and \textit{HSP12} increases the freeze–thaw resistance of baker’s yeast in bread dough

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**Abstract**

**Background:** The intracellular molecule trehalose in \textit{Saccharomyces cerevisiae} may have a major protective function under extreme environmental conditions. \textit{NTH1} is one gene which expresses trehalase to degrade trehalose. Small heat shock protein 12 (\textit{HSP12} expressed) plays a role in protecting membranes and enhancing freezing stress tolerance.

**Results:** An optimized \textit{S. cerevisiae} CRISPR-Cpf1 genome-editing system was constructed. Multiplex genome editing using a single crRNA array was shown to be functional. \textit{NTH1} or/and \textit{HSP12} knockout in \textit{S. cerevisiae} enhanced the freezing stress tolerance and improved the leavening ability after freezing and thawing.

**Conclusions:** Deleting \textit{NTH1} in the combination with deleting \textit{HSP12} would strengthen the freezing tolerance and protect the cell viability from high rates of death in longer-term freezing. It provides valuable insights for breeding novel \textit{S. cerevisiae} strains for the baking industry through a more precise, speedy, and economic genome-editing system.

**Keywords:** \textit{S. cerevisiae}, \textit{NTH1}, \textit{HSP12}, Freeze–thaw resistance, CRISPR, Cpf1

**Background**

Frozen dough technology is important to the development of the bakery industry because the frozen dough can be delivered to downstream sales at low temperatures, and through simple thawing, fermentation, and baking, it is convenient to supply freshly baked bread to consumers. The technology not only can provide consumers with freshly baked products at any time, but also reduce economic losses caused by unstable quality, short shelf life, and aging of baked products. However, a disadvantage of the frozen dough technology is the low resistance of baker’s yeast, \textit{Saccharomyces cerevisiae}, to freezing stress [1]. Considerable evidence indicates that the intracellular molecule trehalose in \textit{S. cerevisiae} may have a major protective function under extreme environmental conditions [2]. Trehalose in \textit{S. cerevisiae} can maintain the membrane integrity by combining with phospholipids and the native conformation of proteins, preventing the aggregation of partially denatured proteins to achieve the effect of freezing resistance [3, 4].

The biosynthesis of trehalose is a two-step process, involving the production of trehalose-6-phosphate (T6p) catalyzed by trehalose-6-phosphate synthase (Tps) and its consecutive dephosphorylation to trehalose, catalyzed by trehalose-6-phosphate phosphatase (Tpp) [5–7]. However, the intracellular level of trehalose in yeast cells is the result of a well-regulated balance between enzymatic synthesis and degradation [8]. Trehalose is hydrolyzed into glucose by a cytosolic/neutral trehalase encoded by \textit{NTH1} [9, 10]. Deletion of \textit{NTH1} results in decreased trehalase activity, enhanced accumulation of trehalose, and...
increased freezing resistance [11–13]. Except for NTH1, there are two genes, NTH2 and ATH1, also involved in the hydrolysis of trehalose. The NTH2 gene, a paralog of NTH1, encodes a functional trehalase involved in trehalose mobilization [14]. An acid trehalase encoded by ATH1, whose major activity is measured extracellularly because of the vacuolar localization. Lack of Ath1p does not alter intracellular levels of trehalose [15, 16].

Accumulated evidence indicates that molecular chaperones such as the heat shock family of stress proteins (HSPs) actively participate in an array of cellular processes, including cryoprotection [17]. Small heat shock protein 12 (Hsp12p) plays a role in protecting membranes against desiccation and ethanol-induced stress [18], enhancing osmotolerance [19], and freezing tolerance [20]. The HSP12 deletion mutant is more resistant to freezing, and overexpression of Hsp12p in the TPS1 deletion strain increases in resistance to freezing storage and heat stress [20].

The clustered regularly interspaced short palindromic repeats (CRISPR) as a bacterial immune defense system that has been developed for genome-editing tools in a variety of organisms [21]. The class 2 CRISPR-Cas9 system is widely used for genome editing due to the inherent simplicity and flexibility in sequence requirements for single guide RNA (sgRNA). Using Cas9 endonuclease from Streptococcus pyogenes, a sgRNA containing 20 bp complementary sequence of the target sequence as utilizing a T-rich PAM [26, 27], guided by one single crRNA [28, 29], and DNA cleavage results in sticky ends [27].

In the present study, we first generated a CRISPR-Cpf1 genome-editing system in S. cerevisiae using a visual recognition model that presents red colonies when the ADE2 gene is deleted [30]. Furthermore, we constructed baker's yeast strains with deleted trehalase gene and/or heat shock protein 12 gene by CRISPR-Cpf1 system to investigate the content of intracellular trehalose, and cell viability after freezing and thawing. Finally, the leavening ability of the frozen bread dough with edited yeast strains was analyzed.

Results
Optimization of the CRISPR-Cpf1 genome-editing system in S. cerevisiae
To optimize the CRISPR-Cpf1 genome-editing system in S. cerevisiae, we targeted for deletion of the ADE2 gene, which has coloring properties to visually confirm the efficiency of genome editing. Three types of homologous DNA template donors for the CRISPR-Cpf1 editing system were designed, such as 1 kb double-strand DNA (dsDNA), 120 bp dsDNA, and 120 bp single-strand DNA (ssDNA). The results showed that the editing efficiency of the longer dsDNA template donor (1 kb) was significantly higher than that of the shorter (120 bp). Comparing the editing efficiency between different types of homologous DNA template donors, the ssDNA template donor was 12.5 times higher than the dsDNA template donor (Fig. 1). The results indicated that the ssDNA template donor for the CRISPR-Cpf1 system in S. cerevisiae could significantly improve the efficiency of genome editing.

Furthermore, we examined the electroporation parameters for genome editing in S. cerevisiae, including recovery period and plasmid concentration. The editing efficiency improved to 67.37 ± 2.34%, when using 500 ng plasmid amounts for electroporation and incubating for 48 h for cell recovery after electroporation (Table 1). These results indicated that using ssDNA as homologous template donors and recovering cells for longer periods had significant effects on genome-editing efficiency in the CRISPR-Cpf1 system in S. cerevisiae.

Generation of NTH1 and/or HSP12 deletion strains by CRISPR-Cpf1
Storage of frozen bread dough may lead to loss of cell viability of baker’s yeast and its baking capacity [1]. Trehalase can maintain the integrity of cell membranes and the native conformation of proteins in baker’s yeast under freezing stress [11]. Studies have demonstrated the correlation between the small heat-shock protein Hsp12p and yeast survival under freezing stress conditions [20]. To improve the baking capacity in baker’s yeast after long freezing storage in bread dough, the NTH1 and/or HSP12 genes of S. cerevisiae were deleted using the CRISPR-Cpf1 genome-editing system (Fig. 2). The NTH1 deleted strain, the HSP12 deleted strain, and the NTH1/HSP12 double deleted strain were generated in this study. PCR analysis was used for the verification of NTH1 and HSP12 deletions (Fig. 3). As expected, PCR products in the deletion strains had smaller sizes than in WT strain. The trehalase activity of the edited strains was also analyzed. Compared with the WT strain, the trehalase activity of the NTH1 deleted strains (Δnth1 and Δnth1/Δhsp12) were significantly reduced, but the trehalase activity of the HSP12 deleted strain was not...
significantly altered (Table 2). Deletion of NTH1 did not cause the complete abolishment of trehalase activity because other trehalase such as NTH2 still presented in the NTH1 deleted strains.

Deletion NTH1 increased intracellular trehalose contents
Trehalose is related to freezing-thawing stress tolerance in baker’s yeast [2]. To examine the intracellular trehalose contents in different knockout strains, cells were harvested in the stationary phase when trehalose synthesis is particularly intensive [31–33]. The results showed that the trehalose content of the NTH1 deleted strain was 104 ± 2.3 mg/g cell dry weight (CDW), which was 2.3 times higher than that of the wild-type strain 45.4 ± 2.3 mg/g CDW. However, the trehalose content was not increased in the HSP12 deleted strain. Furthermore, the intracellular trehalose content of the NTH1/HSP12 double knockout strain was 112.9 ± 5.4 mg/g CDW, which was 2.5 times higher than that of the wild-type strain (Table 2). The levels of trehalose were significantly higher in all NTH1 deleted strains (Δnth1 and Δnth1/Δhsp12). It indicates that NTH1, but not HSP12, is important for the level of trehalose.

NTH1/HSP12 double deleted strains confer long-term freezing tolerance
To investigate the freezing tolerance of the NTH1 deleted and/or the HSP12 deleted strains, the cell viability was analyzed after 7, 14, and 21 days of frozen storage. After
7 days of frozen storage, the cell viability of the wild-type strain was 42.6 ± 1.58% and 74.7 ± 4.26% without and with 30% glycerol as a cryoprotectant, respectively. However, it dropped to 9.15 ± 1.82% after 21 days of frozen storage without cryoprotectant. The cell viability of the NTH1 deleted strain was 87.17 ± 8.32% and 65.46 ± 6.41% after 7 and 14 days of frozen storage, respectively; however, it dramatically decreased to 19.8 ± 1.85% after 21 days. It is suggested that the NTH1 deletion strains could maintain cell viability for short-term frozen storage, but not for long-term frozen storage. The cell viability of the HSP12 deleted strain was 61.05 ± 7.45%, 58.39 ± 6.02%, and 47.81 ± 3.46 after 7, 14, and 21 days of freezing, respectively. Moreover, the cell viability of double genes deleted strains was 88.35 ± 9.41%, 83.33 ± 2.14%, and 62.87 ± 6.29% after 7, 14, and 21 days of freezing, respectively (Fig. 4). It is suggested that double deletion of NTH1 and HSP12 genes improved cell viability for long-term freezing. These data indicated that the trehalose content has a positive correlation with the viability of yeast cells for short-term freezing, and the absence of Hsp12p resulted in higher resistance to long-term freezing.

**NTH1/HSP12 double deleted strain had enhanced leavening ability of frozen dough**

An important fermentation characteristic of baker’s yeast used for frozen dough is leavening ability. We analyzed the leavening ability of bread dough
containing different edited yeast strains after 7 and 21 days of freezing (Fig. 5). Our results showed that the bread dough lost the leavening ability after freezing and thawing compared to the fresh bread dough, suggesting that wild-type yeast died in frozen dough. The \textit{NTH1} gene deletion alone and the \textit{HSP12} deletion also slightly enhanced the leavening ability. Moreover, after 7 and 21 days of freezing, the bread dough with \textit{NTH1/}

\textit{HSP12} double knockout strain significantly enhanced the leavening ability. These data suggested that \textit{NTH1/}

\textit{HSP12} double deletion provides the best improvement of leavening ability upon freezing–thaw stress.
Discussion

In this study, we established the high-efficiency CRISPR-Cpf1 genome-editing system in *S. cerevisiae*, and also constructed the edited strains with a double-gene deletion including the genes encoding the trehalose degradation enzyme and the membrane chaperone. The edited yeast increased the trehalose contents and the cell viability 21 days after freezing. The bread dough with edited yeast showed leavening ability after freezing, and it is indicated that precise editing of specific genes can improve freezing tolerance in yeast.

The quality of frozen dough depends on the ability of the yeast to generate carbon dioxide and the ability of the bread to retain carbon dioxide after fermentation. Decreased yeast viability is considered to be one of the main factors leading to the deterioration of dough quality [34]. Therefore, many studies have focused...
on generating yeast strains with improved growth or higher fermentation rates for frozen dough.

Maltose metabolism is related to the leavening ability in lean dough. Overexpression of maltose permease (encoded by MAL62) increases maltase activity and maltose metabolism, resulting in improved fermentation capacity of industrial baker’s yeast in lean dough [35]. Deletion of NTH1 combined with MAL62 overexpression enhances freezing tolerance and improves leavening ability after freezing [12]. Overexpression of MAL62 and TPS1 in an NTH1-deletion strain also enhances freezing tolerance and improves leavening ability [13]. The viability of yeast is also related to the content of several amino acids, such as glutamic acid, arginine, and proline [36]. Deletion of both NTH1 and PUT1 (a proline oxidase encoding gene) led to elevated levels of trehalose and proline, higher cell survival rate and higher dough-leavening ability after freezing [37]. The serine/threonine protein kinase (SNF1 expressed) is an important regulator of yeast in response to stress. Overexpression of SNF1 is effective in enhancing the cell tolerance and fermentation capacity of baker’s yeast in freezing, which may be related to the upregulated proteasome, altered metabolism of carbon sources and protectant molecules, and changed cell membrane components [38, 39]. Ycp4p is predicted to be palmitoylated, a posttranslational modification typical of membrane-binding proteins involved in signal transduction. Disruption of YCP4 enhances freeze–thaw tolerance in baker’s yeast [40]. In this study, the yeast strains were manipulated with NTH1 and/or HSP12 deletion by genome-editing technology, and the leavening ability was increased in the bread dough with NTH1/HSP12 double deletion strain after freezing (Fig. 5).

Chaperone proteins are essential for maintaining the functionality of the cellular proteome to prevent the aggregation of unfolded protein under stress conditions, such as heat, low oxygen, heavy metals, UV radiation, freezing and thawing. The HSP12 deletion mutant is more resistant to freezing, and overexpression of Hsp12p in the TPS1 deletion strain increases the resistance to freezing stress [20]. It is contradictory. Both deletion and overexpression of HSP12 gene improve resistance to freezing. Hsp12p is monomeric and unfolded in solution, and it is most likely a membrane chaperone expressed during stresses. Hsp12p gains in structure in the presence of specific lipids (PiP2) leading to membrane rigidification [41]. Membrane fluidity is higher, yeast cells survive better under freeze–thaw stress [42]. The freeze/thaw process results in a rigidifying effect on the cell membrane and cell adaptability to freeze/thaw-induced stress could be dependent on their initial membrane fluidity. Hsp104p contributes to freeze–thaw tolerance by maintaining ubiquitin–proteasome system activity via the disaggregation of aggregated proteins. Disruption of HSP104 caused a reduction in cell viability [43].

The NTH1 deletion strain with higher trehalose levels (Table 2) could maintain cell viability for short-term frozen storage, but not for long-term frozen storage (Fig. 4). The HSP12 deleted strain, without increased trehalose levels (Table 2), was less viable than the NTH1 deletion strain for short-term frozen storage, but more viable for long-term frozen storage (Fig. 4). Disruption of NTH1 contributed to an accumulation of trehalose and disruption of HSP12 contributed to long-term survival upon freezing stress. In this study, we demonstrated that the NTH1/HSP12 double deleted strain, with high levels of trehalose content and the long-term viability under frozen conditions, enhanced the leavening ability of frozen dough (Fig. 5d). However, compared to fresh dough with the wild-type strain (Fig. 5a), the leavening ability and fermentation time still needed to be improved (Fig. 5d).

In this study, we employed the CRISPR-Cpf1 system to generate mutant strains with precise gene deletion. We manipulated the NTH1 and HSP12 gene double deletions by simultaneously expressing Cpf1 and crRNAs based editing on two loci. It was successfully achieved and it was efficient. CRISPR has been hailed as a promising technology because it can accurately insert and alter DNA with targeted specificity and relatively easy implementation. However, recent advances in genome-editing technologies have led to a new era, and the technologies involve precise gene editing without the transfer of foreign genes. The genome-editing technology can be applied to improve strain characteristics and it will enhance the application and development of yeast in the food industry.

Conclusions

In summary, we constructed an efficient CRISPR-Cpf1 genome-editing system, including two direct repeats flanking the crRNA sequence, 120 bp single-strand homologous templates, 48 h recovery period after electroporation, and higher transformed plasmids concentration. NTH1or HSP12 knockout in S. cerevisiae by the optimized CRISPR-Cpf1 genome-editing system enhanced the freezing tolerance and improved the leavening ability after freezing and thawing stress. Deleting NTH1 would introduce the increase of the trehalose contents and achieve a protective role in shorter-term freezing storage. Deleting HSP12 in the combination with deleting NTH1 would strengthen the freezing tolerance and protect the cell viability from higher levels of death in longer-term freezing. It provides valuable insights for breeding novel S. cerevisiae strains for the baking industry through a more precise, speedy, and economic genome-editing system.
Methods and materials

Strains and plasmids
The *S. cerevisiae* wild-type strain BCRC 21447 used in this study was obtained from Bioresource Collection and Research Center. The plasmid vectors pUDC175 and pUDE710 were generated by Dr. Jean-Marc Daran (Addgene plasmid #103019 and #103020) [44]. The genetic properties of *S. cerevisiae* strains and plasmids used in the present study are summarized in Table 3.

Media and growth conditions
*Escherichia coli* Top10 strain, used for DNA amplification, was grown at 37°C in a LB medium (10 g/L tryptone, 5 g/L yeast extract, and 10 g/L NaCl). *S. cerevisiae* was grown at 30°C in a YPD medium (1% yeast extract, 2% peptone, and 2% glucose). When the selection was required, antibiotics were added with 100 mg/L ampicillin for *E. coli*, and 200 mg/L G418 for *S. cerevisiae*.

Plasmid construction
Yeast genomic DNA was separated from *S. cerevisiae* BCRC 21447 using a DNA microprep kit (D4301, Zymo Research Corp.) Primers used in this study were purchased from Genomics BioSci &Tech. Co., Ltd. The plasmid pBCo1 was applied to express *FnCpf1* cassette and contained crRNA sequence crADE2 targeting gene for *ADE2* gene deletion. The plasmid pBCo1N1 was applied to express *FnCpf1* cassette and contained crRNA sequence crNTH1.2 targeting gene for *NTH1* gene deletion. The plasmid pBCoH1 was applied to express *FnCpf1* cassette and contained crRNA sequences crNTH1.2 and crHSP12.1 targeting gene for *HSP12* gene deletion. The plasmid pBCoNH1 was applied to express *FnCpf1* cassette and contained crRNA sequences crNTH1.2 and crHSP12.1 targeting gene for *NTH1* and *HSP12* gene deletion, respectively. The crRNA sequences were designed by CHOPCHOP website (http://chopchop.cbu.uib.no/) and based on the following principles: (1) the PAM sequence of *FnCpf1* for crRNA is 5′-TTTV-3′ (V = A/G/C), (2) the length of direct repeats (DR) is 19 nt, and (3) crRNA is flanked by two DRs at 5′ and 3′ end [23].

Transformation and genome editing in yeast
All yeast transformations were performed using the lithium acetate protocol as previously described [44]. The synthesized homologous DNA templates contained 60 bp of DNA upstream and downstream of the target gene (Table 4). Oligonucleotides were purchased from FnCpf1.

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### Table 3 Characteristics of strains and plasmids used in this study

| Strains or plasmids | Characteristics | Reference or source |
|---------------------|-----------------|---------------------|
| *S. cerevisiae* BCRC21447 | MATa/a industrial brewer’s top yeast | Bioresource collection and research center |
| *S. cerevisiae* Δnth1 | MATa/a, Δnth1 + pBCoN1 | This study |
| *S. cerevisiae* Δhsp12 | MATa/a, Δhsp12 + pBCoH1 | This study |
| *S. cerevisiae* Δnth1/Δhsp12 | MATa/a, Δhsp12 + pBCoNH1 | This study |
| pUDC175 | *E. coli/S. cerevisiae* shuttle vector, containing Amp<sup>+</sup>, TRP1<sup>+</sup>, *FnCpf1* | Świat et al. [23] |
| pUDE710 | *E. coli/S. cerevisiae* shuttle vector, containing Amp<sup>+</sup>, KanMX<sup>+</sup>, crADE2-crHIS4 | Świat et al. [23] |
| pBCo1 | *E. coli/S. cerevisiae* shuttle vector, containing Amp<sup>+</sup>, KanMX<sup>+</sup>, *FnCpf1*, crADE2 | This study |
| pBCoN1 | *E. coli/S. cerevisiae* shuttle vector, containing Amp<sup>+</sup>, KanMX<sup>+</sup>, *FnCpf1*, crNTH1.2 | This study |
| pBCoH1 | *E. coli/S. cerevisiae* shuttle vector, containing Amp<sup>+</sup>, KanMX<sup>+</sup>, *FnCpf1*, crHsp12.1 | This study |
| pBCoNH1 | *E. coli/S. cerevisiae* shuttle vector, containing Amp<sup>+</sup>, KanMX<sup>+</sup>, *FnCpf1*, crNTH1.2-crHsp12.1 | This study |

### Table 4 Target genes, primers, and homologous templates used in this study

| Gene | Targeted sequence | PAM | Primer sequence for colony PCR | ssDNA template sequence |
|------|-------------------|-----|--------------------------------|-------------------------|
| ADE2 | CCGGTGTTGGTATATTTTGGTGGA | TTTT | F: TCTAAGTACATCTCCTATATAAACACT | TTACTTGGTTTTTCTAGAAAGCTCGGGAACACGTTTTTTCTACTTTGTTTCGATCGTCAGGCTGGATTCTT |
|       |                   |     | R: GGACACCTATATGTGAGCAAGAAGA | AATTAGTCAACTGTTGTCTAGATTCAT |
| NTH1 | CACATAGCTGAGGTGGTATAGAAT | TTTG | F: GACAGGGACACCTGGTGAGGGA | CTATGCTAGAAAGGTTTTTCTTCGCTAGTTAACTGCTAAA |
|       |                   |     | R: CTACCTAGGAGCCGCACCA | GAATGGATATTGTCGTTGATACCTTCTTCTGGGGCTTGCATGTTACGGTCCGTAGTCAGTTC |
| HSP12 | GGCAGAGTCTGAGCAGCTGGGAA | TTTT | F: CCCCAAAACACGACACGAGAGA | ATGTCGCTCTGGAGCTGAATAGACTGCTTCTGCAGGGGCTTGCAGG |
|       |                   |     | R: CGCGGGAAATTGAGCAGAAG | GAGCTCTCTGAGCGCTGAAGGGACTGCTTCTGCAGG |

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Integrated DNA Technologies (IDT) Int. The edited strains with deleted ADE2 were visible colored colonies since the ADE2 gene was essential for adenine biosynthesis, and its deletion resulted in adenine auxotrophy and red colonies [45]. The efficiency of genome editing was calculated by the percentage of red colonies to total colony numbers. The genome editing of NTH1 and HSP12 was confirmed by colony PCR analysis with nth1CK1/nth1CK2 and hsp12CK1/hsp12CK2 primer sets and DNA sequencing.

Trehalase activity assay
Trehalase activity was measured by monitoring the production of glucose from trehalose. Yeast cells were harvested by centrifugation and washed twice with distilled water. The cells were suspended in extraction buffer and disrupted with glass beads. The supernatant was used as a crude extract after removing debris by centrifugation at 12,000×g for 10 min at 4 °C. Trehalase activity in supernatants was measured using the trehalase assay kit (ARG82017, Arigo Biolaboratories Corp.). One unit of trehalase activity was the enzyme that generated 1 µmol of glucose per minute.

Assay of the intracellular trehalose content
Intracellular trehalose of yeast cells was extracted as briefly described as follows [12]. Yeast cells were harvested by centrifugation and washed twice with distilled water. Trehalose was extracted from 0.1 g cell pellets with 4 mL of 5% (w/v) cold trichloroacetic acid for 45 min with shaking. Extraction was repeated once more, and supernatants from the two extractions were combined and used for measuring the trehalose content by the trehalase assay kit (K-TREH, Megazyme ltd.). The CDW was determined by drying fresh yeast cells overnight at 85 °C.

Determination of cell viability
For the freeze–thaw stress, yeast cells were cultured in a YPD medium at 30 °C and harvested in the stationary phase. After microscopic counting of viable cells by trypan blue staining using a hemocytometer, cells were aliquoted into cryotubes and frozen at −20 °C for various periods. The number of cells was counted after thawing the frozen cell samples at 30 °C for 30 min. The survival ratio was calculated from the number of living cells before and after freezing.

Determination of leavening ability
The composite of bread dough was 10 g of wheat flour, 5 mL of water, 1.25 g of sugar, 0.25 g of salt, and 0.1 mL of fresh yeast cells at 0.3 g/mL. All the ingredients were mixed properly, and each dough was put into a 50 mL graduated cylinder. The height of the dough was recorded every 30 min up to 3 h. One group of dough was prepared with fresh yeast samples as a positive control, while another group of dough was prepared without any yeast samples as a negative control. The experimental groups of dough with edited yeast cells were stored at −20 °C for 7 or 21 days. The frozen dough was thawed at 30 °C for 30 min and then tested for the leavening ability. The height of the dough was measured from the graduated surface of the graduated cylinder before and after fermentation, and the net increased height was calculated [46].

Statistical analysis
All the experiments were performed individually at least three times, and the data reported were mean values±SD. The differences between the edited strains and the parental strain were confirmed by Student’s t-test. Differences at p<0.05 were considered statistically significant.

Abbreviations
ADE2: Phosphoribosylaminoimidazole carboxylase 2; CDW: Cell dry weight; CRISPR: Clustered regularly interspaced short palindromic repeats; crRNA: crispr RNA; DR: Direct repeats; FnCpf1: Cpf1 variants from Francisella novicida; HSP12: Heat shock protein 12; NTH1: Neutral trehalase 1; PAM: Protospacer adjacent motif; SD: Standard deviation.

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Author contributions
BCC carried out the experiments and drafted the manuscript. HYL conceived the study and reviewed the final manuscript. All authors reviewed and approved the final manuscript.

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Not applicable.

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The authors declare that they have no competing interests.

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