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GENETICS AND MOLECULAR BIOLOGY OF INDUSTRIAL ORGANISMS

# Enhanced leavening ability of baker's yeast by overexpression of *SNR84* with *PGM2* deletion

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**Abstract** Dough-leavening ability is one of the main aspects considered when selecting a baker's yeast strain for baking industry. Generally, modification of maltose metabolic pathway and known regulatory networks of maltose metabolism were used to increase maltose metabolism to improve leavening ability in lean dough. In this study, we focus on the effects of *PGM2* (encoding for the phosphoglucomutase) and *SNR84* (encoding for the H/ACA snoRNA) that are not directly related to both the maltose metabolic pathway and known regulatory networks of maltose metabolism on the leavening ability of baker's yeast in lean dough. The results show that the modifications on *PGM2* and/or *SNR84* are effective ways in improving leavening ability of baker's yeast in lean dough. Deletion of *PGM2* decreased cellular glucose-1-phosphate and overexpression of *SNR84* increased the maltose permease activity. These changes resulted in 11, 19 and 21 % increases of the leavening ability for *PGM2* deletion, *SNR84* overexpression and *SNR84* overexpression combining deleted *PGM2*, respectively.

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**Keywords** Baker's yeast · Leavening ability · *PGM2* · *SNR84*

## Introduction

Baker's yeast (*Saccharomyces cerevisiae*) is an important ingredient in baking process, and is of crucial importance in lean dough (nonsugared dough) leavening [2, 32]. The main function of baker's yeast in lean dough is the production of $CO_2$ from sugars that results in dough leavening, and contributes to the flavour and crumb structure of dough products [29, 30]. A high leavening ability is the most important characteristic in a baking strain to produce baker's yeast production of good quality and save baking time [13]. Given that maltose is the most abundant fermentable sugar in lean dough, the maltose metabolism level is a major factor for the leavening ability [3, 6]. Therefore, many studies intending to improve the leavening ability of baker's yeast are limited to the direct modification of the maltose metabolic pathway and the known regulatory networks of maltose metabolism [24, 35]. Previous work showed that combination mutation of *MIG1* and *SSN6*, which break the regulatory pathway of glucose repression, produced a 12 % increase in the leavening ability compared with the wild type [24]. *MIG1* deletion combining *MAL62* overexpression, which alleviates the glucose repression and enhances expression level of gene directly involved in maltose metabolism, increased the leavening ability by 40 % [35]. However, the effects of modification on global regulatory factors in maltose metabolism and leavening ability of baker's yeast are unclear.

Dough is leavened by carbon dioxide evolved from fermentation through the glycolytic pathway in baker's yeast [27]. Glucose-6-phosphate is the form of glucose that is

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

| Strains or plasmids | Relevant characteristic | Reference or source |
|---|---|---|
| Strains | | |
| *Escherichia coli* DH5α | Φ80 *lacZ*ΔM15 Δ*lacU169 recA1 endA1 hsdR17 supE44 thi-1 gyrA relA1* | [35] |
| BY14α | *MAT α*, Industrial baker's yeast | [17] |
| B-PGM2 | *MAT α*, Δ*PGM2*:: *loxP* | This study |
| B+K | *MAT α*, Yep-K | This study |
| B+SNR84 | *MAT α*, Yep-KPS | This study |
| B-PGM2+SNR84 | *MAT α*, Δ*PGM2*:: *loxP*, Yep-KPS | This study |
| Plasmids | | |
| pPGK1 | *bla LEU2 PGK1*$_P$-*PGK1*$_T$ | [23] |
| Yep352 | *URA3*$^+$, *Amp*$^R$ *ori* control vector | Invitrogen, Carlsbad, Ca, USA |
| Yep-K | *KanMX ARS URA3*$^+$, *Amp*$^R$ *ori* control vector | This study |
| pPGKS | *bla LEU2 PGK1*$_P$-*SNR84-PGK1*$_T$ | This study |
| pUG6 | *E. coli/Saccharomyces cerevisiae* shuttle vector, containing *Amp*$^+$, *loxP-kanMX-loxP* disruption cassette | [10] |
| pSH-Zeocin | Zeo$^r$, Cre expression vector | [25] |

utilized by the enzymes of the glycolytic pathway to produce $CO_2$, $H_2O$ and ATP under aerobic condition [4, 15]. Simultaneously, glucose-6-phosphate could be converted to glucose-1-phosphate (Glc-1-P) for glycogen synthesis through the phosphoglucomutase encoded by the gene *PGM2*, thereby decreasing the direction to glycolytic pathway [8, 28]. Hence, it was feasible to accelerate the direction to glycolytic pathway to produce $CO_2$ through *PGM2* deletion under aerobic condition.

The regulation of baker's yeast to utilize maltose to produce $CO_2$ could involve encoding RNA transcription control and non-coding RNA modification (for instance, 2′-*O*-ribose methylation and pseudouridylation) of related genes. The small nucleolar RNAs (snoRNA) define one of the largest families of small non-coding RNAs known in eukaryotes [33]. The *SNR84* gene encodes H/ACA snoRNA, which is known to guide RNAs in pseudouridylation of specific nucleotides of ribosomal RNA (rRNA) [5, 18, 36]. With regard to the function of the *SNR84* gene, Lee et al. [21] showed the significant beneficial effect of *SNR84* amplification in the yeast cells during galactose catabolism. Like with galactose catabolism, cleaved maltose also enters the glycolytic pathway. Nevertheless, the function of *SNR84* in maltose metabolism remains unknown.

The objectives of the current study are (1) to provide new ideas rather than the direct modification on genes involved in maltose metabolic pathway and the known regulatory networks of maltose metabolism for improving the leavening ability of baker's yeast and (2) to study the effects of *PGM2* deletion and *SNR84* overexpression on the leavening ability of baker's yeast in lean dough.

## Materials and methods

### Strains and vectors

The genetic properties of all strains and plasmids used in this study are summarized in Table 1.

### Growth, cultivation, and fermentation conditions

Recombinant DNA was amplified in *Escherichia coli* DH5α, which was grown at 37 °C in Luria–Bertani medium (10 g/L tryptone, 5 g/L yeast extract, and 10 g/L NaCl) supplemented with 100 μg/mL ampicillin. The plasmid was obtained using a Plasmid Mini Kit II (D6945, Omega, Norcross, GA, USA).

Yeast cells grown in yeast extract peptone dextrose (YEPD) medium (10 g/L yeast extract, 20 g/L peptone, and 20 g/L glucose) to the stationary phase were inoculated into 200 mL of cane molasses medium [5 g/L yeast extract, 0.5 g/L $(NH_4)_2$ $SO_4$ and 12 °Brix (1 °Brix represents 1 g sugar per 100 g solution) cane molasses] at the initial $OD_{600}$ = 0.4, and cultivated for 24 h at 30 °C with 180 rpm rotary shaking to the final $OD_{600}$ = 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 effects of *PGM2* and *SNR84* on maltose metabolism under different concentrations of extracellular maltose, we used the low sugar model liquid dough fermentation medium [LSMLD fermentation medium, 2.5 g/L $(NH_4)_2$ $SO_4$, 5 g/L urea, 16 g/L $KH_2PO_4$, 5 g/L $Na_2HPO_4$, 0.6 g/L $MgSO_4$,

**Table 2** Primers used in the present study (restriction sites are italics)

| Primer | Sequence (5′ → 3′) |
|---|---|
| PGM2-Kan-F | AACAATAGGATAATAAGAAGAAGATCAACCAATCTTTCTCAGTAAAAAAA GTAACAAAAGTTAACATAACCAGCTGAAGCTTCGTACGC |
| PGM2-Kan-R | TTCTTCTTTACCGTTAATATTCATTGAAAAAGGTGAAAATCATTAAGCCATT AGTAAATCATTCGTTGCATAGGCCACTAGTGGATCTG |
| SNR84-F | GGA*AGATCT*ATTGCACAACTTAAGTTTGTCGAGG |
| SNR84-R | GGA*AGATCT*TAATGTGTCTCTTTGAGTCATGTTCCTT |
| PGK-F | CCC*AAGCTT*TCTAACTGATCTATCCAAAACTGA |
| PGK-R | CCC*AAGCTT*TAACGAACGCAGAATTTTC |
| Kan-F | CGC*GGATCC*CAGCTGAAGCTTCGTACGC |
| Kan-R | CGC*GGATCC*GCATAGGCCACTAGTGGATCTG |
| K-F | CTTGCTAGGATACAGTTCTCACATCA |
| K-R | CGCATCAACCAAACCGTTATTCATTC |
| P-F | GATTCTTTCAGAATCTGGATGACTGC |
| P-R | AACTCGGGGTAGGTAATCTGAATTC |
| P-U | ATGTCTTTCTTCAGCGTTGCTTG |
| P-D | TTAAGTACGAACCGTTGGTTCTTC |
| Z-U | CCCACACACCATAGCTTCA |
| Z-D | AGCTTGCAAATTAAAGCCTT |

0.0225 g/L nicotinic acid, 0.005 g/L Ca-pantothenate, 0.0025 g/L thiamine, 0.00125 g/L pyridoxine, 0.001 g/L riboflavin, and 0.0005 g/L folic acid], which contained one of the three specified carbon sources (38 g/L maltose, 40 g/L glucose, and 33.25 g/L maltose mixed with 5 g/L glucose) and was supplemented with 800 mg/L G418 (AG138, Genview, Tallahassee, FL, USA).

### Construction of plasmid and yeast transformants

Genomic yeast DNA was prepared from the industrial baker's yeast strain BY14α using a yeast DNA kit (D3370-01, Omega, Norcross, GA, USA). Table 2 shows the PCR primers used in this study.

We constructed the *PGM2*-deleted baker's yeast as follows: PCR was performed to amplify the fragment *KanMX* (antibiotic gene) for disruption of the *PGM2* gene using pUG6 as template with long-chain primers PGM2-Kan-F and PGM2-Kan-R. Then, the amplified fragment was integrated into the parental strain BY14α by homologous recombination to delete the *PGM2* gene. After G418 selection, the *KanMX* marker gene was removed from the *PGM2* deletion yeast strains using the Cre/*loxP* recombination system [25], resulting in *PGM2*-deleted strain B-PGM2. To select Zeocin-resistant yeast strains, 500 mg/L Zeocin (R25001, Invitrogen, Carlsbad, CA, USA) was added to the YEPD plates for yeast culture. Then, the YEPG medium (10 g/L yeast extract, 20 g/L peptone, and 20 g/L galactose) was used for Cre expression in the yeast transformants. The primers Z-U and Z-D were used to verify the transformation of pSH-Zeocin using to knock out *KanMX*.

Plasmid Yep-KPS (Yep-KanMX-PGK1-SNR84), an episomal plasmid with *SNR84* under the control of the constitutive yeast phosphoglycerate kinase gene (*PGK1*) promoter ($PGK1_P$) and terminator ($PGK1_T$), was constructed as follows: a *Bam*HI *KanMX* fragment, which was the dominant selection marker during yeast conversion, was amplified through PCR using pUG6 as template with Kan-F and Kan-R primers, and was cloned to the Yep352 vector to construct the empty plasmid Yep-K (Yep-KanMX). A *Bgl*II fragment of *SNR84* amplified with SNR84-F and SNR84-R primers from the genomes of the parental strain BY14α was inserted into the *PGK1* fragment of pPGK1 vector and resulted in plasmid pPGKS. Then, the *Hin*dIII fragment of PS (the entire *PGK1* and the inserted *SNR84*) amplified from pPGKS was cloned to Yep-K to produce the final plasmid Yep-KPS.

Baker's yeast transformation was achieved through lithium acetate/PEG procedure [9]. The YEPD plates were supplemented with 800 mg/L G418 to select the geneticin (G418)-resistant strains BY14α-PGM2 (B-PGM2), BY14α+KanMX (B+K), BY14α+SNR84 (B+SNR84) and BY14α-PGM2+SNR84 (B-PGM2+SNR84) after transformation. All the transformants were verified through PCR with the primers listed in Table 2. The transformant B-PGM2 were verified with the primers P-F/K-F, P-R/K-R and P-U/P-D, respectively. The transformant B+K were verified with the primers Kan-F and Kan-R. The transformants B+SNR84 and B-PGM2+SNR84 were verified with the primers PGK-F and PGK-R.

### Determination of specific growth rate and biomass yield

After incubating for 24 h, the mixtures of cell culture and medium were mixed in a deep well plate in appropriate proportions, and the growth curve was detected using bioscreen automated growth curves (Type Bioscreen C, Finland). The specific growth rate was determined with the change in the cell dry weight logarithm versus the time during exponential growth.

Nitrocellulose filters with a pore size of 0.45 mm (Gelman Sciences, Ann Arbor, MI, USA) were pre-dried in a microwave oven at 150 W for 10 min and were subsequently weighed. Harvested cells were obtained from 10 mL of cell culture, washed twice with isometric distilled water and dried at 105 °C for 24 h. The biomass yield was determined from the slopes of the plots of biomass dry weight versus the amount of consumed sugar during exponential growth. Experiments were conducted thrice.

### Determination of leavening ability

The leavening ability of yeast cells in lean dough was based on the Chinese National Standards for yeast used in food processing. Leavening ability was determined by $CO_2$ production per hour per gram (dry weight) of yeast cells. Lean dough consisted of 280 g of flour, 150 mL water, 4 g salt, and 8 g fresh yeast. The dough was evenly and quickly mixed for 5 min at 30 ± 0.2 °C, and placed inside the box of a fermentograph (Type JM451, Sweden). $CO_2$ production was recorded at 30 °C. Experiments were conducted thrice.

### Analysis of sugar consumption

For extracellular sugar measurements, cultures were sampled at 30 °C at suitable intervals for 4 h. High-performance liquid chromatography (HPLC) with a refractive index detector and Aminex® HPX-87H column (Bio-Rad, Hercules, CA, USA) was utilized at 65 °C with 5 mM $H_2SO_4$ as the mobile phase at a flow rate of 0.6 mL/min [11] to analyze the sugars filtered through 0.45 μm pore size cellulose acetate filters (Millipore Corp, Danvers, MA, USA). Experiments were conducted thrice.

### Maltase activity assays

Crude extracts were prepared according to the Salema-Oom [31] procedure to determine enzyme activities. The cells were broken by pulp refiner (Type Precellys 24, Bertin, France) with sterile glass beads (0.5 mm, 1.5 g/mL) at 5500 oscillations $min^{-1}$ for 16 s. Disrupted samples was centrifuged at 2500×*g* for 10 min at 4 °C, and the supernatants were used as a cell extract. Maltase activity was measured using *p*-nitrophenyl-alpha-D-glucopyranoside as substrate in a spectrophotometric assay. For every strain, three independent biological replicates and technical duplicates were assayed.

### Maltose permease assay

Maltose permease was determined by measuring uptake of [$^{14}$C]maltose as described by Houghton-Larsen and Brandt [14]. For every strain, three independent biological replicates and technical duplicates were assayed.

### Analysis of glucose-1-phosphate levels

Cells were broken by glass beads as above-mentioned method. Glc-1-P levels were assayed using a Glucose-1-Phosphate Colorimetric Assay Kit (K697-100, BioVision, Milpitas, CA, USA). For every strain, three independent biological replicates and technical duplicates were assayed.

### Statistical analysis

Data were expressed as mean ± SD and were accompanied by the number of experiments independently performed. The differences of the transformants compared with the parental strain were confirmed by Student's *t* test. Differences at $P < 0.05$ were considered significant differences in statistics.

## Results

### $CO_2$ production of *PGM2* deletion and *SNR84* overexpression in lean dough

The *PGM2* deletion, *SNR84* overexpression and *SNR84* overexpression combined with *PGM2* deletion were established in the industrial baker's yeast cells. The strain B+K was used as a blank control to avoid any possible effects of the empty vector. The impacts of *PGM2* deletion and *SNR84* overexpression on $CO_2$ production were assayed in lean dough. When the mixed dough was placed inside the fermentograph box, the *PGM2* deletion strain B-PGM2 exhibited stronger dough-raising ability than the parental strain BY14α (Fig. 1a). Similar effect, but with higher level of gas evolution was observed in the *SNR84* overexpression strain B+SNR84 (Fig. 1a). Compared with the parental strain BY14α, the amounts of $CO_2$ produced by B-PGM2 and B+SNR84 within 60 min increased from 675 to 750 and 800 mL, respectively (Fig. 1a), and the leavening ability increased by 11 and 19 %, respectively (Fig. 1c). Moreover, the fermentation time in B-PGM2 and B+SNR84

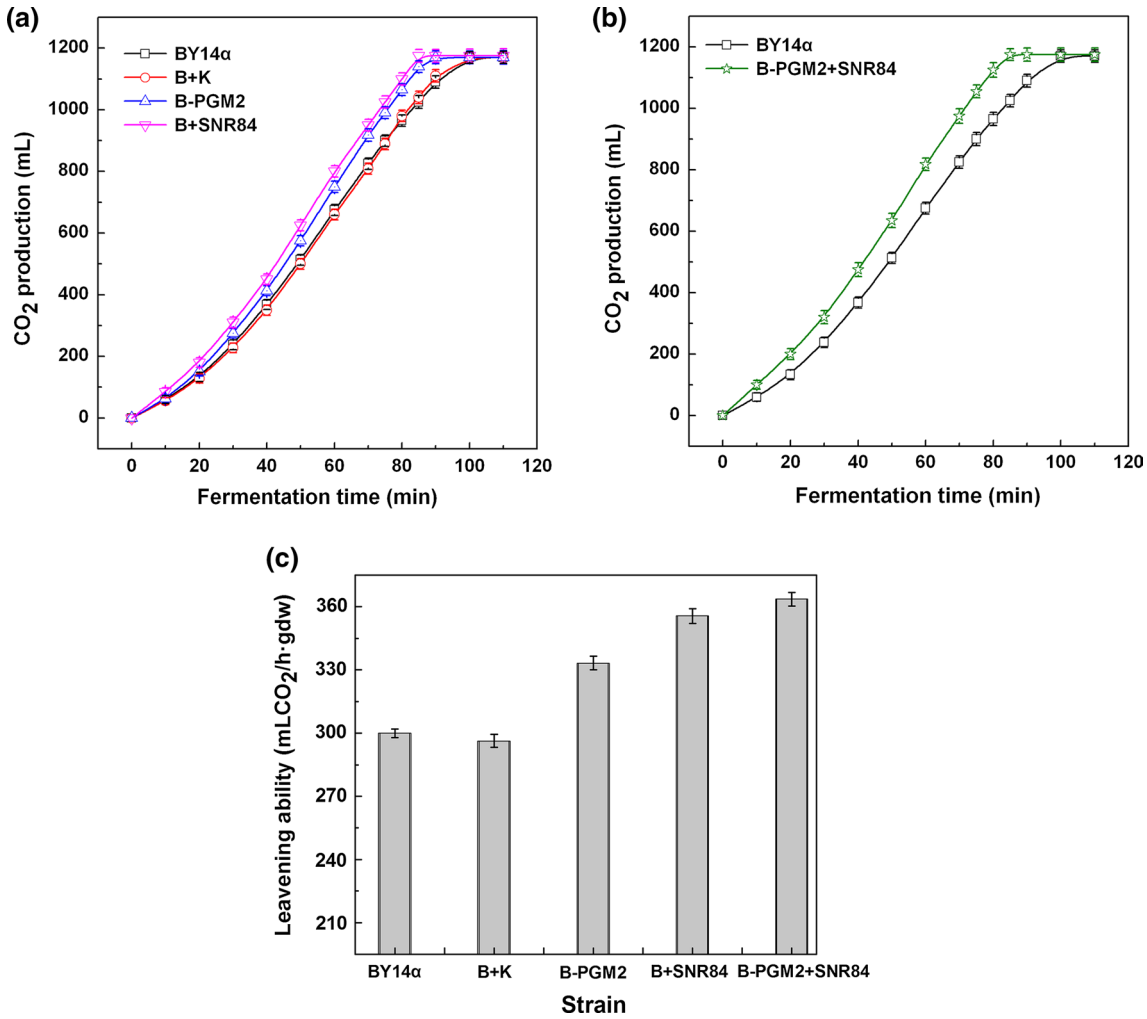


**Fig. 1** $CO_2$ production by parental strains and transformants in lean dough. Flour (280 g), water (150 mL), salt (4 g) and fresh yeast (8 g) were mixed into the fermentograph until steady $CO_2$ formations were achieved by **a** the parental strains BY14α and B+K, and the strains B-PGM2 and B+SNR84, **b** the parental strain BY14α and the strain B-PGM2+SNR84, and **c** the leavening ability was determined by $CO_2$ production per hour per gram (dry weight) of yeast cells. Data are average of three independent experiments and *error bars* represent ± SD. Significant difference of the transformants (the strains B-PGM2, B+SNR84 and B-PGM2+SNR84) from the parental strain BY14α was confirmed by Student's *t* test ($P < 0.05$)

evidently shortened by 10.0 and 15.0 %, respectively, compared with the parental strain (Fig. 1a). Similar results with the parental strain BY14α were observed in the blank control strain B+K.

The strain B-PGM2+SNR84, which carried the *SNR84* overexpression with deleted *PGM2*, showed better improvement in the production of $CO_2$. The $CO_2$ production within 60 min of the strain B-PGM2+SNR84 increased to 818 mL, which was 21 % higher than the parental strain (Fig. 1b), and the leavening ability increased by 21 % compared with the parental strain (Fig. 1c). Furthermore, the fermentation time decreased from 100 to 82 min (Fig. 1b).

These findings suggest that the deletion of *PGM2* and overexpression of *SNR84* resulted in positive effect on the $CO_2$ production of baker's yeast in lean dough. Furthermore, overexpression of *SNR84* could lead to better gassing performance than deletion of *PGM2*. Particularly, *SNR84* overexpression combined with *PGM2* deletion was the most effective way to improve leavening ability of baker's yeast.

## Sugar consumption in LSMLD medium

Based on the results regarding $CO_2$ production, the sugar consumption of the strains was investigated in LSMLD medium. The strain B+K exhibited similar sugar consumption to the parental strain BY14α. In glucose LSMLD medium, no obvious changes were observed during fermentation, and hardly any glucose was present at the end

of the process for these five strains (Fig. 2a, b). Compared with the parental strain BY14α, the maltose utilization efficiency increased by 11 and 19 %, respectively, for the *SNR84* overexpression strain B+SNR84 in maltose and glucose–maltose LSMLD media (Fig. 2c, e; Table 3). Simultaneously, the time span used to describe the glucose repression level decreased by 11 % in the strain B+SNR84 (Table 3). A similar trend with similar effect was observed in the strain B-PGM2+SNR84. The maltose utilization efficiency of B-PGM2+SNR84 was 11 and 22 % higher than the parental strain in maltose and glucose–maltose LSMLD media, respectively (Fig. 2d, f; Table 3), and the time span decreased from 2.21 to 1.94 h compared with the parental strain (Table 3). For the *PGM2* deletion strain B-PGM2, negligible differences of sugar consumption were observed compared with the parental strain (Fig. 2a, c, e).

These findings demonstrate that deletion of *PGM2* and overexpression of *SNR84* produced different effects on maltose utilization of baker's yeast cells. *SNR84* overexpression was efficient to alleviate glucose repression with decreased time span, resulting in the enhancement of maltose utilization. However, deletion of *PGM2* alone did not promote the maltose utilization.

### Enzyme activities

The maltase and maltose permease activities were determined in three LSMLD media. The strain B+K displayed no significant differences from the parental strain BY14α. In the three LSMLD media, all the five strains showed negligible changes for the maltase activity (date not shown). Surprisingly, the maltose permease activities of the strain B+SNR84 increased by 8, 31 and 36 %, respectively, in glucose, maltose and glucose–maltose LSMLD media compared with the parental strain, and the almost same result to strain B+SNR84 was found in the strain B-PGM2+SNR84 (Fig. 3). By comparison, the strain B-PGM2 exhibited similar maltose permease activity to the parental strain (Fig. 3).

These results corresponded directly with sugar consumption in LSMLD medium reflecting that overexpression of *SNR84* was an effective method to elevate maltose permease activity. However, deletion of *PGM2* did not affect the activity of maltose permease.

### Glc-1-P levels

The cellular concentrations of Glc-1-P in these five strains were determined in three LSMLD media. Similar Glc-1-P level to the parental strain BY14α was found in the strain B+K. The cellular level of Glc-1-P evidently decreased by 33, 33 and 15 % in glucose, maltose and glucose–maltose LSMLD media, respectively, in the *PGM2* deletion strain B-PGM2 compared with the parental strain BY14α (Fig. 4). The observed Glc-1-P in the strain B-PGM2 could originate from phosphoglucomutase encoded by *PGM1* [15]. Similar effect, but with higher level of cellular Glc-1-P than the strain B-PGM2 was observed in the strain B-PGM2+SNR84 (Fig. 4). By contrast, the Glc-1-P accumulated in the *SNR84* overexpression strain B+SNR84 was higher than that of the parental strain in three LSMLD media (Fig. 4).

These results suggest that overexpression of *SNR84* increased the cellular level of Glc-1-P. Instead, deletion of *PGM2* was efficient to decrease intracellular Glc-1-P of baker's yeast cells.

### Growth characteristics

Stable performance is important for an industrial strain in fermentation application. We investigated the growth characteristics (specific growth rate and biomass yield) for these five strains. As shown in Table 4, the strain B-PGM2 showed similar specific growth rate to the parental strain, but exhibited a decrease in the biomass yield. The specific growth rate and biomass yield of the strain B+SNR84 increased from 0.23 to 0.26/h and 6.2 to 6.7 g/L, respectively, compared with the parental strain (Table 4). Compared with the parental strain, the strain B-PGM2+SNR84 did not obviously change the specific growth rate, while slightly changed the biomass yield.

These results indicate that deletion of *PGM2* and overexpression of *SNR84* could slightly influence the growth of baker's yeast. The *SNR84* overexpression conferred growth abilities well on the baker's yeast cells, but the *PGM2* deletion was opposite to that.

## Discussion

The development of baker's yeast strains capable of producing high amounts of $CO_2$ during dough leavening is an important biotechnological objective of the baking industry [1]. Two different approaches were successfully applied to improve leavening ability of baker's yeast via improving the maltose metabolism. The first approach aimed to enhance the production of the enzymes involved in maltose utilization through *MAL* genes overexpression [34, 35]. The second approach was to alleviate glucose repression by silencing the repressor [16, 19, 20, 24]. Both of the two approaches were directly associated with the maltose metabolic pathway and the known regulatory networks of maltose metabolism. In this study, the phosphoglucomutase (*PGM2*) and H/ACA snoRNA (*SNR84*) were targeted to improve leavening ability of baker's yeast in lean dough. However, none of the targets are directly related to the

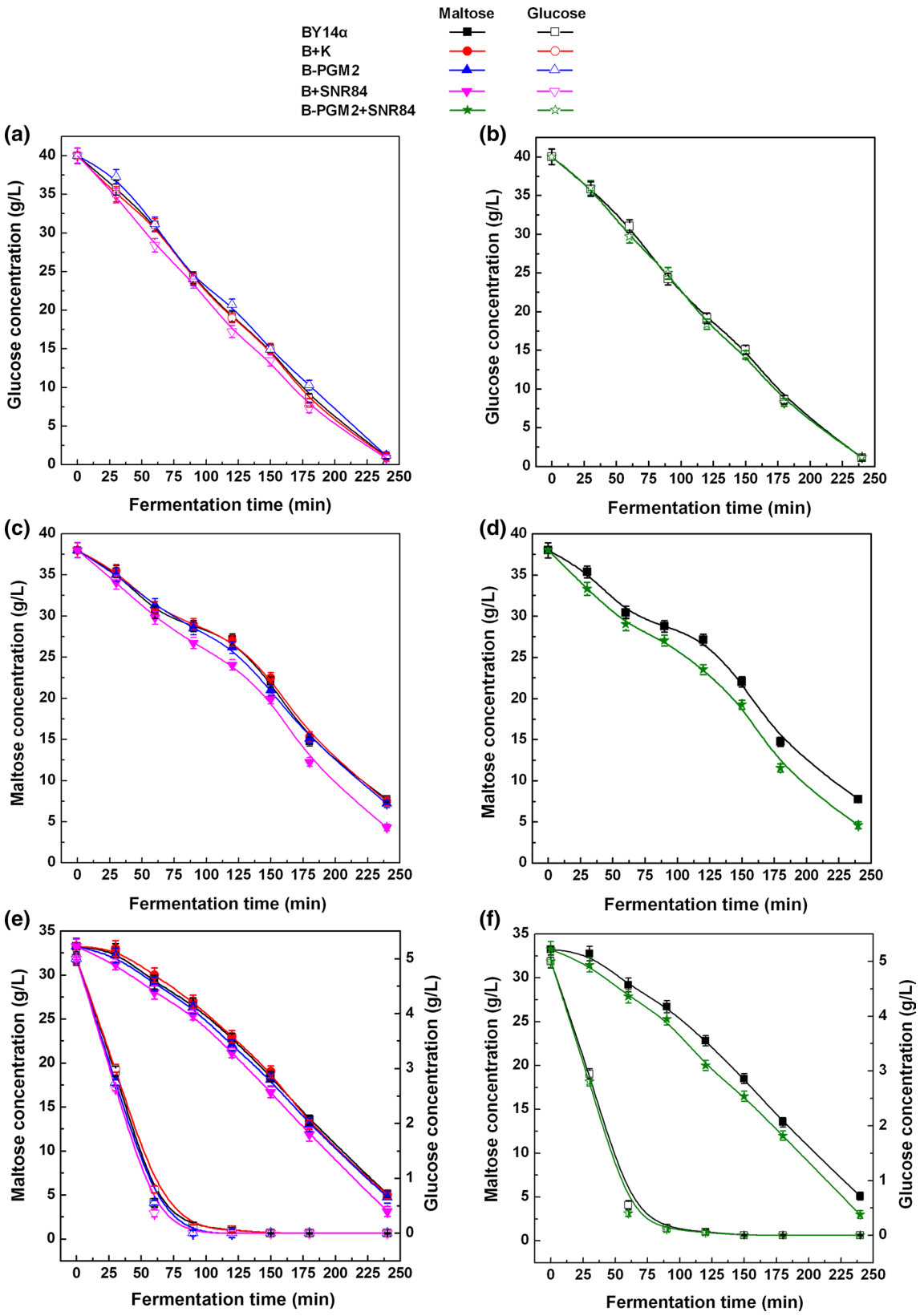


**Fig. 2** Concentration of residual sugar in parental strain and transformants in LSMLD medium. Fresh yeast cells were inoculated into **a**, **b** glucose LSMLD medium, **c**, **d** maltose LSMLD medium and **e**, **f** glucose–maltose LSMLD medium, and sampled at suitable intervals. Data are average of three independent experiments and *error bars* represent ± SD

**Table 3** Data from cultivation in LSMLD media

| Strains | BY14α | B+K | B-PGM2 | B+SNR84 | B-PGM2+SNR84 |
|---|---|---|---|---|---|
| Maltose utilization efficiency (%) | 79.6 ± 2.4[a] | 80.1 ± 2.2[a] | 81.0 ± 1.9[a] | 88.7 ± 2.6[a]* | 88.0 ± 2.0[a]* |
| | 19.6 ± 0.6[b] | 18.9 ± 0.5[b] | 20.2 ± 0.5[b] | 23.4 ± 0.4[b]* | 23.9 ± 0.5[b]* |
| Time span (h)[c] | 2.21 ± 0.12 | 2.20 ± 0.10 | 2.20 ± 0.09 | 1.96 ± 0.10* | 1.94 ± 0.11* |

Values shown represent averages of three independent experiments (data are mean ± SD). Significant difference of the transformants (B+SNR84 and B-PGM2+SNR84) from the parental strain was confirmed by Student's *t* test (* $P < 0.05$, $n = 3$)

[a] Data from cultivation in maltose LSMLD medium and the maltose utilization efficiency in maltose LSMLD medium was determined by the ratio of the consumed maltose in 240 min with the total maltose

[b] Data from cultivation in glucose–maltose LSMLD medium and the maltose utilization efficiency in glucose–maltose LSMLD medium was determined by the ratio of consumed maltose, when glucose was exhausted, with the total maltose

[c] Time span was determined between the point when half of the glucose and that of the maltose had been consumed in the glucose–maltose LSMLD medium

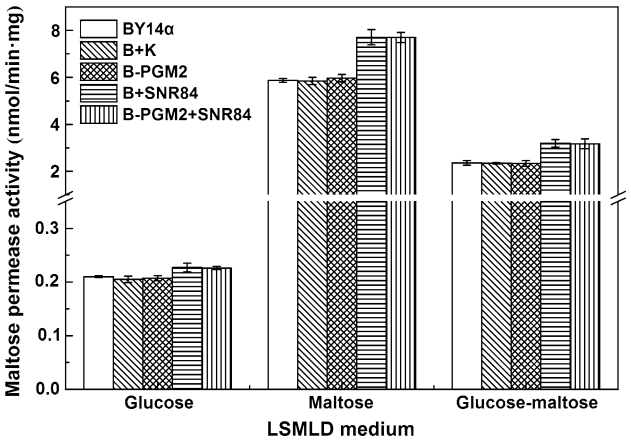


**Fig. 3** Maltose permease activity for parental strains and transformants in LSMLD medium. Cells were inoculated into three LSMLD media, and were sampled at 1 h. Data are averages from three independent experiments, and *error bars* represent ± SD. Significant difference of the transformants (the strains B+SNR84 and B-PGM2+SNR84) from the parental strain BY14α was confirmed by Student's *t* test ($P < 0.05$)

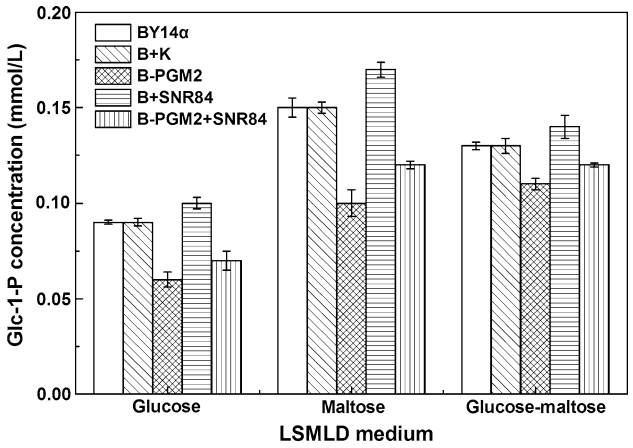


**Fig. 4** Glucose-1-phosphate levels for parental strains and transformants in LSMLD medium. Cells were inoculated into three LSMLD media, and were sampled at 1 h. Data are averages from three independent experiments, and *error bars* represent ± SD. Significant difference of the transformants (the strains B-PGM2, B+SNR84 and B-PGM2+SNR84) from the parental strain BY14α was confirmed by Student's *t* test ($P < 0.05$)

maltose metabolic pathway and the known regulatory networks of maltose metabolism.

*PGM2* deletion and/or *SNR84* overexpression presented different levels of positive effects on the leavening ability of baker's yeast. Compared with the parental strain, the dough-leavening abilities of all three transformants increased (Fig. 1). The leavening ability was significantly improved by *PGM2* deletion with decreased cellular Glc-1-P, supporting the point that the yield of target product could be enhanced through increasing the direction to glycolytic pathway [7, 22]. A higher improvement was obtained through the *SNR84* overexpression. The increasing $CO_2$ evolution could be caused by accelerating maltose utilization (Fig. 2). Maltose was rapidly converted to glucose to $CO_2$, which resulted in a remarkable improvement

**Table 4** Growth and fermentation characteristics of parental strain and transformants

| Strains | Specific growth rate (/h)[a] | Biomass yield (g/L)[a] |
|---|---|---|
| BY14α | 0.23 ± 0.02 | 6.2 ± 0.20 |
| B+K | 0.23 ± 0.03 | 6.1 ± 0.21 |
| B-PGM2 | 0.21 ± 0.01 | 5.8 ± 0.20* |
| B+SNR84 | 0.26 ± 0.04* | 6.7 ± 0.22* |
| B-PGM2+SNR84 | 0.24 ± 0.02 | 6.4 ± 0.19* |

Values shown represent averages of three independent experiments (data are mean ± SD). Significant difference of the transformants from the parental strain was confirmed by Student's *t* test (* $P < 0.05$, $n = 3$)

[a] The initial sugar concentrations are 33.25 g/L of maltose and 5 g/L of glucose

in the leavening ability [12]. Overexpression of *SNR84* with deleted *PGM2* further increased the leavening ability, which demonstrates that *PGM2* deletion and *SNR84* overexpression collectively act for the improvement of $CO_2$ production of baker's yeast in lean dough.

The ability of baker's yeast to leaven dough has a strong correlation with the levels of sugar metabolism. In the present study, deletion of *PGM2* and overexpression of *SNR84* led to different effects on the maltose metabolism (Fig. 2). The maltose utilization level was notably improved for the *SNR84* overexpression (Fig. 2). Nucleotide modification is the main function of the snoRNAs at large [26]. It is presumed that the function of the *SNR84* gene involved in the pseudouridylation of large subunit rRNA is activated, which results in an improved maturation rate and production of a greater quantity of large subunit rRNA. When mature large subunit rRNA is formed rapidly in an initial exponential phase, the production rate of most enzymes becomes higher. Therefore, related enzymes involved in maltose metabolism are probable to be rapidly produced through overexpression of *SNR84*. The results were verified by the test of enzyme activities (Fig. 3). The enhancement of maltose permease activity is capable to promote the maltose metabolism to improve the leavening ability. In addition, the production rate of related enzymes involved in glucose derepression and glucose catabolism could be increased by *SNR84* overexpression, thereby increasing the $CO_2$ production as well as glucose-1-phosphate level (Table 3; Figs. 1, 4). The sugar consumption trend of the strain B-PGM2 was parallel to the parental strain, and the strain B-PGM2+SNR84 displayed similar sugar consumption to the strain B+SNR84 (Fig. 2). These results indicate that *PGM2* deletion made no changes in maltose metabolism. It could be only efficient to reduce the direction to glucose-1-phosphate and accelerate the glycolytic pathway direction to end product ($CO_2$) yield by deletion of *PGM2*. In addition, deletion of *PGM2* could partially compromise the accumulation of cellular Glc-1-P in *SNR84* overexpression (Fig. 4). Thus, overexpression of *SNR84* with *PGM2* deletion could possess the maximum leavening ability.

*PGM2* deletion negatively influenced the growth characteristics (Table 4). However, the defects of growth abilities were insufficient to affect the maltose metabolism and leavening ability. Overexpression of *SNR84* was beneficial to the specific growth rate and biomass yield of baker's yeast (Table 4). The improvement of growth characteristics was also likely to increase maltose metabolism and leavening ability for *SNR84* overexpression. Moreover, overexpression of *SNR84* could compensate for the reduction in biomass yield of the strain B-PGM2 (Table 4). The strain B-PGM2+SNR84 could rapidly utilize maltose and acquire the maximum leavening ability. These characteristics are consistent with the requirement of an industrial strain in terms of yield and leavening ability.

Based on the results of this study, the modifications on *PGM2* and/or *SNR84*, which are indirectly involved in the maltose metabolic pathway and the known regulatory networks of maltose metabolism, appear to be effective ways in improving leavening ability of baker's yeast in lean dough. Such approach combining with modification on genes directly involved in maltose metabolism would produce better improvement in the leavening ability, which thus have practical implications for the industrial application and suggest a novel perspective for the optimization of industrial baker's yeast in dough leavening.

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