GENETICS AND MOLECULAR BIOLOGY OF INDUSTRIAL ORGANISMS

Enhanced leavening properties of baker's yeast overexpressing *MAL62* with deletion of *MIG1* in lean dough

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Abstract This study aimed to increase maltose fermentation in industrial baker's yeast to increase its leavening properties. To this end, we overexpressed *MAL62* encoding alpha-glucosidase (maltase) and deleted *MIG1* encoding a transcriptional repressor that regulates *MAL* gene expression. Strain overexpressing *MAL62* showed 46.3 % higher alpha-glucosidase activity and enhanced leaving activity than the parental strain when tested in glucose–maltose low sugar model liquid dough (LSMLD). Deleting *MIG1* was much less effective, but it could further strengthen leavening properties in a strain overexpressing *MAL62*. The relationship between maltose permease and alpha-glucosidase was further dissected by transforming the two genes. The results indicated that without increasing the maltose permease activity, maltose metabolism could also be enhanced by the increased alpha-glucosidase activity. Previous strategies for strain improvement have targeted the enhancement of alpha-glucosidase and maltose permease activities in concert. Our results suggest that increasing alpha-glucosidase activity is sufficient to improve maltose fermentation in lean dough.

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Introduction

The main component of lean dough is starch, which is hydrolyzed to maltose by amylases. Therefore, to improve the leavening ability in lean dough, it is essential to enhance maltose metabolism [2, 24]. Yeast cells require at least one of the five unlinked *MAL* loci (*MAL1* through *MAL4* and *MAL6*) in order to utilize maltose. Therefore, the key factor in increasing leavening ability is regulation of the *MAL* system [20]. 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]. In previous studies, maltose permease was considered to have a crucial role in both maltose uptake and maltose induction of the *MAL* genes [4, 6, 25]. Hence, many improvements intended to increase the leavening ability of yeast have concentrated on the modification of maltose permease [21].

The presence of glucose inhibits the transcription of genes specifically related to the consumption of other carbon sources, thereby delaying maltose uptake [18, 22]. An effective alleviation of glucose control would help to achieve a better process economy for bread-making. Several lines of evidence suggest that *MIG1* encodes a repressor protein (MIG1p), which represses the transcription of all three *MAL* genes by binding upstream of these genes [3, 19]. Previous studies have shown that deleting *MIG1* can enhance transcription of the *MAL* gene, thereby resulting in increased synthesis of proteins involved in maltose utilization [12].

Our objectives are (1) to gain better insight into the relationship between alpha-glucosidase and maltose permease at different extracellular maltose concentrations and

(2) to study the effects of *MAL62* overexpression and *MIG1* deletion in industrial baker's yeast.

Materials and methods

Strains, plasmids, and conditions of culture and fermentation

The genetic properties of all *Saccharomyces cerevisiae* strains and plasmids used in the present study are summarized in Table 1. Industrial baker's yeast strain BY14 was obtained from the Yeast Collection Center of the Tianjin Key Laboratory of Industrial Microbiology.

Recombinant DNA was amplified in *Escherichia coli* DH5α. Transformants were grown in Luria–Bertani medium (10 g l^{-1} tryptone, 5 g l^{-1} yeast extract, and 10 g l^{-1} NaCl) with 100 μg ml^{-1} ampicillin. The plasmid was obtained using Plasmid Mini Kit II (D6945, Omega, USA).

Yeast strains were routinely cultivated in yeast extract peptone dextrose (YEPD) medium (10 g l^{-1} yeast extract, 20 g l^{-1} peptone, and 20 g l^{-1} glucose) in a shake-flask culture at 30 °C and shaken at 200 rpm in a rotary shaker. Cells were grown to stationary phase and inoculated into a LSMLD fermentation medium (2.5 g l^{-1} (NH$_4$)$_2$SO$_4$, 5 g l^{-1} urea, 16 g l^{-1} KH$_2$PO$_6$, 5 g l^{-1} Na$_2$HPO$_4$, 0.6 g l^{-1} MgSO$_4$, 0.0225 g l^{-1} nicotinic acid, 0.005 g l^{-1} Ca-pantothenate, 0.0025 g l^{-1} thiamine, 0.00125 g l^{-1} pyridoxine, 0.001 g l^{-1} riboflavin, and 0.0005 g l^{-1} folic acid) containing one of three specified carbon sources to further investigate the degree of glucose repression and determine the relationship between two *MAL* enzymes under different extracellular maltose concentrations. The C-mol of the three specified carbon sources (38 g l^{-1} maltose, 40 g l^{-1} glucose, and 33.25 g l^{-1} maltose mixed with 5 g l^{-1} glucose) was 1.33 C-mol. Cells were harvested by centrifugation (4 °C, 5,000 rpm, 5 min) and washed twice with sterile water at 4 °C for use in succeeding experiments. Episomal plasmids and yeast genomes were obtained using a yeast plasmid kit (PD1211-02, Omega, USA) and yeast DNA kit (D3370-01, Omega, USA), respectively.

Plasmid construction and yeast transformation

PCR primers used in the current work are listed in Table 2. Plasmid Yep-CPM, an episomal plasmid with *MAL62* under the control of the *PGK* promoter, was constructed as follows: an *Xho*I fragment of *MAL62* that was amplified with the primers *MAL62*-F and *MAL62*-R from genomes of the parent strain (BY14a) was cloned into vector pPGK1, resulting in plasmid pPGKM. A *Bam*HI fragment of pPGKM containing the entire *MAL62*, the entire promoter, and the entire terminator of *PGK1* was amplified with the primers *PGK*-F and *PGK*-R and then cloned into the *Bam*HI linearized Yep-C plasmid, resulting in Yep-CPM plasmid. Yep-C is the control vector for Yep-CPM and contains the *Cu* resistant gene as the dominant selection marker when integrated into yeast. The pKAB plasmid used for deletion of *MIG1* was created by the following process: an *Eco*RI upstream homologous fragment of the *MIG1* gene, named A and amplified with A-F and A-R primers, was inserted into the multicopy episomal shuttle vector pUC19, resulting in the pUC19-A plasmid. The

Table 1 Characteristics of strains and plasmids used in the current study

Strains and plasmids	Relevant features	Reference or source
S. cerevisiae yeast strain		
BY14	Industrial baker's yeast	Jiang [15]
BY14a[a]	*MATa* (haploid derived from BY14 strain)	This study
BY-C	*MATa* Yep-C	This study
BY14a + MAL62	*MATa* Yep-CPM	This study
BY14a-MIG1	*MATa mig1*::pKAB	This study
BY14a + MAL62-MIG1	*MATa mig1*::pKAB Yep-CPM	This study
Plasmids		
Yep-C	*CUP1 ARS URA3$^+$ AmpR ori* control vector	This study
pPGK1	*bla LEU2 PGK1$_P$-PGK1$_T$*	[17]; Bauer F, University of Stellenbosch
Yep-CPM	*CUP1 PGK1$_P$-MAL62-PGK1$_T$*	This study
pKAB	A-*Kan*-B	This study
pMKAB	A-*Kan-PGK1$_P$-MAL62-PGK1$_T$*-B	This study

[a] 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′
MAL62-F	CCG<u>CTCGAG</u>ATGACTATTTCTGATCATCC
MAL62-R	CCG<u>CTCGAG</u>TTATTTGACGAGGTAGATT
PGK-F	CGC<u>GGATCC</u>AAGCTTTCTAACTGATCTATCCAAAACTGA
PGK-R	CGC<u>GGATCC</u>AAGCTTTAACGAACGCAGAATTTTC
Kan-F	CGG<u>GGTACC</u>CAGCTGAAGCTTCGTACGC
Kan-R	CGG<u>GGTACC</u>GCATAGGCCACTAGTGGATCTG
A-F	CCG<u>GAATTC</u>GGATTTTATGGAGTGTTGATG
A-R	CCG<u>GAATTC</u>GGCTATGGTAGTATGTCGTCT
B-F	CCC<u>AAGCTT</u>GCTGACAAGTTTTTGGCGG
B-R	CCC<u>AAGCTT</u>GCTATGACTACGGCACCTACGA
MIG1-U	TAGAAGTTAGCGAGCCAGCAC
MIG1-D	CAACCAACCACCTTAACT
Cu-F	CAG<u>GTCGAC</u>TCTAGAGGATCCCATTACCG
Cu-R	CAG<u>GTCGAC</u>CTCCCAGAGCTGTTCGTC

*Hin*dIII downstream homologous fragment of *MIG1*, named B and amplified with primers B–F and B-R, was also isolated from genomic DNA of the parent strain (BY14a) and subcloned into the *Hin*dIII site of pUC19-A, resulting in a plasmid named pUC-AB. The marker excised with primers *Kan*-F and *Kan*-R from pUG6 as a *Kpn*I fragment was inserted into pUC-AB to obtain a plasmid called pKAB.

Yeast transformation was carried out using a lithium acetate procedure described previously [15]. Selection of strains BY-C, BY14a + MAL62, and BY14a + MAL62-MIG1 were performed in YEPD medium supplemented with 4 mmol l^{-1} CuSO$_4$. Selection of BY14a-MIG1 strain was performed in YEPD medium supplemented with 0.8 mg ml^{-1} G418. After selection, recombinant strains were verified by primers Cu-F and Cu-R (BY-C), primers MIG1-U and MIG1-D (BY14a-MIG1), and primers PGK-F and PGK-R (BY14a + MAL62, BY14a + MAL62-MIG1).

Determination of cell dry weight and biomass yield

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 subsequently weighed. Next, 5 ml of the cell culture was filtered, washed three times with 5 ml of distilled water, and dried at 110 °C for 24 h. Cell dry weight was determined with an accuracy of 2 %. Biomass yield was determined from the slopes of plots of biomass dry weight versus consumed sugar during exponential growth.

Analysis of sugars

For measurements of extracellular glucose and maltose, the cultivation medium was sampled, immediately filtered through a 0.45-μm-pore-size cellulose acetate filter (Millipore Corp., Danvers, MA, USA), and subsequently frozen and kept at −20 °C until analysis.

The equipment used in the experiment included a differential refractometer detector (Waters 410 RI), Aminex HPX-87H columns (Bio-Rad, Hercules, CA, USA), and an HPLC pump (Waters 515). Samples were measured at 65 °C with 5 mM H$_2$SO$_4$ as the mobile phase at a flow rate of 0.6 ml min^{-1} [8]. Concentrations were determined with accuracy better than 5 %.

Determination of leavening ability of lean dough

The leavening ability of lean dough of yeast was determined according to the Chinese National Standards for yeast used in food processing. Lean dough was composed of 280 g of 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 and placed in a fermentograph box (Type JM451, Sweden). CO$_2$ production was recorded for 60 min at 30 °C. Experiments were conducted three times.

Enzyme activity assays

Crude extracts were prepared using the Salema-Oom method to determine enzyme activities [24]. Alpha-glucosidase and maltose permease activities were determined following the Houghton-Larsen method [11]. Standard errors were less than 10 %.

Results

Overexpressed *MAL62* gene results in enhanced maltose fermentation in three LSMLD

We tested the effects of *MAL62* gene overexpression on maltose fermentation. Alpha-glucosidase and maltose permease activities were assayed in three LSMLD, and the amounts of CO$_2$ produced in lean dough were measured. Alpha-glucosidase activities of strains BY14a + MAL62 and BY14a + MAL62-MIG1 were approximately 46.3 % and 82.2 %, respectively, higher than the parental strain (BY14a) in the glucose-maltose LSMLD (Fig. 1c), resulting in 33.9 and 39.5 % increases in 1-h CO$_2$ production (Fig. 2) and 34 and 40 % enhancements in leavening ability (Table 3), respectively. Similar effects, but with higher levels of alpha-glucosidase activity, were observed in experiments utilizing the maltose LSMLD (Fig. 1b). In the glucose LSMLD, the activities of alpha-glucosidase in all strains were very low.
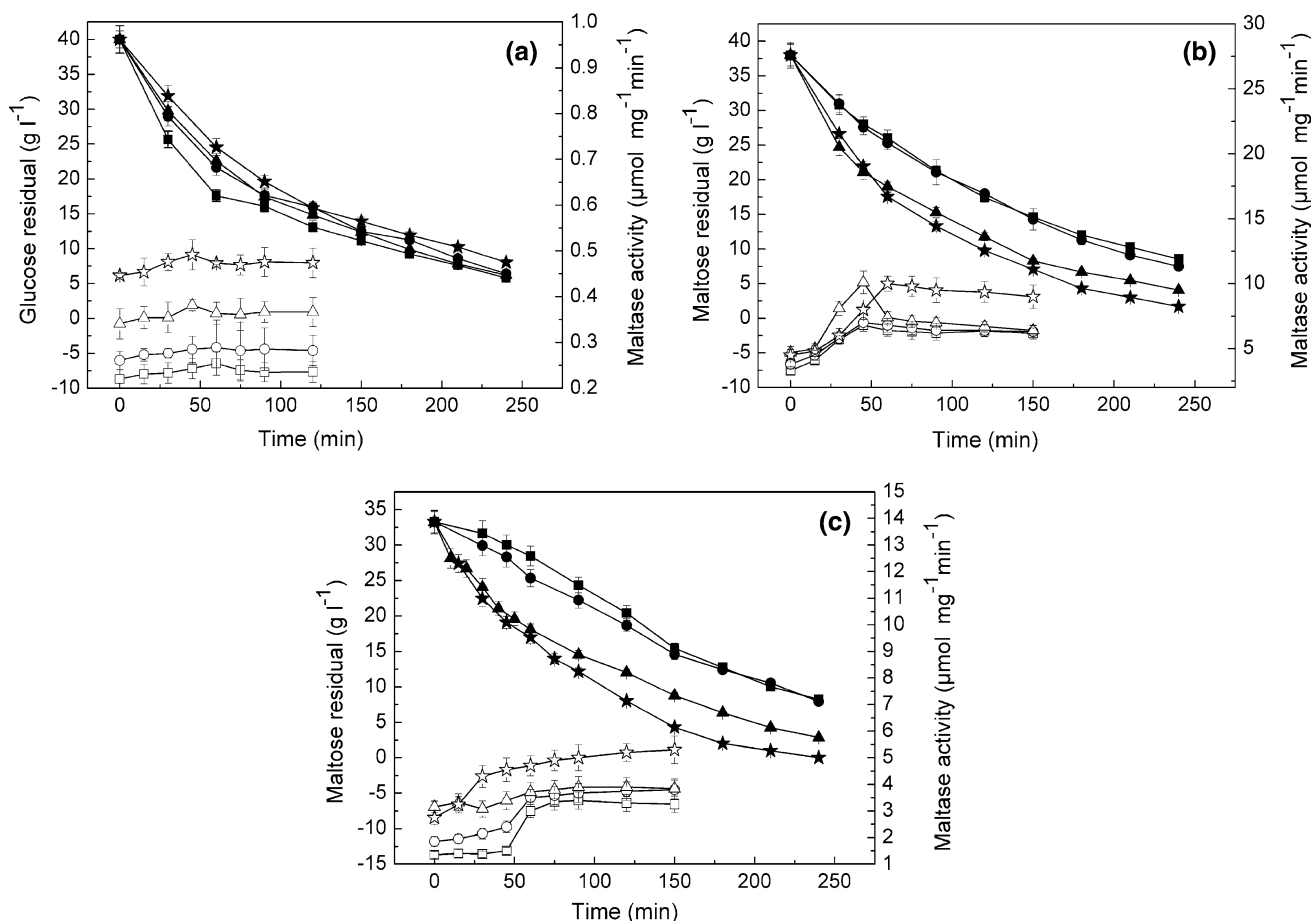
Fig. 1 Alpha-glucosidase activity and concentration of residual sugar in four yeast strains during shaking cultivation in three LSMLD: **a** glucose, **b** maltose, and **c** glucose-maltose. *Filled square*: residual sugar concentration of parent strain BY14a; *filled circle*: residual sugar concentration of BY14a-MIG1; *Filled triangle*: residual sugar concentration of BY14a + MAL62; *Filled star*: residual sugar concentration of BY14a + MAL62-MIG1; *open square*: alpha-glucosidase activity of parent strain BY14a; *open circle*: alpha-glucosidase activity of BY14a-MIG1; *open triangle*: alpha-glucosidase activity of BY14a + MAL62; and *open star*: alpha-glucosidase activity of BY14a + MAL62-MIG1. Data are averages from three independent experiments and *error bars* represent ± SD. BY-C was used as the blank control for the BY14a + MAL62 strain to avoid the possible effects of an empty vector on enzyme activities, fermentation characteristics, and leavening activity. Since no significant distinctions were observed (data not shown), BY14a was used as the control strain for the BY14a + MAL62 strain

However, an increase of at least 52.8 % (Fig. 1a) was observed in strains carrying the overexpressed *MAL62*. Both strains overexpressing the *MAL62* gene showed enhanced alpha-glucosidase activities in the three LSMLD but displayed no increase in maltose permease activity (data not shown). These results suggest that, although it does not bring about increases in maltose permease activity, overexpression of the *MAL62* gene could positively affect the level of alpha-glucosidase activity and production of gas.

MIG1 deletion was much less effective than MAL62 overexpression in enhancing maltose fermentation

MIG1 is a major mediator of glucose repression in *S. cerevisiae* [1]. We measured alpha-glucosidase and maltose permease activities in the *MIG1*-deleted and parent yeast strains to examine whether *MIG1* deletion is effective for *MAL* gene derepression. Loss of *MIG1* in strain BY14a-MIG1 led to a slightly elevated level of alpha-glucosidase activity in three conditions (Fig. 1a–c) but no effect on maltose permease activity (data not shown). These findings confirm the role of *MIG1* in the glucose repression of alpha-glucosidase expression in industrial baker's yeast. In the glucose-maltose LSMLD, as glucose was consumed, a reduction in maltose permease activity was observed in the BY14a-MIG1 strain (Fig. 3). This result indicates that deletion of *MIG1* in the industrial yeast strain does not entirely alleviate glucose control of the genes involved in maltose utilization.

Fermentation characteristics

We explored the physiological characteristics (specific growth rate and biomass yield) and leavening ability of
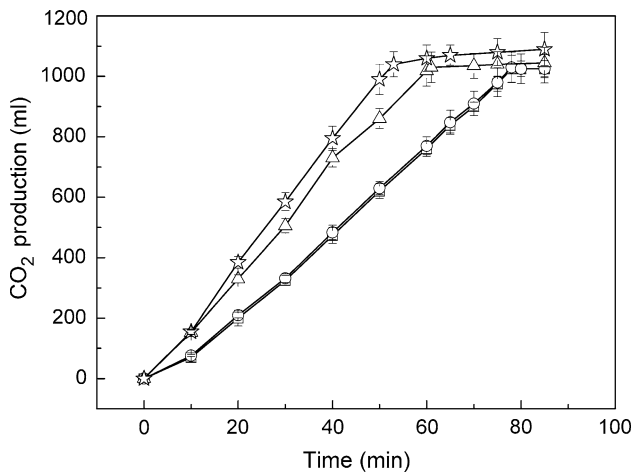
Fig. 2 CO_2 production in lean dough by four strains of yeast. *Open square*: parent strain BY14a; *open circle*: BY14a-MIG1; *open triangle*: BY14a + MAL62; and *open star*: BY14a + MAL62-MIG1. Data are averages from three independent experiments and *error bars* represent ± SD

four yeast strains to investigate their fermentation characteristics (Table 3). The physiological characteristics of strain BY14a-MIG1 were strongly affected by *MIG1* deletion compared with the parent strain, BY14a. In the BY14a-MIG1 strain, less tightly controlled maltose metabolism increased the specific growth rate from 0.29 h^{-1} to 1.21 h^{-1} and the biomass yield from 0.62 g C-mol^{-1} to 0.68 g C-mol^{-1} in comparison to the parent strain. In contrast, overexpression of the *MAL62* gene in strain BY14a + MAL62 had a slightly negative physiological effect and reduced the biomass yield from 0.62 g C-mol^{-1} to 0.58 g C-mol^{-1}. Two strains overexpressing *MAL62*, BY14a + MAL62 and BY14a + MAL62-MIG1, showed significantly higher leavening abilities than strain BY14a-MIG1 (Table 3). These results suggest that only strains with a marked increase in alpha-glucosidase activity exhibit increased leavening ability.

Discussion

Previous studies have reported that maltose permease is the determining factor in leavening lean dough [6, 13]. In this study, we showed that maltose permease is less necessary than alpha-glucosidase, which is capable of enhancing maltose fermentation under any maltose concentration in LSMLD (i.e., glucose, maltose, or glucose-maltose). When cultivated in glucose medium and no extracellular maltose is available, intracellular maltose is sufficient to induce maltose-utilizing gene expression [25], rendering maltose transportation by maltose permease unnecessary. When yeast cells are grown in high extracellular maltose concentrations, alpha-glucosidase activity could also increase due to the presence of distinct maltose uptake systems that differ from maltose permease [5, 7, 23, 25]. Maltose permease does not play an exclusive role in either triggering *MAL* gene expression or maltose uptake. Hence, only overexpressed *MAL62* could respond to maltose and bring about further increases in alpha-glucosidase activity. Furthermore, the increased alpha-glucosidase activity in yeast could have a strong correlation with the ability of the strain to leaven lean dough. These results support the conclusion of Jiang [15], who believed that alpha-glucosidase is the essential enzyme in maltose fermentation for high extracellular maltose concentrations. In this study, we verified that regardless of the extracellular maltose concentration, alpha-glucosidase, rather than maltose permease, is the most relevant feature in maltose fermentation.

Glucose, which is most commonly present in industrial carbon sources, delays the uptake of sugars and prolongs the production process unnecessarily [16]. Effective alleviation of glucose repression, or the rapid transition from glucose to maltose metabolism, is essential to improve the leavening ability of yeast during bread-making. In laboratory strains, glucose repression could partially be relieved by deletion of *MIG1* [3, 19], whereas in industrial strains, *MIG1* deletion was much less effective to yields high levels

Table 3 Fermentation characteristics of the four strains when cultivated in glucose–maltose mixture LSMLD medium and lean dough

Strains	Specific growth rate (h^{-1})[a]	Biomass yield (g C-mol^{-1})[a]	Leavening ability ($mlCO_2$ h^{-1} g^{-1} dw)[b]
BY14a	0.29 ± 0.00	0.62 ± 0.011	108.5 ± 3.5
BY14a + MAL62	0.28 ± 0.01	0.58 ± 0.010	145.4 ± 4.6
BY14a-MIG1	1.21 ± 0.03	0.68 ± 0.012	110.0 ± 3.1
BY14a + MAL62-MIG1	0.63 ± 0.03	0.73 ± 0.014	151.4 ± 5.0

Values shown represent averages of at least three independent experiments (data are means ± SD)

[a] The specific growth rate and biomass yield were calculated from the cells grown in glucose–maltose mixture LSMLD medium

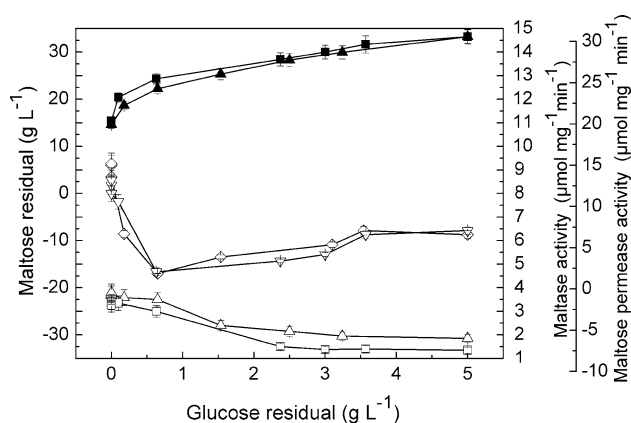[b] Leavening ability were calculated from lean dough

Fig. 3 Alpha-glucosidase activity, maltose permease activity, and concentration of residual sugar in parent strains BY14a and BY14a-MIG1 during shaking cultivation in the glucose-maltose LSMLD. *Filled square*: residual maltose concentration of parent strain BY14a; *Filled triangle*: residual maltose concentration of BY14a-MIG1; *open square*: alpha-glucosidase activity of parent strain BY14a; *open triangle*: alpha-glucosidase activity of BY14a-MIG1; *open diamond*: maltose permease activity of parent strain BY14a; and *open inverted triangle*: maltose permease activity of BY14a-MIG1. Data are averages from three independent experiments and *error bars* represent ± SD

of alpha-glucosidase and maltose permease activities [10, 14, 26, 27]. These results suggest that *MAL* gene control in industrial baker's yeast occurs through a dual-level control mechanism (i.e., *MIG1*-dependent and *MIG1*-independent) [16]; thus, deletion of *MIG1* in industrial yeast strains cannot completely eliminate glucose repression.

MAL62 overexpression negatively affected the specific growth rate and biomass yields. Deletion of *MIG1* could compensate for the reduction in specific growth rate and biomass yield of the BY14a + MAL62 strain. BY14a + MAL62-MIG1 can readily ferment maltose and instantly produce CO_2 when it enters the lean dough. These characteristics are consistent with what is required of a strain used commercially in terms of yield and leavening ability.

Based on the results of the present study, metabolic engineering of glucose derepression appears to be a greater challenge than maltose fermentation. Baker's yeast containing overexpressed-*MAL62* and featuring a deleted *MIG1* genetic background that provides high levels of alpha-glucosidase activity is sufficient to enable the strain to rapidly ferment maltose and enhance its leavening properties. Such a strain could be useful for leavening lean dough.

References

1. Ahuatzi D, Riera A, Peláez R, Herrero P, Moreno F (2007) Hxk2 regulates the phosphorylation state of Mig1 and therefore its nucleocytoplasmic distribution. J Biol Chem 282:4485–4493
2. Bell PJ, Higgins VJ, Attfield PV (2001) Comparison of fermentative capacities of industrial baking and wild-type yeasts of the species Saccharomyces cerevisiae in different sugar media. Lett Appl Microbiol 32:224–229
3. Cao HL, Yue M, Li SG, Bai XF, Zhao XM, Du YG (2011) The impact of MIG1 and/or MIG2 disruption on aerobic metabolism of succinate dehydrogenase negative Saccharomyces cerevisiae. Appl Microbiol Biotechnol 89:733–738
4. Day RE, Higgins VJ, Rogers PJ, Dawes IW (2002) Characterization of the putative maltose transporters encoded by YDL247w and YJR160c. Yeast 19:1015–1027
5. Dietvorst J, Walsh MC, van Heusden GP, Steensma HY (2010) Comparison of the MTT1- and MAL31-like maltose transporter genes in lager yeast strains. FEMS Microbiol Lett 310:152–157
6. Goldenthal MJ, Vanoni M, Buchferer B, Marmur J (1987) Regulation of MAL gene expression in yeast: gene dosage effects. Mol Gen Genet 209:508–517
7. Hach A, Hon T, Zhang L (2000) The coiled coil dimerization element of the yeast transcriptional activator Hap1, a Gal4 family member, is dispensable for DNA binding but differentially affects transcriptional activation. J Biol Chem 275:248–254
8. Hauf J, Zimmermann FK, Müller S (2000) Simultaneous genomic overexpression of seven glycolytic enzymes in the yeast Saccharomyces cerevisiae. Enzyme Microb Technol 26:688–698
9. Higgins VJ, Braidwood M, Bell P, Bissinger P, Dawes IW, Attfield PV (1999) Genetic evidence that high noninduced maltase and maltose permease activities, governed by MALx3-encoded transcriptional regulators, determine efficiency of gas production by baker's yeast in unsugared dough. Appl Environ Microbiol 65:680–685
10. Higgins VJ, Braidwood M, Bissinger P, Dawes IW, Attfield PV (1999) Leu343Phe substitution in the Malx3 protein of Saccharomyces cerevisiae increases the constitutivity and glucose insensitivity of MAL gene expression. Curr Genet 35:491–498
11. Houghton-Larsen J, Brandt A (2006) Fermentation of high concentrations of maltose by Saccharomyces cerevisiae is limited by the COMPASS methylation complex. Appl Environ Microbiol 72:7176–7182
12. Hu Z, Nehlin JO, Ronne H, Michels CA (1995) MIG1-dependent and MIG1-independent glucose regulation of MAL gene expression in Saccharomyces cerevisiae. Curr Genet 28:258–266
13. Hu Z, Yue Y, Jiang H, Zhang B, Sherwood PW, Michels CA (2000) Analysis of the mechanism by which glucose inhibits maltose induction of MAL gene expression in Saccharomyces. Genetics 154:121–132
14. Jiang H, Tatchell K, Liu S, Michels CA (2000) Protein phosphatase type-1 regulatory subunits Reg1p and Reg2p act as signal transducers in the glucose-induced inactivation of maltose permease in Saccharomyces cerevisiae. Mol Gen Genet 263:411–422
15. Jiang TX, Xiao DG, Gao Q (2008) Characterisation of maltose metabolism in lean dough by lagging and non-lagging baker's yeast strains. Ann Microbiol 58:655–660
16. Klein CJL, Olsson L, Nielsen J (1998) Glucose control in Saccharomyces cerevisiae: the role of MIG1 in metabolic functions. Microbiol Sgm 144:13–24
17. Lilly M, Lambrechts MG, Pretorius IS (2000) Effect of increased yeast alcohol acetyltransferase activity on flavor profiles of wine and distillates. Appl Environ Microbiol 66:744–753
18. Lucero P, Herweijer M, Lagunas R (1993) Catabolite inactivation of the yeast maltose transporter is due to proteolysis. FEBS Lett 333:165–168

19. Nehlin JO, Carlberg M, Ronne H (1991) Control of yeast *GAL* genes by *MIG1* repressor: a transcriptional cascade in the glucose response. EMBO J 10:3373–3377

20. Oda Y, Ouchi K (1990) Role of the yeast maltose fermentation genes in CO_2 production rate from sponge dough. Food Microbiol 7:43–47

21. Peinado JM, Barbero A, van Uden N (1987) Repression and inactivation by glucose of the maltose transport system of *Candida utilis*. Appl Microbiol Biotechnol 26:154–157

22. Randez-Gil F, Sanz P (1994) Construction of industrial baker's yeast strains able to assimilate maltose under catabolite repression conditions. Appl Microbiol Biotechnol 42:581–586

23. Richter K, Buchner J (2001) Hsp90: chaperoning signal transduction. J Cell Physiol 188:281–290

24. Salema-Oom M, de Sousa HR, Assuncao M, Goncalves P, Spencer-Martins I (2011) Derepression of a baker's yeast strain for maltose utilization is associated with severe deregulation of *HXT* gene expression. J Appl Microbiol 110:364–374

25. Wang X, Bali M, Medintz I, Michels CA (2002) Intracellular maltose is sufficient to induce *MAL* gene expression in *Saccharomyces cerevisiae*. Eukaryot Cell 1:696–703

26. Wanke V, Vavassori M, Thevelein JM, Tortora P, Vanoni M (1997) Regulation of maltose utilization in *Saccharomyces cerevisiae* by genes of the RAS/protein kinase A pathway. FEBS Lett 402:251–255

27. Westergaarda SL, Broa C, Olssona L, Nielsena J (2004) Elucidation of the role of Grr1p in glucose sensing by *Saccharomyces cerevisiae* through genome-wide transcription analysis. FEMS Yeast Res 5:193–204