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# Improve carbon metabolic flux in *Saccharomyces cerevisiae* at high temperature by overexpressed *TSL1* gene

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**Abstract** This study describes a novel strategy to improve the glycolysis flux of *Saccharomyces cerevisiae* at high temperature. The *TSL1* gene-encoding regulatory subunit of the trehalose synthase complex was overexpressed in *S. cerevisiae* Z-06, which increased levels of trehalose synthase activity in extracts, enhanced stress tolerance and glucose consuming rate of the yeast cells. As a consequence, the final ethanol concentration of 185.5 g/L was obtained at 38 °C for 36 h (with productivity up to 5.2 g/L/h) in 7-L fermentor, and the ethanol productivity was 92.7 % higher than that of the parent strain. The results presented here provide a novel way to enhance the carbon metabolic flux at high temperature, which will be available for the purposes of producing other primary metabolites of commercial interest using *S. cerevisiae* as a host.

**Keywords** *Saccharomyces cerevisiae* · *TSL1* gene · Glycolysis flux · High temperature

## Introduction

Microbial-based ethanol production serves large and diverse industries, from alcoholic beverages to biofuel production. Therefore, high concentration, yield and productivity are required for a cost-effective biotechnological production of ethanol. In order to decrease the ethanol production cost, the final ethanol concentration must be increased; however, such an increase will significantly inhibit yeast growth and viability [9, 23]. A high fermentation temperature would also be attractive to the ethanol industry, as it would dramatically decrease the cost of cooling in the summer. However, increasing the culture temperature enhances the yeast ethanol toxicity and decreases its osmotic stress tolerance. Therefore, much work has been done to improve the ethanol production using genetically modified organisms and multistage fermentation systems in recent years [12, 16, 17, 19, 28, 31, 34].

Trehalose (α-D-glucopyranosyl-α-D-glucopyranoside), a nonreducing disaccharide participating in tolerance to various environmental stresses, occurs in many organisms, including bacteria, fungi, insects and plants [7, 26, 35]. In yeast, trehalose is synthesized by a large enzyme complex comprising four subunits which are encoded by the genes *TPS1*, *TPS2*, *TPS3* and *TSL1*, respectively. Among the four proteins, *TSL1* not only acts as a regulatory subunit in the complex but is also involved in glucose signaling [1, 3, 4]. Trehalose can be hydrolyzed into two glucose molecules by trehalase: neutral trehalase (Nth1) and acid trehalase (Ath1). Depending on the environmental conditions, trehalose can consist of up to 20 % of *S. cerevisiae* cell dry weight [14]. Over the past 20 years, a number of studies have demonstrated a correlation between intracellular trehalose levels and the ability of yeast to survive various environmental stresses, such as starvation, desiccation, dehydration, osmotic and oxidative stress and extremes in temperature [10, 15, 27, 32].

In order to enhance the trehalose concentration in yeast cells, Jung and Park decreased the expression of acid trehalase gene in *S. cerevisiae*, and the recombinant yeast had a high viability with 8 % (v/v) ethanol [18]. However, very few studies investigating the effect of overexpressed *TSL1*

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gene on trehalose and on ethanol production have been reported. In this study, with the aim of increasing the fermentation temperature and ethanol productivity, the *TSL1* gene encoding the regulatory subunit of the trehalose synthase complex in *S. cerevisiae* Z-06 was overexpressed, and the influence of regulatory subunit on the trehalose synthesis as well as the carbon metabolic flux at high temperature was carefully investigated. Our results showed that overexpressing the *TSL1* gene could enhance cell stress tolerance and glycolysis flux of the recombinant *S. cerevisiae* Z-061 dramatically. Furthermore, the strategy for enhancing carbon metabolic flux and stress tolerance of the *S. cerevisiae* cells may be applied to other primary metabolite fermentation processes using this strain.

## Materials and methods

### Chemicals

Pyrobest polymerase, restriction enzymes *Bam*H I and *Eco*R I, proteinase K, lysozyme, cetyltrimethyl ammonium bromide, and saturated phenol were obtained from Takara (Dalian, China). Carbobenzoxy-L-glutaminylglycine (CBZ-Gln-Gly), valinomycin, ampicillin, and nigericin were obtained from Sigma–Aldrich (Steinheim, Germany). All inorganic compounds were of reagent grade or higher quality.

### Microorganism, plasmids and culture condition (*S. cerevisiae* and *E. coli*)

*Saccharomyces cerevisiae* Z-06 (ura-) is a laboratory strain screened and constructed by our lab with the marker URA3, and has been deposited in the Culture and Information Center of Industrial Microorganisms of China University (CICIM-CU) at Jiang Nan university as CICIM Y0086 (T). This strain was maintained on a YPD medium containing (per liter) 20 g of glucose, 20 g of peptone, and 10 g of yeast extract, at pH 6.8, and 4 °C. *E. coli* JM109 was used for the constructions of the plasmids of pYES2-*TSL1*, grown in LB media at 200 rpm and 37 °C. Plasmid pYES2 was presented by Invitrogen (Shanghai) Co., Ltd. This vector contains the *URA3* selection in yeast, *GAL1* gene for promoter and 2μ origin for high-copy maintenance. The strains, plasmids and oligonucleotides used in this study were presented in Table 1.

### DNA manipulations

Isolation of chromosomal DNA from *S. cerevisiae* and standard recombinant DNA techniques were performed as described by Sambrook et al. [25]. Isolation and transformation of *S. cerevisiae* plasmid DNA were performed as described in the literature [11, 20].

### Construction of strains and plasmids

To make a transcriptional fusion of the *TSL1* gene to the GAL promoter, the *TSL1* gene with ATG start codon was introduced at the *Bam*H I-*Eco*R I site of the pYES2 plasmid. The gene was amplified by using *S. cerevisiae* Z-06 chromosome DNA as a template and two primers designed according to the published nucleotide sequence of the *TSL1* gene from *S. cerevisiae* S288c [5]: 5′-ACCGGGATCCATGGCTCTCATCGTGGCATC-3′(containing a *Bam*H I site [underlined]) and 5′-ACCGGAATTCTTAATCTTCAATGATTCTTG-3′(containing an *Eco*R I site [underlined]). The PCR-amplified *TSL1* gene fragment digested by *Bam*H I-*Eco*R I was inserted into the pYES2 vector to create plasmid pYES2-*TSL1*. The plasmid pYES2-*TSL1* was extracted after transforming into *E. coli* to culture the transformants, and was then introduced into *S. cerevisiae* Z-06 using the *S.c* EasyComp$^{TM}$ Transformation Kit, yielding *S. cerevisiae* Z-061. Meanwhile, the plasmids pYES2 without an insert was also introduced into the host to obtain the control strain of *S. cerevisiae* Z-060. The plasmid pYES2-*TSL1* was extracted from *E. coli* transformants, and the inserted *TSL1* gene was sequenced by Sangon Biotechnology Company (Shanghai, China). Analysis revealed that the amino acid sequence encoded by the *TSL1* gene from *S. cerevisiae* Z-061 was identical to that of the template strain S. *cerevisiae* S288c [5].

### Batch fermentations

Batch fermentations were carried out in a 7-L jar fermentor (KF-7 L; Korea Fermentor Co., Inchon, South Korea) with a working volume of 4 L at 30 °C for 40 h after the addition of the *S. cerevisiae* cells at the concentration of $10^8$/mL. Cultivations of strain Z-061 were carried out in YPD medium to exponential growth phase culture with $OD_{600}$ nm of 10.0. Then, the cells were recovered by centrifugation of this culture at 8,000×*g* for 5 min and resuspended to obtain an $OD_{600\ nm}$ of 0.4 in induction medium containing (per liter) 20 g of galactose, 20 g of peptone, 10 g of yeast extract, and induced for 3 h. Then, the cells recovered after induction were used to inoculate the medium supplemented with 2.0 g $(NH_4)_2SO_4$, 1.0 g $KH_2PO_4$, and 0.2 g $MgSO_4{\cdot}7H_2O$ at the yeast cells concentration of $10^8$/mL. Glucose solution (800 g/L) was used as a carbon source to allow adjustment of the feeding speed according to glucose concentration measurements checked every 4 h, which allowed the maintaining of a glucose concentration between 20 and 30 g/L in the late fermentation phase. All the experiments were repeated three

**Table 1** Strains, Plasmids and Oligonucleotides used in this study

| Strain, plasmid, or oligonucleotide | Relevant characteristic(s) or sequence | Reference or purpose |
|---|---|---|
| Strains | | |
| *E.coli* JM109 | recA1 end1 gyrA96 thi hsdR17 supE44 relA1 Δ(lac-proAB)/ F′(traD36 proAB$^+$ lac$^q$ lacZ ΔM15) | Stratagene |
| *S. cerevisiae* Z-06 | *S. cerevisiae* Z-06 (ura-) | CICIM-CU |
| *S. cerevisiae* Z-060 | Z-06 harboring pYES2 | This work |
| *S. cerevisiae* Z-061 | Z-06 harboring pYES2-*TSL1* | This work |
| Plasmids | | |
| pYES2 | Amp$^r$ *E. coli- S. cerevisiae* shuttle vector | Invitrogen |
| pYES2-*TSL1* | Plasmid carrying the *TSL1* genes | This work |
| Oligonucleotides | 5′ → 3′ sequence | |
| *TSL1*-F | ACCGGGATCCATGGCTCTCATCGTGGCATC | Amplifying *TSL1* |
| *TSL1*-R | ACCGGAATTCTTAATCTTCAATGATTCTTG | Amplifying *TSL1* |

Restriction sites (*Bam*HI, *Eco*RI) are underlined

times, and the values are expressed as the means of duplicate measurements on three independent samples. The data were analyzed by SAS software (USA).

## Stress conditions

To determine the environmental stresses on the growth and metabolism of *S. cerevisiae* Z-06, experiments were carried out in the YPD medium supplemented with 240 g/L of glucose and various concentrations of NaCl (from 20 to 80 g/L) at 30 °C to 38 °C with a 2 °C interval. Meanwhile, to compare the growth of strain Z-06 and Z-061 at 34 °C, 38 °C, and 40 °C, all of the tests were carried out in the YPD media at 34 °C, 38 °C, and 40 °C, respectively. All of the above experiments were carried out in a 7-L jar fermentor containing 4 liters of the liquid culture with 240 g/L of glucose without pH control.

## Influence of overexpressed *TSL1* gene on glycolysis

To determine the effect of the overexpressed *TSL1* gene on the carbon flux distribution at 38 °C, samples were aseptically withdrawn from the fermentation vessel at 12 h and 15 h. All the samples were immediately cooled on ice to determine the extracellular and intracellular metabolites [30].

To determine the intracellular TPS activity and trehalose concentrations, the above cultures were filtered through cheese-cloth to obtain a clear supernatant, and then centrifuged at 5,000×*g* for 15 min at 4 °C to harvest the cells. The cell pellets were washed twice with ice-cold saline (0.85 % NaCl, wt/vol) and re-suspended in 25 ml of a 200 mM potassium phosphate buffer (pH 7.0) containing 2 mM EDTA. Next, the cells were disrupted ultrasonically at 4 °C for 60 cycles of 4 s (ACX 400 sonicator at 20 kHz; Sonic and Materials, Newton, Mass.), and then the cell debris was removed by centrifugation (10,000×*g* for 10 min at 4 °C), leaving the cell extract (CE). The intracellular and extracellular metabolites (trehalose, fructose, lactate, acetate, and pyruvate) were analyzed using high performance liquid chromatography (HPLC, Waters Associates model 209, equipped with a differential refractive index R1401 detector; using a Merck Lichrosorb-NH2 column (4.6 × 250 mm, 5 μm), at a temperature of 30 °C. A mixture of acetonitrile–water (60:40, V/V) was used as the mobile phase at a flow rate of 1.0 mL/min). All the samples were injected twice.

## Assay of trehalose synthase activity (TPS)

TPS activity was assayed at 37 °C by a colorimetric method using 5 mM of UDPG (uridine diphosphate glucose) and G-6-P (Glucose-6-phosphate) as substrates [21, 24]. The assay mixture contained 50 mM Tris–HCl buffer, pH 8.5, 10 mM $MnCl_2$ and 1 μg heparin salt, apart from the substrate and the enzyme. $MnCl_2$ and heparin were used as co-factors and lower concentrations were seen to increase enzyme activity by three–fourfold. At the end of incubation, HCl was added to a final concentration of 100 mM and the tubes were heated at 100 °C for 10 min in a boiling water bath to destroy all remaining UDPG. NaOH was next added to a final concentration of 150 mM and tubes were again heated similarly at 100 °C for 10 min to destroy all remaining reducing sugars. Units of enzyme activity (U) for TPS were expressed as micromoles (μmol) of product synthesized per min under assay conditions.

Traditionally, in vitro assays of enzyme kinetics are performed after some attempts at protein purification, and the percent purity of active sample is far from 100 %. Here, however, the trehalose synthase activity is quantified directly from cell extract and described with units of U/mg of cell extract.

### Analytical methods

Reducing sugars were estimated with 3,5-dinitrosalicylic acid (DNS) using glucose as standard [13]. Yeast cell populations were determined by direct microscopic count in a counting chamber. The protein concentrations of the cells mass were determined using the Bradford method with bovine serum albumin as the standard [6]. The pH was measured by pH-meter. Ethanol was assayed by gas liquid chromatography. Biomass concentration was determined by harvesting the yeast cells by filtration and freeze-drying them to a constant mass, the dry cell weight (DCW) was expressed as gram per liter of the culture.

## Results

### Effect of fermentation temperature and osmotic stress on the growth of *S. cerevisiae*

The effects of fermentation temperature and NaCl concentration on the ethanol production of *S. cerevisiae* Z-06 were presented in Fig. 1. In the medium with 240 g/L of glucose, increasing the fermentation temperature from 30 °C to 34 °C resulted in an enhanced ethanol concentration (from 93.2 g/L to 105.1 g/L) (Fig. 1a). However, the concentrations of ethanol decreased to 67.9 g/L when the fermentation temperature increased to 38 °C. On the other hand, the concentrations of ethanol decreased with the increase of the NaCl concentration in culture broth (Fig. 1b). These results demonstrated that the ethanol production and growth of *S. cerevisiae* could be adjusted and controlled by fermentation medium osmolality, and cell growth and ethanol production would decrease under the higher environmental stresses osmolality.

### Improvement of the *S. cerevisiae* growth by overexpressed *TSL1* gene

Above investigation indicated that it is necessary to enhance stress tolerance of the yeast cells for ethanol production. As recent studies found that *TSL1* gene involved in not only trehalose synthesis but also the glucose regulatory system [1], therefore, to enhance the fermentation activity of *S. cerevisiae* at high temperature, the plasmid pYES2-*TSL1* was introduced into *S. cerevisiae* Z-06. It was found that the maximal DCW and specific growth rate obtained at 34 °C for Z-061 and strains Z-06 were 22.34 ± 0.74 and 21.70 ± 0.72 g/L, 0.306 $h^{-1}$ at 6 h and 0.298 $h^{-1}$ at 6.5 h, respectively (Fig. 2). At elevated temperatures for strains Z-061, the maximal DCW was 22.05 ± 0.66 g/L for 38 °C, and 14.42 ± 0.58 g/L for

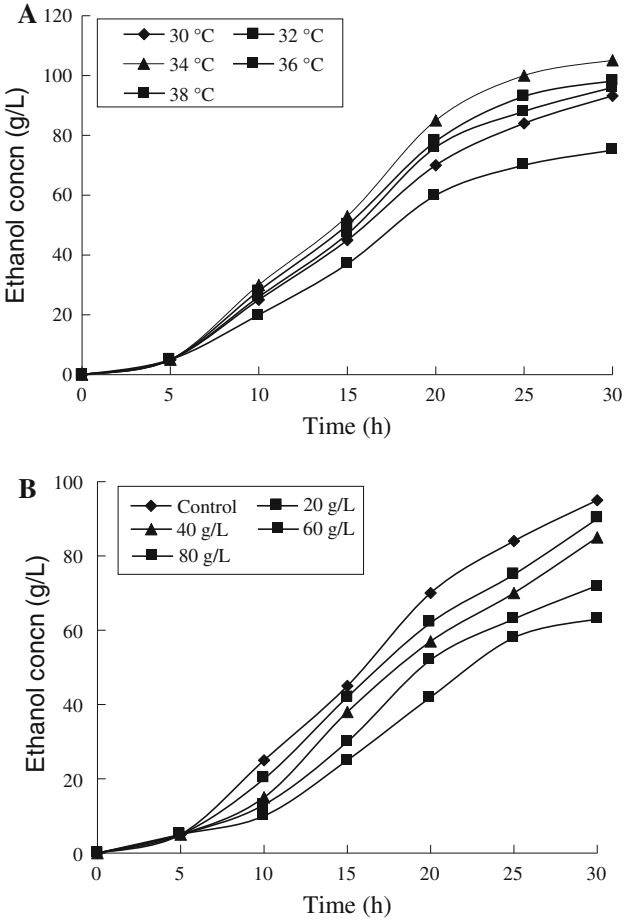


**Fig. 1** Effect of fermentation temperature (**a**) and NaCl concentration (**b**) on ethanol production of strain *S. cerevisiae* Z-06. The experiments were carried out in a 7-L jar fermentor containing 4 L of the liquid culture with 240 g/L of glucose without pH control

40 °C, the maximal specific growth rate for strains Z-061 at different temperatures was 0.372 $h^{-1}$ for 38 °C, and 0.287 $h^{-1}$ for 40 °C. However, for strain Z-06, the maximum DCW and specific growth rate at 38 °C were 17.80 ± 0.51 g/L and 0.281 $h^{-1}$, respectively, and a similar result was found for strain Z-060. These results demonstrated the optimum growth temperature for strain Z-061 was near to 38 °C, which was 4 °C higher than that for the strain Z-06.

Figure 3 shows the influence of overexpressed *TSL1* genes on TPS activity and trehalose production after in the yeast strains at 38 °C. It was found that maximum TPS activities was enhanced from 1.48 U/mg of cell extract (strain Z-06) and 1.42 U/mg of cell extract (strain Z-060) to 5.62 U/mg of cell extract (strain Z-061), which with near fourfold higher increased. As a result, the trehalose concentration was increased from 0.151 g/gDCW (strain Z-06) and 0.146 g/gDCW (strain Z-060) to 0.461 g/gDCW (strain Z-061), which with over threefold increased. These results signified that overexpressed *TSL1* gene

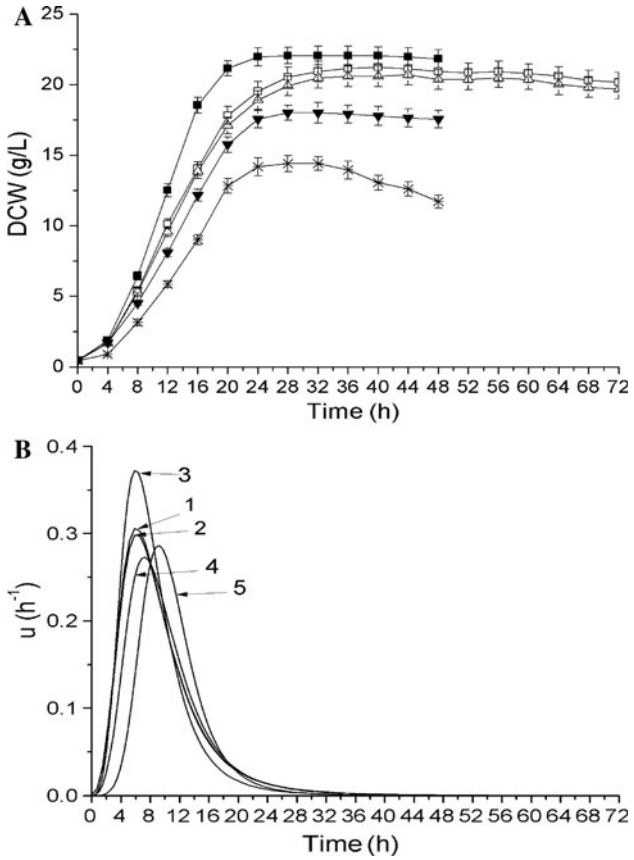


**Fig. 2** Parameters in different fermentation models of strain *S. cerevisiae* Z-061 and *S. cerevisiae* Z-06. **a** DCW (dry cell weight), **b** specific growth rate. The experiments were carried out in a 7-liter jar fermentor containing 4 L of the liquid culture with 240 g/L of glucose without pH control. The inoculations and induction of strain Z-061 were described in Batch Fermentations under "Materials and Methods". *Open square* and *curve* 1: strain Z-061 in 34 °C for 72 h; *Large open square* and *curve* 2: strain Z-06 in 34 °C for 72 h; *Filled square* and *curve* 3: strain Z-061 in 38 °C for 48 h; *Filled inverted triangle* and *curve* 4: strain Z-06 in 38 °C for 48 h; * and curve 5: strain Z-061 in 40 °C for 48 h

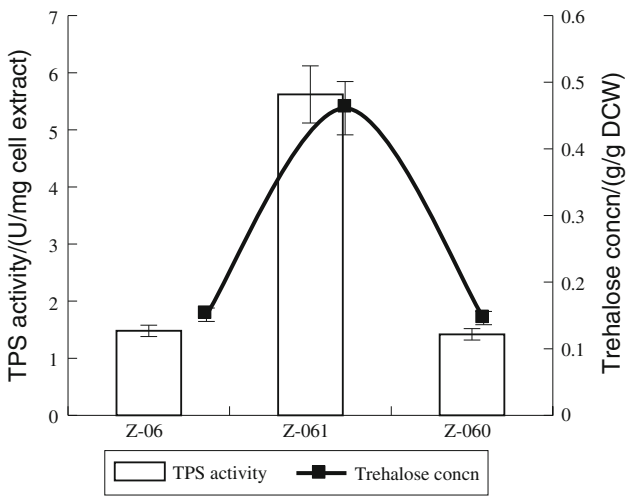


**Fig. 3** Effect of overexpressed *TSL1* genes on TPS activity and trehalose production of *S. cerevisiae*. The experiments were carried out in the YPD medium for at 38 °C for 24 h, and then cells were recovered and resuspended in induction medium for 3 h as described in Batch Fermentations under "Materials and Methods"

resulted into an increased TPS activity, which enhanced the trehalose synthesis in the yeast cells.

## Comparison of carbon metabolic flux in *S. cerevisiae* strains

To determine the influences of the overexpressed *TSL1* gene on ethanol production, fed-batch were used at 38 °C in the media with initial glucose concentration of 210 g/L, and 800 g of glucose was supplemented after 16 h of fermentation. Fig. 4a shows the time course of ethanol production by strain Z-06, Z-061 and Z-060 with 410 g/L of total glucose at 38 °C for 36 h. The results indicated that significant changes took place in carbon metabolism of the strain Z-061. The highest ethanol concentration of 185.5 g/L was achieved at 36 h, which was over twofold higher than that of parent strain (96.3 g/L). Moreover, for the control strains of Z-06 and Z-060, residual glucose concentration was still over 200 g/L at 36 h of fermentation, and less than 20 g/L in the medium for the strain Z-061 at this stage of fermentation (Fig. 4b). These results suggest that the ethanol production ability of *S. cerevisiae* could be increased by overexpressed *TSL1* gene for enhanced TPS activity in the cells.

To determine the influence of overexpressed *TSL1* gene on metabolism of Z-061 at 38 °C, the extracellular metabolite concentration of Z-061 after 12 h and 15 h is presented in Table 2, and the metabolite accumulation rates were calculated using the following equation (Table 3):

$$V = \frac{(C_{15h} - C_{12h}) \times 1000}{\mathrm{MW} \times 3} \quad (1)$$

where $V$ (mmol/L/h) is the metabolite accumulation rate, $C_{15h}$ and $C_{12h}$ are the metabolite concentrations measured after 15 and 12 h, respectively, and MW is the molecular weight of the metabolites. As seen, the glucose consumption and ethanol accumulating rate of Z-061 were enhanced by 19.59 and 153.54 % more than that of Z-06, respectively. Summarizing the results in Tables 3 and 4, the extracellular pyruvate and acetic acid, as well as glycerol, accumulation rates of Z-061 after 15 h were both significantly decreased by the overexpressed *TSL1* gene, and the pH value of strain Z-061 was increased to near 4.52, which was about 0.5 pH units higher than that of the strain Z-06 (pH 4.16).

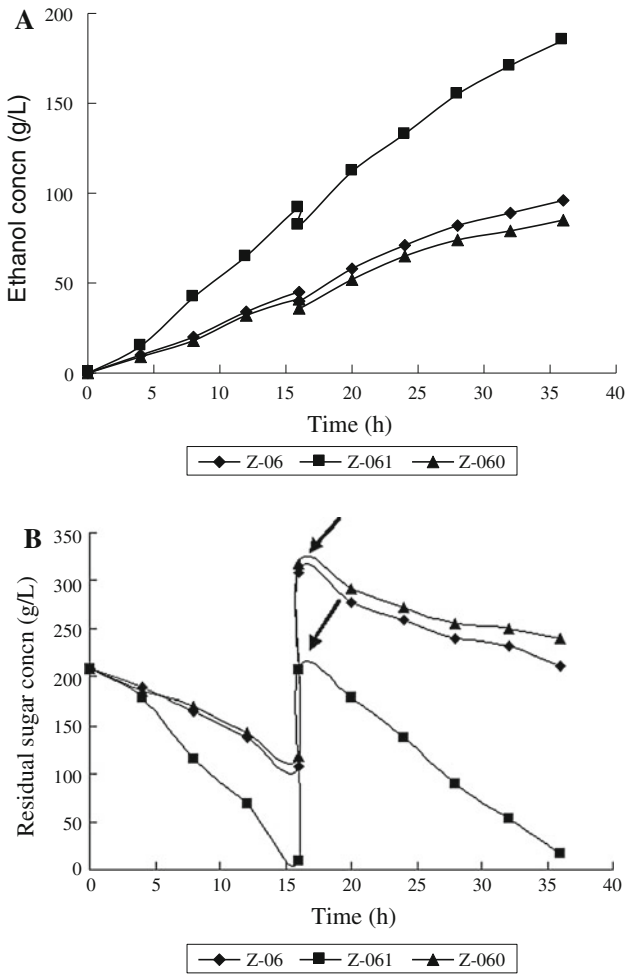


**Fig. 4** Time course of ethanol production and residual sugar in the fermentation media of *S. cerevisiae*. The experiments were carried out in a 7-L jar fermentor containing 4 L of the liquid culture at 38 °C with 210 g/L of initial glucose without pH control. The inoculations of strain Z-06 and Z-061, and the induction of strain Z-061 were prepared as described in Batch Fermentations under Materials and Methods. The *arrows* indicate the addition of 800 g glucose to media after 16 h of the fermentation

## Discussion

In fermentation medium, an osmotic stress increase in fermentation broth is always observed if the addition of substrate continues in order to obtain high concentration of product. On the other hand, enhancement of the fermentation temperature not only decreased the cost of cooling in the process dramatically, but also conferred upon the yeast a competitive advantage over undesirable mesophilic bacteria. Nevertheless, increasing fermentation temperature results in a significantly decreased resistance on the osmotic stress and ethanol toxicity of the cells [8, 22, 29, 33, 36]. Previous studies signified *TSL1* gene involved in the synthesis of the regulatory subunit for trehalose synthase complex, which participates in the tolerance to

**Table 2** Extracellular parameters of Z-06 and Z-061 at 12 and 15 h in the medium under 38 °C

| Parameter | Time (h) | Z-06 | Z-061 |
|---|---|---|---|
| Glucose[a] | 12 | 138.67 ± 0.53 | 69.84 ± 0.55 |
| 180.00[b] | 15 | 100.19 ± 0.65 | 23.83 ± 0.28 |
| Pyruvic acid[a] | 12 | 2.48 ± 0.02 | 1.84 ± 0.01 |
| 88.06[b] | 15 | 3.13 ± 0.02 | 2.26 ± 0.01 |
| Ethanol[a] | 12 | 34.17 ± 0.43 | 65.63 ± 0.97 |
| 46.07[b] | 15 | 43.15 ± 0.86 | 86.78 ± 1.18 |
| Acetic acid[a] | 12 | 4.31 ± 0.05 | 2.48 ± 0.05 |
| 60.05[b] | 15 | 5.45 ± 0.05 | 3.12 ± 0.07 |
| Glycerol[a] | 12 | 7.24 ± 0.17 | 5.13 ± 0.12 |
| 92.09 | 15 | 8.96 ± 0.15 | 6.71 ± 0.18 |
| pH[c] | 15 | 4.16 ± 0.10 | 4.52 ± 0.10 |

After incubation for 12 and 15 h, 20 ml of culture was removed from the fermentation vessel, frozen immediately in liquid nitrogen for 60 s, filtrated on cheese-cloth, and centrifuged at 5,000×g for 15 min at 4 °C to get a clear supernatant as described under Materials and Methods. Extracellular metabolite concentrations are expressed as the amount present in 1 ml of incubation mix just before quenching. Measurements are averages of three supernatant. The standard deviations were lower than 10 % of the values

[a] Concentrations of the metabolites expressed in g/L

[b] Molecular weight of the metabolites

[c] pH of the liquid culture at 15 h of the fermentation

**Table 3** Extracellular metabolites accumulating/consuming rate of Z-061 and Z-06 at 15 h under 38 °C

| Metabolites* | Z-06 | Z-061 | Enhancement (%) |
|---|---|---|---|
| Glucose | 71.25 | 85.21 | 19.59 |
| Pyruvic acid | 2.46 | 1.58 | −35.77 |
| Ethanol | 64.97 | 153.03 | 135.54 |
| Acetic acid | 6.32 | 3.55 | −43.83 |
| Glycerol | 6.22 | 5.72 | −8.04 |

Values of extracellular metabolite concentrations between Z-061 and Z-06 differ significantly ($\alpha = 0.05$)

* Glucose expressed as consuming rate in mmol/L/h, and others expressed as accumulating rate in mmol/L/h

**Table 4** Extracellular metabolites distribution of strain Z-06 and Z-061 at 15 h under 38 °C

| Metabolites* | Z-06 | Z-061 | Enhancement (%) |
|---|---|---|---|
| Glucose | 100.00 | 100.00 | – |
| Pyruvic acid | 1.69 | 0.91 | −46.15 |
| Ethanol | 26.05 | 45.96 | 76.43 |
| Acetic acid | 2.96 | 1.39 | −53.04 |
| Glycerol | 4.46 | 3.43 | −23.09 |

* Calculation by regarding the glucose as 100

various environmental stresses occurs in the *S. cerevisiae* [2–4]. However, little research has been done investigating the relations between the *TSL1* gene, stress tolerance, and the carbon metabolic flux of the yeast.

This study clearly demonstrated that the ethanol production capability of the yeast decreased with high osmotic stress caused by NaCl in fermentation broth (Fig. 1b). In order to alleviate the osmotic stress inhibition in ethanol fermentations, multiple metabolic and process engineering techniques have been developed. These process engineering strategies are mainly involved in the substrate fed-batch and situ product removal systems. However, compared to producing the osmotic-resistant mutants, the application of substrate fed-batch and situ product removal processes are of higher complexity and cost, and also require detailed process knowledge of the application. Therefore, utilizing osmotic-resistant mutants at high temperatures is desired within the industry, and has been proven to be successful in biotechnological production, such as for amino acids and organic acids.

With the target of increasing the stress tolerance and glycolysis flux, the *TSL1* gene in *S. cerevisiae* Z-06 was overexpressed in this investigation. As a result, TPS activity of the yeast increased nearly fourfold higher than that of parent strain, and the trehalose concentration was enhanced from 0.151 g/gDCW to 0.461 g/gDCW. The carbon metabolic flux analysis in Tables 2, 3 and 4 signify that as the byproducts such as pyruvate, acetic acid and glycerol were decreased, the media pH value was increased from 4.16 to 4.52, which alleviated the acid toxicity of the culture. As a consequence, the carbon flux of mutant Z-061 was enhanced significantly, and 185.5 g/L ethanol accumulated at 38 °C, with the maximum productivity of 5.2 g/L/h. These results were consistent with a very recent publication which indicated that the *TSL1* gene is involved in not only trehalose synthesis but also in the glucose regulatory system; nevertheless, all changes in perceived glucose levels ultimately lead to a shift in trehalose biosynthesis [1].

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