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# gTME for Improved Xylose Fermentation of *Saccharomyces cerevisiae*

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**Abstract** Global transcription machinery engineering (gTME) is an approach for reprogramming gene transcription to elicit cellular phenotypes important for technological applications. In our study, the application of gTME to *Saccharomyces cerevisiae* was to improve xylose utilization and tolerance, which is a key trait for many biofuel programs. Mutation of the transcription factor *spt15* was introduced by error-prone polymerase chain reaction and then screened on media using xylose as the sole carbon source. The selected out strain spt15-25 showed modest growth rates in the media containing 50, 100, and 150 g/L of xylose or glucose. Under the following fermentation condition: 30 °C, rotating speed of 200 r/min, 500-mL Erlenmeyer flask containing 100-mL media, after 109 h, 93.5% of xylose was consumed in 50 g/L xylose medium. Meanwhile, 98.3% glucose can be metabolized in 50-g/L glucose medium. And the carbon source was 50 g/L glucose–xylose ($w/w=1$); the utilization ratio of xylose and glucose was 90.8% and 97.3%, respectively. And all the xylitol concentration was below 2.48 g/L.

**Keywords** gTME · Xylose uptake · *Saccharomyces cerevisiae*

## Introduction

Considerable efforts have been focused on ethanol production in the past decades from plant biomass for usage in gasoline blends to reduce petroleum consumption and air pollution. However, the worldwide shortage of grain has made ethanol production unsustainable, which was traditionally produced from grain. Lignocellulose is considered to be the most abundant biomass resources for their huge quantities and low cost, which constituted 50% weight of all the biomass resources. The monosaccharides in the hydrolysate of lignocellulose were glucose, xylose, mannose, galactose, and arabinose [1], of which two thirds were hexoses and one third were pentoses [2]. The output of

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ethanol will be increased by 25% if pentoses, especially xylose, were fully utilized to produce ethanol.

*Saccharomyces cerevisiae* is a generally recognized as safe microorganism, having the high ethanol and toxical factors tolerance in the hydrolyzate of lignocellulose [3], which is the first eukaryotic organism with completely determined genome sequence [4]. It has been wildly used in ethanol production. Unfortunately, native *S. cerevisiae* is unable to efficiently utilize xylose as sole carbon source and cannot ferment xylose to ethanol despite having a full xylose metabolic pathway [5]. We expect to reconstitute its metabolic network with metabolic engineering and develop a new technique to obtain desirable phenotype.

In the past few years, two approaches have been used to construct recombinant strains which are able to efficiently ferment both pentoses and hexoses to ethanol. Firstly, *S. cerevisiae* cells have been conjugated with other yeast cells which could utilize xylose and the fusion cells with the ability to utilize xylose have been selected out [6]; secondly, plasmids carrying xylose exogenous gene have been transformed into *S. cerevisiae* with gene engineering methods; consequently, the recombinant strains have the ability to ferment mixed sugars to ethanol. Multiple genetic modifications are often required to unlock the desirable cellular phenotypes. However, most current cellular and metabolic engineering approaches exclusively rely on the deletion or overexpression of single gene due to experimental limitations in vector construction, transformation efficiency, and screening capacity [7]. These limitations preclude the simultaneous exploration of multiple gene modifications and confine gene modification searches to restricted sequential approaches that often have difficulties reaching a global phenotype optimum due to the complexity of metabolic landscapes [8, 9].

Global transcription machinery engineering (gTME) is an approach for reprogramming gene transcription to elicit cellular phenotypes, which change the whole transcriptional control process and improve the transcription and expression of target genes [10]. The gTME has already been demonstrated by engineering TFIID (TFIID is a multi-component (>5 subunits) transcription factor that recognizes and binds to the promoter DNA) in *S. cerevisiae* to reprogram the transcription. TFIID consists of TATA-binding protein and 14 other associated factors [7, 11]. Transcription factor spt15 in transcription initiation complex is a TATA-binding protein, which can transcript most of the mRNA gene in the genome when binding to RNA Pol II and more than ten kinds of general transcription factors. Mutations in the spt15 lead to overexpression of related genes and improve the tolerance of *S. cerevisiae* to ethanol [11].

In this report, we cloned and mutated global transcription factor spt15 with gTME method and constructed a spt15 mutant library. Mutant gene was linked to the expression vector pYX212, which contained strong promoter TPI, and transformed into the xylose unavailable *S. cerevisiae* YPH499 using lithium acetate transformation method and screened in the specified media. After the preliminary screening, the obtained recombinant *S. cerevisiae* spt15-25 could efficiently utilize xylose and co-ferment xylose and glucose. Regarding these good qualities, the construction of this recombinant stain provided favorable basis for further investigation.

## Materials and Methods

### Strains and Vectors

*S. cerevisiae* YPH499 (Stratagene, USA) was able to grow on glucose but not on xylose.

The expression vector used for the *spt15* was pYX212 (Jiangnan University, Wuxi, China).

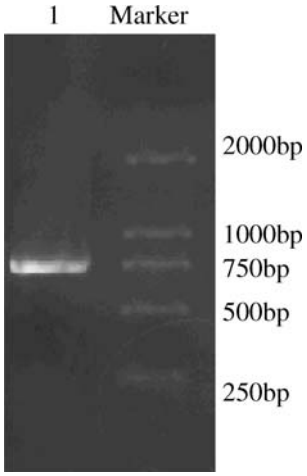


**Fig. 1** PCR product of *spt15* gene. *Lane 1* was PCR product and *lane Marker* was DNA Marker DL2000

Media and Culture Conditions

Add T at blunt end [12] of pYX212, subsequently ligated to *spt15* then transformed into YPH499 by lithium acetate transformation [13]. Plasmid-contained yeast strains were grown on YPAD media supplemented with an appropriate carbon source. Yeast extract–peptone medium (10 g/L yeast extract and 20 g/L peptone; 0.075 g/L adenine sulfate). Carbon sources used for YPH499-derived yeast spt15-25 were 50, 100, and 150 g/L xylose and glucose also with the mixture of the two sugars ($w/w=1$).

Precultures for liquid culturing were prepared as follows: a few colonies from pure cultures on plates were inoculated into 5 mL of YPAD medium supplemented with 20 g/L xylose and grew over one to two nights at 30 °C, 200 r/min; 30-mL precultures were inoculated from the previous preculture using the same medium and grown similarly. The 30-mL precultures were centrifuged, washed with distilled water, and used as inocula. The initial OD of the actual cultures (optical density at 600 nm; $OD_{600}$) was usually 0.5.

Culture conditions for the fermentation experiments were as follows: the actual cultures were inoculated without washing of the precultured cells to $OD_{600}$ of 0.2–0.5 from the above-mentioned 30-mL precultures inoculated to flasks of 100-mL YPX media with 50 g/L

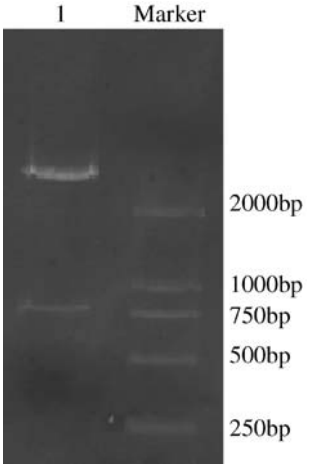


**Fig. 2** *Nco*I and *Not*I digest of pGM-T-spt15. *Lane 1* was pGM-T-spt15/*Nco*I + *Not*I and *lane Marker* was DNA Marker DL2000

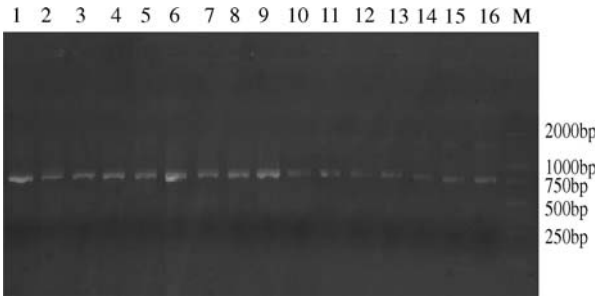


**Fig. 3** Error-prone PCR of different spt15 concentrations. *Lanes 1–16* were error-prone PCR product of different spt15 concentrations and *lane M* was DNA Marker DL2000

xylose and glucose also with the mixture of the two sugars (*w*/*w*=1). Culture condition was 30 °C, 200 r/min for 72 h. The YPH499 control strain was grown similarly on YPAD media containing 50 g/L of glucose.

Assay Techniques

Sugars and xylitol in culture supernatants were analyzed with Dionex high-performance liquid chromatography (HPLC) system consisting of an UltiMate 3000 pump, an UltiMate 3000 autosampler, an UltiMate 3000 column compartment, and a Lichrospher Nh2 column (4.6×250 nm; Hanbang Technology Ltd. Jiangsu, China), maintained at 30 °C (equilibrated and samples eluted with acetonitrile and purified water with the volume ratio of 80:20, at the flow rate of 1.0 mL/min), a Shodex 101 refractive index (RI) detector. Ethanol concentrations were determined by enzymatic assay kit (Huili Biotech Co. Ltd, Changchun, China).

## Results and Discussion

The Clone of *spt15* and Gene Library Construction of Error-Prone PCR Products [14]

The template was isolated from BY4742 yeast using the TIANamp Yeast DNA Kit (TianGen Biotech, Beijing, China). Amplification was performed with the template using Taq polymerase (Shenergy Biocolor, Shanghai, China) using the following primers:

SPT15-Sense: TCGAGTGCTAGCAAAATGGCCGATGAGGAACGTTTAAAGG
SPT15_Anti: CTAGCGGTCGACTCACATTTTTCTAAATTCACTTAGCACA

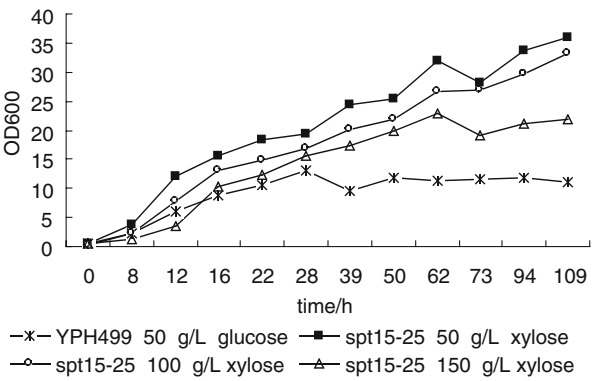


**Fig. 4** The growth curve of YPH499 and spt15-25 in the media with different xylose concentrations

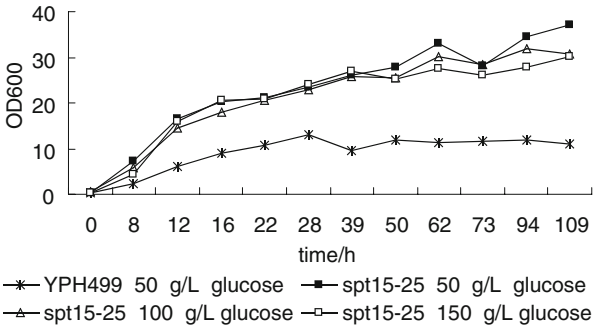


**Fig. 5** The growth curve of YPH499 and spt15-25 in the media with different glucose concentrations

The PCR products had an obvious fragment about 0.75 kb in the electrophoretic lane (Fig. 1). The nonspecific amplification was not obvious.

PCR products were purified and then ligated overnight at 16 °C to the vector pGM-T. The plasmids were transformed into *Escherichia coli* DH5α. The blue–white selection was performed to screen the positive transformants. The purified plasmids were digested with *Nco*I and *Nol*I. The digested fragments were determined by electrophoresis, which were identical to the PCR products (Fig. 2). The plasmid sequence was in accordance with the *spt15*. And the homology was 100% similar to that of the protein and the sequence in the gene library.

The error-prone PCR was carried out under the template of *spt15* using GenemorphII Random Mutagenesis kit. The error-prone PCR products were identical to the PCR products determined by electrophoresis (Fig. 3).

## Construction and Selection of the Recombinant Yeast Expression Vector pYX212-spt15

The recombinant plasmid pGM-spt15 was amplified and then purified by agarose gel electrophoresis with Takara Agarose Gel DNA Purification Kit Ver. 2.0. *Spt15* was ligated to pYX212 to constitute the recombinant vector pYX212-spt15 with T4DNA ligase. PYX212-spt15 was then transformed into *S. cerevisiae* YPH499, which could not utilize

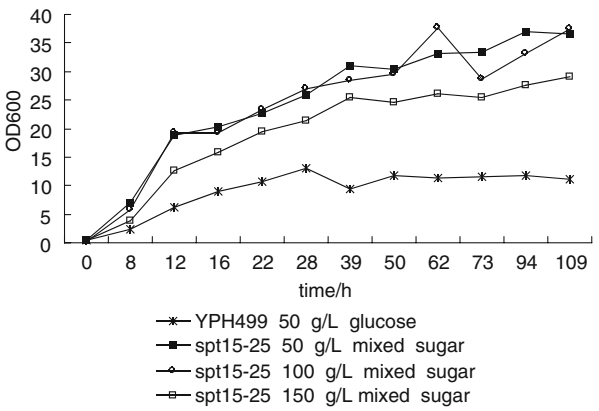


**Fig. 6** The growth curve of YPH499 and spt15-25 in the media with different concentrations of mixed sugars

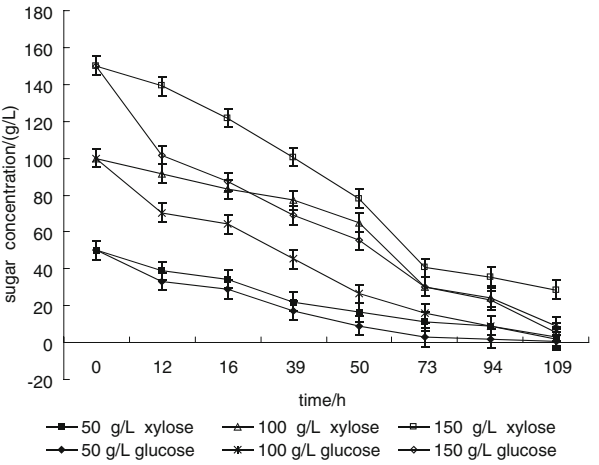


**Fig. 7** Utilization of xylose and glucose in the media of different sugar concentrations by spt15-25

the xylose. Subsequently, the strain was plated on the SD medium consisting of YNB (without amino acids), amino acids (without uracil), and xylose. The combinant yeast spt15-25 was isolated from the plate containing xylose as the sole carbon source.

## Analysis of the Fermentation Products by spt15-25

### *Growth Curve of spt15-25 by Different Concentrations of Sugar*

Comparing with the parent strain of YPH499, spt15-25 had higher growth rate in the medium with varying xylose and glucose concentrations of 50, 100, and 150 g/L and the mixture of the two sugars (*w*/*w*=1). The growth rates of the recombinants spt15-25 with varying carbon sources were shown in Figs. 4, 5, and 6. With the sugar concentration increasing, the value of $OD_{600}$ was decreased, which reveals the substrate inhibition for growth rate. (Figs. 4, 5, and 6).

### *Effect of the Growth of spt15-25 by Different Concentration of Xylose and Glucose*

Low inoculum cultures were started using an overnight culture of yeast at an $OD_{600}$ of 0.1 in 50 mL of medium containing 20 g/L of glucose. High inoculum cultures were created by growing 250 mL of yeast in a 1,000-mL flask for 2 days and then collected by centrifugation at 500×*g* for 25 min. The cell pellet was then resuspended in 3 mL of YPD without glucose. This solution was then appropriately inoculated into 100 mL of the medium in a 500-mL flask to obtain a starting $OD_{600}$ of around 0.5. The carbon source of the culture was as follows: 50, 100, and 150 g/L of xylose, 50, 100, and 150 g/L of glucose, or the mixture of the two (*w*/*w*=1). Fermentations were run at 30 °C, 200 r/min. Samples were taken and supernatant analysis was conducted to measure sugar concentrations. After 109 h, sugar utilization rate of the supernatant were as follows: 93.5% (50 g/L xylose), 91.1% (100 g/L xylose), 80.9% (150 g/L xylose), 98.3% (50 g/L glucose), 97.9% (100 g/L glucose), 96.4% (150 g/L glucose), 90.8% (xylose in the mixture sugar of 25 g/L), 97.3%[1]

[1] This refers to the utilization rate of 25 g/L glucose in mixed sugar after 50 h which was 97.3% in the paper.

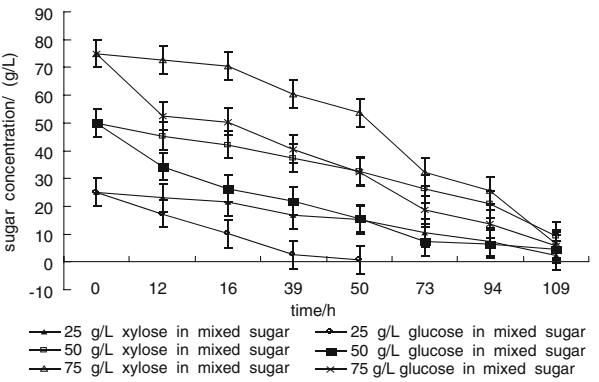


**Fig. 8** Utilization of xylose and glucose in the media of different mixed sugar concentrations by spt15-25

(glucose in the mixture sugar of 25 g/L), 81.2% (xylose in the mixture sugar of 50 g/L), 91.1% (glucose in the mixture sugar of 50 g/L), 91.6% (xylose in the mixture sugar of 150 and 75 g/L), 92.4% (glucose in the mixture sugar of 75 g/L). The utilization rates of xylose and glucose were beyond 80% (see Figs. 7 and 8).

*The Concentration of Xylitol by spt15-25*

The xylitol concentration in the supernatant was detected by HPLC mentioned in assay techniques. In the results shown in Table 1, the maximum xylitol concentration was 2.84 g/L after 109 h in the medium contained 100 g/L xylose. The xylitol concentration in the xylose media was more than in the glucose and the mixture of the two sugars.

*The Concentration of Ethanol by spt15-25 Anaerobic Fermentation*

The parent strain YPH499 and the recombinant strain spt15-25 were grown in the medium containing 50 g/L glucose, xylose, and the mixture of the two at 30 °C, 200 r/min for 96 h.

**Table 1** Xylitol yield of the spt15-25.

| Time/h | Xylitol (g/L) | | | | | |
|---|---|---|---|---|---|---|
| | 16 | 39 | 50 | 73 | 94 | 109 |
| 50 g/L xylose | a[a] | a[a] | a[a] | a[a] | 0.82 | 1.315 |
| 100 g/L xylose | a[a] | a[a] | a[a] | a[a] | 2.23 | 2.84 |
| 150 g/L xylose | a[a] | a[a] | a[a] | 0.74 | 1.73 | 2.69 |
| 50 g/L glucose | a[a] | a[a] | 0.8 | 1.39 | 1.79 | 1.91 |
| 100 g/L glucose | a[a] | a[a] | 0.93 | 1.45 | 1.95 | 2.48 |
| 150 g/L glucose | a[a] | a[a] | a[a] | a[a] | 1.57 | 2.28 |
| 50 g/L mixed sugar | a[a] | a[a] | a[a] | a[a] | 0.81 | 1.56 |
| 100 g/L mixed sugar | a[a] | a[a] | a[a] | a[a] | 0.99 | 1.73 |
| 150 g/L mixed sugar | a[a] | a[a] | a[a] | 0.83 | 1.62 | 2.37 |

[a] Refer to the xylitol concentration that was lower than the RI detector minimum of 0.7 g/L

**Table 2** Comparison of the ethanol yield with the recombinant yeast and control yeast.

| Strain | Carbon source/(g/L) | Ethanol mass concentration (g/L) | Ethanol yield (%) |
|---|---|---|---|
| YPH499 | 50 (glucose) | 21.63 | 43.26 |
| SPT15-25 | 50 (glucose) | 16.9 | 33.8 |
| SPT15-25 | 50 (xylose) | 15.3 | 30.6 |
| SPT15-25 | 50 (mixed sugars) | 14.6 | 29.2 |

The results (Table 2) showed that spt15-25 was able to convert xylose to ethanol efficiently and the concentration of ethanol produced by the recombinant in the culture was higher than that of the glucose or mixed sugar. But the concentration of ethanol was lower than YPH 499. This was not in accordance with others; this also revealed that the pathways of this strain were different from others and need to be highly investigated.

Gene expression regulation plays a part in all levels in the course of the genetic information transmission, whereas the transcriptional regulation is one of the most important parts in the gene expression regulation and it is also the focus nowadays. Recently, a tool termed gTME was created that enables broad perturbations of the whole transcription through the modification of the proteins responsible for orchestrating transcription. This methodology permits changes in the expression of many distal genes, and it can easily be linked back to a single transcriptional protein. It had been reported that the MIT research group used gTME to engineer the transcription factor of *spt15* then transformed into *S. cerevisiae*. In doing so, several proof-of-concept studies have been obtained, including increased cellular tolerances (including increased ethanol and glucose tolerance) and the rate and efficiency of the conversion glucose to ethanol.

In this article, the tool of gTME outperformed traditional approaches by much more quickly and effectively optimizing phenotypes. We demonstrate that the components of global cellular transcription machinery (spt15) can be engineered to allow global perturbations of the transcription, which can help unlock complex phenotypes.

These results showed the applicability of gTME to alter cellular eukaryotic phenotypes. The isolation of dominant mutations permits the modification of vital functions for novel tasks, whereas the unmodified allele carries out the functions critical for viability. An examination of further modifications of other transcription factors through gTME could additionally have the potential for drastically improving xylose fermentation and the prospects of ethanol production. For the mutants analyzed, altered fermentation conditions and additional pathway engineering are likely to increase xylose utilization. Finally, the mechanism of gTME and how to engineer the *S. cerevisiae* to utilize xylose are still unknown.

Application of gTME to *S. cerevisiae* for improved xylose fermentation is feasible theoretically. In practice, ethanol production of the recombinant strain through co-fermentation of glucose and xylose was improved under anaerobic condition in this paper, which gives the supplementary for the theory of biotransformation of biomass to produce ethanol. However, a lot of work needs to be done to realize commercialization of the recombinant strain to produce ethanol. Such as improving the tolerance of depressor during fermentation, cutting down the production cost, and so on; this will be meaningful for biotransformation of vegetable fiber to ethanol.

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