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# Simultaneous fermentation of glucose and xylose at elevated temperatures co-produces ethanol and xylitol through overexpression of a xylose-specific transporter in engineered *Kluyveromyces marxianus*

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## HIGHLIGHTS

- A glucose–xylose simultaneous co-fermentation *K. marxianus* strain was constructed.
- Higher value product xylitol was produced from xylose instead of ethanol.
- More than 98% yield of xylitol was achieved through co-fermentation.
- Strain YZJ119 efficiently produced xylitol & ethanol at 42 °C through corncob SSCF.

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## ABSTRACT

Engineered *Kluyveromyces marxianus* strains were constructed through over-expression of various transporters for simultaneous co-fermentation of glucose and xylose. The glucose was converted into ethanol, whereas xylose was converted into xylitol which has higher value than ethanol. Over-expressing xylose-specific transporter *Sc*GAL2-N376F mutant enabled yeast to co-ferment glucose and xylose and the co-fermentation ability was obviously improved through increasing *Sc*GAL2-N376F expression. The production of glycerol was blocked and acetate production was reduced by disrupting gene *KmGPD1*. The obtained *K. marxianus* YZJ119 utilized 120 g/L glucose and 60 g/L xylose simultaneously and produced 50.10 g/L ethanol and 55.88 g/L xylitol at 42 °C. The yield of xylitol from consumed xylose was over 98% (0.99 g/g). Through simultaneous saccharification and co-fermentation at 42 °C, YZJ119 produced a maximal concentration of 44.58 g/L ethanol and 32.03 g/L xylitol or 29.82 g/L ethanol and 31.72 g/L xylitol, respectively, from detoxified or non-detoxified diluted acid pretreated corncob.



## 1. Introduction

A major issue in the conversion of saccharified cellulosic biomass into biofuel is the utilization of D-xylose, one of the primary hydrolysis products of lignocellulosic biomass and the second most abundant fermentable material. Thus, microbial strains that can utilize glucose and xylose simultaneously and efficiently are crucial for exploitation of lignocellulosic biomass as feedstock (Hu et al., 2011). Contrary to the efficient glucose fermentation in yeast, D-xylose fermentation is challenging, as very few ethanol-producing microorganisms can readily ferment xylose despite several microorganisms possessing the ability to utilize xylose as a carbon source (Nduko et al., 2013; Zhang et al., 2013). Carbon catabolite repression was bypassed by constructing a cellobiose transporter pathway in *Saccharomyces cerevisiae* (Oh et al., 2013); however, the main components of cellulase digested cellulose and hemicellulose hydrolyzate are glucose and xylose, which can still lead to catabolite repression. A few native microorganisms, such as the oleaginous yeast *Trichosporon cutaneum* AS 2.571 can simultaneously consume glucose and xylose (Hu et al., 2011). This strain showed no preference between glucose and xylose, likely due to the similar efficiency of specific transporters for both sugars (Hu et al., 2011). However, the underlying mechanism of

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simultaneous assimilation of glucose and xylose by *T. cutaneum* awaits further study.

Converting xylose into xylitol is a more economical strategy. The low efficiency of xylose fermentation is also a limitation in bioethanol production. A lot of works were tried to convert xylose into ethanol and avoid the byproduct xylitol. However, xylitol is also a very important chemical which has higher value than ethanol and is widely used as food additive (Guo et al., 2013; Li et al., 2013). Moreover, as a valuable synthetic building block, xylitol is also classified as one of the most promising chemicals among the 12 bio-based chemicals by the US Department of Energy and thereby serves as a key economic driver of the biorefinery concept (Pal et al., 2013; Prakash et al., 2011; Zhang et al., 2014; Zhang et al., 2015a). To convert glucose into ethanol and xylose into xylitol may be more economical in the lignocellulosical biofuel production.

For bioethanol production using lignocellulose, the optimal temperature of cellulase used in biomass saccharification is 45–50 °C, and hence a higher temperature is preferred in the biomass simultaneous saccharification and fermentation (SSF), as well as simultaneous saccharification and co-fermentation (SSCF) (Alvira et al., 2010; Hickert et al., 2013; Suriyachai et al., 2013). Moreover, thermophilic and thermotolerant ethanologens have certain advantages over mesophiles, such as solvent tolerance, saving energy through reduced cooling cost, higher saccharification and fermentation rates, easier stripping of ethanol from the broth, and minimal risk of contamination (Zeng et al., 2013). *Kluyveromyces marxianus*, a 'generally regarded as safe' (GRAS) microorganism, has attracted increased attention because of its thermotolerance, high growth rate, and a broad substrate spectrum, including xylose (Fonseca et al., 2008). Some *K. marxianus* strains can grow with a growth rate of 0.86–0.99 $h^{-1}$ at 40 °C, and can grow at temperatures as high as 52 °C (Banat and Marchant, 1995; Zhang et al., 2011). Even at 45 °C, *K. marxianus* can still produce ethanol well with glucose, while most yeasts cannot survive at this temperature (Zhang et al., 2011). Therefore, engineered *K. marxianus* would allow increased SSF or SSCF temperatures, and the high fermentation temperatures would allow more rapid and efficient enzymatic cellulose hydrolysis (Fonseca et al., 2008; Zhang et al., 2011; Zhu et al., 2014).

In this study, a *K. marxianus* strain YZJ119 was constructed that simultaneously utilizes glucose and xylose and produces ethanol and xylitol through overexpression of a xylose-specific transporter *Sc*GAL2N376F. The *KmGPD1* gene was disrupted to reduce its byproduct (Fig. 1). The fermentation ability of *K. marxianus* YZJ119 was also evaluated using detoxified or non-detoxified diluted acid pretreated corncob to produce xylitol and ethanol through SSCF at 42 °C.

## 2. Materials and methods

### 2.1. Reagents and microorganisms

All chemicals used were of analytical grade or higher. D-glucose, D-xylose and yeast nitrogen base without amino acids (YNB) were obtained from Sangon Biotech Co. (Shanghai, China). Restriction enzymes and modifying enzymes were obtained from Thermo Fisher Scientific (Thermo Fisher Scientific, West Palm Beach, Florida, USA). The yeast extract and peptone were purchased from Oxoid (Oxoid Ltd., Basingstoke, Hampshire, England). Cellulase UTE-1500 was obtained from Youtell Bio (Youtell Bio, Hunan, China). *K. marxianus* NBRC1777 was obtained from NITE Biological Resource Center (Tokyo, Japan). *K. marxianus* YHJ010 was the *TRP1*, *LEU2* and *URA3* auxotrophic strain derived from NBRC1777 (Hong et al., 2007). Synthetic dropout (SD) medium (6.7 g/L yeast nitrogen base without amino acids, 20 g/L glucose) supplemented with appropriate amino acids was used to select the transformants. Yeast extract/peptone-dextrose (YPD) medium (10 g/L yeast extract, 20 g/L peptone and 20 g/L glucose) was used to aerobically culture *K. marxianus* strains. Fermentation and xylose assimilation ability was determined in YPX medium (10 g/L yeast extract, 20 g/L bacterial peptone and 20 g/L D-xylose). To prepare solid plates of each medium, 15 g/L agar was added. *Escherichia coli* XL10-Gold was used for cloning, and it was grown in a lysogeny broth (LB) medium.

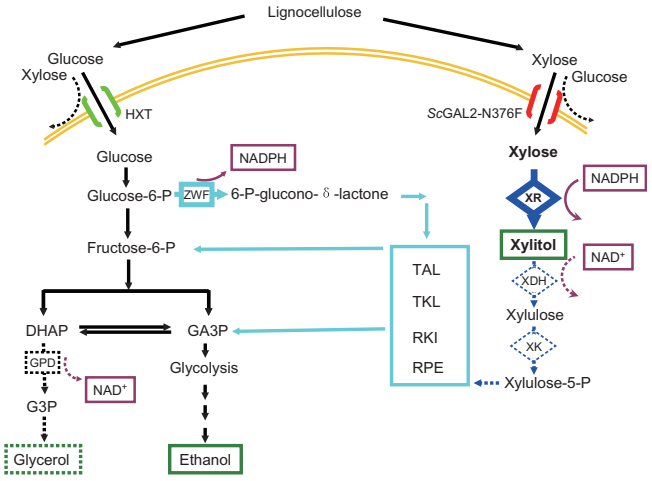


**Fig. 1.** Schematic presentation of the glucose and xylose metabolic pathway in *K. marxianus*. Boxes with dashed lines indicate the pathways that were blocked. *Abbreviations:* XR, xylose reductase; XDH, xylitol dehydrogenase; XK, xylulokinase; TKL transketolase; TAL, transaldolase; RKI, ribose-5-phosphate isomerase; RPE, L-ribulose-5-phosphate 4-epimerase; HXK, hexokinase.

### 2.2. Plasmids and strains

The plasmids used in this study are summarized in Fig. S1 and Table S2. The plasmid constructions are described in detail in the supplementary material. Briefly, pZJ041 was constructed for the expression of *K. marxianus* glucose 6-phosphate dehydrogenase gene (*KmZWF1*, GenBank: BAO41285.1); pZJ050 was constructed for the expression of *S. cerevisiae* galactose permease gene (*ScGAL2*) which was amplified from the genomic DNA of strain W303-1A (ATCC 208352); pZJ061 or pZJ063 was constructed for the expression of the xylose specific transporter mutant *Sc*GAL2-N376F which was discovered by Farwick et al. (2014) with the selection marker of *URA3* or *Zeocin*$^R$; pZJ066 was constructed for the disruption of hexose transporter 2 gene (*KmHXT2*).

The above plasmids were linearized and transformed into *K. marxianus* by the lithium acetate method (Abdel-Banat et al., 2010). To express heterogeneous genes in YZJ015, a *ScURA3* disruption cassette was amplified from pMD18T-Δ*ScURA3* (Zhang et al., 2015a) and transformed to disrupt *ScURA3* in YZJ015 (Zhang et al., 2014). The *URA3* disrupted strain was selected on an SD plate containing uracil and 0.1% 5′-fluoro-orotic acid (5′-FOA) and named YZJ037 (Table S3). Plasmids pZJ023, pZJ039, pZJ040 (Zhang et al., 2015a), pZJ050 and pZJ061 were digested with *Sma*I and transformed into strain YZJ037 to obtain strains YZJ041, YZJ072, YZJ073, YZJ106 and YZJ103, respectively (Table S3). These strains harbored different sugar transporters and were used to evaluate their function in the improvement of glucose and xylose co-fermentation. The yeast transformants were isolated on SD supplemented with suitable amino acids and validated by a polymerase chain reaction (PCR). After linearized by *Pme*I digestion, plasmid pZJ063 was transformed into YZJ103 and selected on the

plate contains 400 μg/ml *Zeocin* to obtain YZJ109, which overexpressed another copy of *ScGAL2-N376F* (Table S3). The *URA3* gene in YZJ109 was disrupted again as described above to recycle the selection marker and obtain the strain YZJ114 (Table S3). The *KmGPD1* (GenBank: BAP69368.1) gene in YZJ114 was then disrupted. The construction of disruption cassettes is described in the supplementary material. The gene fragments whose ORFs were partially substituted by the *ScURA3* expression cassette were amplified and transformed into *K. marxianus* YZJ114. Additionally, gene disruption was confirmed by PCR with the genomic DNA of the obtained strains as a template. The obtained strains were named YZJ115 (Table S3). The *ScURA3* of YZJ115 was disrupted again to obtain strain YZJ116 (Table S3). Plasmid pZJ041 was linearized by *Sma*I digestion and transformed into strain YZJ116 to obtain strain YZJ117 (Table S3), which overexpressed the *KmZWF1* gene for NADPH regeneration. Plasmid pZJ061 was linearized by *Sma*I digestion and transformed into strain YZJ116 to obtain strain YZJ118 (Table S3), which overexpressed another copy of *ScGAL2*-N376F. Plasmid pZJ063 was linearized by *Pme*I digestion and transformed into YZJ118 and selected on the plate contains 1000 μg/ml *Zeocin* to obtain strain YZJ119 (Table S3), which overexpressed more copy of *ScGAL2*-N376F. The *ScURA3* of YZJ119 was disrupted to obtain strain YZJ120 (Table S3). This was followed by disruption of gene *KmHXT2* (GenBank: BAP72475.1) of strain YZJ120 to reduce glucose transformation, and the obtained strain was named YZJ121 (Table S3). From strains YZJ103, YZJ109, YZJ118, and YZJ119, the copy number of the gene*ScGAL2*-N376F was increased from 1 to about 6.

### 2.3. Comparison of the functions of various sugar transporters in glucose and xylose co-fermentation

Strains YZJ015, YZJ041, YZJ072, YZJ073, YZJ106 and YZJ103 harboring different sugar transporters (FPS1 (GenBank: AY541009.1), *Ci*GXF1 (GenBank: AJ937350.1), *Ci*GXS1 (GenBank: AJ875406.1), *Sc*GAL1 (GenBank: NP_013182.1) and *Sc*GAL2-N376F) which were reported that promoted the xylose utilization in yeast (Farwick et al., 2014; Leandro et al., 2006; Wei et al., 2013; Zhang et al., 2015a,b) were fermented with a glucose–xylose mixture to compare the effects of the transporter on the glucose–xylose co-utilization ability. After the recombinant yeast strains were precultivated aerobically with YPD medium overnight at 37 °C, the cells were recovered through centrifugation at 5000*g* for 5 min. The yeast cells were then transferred into 50 mL fermentation bottles with 30 mL of YP medium (10 g/L yeast extract, 20 g/L peptone) containing a mixture of 80 g/L glucose and 40 g/L xylose. Unless indicated otherwise, the cell density in the fermentation medium was adjusted to an initial $OD_{600}$ of 1, and the fermentations were performed with 250 rpm shaking at 42 °C under oxygen-limited conditions.

### 2.4. Evaluate the glucose–xylose co-fermentation ability with further engineered K. marxianus

*K. marxianus* YZJ103 and its further engineered strains, including YZJ109, YZJ115, YZJ117, YZJ118, YZJ119 and YZJ121, were fermented with the YP medium containing 80 g/L glucose and 40 g/L xylose.

### 2.5. Co-fermentation with glucose and xylose in various ratios and at different temperatures by YZJ119

As the ratios of glucose to xylose in lignocellulose hydrolyzates range from 1:1 to 2:1, the ratios 1:1, 4:3, 3:2, and 2:1 of glucose/xylose were used in the evaluation of co-fermentation. Mixtures of 80 g/L glucose and 80 g/L xylose, 80 g/L glucose and 60 g/L xylose, 60 g/L glucose and 40 g/L xylose, 80 g/L glucose and 40 g/L xylose, 100 g/L glucose and 50 g/L xylose, 120 g/L glucose and 60 g/L xylose, and 160 g/L glucose and 80 g/L xylose were used for co-fermentation. A mixture of 40 g/L glucose and 40 g/L xylose was used for co-fermentation as lower sugar concentrations to avoid the effect of the sugar tolerance. To evaluate co-fermentation under various temperatures, 80 g/L glucose and 40 g/L xylose were co-fermented by YZJ119 at 37 °C, 42 °C, and 45 °C.

### 2.6. Real-time PCR analysis

The relative expression levels of *ScGAL2*-N376F in different strains were determined using real-time PCR (RT-PCR). Strains YZJ103, YZJ109, YZJ118, YZJ119 and YZJ120 were cultivated in YP medium contained 80 g/L glucose and 40 g/L xylose for 8 h with initial $OD_{600}$ = 1 under oxygen-limited conditions at 42 °C, and the total RNA was isolated using a yeast total RNA extraction kit (Sangon Biotech Co. Shanghai, China). The isolated RNA was then treated with RNase-free DNaseI (Toyobo, Japan) at 37 °C for 15 min to remove potentially contaminated genomic DNA. cDNA was synthesized by ReverTra Ace qPCR RT Master Mix kit (Toyobo, Osaka, Japan). The reverse transcription reaction was performed in an Arktik thermal cycler (Thermo Fisher Scientific, West Palm Beach, FL) at 37 °C for 15 min, 50 °C for 5 min and denatured at 98 °C for 5 min. The synthesized cDNA was quantitatively determined using a Nanodrop 2000 (Thermo Fisher Scientific, West Palm Beach, Florida, USA). RT-PCR was conducted on a LightCycler 480 (Roche Diagnostics Ltd., Forrenstrasse CH-6343 Rotkreuz, Switzerland) with a THUNDERBIRD SYBR qPCR mix kit (Toyobo, Osaka, Japan). Gene *KmACT1* for actin was used as an internal control. The primers for the RT-PCR are described in Supplementary Table S1.

### 2.7. Preparation of corncob hemicellulose hydrolysate

The corncob hydrolysate was prepared as previously reported (Cheng et al., 2014). Acid hydrolysis of corncobs was carried out at 127 °C with dilute acid (0.5% (w/w) $H_2SO_4$ + 1.5% (w/w) $H_3PO_4$) for 1 h using a solid:liquid (quantity:volume) ratio of 1:3. The corncob hydrolysate with or without detoxification was used in fermentation. To prepare the non-detoxified material, the corncob residue (CCR) and hydrolysate were neutralized and used directly for fermentation. The neutralization was conducted by adding lime cream into the mixture of CCR and hydrolysate until the pH reached 6.0. Then, the CCR and hydrolysate were used for fermentation without sterilization. To prepare the detoxified material, the hydrolysate over-limed with $Ca(OH)_2$ as previous reported with some modifications (Martinez et al., 2000; Verbeke et al., 2011). Briefly, the solid CCR and liquid were separated with filtration after pretreatment, following which the acid pretreated corncob solution was heated to 42 °C, and $Ca(OH)_2$ was added until the pH reached 10.0. The alkaline solution was allowed to mix for 30 min leading to precipitation, which was removed via centrifugation at 5000*g*, and the pH of the solution was adjusted to 6.0 and re-centrifuged to remove the precipitation. The solid part (CCR) was washed to remove the acid, and dried at 60 °C. After that, the CCR and over-limed hydrolysate solution were mixed with various ratios for fermentation. After acid-pretreatment, the weight of CCR was 60.98% of the initial corncob.

### 2.8. Xylitol and ethanol co-produced from diluted acid pretreated corncob by engineered K. marxianus YZJ119 through SSCF

When fermented with non-detoxified material, the acid-hydrolyzed corncob containing CCR and hydrolysate was used after

neutralization and dilution. The original corncob was pretreated by diluted acid with a solid/liquid ratio of 1:3. The acid pretreated corncob was then diluted 3.33, 2.50, 2.00 and 1.67 folds for fermentation after neutralization by adding water. When fermented with detoxified corncob hydrolysate, the washed and dried CCR was added with final concentrations of 60, 75, 85, 120 and 150 g/L into 30 mL YP medium containing the detoxified hydrolysate. Cellulase was added as 15 FPU/g CCR. Both CCR and hydrolyzate were used without sterilization. YZJ119 was pre-cultivated aerobically with YPD medium overnight at 37 °C and inoculated into the medium with initial $OD_{600}$ = 1. The fermentations were performed with 250 rpm shaking at 42 °C under oxygen-limited conditions.

### 2.9. K. marxianus YZJ119 fermented with corncob residue and hydrolysate containing various amount of cellulase

As elevated temperatures can enhance the hydrolysis of CCR, along with the amount of cellulase, 15 FPU/g CCR, 30, 10 or 5 FPU/g CCR was also evaluated with the fermentation medium containing non-detoxified corncob hydrolysate and 60.98 g/L CCR in SSCF.

### 2.10. Analytical methods

The concentrations of D-xylose, xylitol, D-glucose, glycerol, acetic acid and ethanol were analyzed using a high-pressure liquid chromatography (HPLC) system (Agilent 1100, USA) with an ROA-Organic Acid $H^+$ (8%) column (Phenomenex, USA); 0.005 N $H_2SO_4$ was used as the mobile phase at a column temperature of 75 °C at a flow rate of 0.3 mL/min (Zhang et al., 2011). The concentrations of furfural and 5-HMF were obtained by appropriately diluting the stock solution with the mobile phase and were analyzed using a C18 column (Agilent 5 TC-C18, 250 × 4.6 mm; Agilent, Burnsville, MN, USA). The mobile phase was a mixture of water to methanol (80:20, v/v) and was delivered at a flow rate of 0.5 mL/min. The detection wavelength was 285 nm (He et al., 2014). The culture was centrifuged at 14,000*g* at 4 °C for 5 min and the supernatant was analyzed by HPLC. Culture growth was monitored at 600 nm using a spectrophotometer. At $OD_{600}$ = 1, the concentration of the cells was equivalent to 0.411 g/L dry cell weight (DCW) (Zhang et al., 2013). All results were performed in triplicate and are shown as the mean values in Figs. 2–4. The bars in the figures indicate the ranges of the standard deviation.

## 3. Results and discussions

### 3.1. Comparison of simultaneous co-fermentation of glucose and xylose by strains harboring various transporters

Overexpression of the xylose-specific transporter improved glucose and xylose co-fermentation. Lignocellulosic materials comprise 30–45% glucan and 20–35% xylan. After acid pretreatment and enzymatic hydrolysis of plant biomass, the concentrations of glucose and xylose in the hydrolyzate solution were about 70% and 30%, with glucose concentrations were about twice as that of xylose (Cheng et al., 2014; Ha et al., 2011). Thus, a mixture containing 80 g/L glucose and 40 g/L xylose was thus used to evaluate co-fermentation ability. The strains YZJ041, YZJ072, YZJ073, YZJ106 and YZJ103 correspondent to recombinant expression of transporters FPS1p, *Ci*GXF1 *Ci*GXS1 *Sc*GAL2 and *Sc*GAL2-N376F in *K. marxianus* YZJ015 were used to evaluate the effect of transporter in glucose and xylose co-fermentation. Strains YZJ041, YZJ072, YZJ073 and YZJ106 overexpressed different transporters produced similar ethanol and xylitol to YZJ015 in 12 h (Table 1 and Fig. S2). Strain YZJ103, which contained the xylose-specific *Sc*GAL2-N376F mutant (Table S3), produced xylitol (20.99 g/L) more than two times as much as that of the others (Table 1 and Fig. S2). It has stronger glucose and xylose simultaneously utilization ability than other strains during the fermentation (Fig. S2). This indicated that xylose specificity might be more important than transport rate for the conversion of xylose to xylitol in a glucose/xylose mixture.

Though the transporters *Ci*GXF1 and *Ci*GXS1 from yeast *Candida intermedia* have high xylose transport abilities (Leandro et al., 2006), and *Sc*GAL2 from *S. cerevisiae* also has xylose transport ability (Farwick et al., 2014), all of them did not improve xylitol production and the co-fermentation. As they are glucose/xylose transporters with a preference for glucose, the xylose-transport could be competitively inhibited by glucose. The N376F mutation of *Sc*GAL2 (*Sc*GAL2-N376F) was shown to be a D-xylose specific transporter without being inhibited by D-glucose (Farwick et al., 2014). YZJ103, which expressed *Sc*GAL2-N376F, produced xylitol in significantly greater amounts than other strains with similar ethanol production (Table 1 and Fig. S2). This result indicated that the *Sc*GAL2-N376F mutant could specifically transport xylose with glucose existence. Therefore, only the xylose specific transporter *Sc*GAL2-N376F could release glucose repression at the sugar transporting stage as shown in Fig. 2(B), and the co-consumption of glucose and xylose thus enables the co-enzymes and energy obtained from glucose fermentation for xylose reduction. Such regulation of the transporter system can also be used in other yeasts for glucose and xylose co-utilization.

FPS1p is a plasma membrane channel and aquaglyceroporin, which is involved in glycerol and xylitol transport by facilitated diffusion, and the deletion of *FPS1* reduced xylitol accumulation (Wei et al., 2013). Therefore, the overexpression of FPS1 was expected to improve xylitol accumulation. However, it is possible that the xylitol production ability was not strong enough, thereby no obvious increase was observed in xylitol production in the *FPS1* overexpressing strain YZJ041.

### 3.2. Determining the copy number and expression of ScGAL2-N376F by RT-PCR

Because multiple copies gene integration can be obtained through screening on high concentration *Zeocin* with *Zeocin* resistance as selection marker, multiple *ScGAL2*-N376F gene integration strains were obtained on medium containing 1000 μg/ml *Zeocin* other than the selection marker of *URA3*. The copy number of *ScGAL2*-N376F was determined by RT-PCR. The results showed that as compared toYZJ103, the abundance of *ScGAL2*-N376F increased to 1.47, 3.10 and 6.46 folds for YZJ109, YZJ118 andYZJ119, respectively. Thus, the copy number of *ScGAL2*-N376F in YZJ119 was around 6 (Fig. 3). RT-PCR was also used to confirm the heterogeneous expression of introduced genes. When fermented with a 80 g/L glucose and 40 g/L xylose mixture, the transcription levels of *ScGAL2*-N376F in YZ109, YZ118 and YZJ119 increased by 3.85, 4.09 and 6.85 folds, respectively, when compared to YZ103 (Fig. 3). The xylitol productions were 24.71, 27.94, 35.62 and 40.43 g/L, respectively. Therefore, the xylitol production was consistent with the transporter expression (Fig. 3).

### 3.3. Improving the glucose–xylose co-fermentation ability in K. marxianus with more genetic modifications

Several metabolic engineering strategies were used to further improve co-fermentation. As shown in Table 1 and Fig. S2, strain YZJ103 showed similar ethanol fermentation ability and the most effective xylitol production ability among other strains. However, after glucose consumption about 50% of xylose remained (Fig. 2B). Overexpressing another copy of *ScGAL2*-N376F, strain YZJ109 produced more xylitol than YZJ103. After 20 h, 26.74 g/L xylitol and

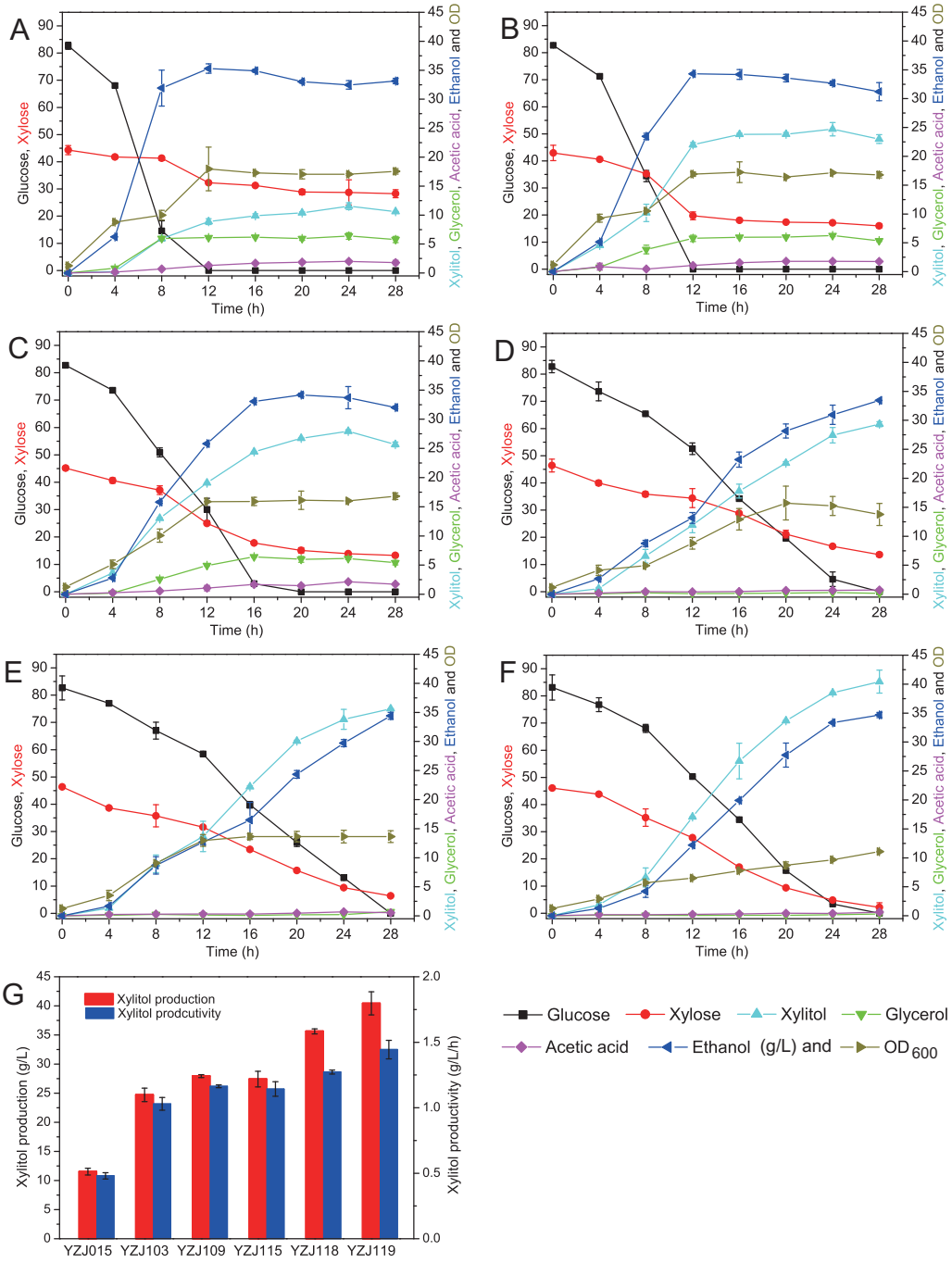


**Fig. 2.** The co-fermentation of 80 g/L glucose and 40 g/L xylose in YP medium at 42 °C under oxygen limited conditions with (A) YZJ015, (B) YZJ103, (C) YZJ109, (D) YZJ115, (E) YZJ118, (F) YZJ119. The xylitol production and productivity of each strain was also compared (G).

34.16 g/L ethanol were produced (Fig. 2C). The *KmGPD1* gene in YZJ109 was then disrupted to block the production of glycerol. The obtained strain YZJ115 produced 29.33 g/L xylitol and 34.44 g/L ethanol in 28 h. Glycerol accumulation was significantly reduced from 6.12 g/L to 0.28 g/L. Though *KmGPD1* disruption also reduced the yeast growth and productivity of xylitol and ethanol, the utilization of glucose and xylose was more synchronous in YZJ115 than in YZJ109 (Fig. 2C and D). Acetate production was also reduced from 2.12 g/L to 0.68 g/L. Co-fermentation ability of YZJ117, obtained through overexpression of *KmZWF1* in YZJ115 was then evaluated. However, a decrease in both fermentation and the growth rate was observed. The results showed that YZJ117 only produced 17.92 g/L xylitol and 26.11 g/L ethanol, which was significantly less than in YZJ115 (Fig. S3A).

The above results indicated that overexpression of the xylose-specific transporter was an effective method to improve xylitol production in co-fermentation. Therefore, YZJ118 and YZJ119 were constructed based on YZJ115 to express more *ScGAL2*-N376F, and the fermentation results show that more xylitol was produced as overexpression of the *ScGAL2*-N376F gene increased in YZJ118 and YZJ119. YZJ118 produced 35.62 g/L xylitol and 34.40 g/L ethanol and YZJ119 produced 40.43 g/L xylitol and 34.66 g/L ethanol in 28 h. YZJ119 consumed almost all of the xylose in the medium and converted it into xylitol (Fig. 2E–G). YZJ121 was constructed through the disruption of the *KmHXT2* gene, which encodes a glucose-preferred sugar transporter. This strain was used to explore whether disruption of the native glucose transporter in YZJ119 could further improve xylitol production. When fermented

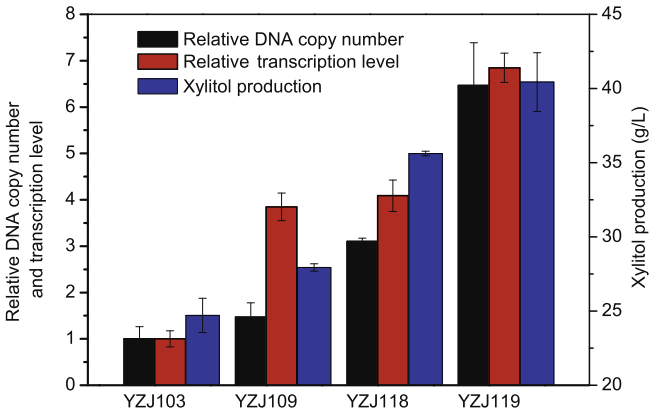


**Fig. 3.** Real time PCR was used to determine the copy number and transcription levels of gene *ScGAL2*-N376F in recombinant strains YZJ103, YZJ109, YZJ118 and YZJ119. The xylitol production correlated with RNA transcription levels and DNA copy number when fermented with 80 g/L glucose and 40 g/L xylose. All values are plotted with reference to YZJ103 and are the means of three biological replicates ± standard deviation ($n$ = 3) at each of the time points.

with 80 g/L glucose and 40 g/L xylose, 37.75 g/L xylitol and 34.42 g/L ethanol were produced in 28 h by YZJ121 (Fig. S3B). Disruption of *KmHXT2* in YZJ121 did not improve xylitol production.

Since expression of the xylose-specific transporter improved the co-consumption of glucose and xylose, we hypothesized that higher expression may further improve co-consumption. From strains YZJ103, YZJ109, YZJ118 to YZJ119, the copy number of *Sc*GAL2-N376F was increased to about 6, and a concurrent 6.85-fold increase in the expression levels was observed (Fig. 3). The co-consumption also improved with increased *Sc*GAL2-N376F expression (Fig. 2). However, with an increase in xylitol production, the rates of glucose consumption and ethanol production decreased (Fig 2). While the reason is not clear, increased expression of the xylose transporter may reduce the native glucose transporter on the cell surface, due to limited yeast cell surface (Kieke et al., 1999), thus leading to reduced glucose consumption. Some engineered yeast strains were constructed by overexpression of the xylose-specific transporter in a transport deficient *S. cerevisiae* strain. However, neither ethanol nor xylitol was produced during glucose and xylose co-fermentation, despite a high inoculum size (Nijland et al., 2014; Wang et al., 2015).

Disruption of the native glucose transporter did not improve co-fermentation. *KmHXT2* is the main hexose transporter in yeast; therefore, *KmHXT2* in YZJ119 was disrupted to evaluate the effect of native transporter disruption on co-fermentation. However the co-fermentation efficiency of obtained strain YZJ121 was not improved. YZJ121 produced similar levels of xylitol as YZJ119 with 80 g/L glucose and 40 g/L xylose (Fig. 2(F) and Fig. S3B). Lower concentrations of glucose were used to evaluate differences in the co-fermentation abilities of YZJ119 and YZJ121. With 40 g/L glucose and 40 g/L xylose, YZJ119 produced 33.02 g/L xylitol and 16.69 g/L ethanol in 18 h, and YZJ121 produced 30.71 g/L xylitol and 16.20 g/L ethanol in 24 h (Fig. 4A and B). The co-fermentation ability of YZJ121 was weaker than YZJ119. Because there are more than 21 possible hexose transporters in *K. marxianus*, and the disruption of *KmHXT2* could be compensated by any other transporter, and therefore no significant change in co-fermentation with strain YZJ121 was observed.

The presence of glucose and the decreased glucose consuming rate benefited xylitol production in YZJ119. An unexpected result was that nearly all consumed xylose was converted into xylitol [yield about 98–99% (0.98–0.99 g/g), Supplementary Table S4] in co-fermentation, while the parental strain YZJ015 has a yield of only 71–83% (Zhang et al., 2014). There are at least 2 possible reasons: firstly, the co-enzyme for xylitol production could be supplied by glucose metabolism, which does not need catabolism of part xylose to obtain the co-enzyme. The decreased glucose consumption rate could provide the co-enzyme along with xylose consumption. Secondly, in the presence of glucose, xylitol dehydrogenase (*KmXYL2*) and other xylose metabolic related gene expression was repressed, xylitol consumption was blocked, while the xylose reductase gene (*NcXYL1*) was expressed under a constitutive promoter (*GAPDHp*) which was not repressed by glucose. Therefore, almost all of the consumed xylose was converted into xylitol.

The disruption of *KmGPD1* reduced glycerol accumulation. In our previous study, disruption of *KmGPD1* reduced glycerol accumulation and enhanced the ethanol fermentation from xylose (Zhang et al., 2015b). Through the disruption of *KmGPD1* in YZJ115, glycerol and acetate accumulation were reduced to very low levels (Fig 2), though the growth rate was somewhat decreased. Although the disruption of *KmGPD1* does not block the acetate producing pathway, acetate accumulation was reduced, a finding consistent with previous reports (Fig2C and D) (Xiong et al., 2011; Zhang et al., 2015b). The overexpression of *KmZWF1* did not enhance co-fermentation, but instead repressed it. *KmZWF1* was overexpressed in YZJ117 to enhance NADPH production. However, the overexpression of *KmZWF1* reduced both the amounts and productivity of ethanol and xylitol. A possible explanation may be that the overexpression of *KmZWF1* led to break the redox balance in the cell (Oh et al., 2013).

### 3.4. Xylitol and ethanol production with different ratios of glucose and xylose in K. marxianus YZJ119

As the ratios of mixed sugars differ in various lignocellulosic biomass hydrolysates, the co-fermentation ability of YZJ119 with glucose and xylose in various ratios was also evaluated. YZJ119 produced 34.66 g/L ethanol and 40.43 g/L xylitol with a productivity of 1.24 and 1.44 g/L/h in 28 h from 80 g/L glucose to 40 g/L xylose (2:1) (Fig. 2F and Supplementary Table S4, entry 3). With 60 g/L glucose and 40 g/L xylose (3:2), YZJ119 produced 26.44 g/L ethanol and 38.50 g/L xylitol in 24 h with productivities of 1.10 and 1.60 g/L/h, respectively (Fig. 4C, H and Table S4 entry 2). With 80 g/L glucose and 60 g/L xylose (4:3), YZJ119 produced 38.41 g/L ethanol and 58.78 g/L xylitol in 36 h with productivities of 1.07 and 1.63 g/L/h, respectively (Fig. 4D, 4H and Table S4 entry 4). With 80 g/L glucose and 80 g/L xylose (1:1), YZJ119 produced 34.10 g/L ethanol and 64.26 g/L xylitol with productivities of 0.95 and 1.79 g/L/h, respectively, in 36 h (Fig. 4E, H and Table S4 entry 3). With the ratio of 2:1 (80 g/mL glucose and 40 g/mL xylose), both glucose and xylose were almost completely consumed, and thus a higher concentration sugar mixture was evaluated for co-fermentation. With 100 g/L glucose and 50 g/L xylose, YZJ119 produced 44.47 g/L ethanol and 48.52 g/L xylitol with a productivity of 1.24 and 1.35 g/L/h, respectively, in 36 h (Fig. 4F, 4H and Table S4 entry 6). With 120 g/L glucose and 60 g/L xylose, YZJ119 produced 50.10 g/L ethanol and 55.88 g/L xylitol with a productivity of 1.04 and 1.16 g/L/h, respectively, in 48 h (Fig. 4G, H and Table S4 entry 7). When fermented with 160 g/L glucose and 80 g/L xylose, YZJ119 produced 53.50 g/L ethanol and 57.39 g/L xylitol with a productivity of 1.11 and 1.20 g/L/h, respectively, in 48 h (Table S4 entry 8). Increase in the sugar concentration resulted in increased xylitol and ethanol production. However, with the combination of 160 g/L glucose and 80 g/L xylose, both glucose and xylose were not completely consumed. It is possible that the high concentrations of sugar or ethanol inhibited fermentation. With a 1:1 ratio, 15.85 g/L xylose was retained in a 80 g/L glucose and 80 g/L xylose mixture. To avoid possible inhibition due to high concentrations of sugar, 40 g/L glucose and 40 g/L

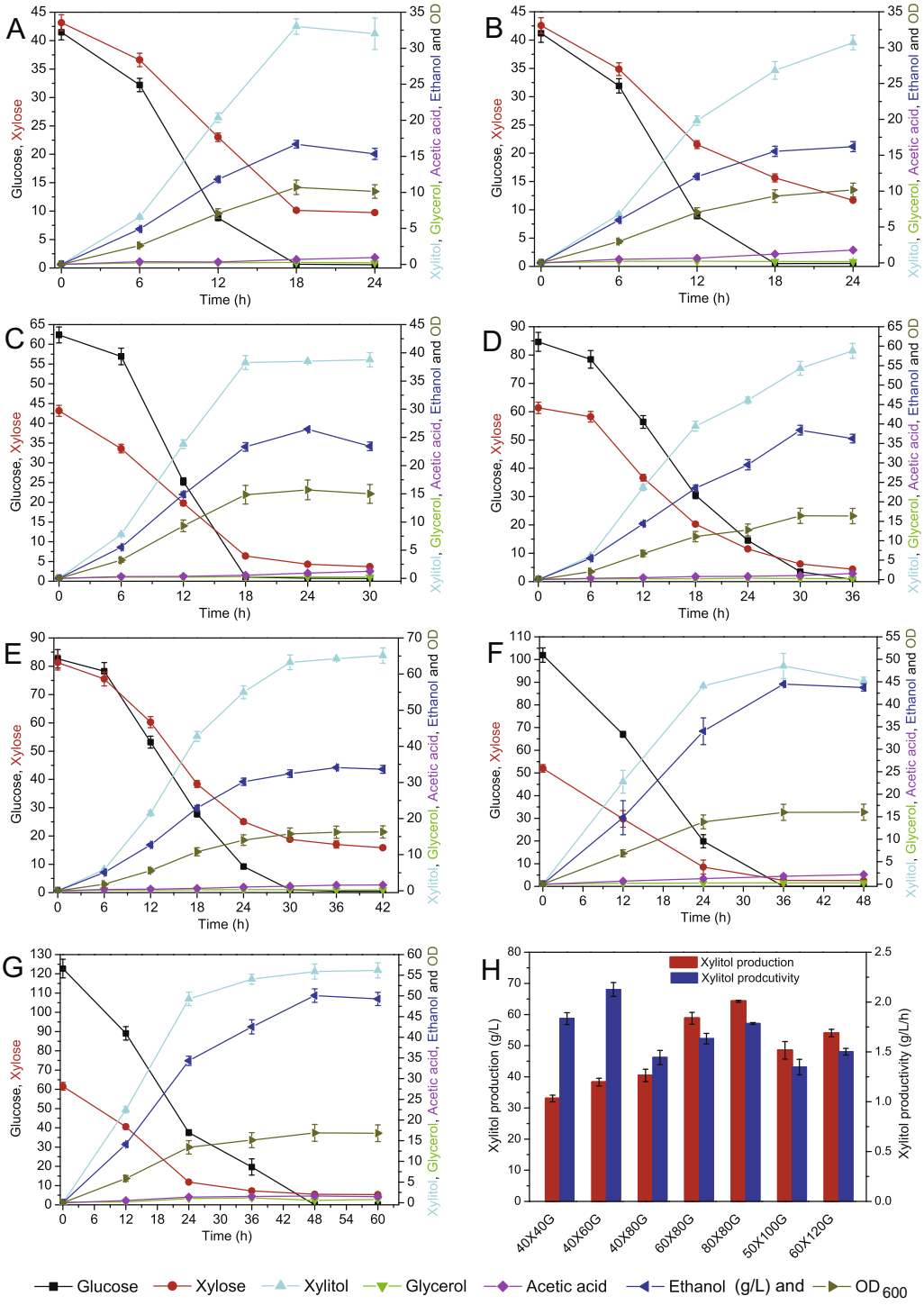


**Fig. 4.** Co-fermentation of glucose and xylose in different ratios at 42 °C under oxygen limited conditions with YZJ119 or YZJ121. (A) YZJ119 and (B) YZJ121 fermented with 40 g/L glucose and 40 g/L xylose, (C) YZJ119 fermented with 60 g/L glucose and 40 g/L xylose, (D) YZJ119 fermented with 80 g/L glucose and 60 g/L xylose, (E) YZJ119 fermented with 80 g/L glucose and 80 g/L xylose, (F) YZJ119 fermented with 100 g/L glucose and 50 g/L xylose, (G) YZJ119 fermented with 120 g/L glucose and 60 g/L xylose and (H) the xylitol production and productivity of YZB119 when fermented with different concentration of sugars, 40X40G means fermentation with 40 g/L xylose and 40 g/L glucose as substrate, as so on.

xylose was also used as a substrate for co-fermentation. YZJ119 produced 33.02 g/L xylitol and 16.69 g/L ethanol in 18 h, and additionally retained 9.73 g/l xylose (Fig. 4A and Supplementary Table S4, entry 1). Therefore, residual xylose was obtained due to the absence of glucose as the source of energy and co-enzymes in metabolism.

### *3.5. Xylitol and ethanol co-production under various temperatures with K. marxianus YZJ119*

Fermentations with 80 g/L glucose and 40 g/L xylose at various temperatures were explored. At 37 °C, YZJ119 produced 39.21 g/L xylitol and 33.18 g/L ethanol in 24 h with a productivity of 1.63

**Table 1**
Comparison of the glucose and xylose co-fermentation abilities of strains with different transporters in 12 h*.

| Strains | Transporters | Residual xylose (g/L) | Xylitol (g/L) | Glycerol (g/L) | Acetic acid (g/L) | Ethanol (g/L) |
|---|---|---|---|---|---|---|
| YZJ015 | – | 32.34 ± 0.59 | 9.01 ± 0.17 | 6.15 ± 0.11 | 1.35 ± 0.02 | 35.53 ± 0.65 |
| YZJ041 | *Km*FPS1 | 33.55 ± 0.65 | 9.26 ± 0.18 | 6.14 ± 0.12 | 1.47 ± 0.03 | 36.22 ± 0.70 |
| YZJ072 | *Ci*GXF1 | 33.02 ± 0.72 | 9.73 ± 0.21 | 5.90 ± 0.01 | 1.30 ± 0.03 | 35.95 ± 0.78 |
| YZJ073 | *Ci*GXS1 | 32.22 ± 0.49 | 8.66 ± 0.13 | 6.30 ± 0.10 | 1.27 ± 0.02 | 36.09 ± 0.55 |
| YZJ103 | *Sc*GAL2-N376F | 20.15 ± 0.23 | 20.99 ± 0.25 | 5.93 ± 0.07 | 1.11 ± 0.01 | 34.33 ± 0.39 |
| YZJ106 | *Sc*GAL2 | 33.51 ± 0.79 | 9.77 ± 0.23 | 7.32 ± 0.17 | 1.72 ± 0.04 | 35.89 ± 0.84 |

* All glucose was consumed.

and 1.38 g/L/h, respectively (Fig. S7A and Supplementary Table S4, entry 9). At 42 °C, YZJ119 produced 40.43 g/L xylitol and 34.66 g/L ethanol in 28 h with a productivity of 1.44 and 1.24 g/L/h, respectively (Fig. 2F and Supplementary Table S4, entry 3). At 45 °C, YZJ119 produced 35.56 g/L xylitol and 35.21 g/L ethanol in 30 h with a productivity of 1.19 and 1.17 g/L/h, respectively (Fig. S7B and Table S4 entry 10). At 42 °C, YZJ119 produced most xylitol and the productivity decreased with an increase in fermentation temperature. Though the ethanol productivity also decreased with increased temperature, the change in ethanol production was not very significant.

### 3.6. Xylitol and ethanol produced from diluted acid pretreated corncob by YZJ119 through SSCF

To integrate the utilization of corncob, the diluted acid pretreated corncob including hydrolysate and solid CCR were used together to evaluate the co-fermentation ability of YZJ119. Fermentation with non-detoxified or detoxified material was performed to determine the effects of the inhibitor on fermentation. The non-detoxified material, i.e., a mixture of CCR and the non-detoxified hydrolysate after neutralization, was used directly for fermentation. With the substrate containing non-detoxified hydrolysate, YZJ119 produced 23.11 g/L, 25.20 g/L, 26.58 g/L, and 31.72 g/L xylitol with a productivity of 0.48 g/L/h, 0.53 g/L/h, 0.55 g/L/h, and 0.66 g/L/h, respectively, and 19.70 g/L, 22.12 g/L, 23.86 g/L, and 29.82 g/L ethanol with a productivity of 0.41 g/L/h, 0.46 g/L/h, 0.50 g/L/h, and 0.62 g/L/h, respectively, at 42 °C, corresponding to CCR concentrations (g/L) of 60.98, 76.23, 101.84, and 121.97 (Fig. S8) upon the addition of 15 FPU/g CCR cellulase.

Furthermore, YZJ119 fermented with CCR and hydrolysate solution derived from 100 g/L corncob with less cellulase at 42 °C was explored. With 10 FPU/g CCR cellulase, 18.07 g/L ethanol and 20.21 g/L xylitol were produced in 60 h with productivities of 0.30 g/L/h and 0.34 g/L/h, respectively (Fig. S6 and Supplementary Table S5, entries 1 and 6). With 5 FPU/g CCR cellulase, fermentation decreased further and 15.02 g/L ethanol and 13.55 g/L xylitol were produced in 60 h with a productivity of 0.25 g/L/h and 0.23 g/L/h, respectively (Fig. S6 and Supplementary Table S5, entry 7). Therefore, SSCF with a higher cellulase loading was evaluated. With 30 FPU/g CCR cellulase, 21.03 g/L ethanol and 24.11 g/L xylitol were produced in 48 h with a productivity of 0.44 and 0.50 g/L/h, respectively (Fig. S6 and Supplementary Table S5, entry 5). In summary, the ethanol and xylitol production and productivity decreased when lesser cellulase was added. This result indicated that even when the temperature was increased to 42 °C and higher enzymatic hydrolysis was achieved, the amount of cellulase cannot be reduced. This may due to inhibitors or retained lignin on CCR. Besides, the saccharification was performed under different temperatures. As the temperature increased from 37 °C to 42 °C and 45 °C, final concentrations of 33.35, 39.49 and 42.50 g/L glucose were produced by adding cellulase 15 FPU/g CCR, with the CCR concentration of 60.98 g/L (Fig. S5). The saccharification ability of cellulase thus increased with a rise in temperature.

Only when the CCR concentration was 60.98 g/L, almost all the xylose transformed into xylitol. This could be explained by a probable increase in the inhibitors in the hydrolysate when the substrate concentration was increased, leading to a decrease in the yeast viability. Therefore, detoxified material was also used to evaluate the SSCF. The substrate contained detoxified hydrolysate and 120 g/L CCR, and the same solid/liquid ratio of non-detoxified material from 200 g/L corncob was used to evaluate the effects of inhibitor removal and fermented with this detoxified hydrolysate. YZJ119 produced 32.17 g/L xylitol and 37.88 g/L ethanol. The detoxification improved fermentation to a certain extent (Table 2 and Supplementary Table S5, entry 8). Ethanol production showed an improvement from 29.82 to 37.88 g/L. The productivities of xylitol and ethanol were improved from 0.66 and 0.62 g/L/h to 0.89 and 1.05 g/L/h, respectively (Table 2 and Supplementary Table S5, entry 8). However, the xylitol concentration remained almost the same as compared to the non-detoxified fermentation result, with a large amount of xylose still retained in medium (Table 2). We assumed that there were not enough CCRs to produce enough glucose and provide co-enzymes and energy for xylitol production. To verify this hypothesis, the hydrolysate with various amounts of CCR was tested and YZJ119 produced 25.20 g/L, 27.02 g/L, 30.68 g/L and 32.03 g/L xylitol, and 23.29 g/L, 24.82 g/L, 28.15 and 44.58 g/L ethanol corresponding to the CCR concentrations of 60 g/L, 75 g/L, 85 g/L and 150 g/L, respectively with a fixed amount of detoxified hydrolysate (Table 2). More the CCR used and more ethanol was produced. However, when the CCR concentrations were higher than 120 g/L, xylitol production did not show further improvement. Therefore, the limitation of xylitol production was not due to shortage of glucose.

Corncob, which represents 20% of the weight of the harvested corn and has high glucan and xylan contents, is one of the most abundant lignocellulosic wastes in Northeast China (Cheng et al., 2014). Co-producing ethanol and xylitol is a promising strategy in lignocellulose. Since it is difficult to completely convert xylose into ethanol and xylitol which has a higher value than ethanol, converting the glucose to ethanol and xylose to xylitol is more economical. In a previous study, *Candida tropicalis* W103 (Cheng et al., 2014) produced 17.1 g/L xylitol and 25.3 g/L ethanol by aerobic and sequential anaerobic fermentation using non-detoxified acid pretreated corncob at 30 °C. In this study, YZJ119 produced 31.72 g/l xylitol and 29.82 g/L ethanol with a productivity of 0.66 and 0.62 g/L/h, respectively with 200 g/L non-detoxified acid pretreated corncob at 42 °C through SSCF (Fig. S8 and Supplementary Table S5, entry 4). The simultaneous consumption of glucose from cellulose and xylose from hemicellulose leading to simultaneous production of ethanol and xylitol is convenient for downstream product purification in which both products are required to be concentrated.

During the acid pretreatment of corncob, large amounts of various inhibitors are produced. These inhibitors mainly include furfural, 5-HMF, and acetic acid. The concentrations of furfural and 5-HMF in diluted acid pretreated corncob hydrolysate before dilution were 487.66 mg/L and 984.08 mg/L, respectively. After overliming

**Table 2**
SSCF of detoxified material with *K. marxianus* YZJ119.

| CCR concentration (g/L) [*] | The composition before fermentation | | | The composition after fermentation[**] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Glucose (g/L) | Xylose (g/L) | Acetic acid (g/L) | Xylose (g/L) | Xylitol (g/L) | Glycerol (g/L) | Acetic acid (g/L) | Ethanol (g/L) | Xylitol productivity (g/L/h) | Ethanol productivity (g/L/h) | Time (h) |
| 60 | 12.85 ± 0.58 | 50.13 ± 1.63 | 8.71 ± 0.28 | 26.04 ± 0.84 | 25.20 ± 0.82 | 0.94 ± 0.03 | 11.42 ± 0.37 | 23.29 ± 0.76 | 1.05 ± 0.03 | 0.97 ± 0.03 | 24 |
| 75 | 11.31 ± 1.24 | 49.32 ± 1.60 | 7.95 ± 0.26 | 25.42 ± 0.82 | 27.02 ± 0.88 | 0.87 ± 0.02 | 10.73 ± 0.35 | 24.82 ± 0.80 | 1.13 ± 0.04 | 1.03 ± 0.04 | 24 |
| 85 | 13.54 ± 1.51 | 48.30 ± 1.57 | 8.33 ± 0.27 | 23.42 ± 0.76 | 30.68 ± 0.99 | 0.98 ± 0.03 | 11.29 ± 0.37 | 28.15 ± 0.91 | 1.02 ± 0.04 | 0.94 ± 0.03 | 30 |
| 120 | 12.27 ± 3.02 | 57.65 ± 1.87 | 7.20 ± 0.23 | 28.34 ± 0.92 | 32.17 ± 1.04 | 1.37 ± 0.04 | 9.51 ± 0.31 | 37.88 ± 1.23 | 0.89 ± 0.04 | 1.05 ± 0.13 | 36 |
| 150 | 12.91 ± 4.29 | 56.91 ± 1.84 | 6.76 ± 0.22 | 30.11 ± 0.98 | 32.03 ± 1.23 | 1.23 ± 0.03 | 9.09 ± 0.29 | 44.58 ± 1.28 | 0.53 ± 0.02 | 0.74 ± 0.02 | 60 |

[*] The hydrolysate was mixed with CCR after detoxification.
[**] All glucose was consumed after fermentation.

**Table 3**
The concentration of furfural and 5-HMF before and after simultaneous saccharification and co-fermentation with *K. marxianus* YZJ119.

| Detoxified or not | CCR concentration (g/L) | Before fermentation | | After fermentation | |
|---|---|---|---|---|---|
| | | Furfural (mg/L) | 5-HMF (mg/L) | Furfural (mg/L) | 5-HMF (mg/L) |
| Detoxified | 60[*] | 81.76 ± 4.28 | 205.46 ± 10.75 | 8.75 ± 0.46 | 27.23 ± 1.42 |
| | 75[*] | 79.42 ± 4.16 | 217.42 ± 11.38 | 7.31 ± 0.38 | 27.14 ± 1.02 |
| | 85[*] | 80.26 ± 4.20 | 217.33 ± 11.37 | 8.45 ± 0.44 | 26.64 ± 1.39 |
| | 120[*] | 70.94 ± 3.71 | 219.05 ± 11.46 | 7.76 ± 0.41 | 27.51 ± 1.44 |
| | 150[*] | 68.71 ± 3.60 | 231.96 ± 12.14 | 7.88 ± 0.21 | 26.99 ± 1.41 |
| Undetoxified | 60.98[**] | 179.88 ± 9.41 | 326.48 ± 17.08 | 9.47 ± 0.50 | 30.75 ± 1.61 |
| | 76.23[**] | 224.85 ± 3.04 | 408.09 ± 5.51 | 9.86 ± 0.13 | 29.56 ± 0.40 |
| | 101.84[**] | 299.80 ± 4.05 | 544.13 ± 7.35 | 9.65 ± 0.13 | 29.01 ± 0.39 |
| | 121.97[**] | 359.76 ± 4.86 | 652.95 ± 8.81 | 11.10 ± 0.15 | 29.83 ± 0.40 |

[*] The CCR was separated from pretreated solution, washed and dried, then added as the indicated amount.
[**] The CCR was not separated from the pretreated solution, and the amount was calculated based on pretreatment.

with $Ca(OH)_2$, they decreased about 73.47% and 72.79%, respectively. Because most of inhibitors are in the hydrolysate fraction, fermentation using hydrolysate with or without detoxification was compared to analyze the effects of inhibitors. Fermentation with detoxified acid pretreated corncob hydrolysate and solid residue, showed an obvious improvement in ethanol production. YZJ119 produced up to 37.88 g/L ethanol with a productivity of 1.05 g/L/h with 120 g/L CCR added (Table 2). With the non-detoxified hydrolysate and 120 g/L CCR, only 29.82 g/L ethanol was produced with a productivity of 0.62 g/L/h. However, whether the hydrolysate was detoxified or not, after the fermentation, the concentrations of furfural and 5-HMF were significantly reduced and the final concentrations were similar (Table 3), which indicated that *K. marxianus* degraded furfural and 5-HMF effectively. Furthermore, not much difference was observed in xylitol production from xylose, which was mainly in the hydrolysate (Supplementary Table S5 and Fig. S8). Thus, *K. marxianus* YZJ119 may have an inhibitor-tolerant ability to an extent, and the decreased fermentation efficiency is possibly due to inhibitors other than furfural and 5-HMF. The inhibition of high level of water-insoluble phenolic compounds in CCR was the most plausible explanation (Cheng et al., 2014). The inhibitor degradation ability of *K. marxianus* YZJ119 is an important property in industrial applications, though its anti-toxin ability to some compounds needs to be further improved. The successful integration of glucose and xylose fermentation in *K. marxianus* is a critical step toward enabling high value-added production from plant biomass in the future.

## 4. Conclusions

In this study, *K. marxianus* yeast strains were constructed through metabolic engineering to obtain the ability of simultaneous fermentation of glucose and xylose and co-produce ethanol and xylitol. The glucose/xylose co-fermentation ability of the recombinant strains was significantly improved by overexpressing a xylose-specific transporter mutant. StrainYZJ119 produced 50.10 g/L ethanol and 55.88 g/L xylitol from 120 g/L glucose to 60 g/L xylose simultaneous at 42 °C. This research will advance the efforts to design a metabolically efficient platform strain for potential use in producing chemicals from lignocellulose.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2016.05.068.

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