Alleviation of catabolite repression in *Kluyveromyces marxianus*: the thermotolerant SBK1 mutant simultaneously coferments glucose and xylose

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**Abstract**

**Background:** Simultaneous cofermentation of glucose and xylose mixtures would be a cost-effective solution for the conversion of cellulosic biomass to high-value products. However, most yeasts ferment glucose and xylose sequentially due to glucose catabolite repression. A well known thermotolerant yeast, *Kluyveromyces marxianus*, was selected for this work because it possesses cost-effective advantages over *Saccharomyces cerevisiae* for biofuel production from cellulosic biomass.

**Results:** In the present study, we employed a directed evolutionary approach using 2-deoxyglucose to develop a thermotolerant mutant capable of simultaneous cofermentation of glucose and xylose by alleviating catabolite repression. The selected mutant, *K. marxianus* SBK1, simultaneously cofermented 40 g/L glucose and 28 g/L xylose to produce 23.82 g/L ethanol at 40 °C. This outcome corresponded to a yield of 0.35 g/g and productivity of 0.33 g/L h, representing an 84% and 129% improvement, respectively, over the parental strain. Interestingly, following mutagenesis the overall transcriptome of the glycolysis pathway was highly downregulated in *K. marxianus* SBK1, except for glucokinase-1 (GLK1) which was 21-fold upregulated. Amino acid sequence of GLK1 from *K. marxianus* SBK1 revealed three amino acid mutations which led to more than 22-fold lower enzymatic activity compared to the parental strain.

**Conclusions:** We herein successfully demonstrated that the cofermentation of a sugar mixture is a promising strategy for the efficient utilization of cellulosic biomass by *K. marxianus* SBK1. Through introduction of additional biosynthetic pathways, *K. marxianus* SBK1 could become a chassis-type strain for the production of fuels and chemicals from cellulosic biomass.

**Keywords:** Simultaneous cofermentation, Cellulosic biomass, Thermotolerant yeast, *Kluyveromyces marxianus* SBK1

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**Background**

Over the past few decades, research has become more focused on alternative fuel resources due to economic and environmental challenges. Among these resources, biofuels, including ethanol, are the most well known substitutes for liquid transportation fuels [1, 2]. Ethanol production is expected to dramatically increase by the year 2030 [3, 4]. Among the renewable feedstocks for ethanol production, cellulosic biomass has various attractive advantages, such as its abundance and relatively low cost [1, 5, 6]. The main components of cellulosic biomass are cellulose and hemicellulose, which are enzymatically hydrolyzed to glucose and xylose [5, 7]. However, one of the main challenges in glucose and xylose mixed sugar utilization is glucose catabolite repression; a universal mechanism in microorganisms whereby glucose inhibits the consumption of other non-glucose sugars [8, 9]. This is considered to be one of the main hurdles for the efficient conversion of cellulosic biomass hydrolysate to ethanol [10].
In recent years, several engineered *Saccharomyces cerevisiae* strains have been reported to efficiently co-utilize mixed sugars. An engineered *S. cerevisiae* DA24-16BT3 cofermented cellobiose and xylose simultaneously and exhibited improved ethanol yield through overexpression of cellobextrin transporter and β-glucosidase [11]. Kahar et al. demonstrated that mutant *S. cerevisiae* (M2) produced 5.5 g/L ethanol from 20 g/L glucose and 16 g/L xylose with a final yield of 0.15 g/g ethanol [12]. Engineered *S. cerevisiae* SR8#22 produced 26 g/L ethanol from 40 g/L glucose and 40 g/L xylose with a final yield of 0.35 g/g ethanol [13]. An engineered *Kluyveromyces marxianus* over expressed the xylose-specific transporters (GAL2-N376F) and simultaneously cofermented glucose and xylose, producing 50.10 g/L ethanol and 55.88 g/L xylitol [14]. Simultaneous saccharification and fermentation (SSF), or simultaneous saccharification and cofermentation (SSCF) processes are time- and cost-effective strategies often utilized today [15, 16]. In these processes, thermostolerant yeasts are necessary, because saccharification, generally considered as the overall rate-determining step in SSF or SSCF, is performed by cellulase enzymes with high optimal reaction temperatures (45–55 °C) [14, 17]. A well known thermostolerant yeast, *K. marxianus*, is a robust strain used for industrial ethanol production [18] and was previously reported to grow and ferment in environments up to 50 °C [19]. *K. marxianus* also has various benefits over mesophilic yeasts, such as high growth rates, reduced cooling costs, and a broad spectrum of substrates, including xylose [20, 21].

Previously, the *K. marxianus* 17694-DH1 mutant was isolated following a random mutagenesis/directed evolutionary approach and shown to be effective for ethanol production from xylose [22]. However, *K. marxianus* 17694-DH1 displays strong glucose catabolite repression when using glucose and xylose as mixed sugars. In the present study, we employed a directed evolutionary approach using 2-deoxyglucose (2-DG) to overcome catabolite repression in *K. marxianus* 17694-DH1. The isolated mutant, *K. marxianus* SBK1, was shown to simultaneously coferment glucose and xylose. Transcriptomic analysis, using RNA-Seq was performed for a deeper understanding of the alleviated catabolite repression.

**Results and discussion**

**A directed evolutionary approach for alleviating catabolite repression**

In previous studies, 2-DG-resistant mutants demonstrated simultaneous glucose and xylose cofermentation capabilities following relief of glucose catabolite repression [12, 13, 23]. A laboratory-directed evolutionary approach was therefore applied to *K. marxianus* 17694-DH1 to alleviate glucose catabolite repression using 2-DG [22]. Pre-cultured cells were initially inoculated into YP media (10 g/L yeast extract, 20 g/L Bacto peptone) containing 15 g/L 2-DG and 40 g/L xylose (2-DG/xylose media). When the residual concentration of xylose was almost zero, the cells were transferred into fresh 2-DG/xylose media. The serial sub-cultures were performed for six cycles. The xylose consumption rate during the first cycle was very low (0.10 g/L h), but gradually increased with repeated sub-culturing (see Additional file 1: Fig. S1). The maximum xylose consumption rate was 0.31 g/L h at the fourth enrichment cycle.

To isolate 2-DG-resistant-mutants, cells from the fourth enrichment cycle were plated onto YPX20 agar media containing g/L 2-DG (see Additional file 2: Fig. S2). Among 104 colonies grown on the plate, ten large colonies were randomly selected for further testing. Their glucose and xylose cofermentation capabilities were then verified. Interestingly, all ten candidates showed improved cofermentation capabilities compared to the parental strain *K. marxianus* 17694-DH1 (see Additional file 3: Fig. S3). The average xylose consumption rates of the ten 2-DG-resistant mutants were more than twofold higher than that of the parental strain. Of those, colony #8 exhibited the highest xylose consumption (0.30 g/L h) and ethanol production rates (0.25 g/L h). Consequently, we selected colony #8 as the best glucose and xylose cofermenting strain and named it *K. marxianus* SBK1.

**Cofermentation capability of *K. marxianus* SBK1**

To compare fermentation capabilities between the parental and *K. marxianus* SBK1 strains, we performed fermentation experiments in YPD40, YPX40, or YPD40X40 media. For glucose fermentation, the parental strain consumed 40 g/L glucose and produced 18.65 g/L ethanol within 7 h (Fig. 1a). The glucose consumption rate, ethanol yield, and productivity were 5.66 g/L h, 0.47 g/g, and 2.66 g/L h, respectively. For xylose fermentation, the parental strain consumed 40 g/L xylose and produced 10.56 g/L ethanol within 120 h (Fig. 1e). The xylose consumption rate, ethanol yield, and productivity were 0.31 g/L h, 0.29 g/g, and 0.09 g/L h, respectively. In the glucose and xylose mixture, the parental strain consumed glucose first, equally as fast as seen for glucose fermentation alone, and thereafter consumed xylose very slowly (only 13.63 g/L xylose was consumed within 120 h) (Fig. 1c). The glucose consumption rate, xylose consumption rate, ethanol yield, and productivity were 4.79 g/L h, 0.11 g/L h, 0.29 g/g, and 0.13 g/L h, respectively. As expected, these results suggest that the parental strain exhibits sequential utilization of the sugar mixture due to glucose catabolite repression.
In the case of *K. marxianus* SBK1, the glucose consumption rate significantly decreased to 0.34 g/L h, whereas the xylose consumption rate was similar to the parental strain over 120 h (Fig. 1e, f). Interestingly, in the glucose and xylose mixture, *K. marxianus* SBK1 simultaneously co-consumed the sugar mixture and produced 25.47 g/L ethanol over 120 h (Fig. 1d). Cell growth (OD$_{600}$), ethanol yield, and productivity were 15.36, 0.35 g/g, and 0.21 g/L h, respectively. Ethanol concentration, yield, and productivity increased by 68%, 19%, and 68% compared to the parental strain, respectively. These cofermentation results suggest that glucose catabolite
repression might be strongly alleviated in *K. marxianus* SBK1, which is consistent with the phenotypes of other 2-DG-resistant mutants [12, 23].

**Verification of catabolite repression in high-glucose concentrations**

*Kluyveromyces marxianus* SBK1 cofermentation experiments were performed with increasing concentrations of glucose and 40 g/L xylose to verify the effect of catabolite repression with high glucose concentrations (see Additional file 4: Fig. S4). When glucose concentrations increased from 20 to 70 g/L, xylose consumption rates gradually decreased from 0.28 to 0.12 g/L h (Fig. 2a). This result suggests that glucose catabolite repression is reduced when glucose concentrations were low; however, catabolite repression persists in *K. marxianus* SBK1 when glucose concentrations were high. As glucose concentrations increased, glucose consumption and ethanol production rates changed in a glucose concentration-dependent manner. Glucose consumption and ethanol production rates increased when the glucose concentration was lower than 40 g/L. However, when the glucose concentration was higher than 40 g/L, glucose consumption and ethanol production rates decreased. Therefore, *K. marxianus* SBK1 exhibited the highest glucose consumption rate (0.48 g/L h) and ethanol production rate (0.28 g/L h) with 40 g/L glucose and 40 g/L xylose.

Xylose concentrations were similarly varied (20–50 g/L), while maintaining 40 g/L glucose constant to investigate the effect of xylose concentration on glucose consumption rates (see Additional file 5: Fig. S5). When xylose concentrations were increased from 20 to 50 g/L, glucose consumption rates remained steady (0.40–0.41 g/L h) (Fig. 2b). Xylose consumption and ethanol production rates gradually increased as xylose concentrations increased. However, with 40 g/L xylose, *K. marxianus* SBK1 exhibited the highest xylose consumption (0.30 g/L h) and ethanol production rates (0.25 g/L h). Therefore, subsequent cofermentation experiments were carried out under these optimal substrate concentrations of 40 g/L glucose and 40 g/L xylose.

**Glycolysis pathway transcripts are highly downregulated except for glucokinase-1**

To further investigate the nature of alleviated glucose catabolite repression in *K. marxianus* SBK1, quantitative RNA-Seq was performed to compare the parental strain and *K. marxianus* SBK1. Among 1542 altered transcripts, 745 were up- and 797 were downregulated in *K. marxianus* SBK1. As *K. marxianus* SBK1 showed reduced glucose consumption rates (Fig. 1b), glycolysis pathway transcripts were analyzed and shown to be downregulated (Table 1). This result suggests the reduced glucose consumption rate of *K. marxianus* SBK1 might be caused by highly downregulated transcripts in the glycolysis pathway. However, glucokinase-1 (GLK1), a glycolysis-initiating enzyme, interestingly showed 20-fold upregulation. Lane et al. reported that catabolite repression can be reduced by modulating the expression of glucose-phosphorylating enzymes, such as GLK1 and hexokinase (HK) [13]. Therefore, *K. marxianus* SBK1 GLK1 and RAG5, which encode GLK1 and HK, respectively, were cloned and sequenced using *K. marxianus* NBRC1777 and *K. marxianus* DMKU3-1042 sequencing data as Refs. [24, 25].

Nucleotide sequences of the respective genes were compared between *K. marxianus* SBK1 and the parental strain. Three nucleotide changes in the *K. marxianus*...
SBK1 GLK1 sequence led to three amino acid changes (see Additional file 6: Table S1), namely the substitution of methionine to isoleucine, serine to proline, and histidine to arginine at positions 13, 301, and 462, respectively. In the K. marxianus SBK1 RAG5 nucleotide sequence, adenine was deleted at position 1141, resulting in a frame-shift mutation in the amino acid sequence at position 381. Enzymatic assays were performed to verify enzymatic activity changes of GLK1 and HK between the parental strain and K. marxianus SBK1. As shown in Fig. 3a, K. marxianus SBK1 GLK1 and HK activities decreased significantly to 0.30 U/mg, compared to 6.47 U/mg in the parental strain. The reduced activities of glycolysis-initiating enzymes are consistent with those observed in other 2-DG-resistant mutants [13, 23]. It was assumed that significantly decreased GLK1 and HK activities might alleviate catabolite repression; therefore, glucokinase-1 was highly upregulated to overcome low GLK1 and HK activities for glucose utilization in K. marxianus SBK1.

Verification of key gluconeogenesis pathway enzymes
Gluconeogenesis, the reverse pathway of glycolysis, is generally suppressed by the presence of glucose [26–28]. However, the K. marxianus SBK1 transcriptomic data indicated phosphoenolpyruvate carboxykinase (PEPCK), a key enzyme in the gluconeogenesis, was highly upregulated (54-fold) [29]. Consequently, the PEPCK gene sequence and enzymatic activity were compared between the parental strain and K. marxianus SBK1. Six nucleotide changes were identified in the K. marxianus SBK1 PCK1 gene (coding for PEPCK) leading to the substitution of serine to threonine and threonine to serine at positions 199 and 386, respectively (Table 2). Four additional nucleotide changes were silent. Since serine and threonine are structurally very similar, it was expected

Table 1 Up- or downregulated transcriptomes in glycolysis pathway by the mutant K. marxianus SBK1 compared to the parental strain

| Gene ID | Description | Fold-change | Volume ratio |
|--------|-------------|-------------|--------------|
| Upregulation | gene36 | Glucokinase-1 | 20.89 | 6.03 |
| Downregulation | gene277 | Probable phosphoglycerate mutase YOR283W | −17.60 | 7.79 |
| | gene2516 | Putative phosphoglycerate mutase DET1 | −5.66 | 5.88 |
| | gene538 | Phosphoglycerate kinase | −4.48 | 11.79 |
| | gene2788 | Fructose-bisphosphate aldolase | −4.38 | 12.34 |
| | gene310 | 6-Phosphofructokinase subunit alpha | −3.87 | 8.55 |
| | gene760 | Glucose-6-phosphate isomerase | −3.44 | 9.36 |
| | gene2517 | Triosephosphate isomerase | −3.40 | 10.58 |
| | gene3891 | Pyruvate kinase | −3.39 | 10.79 |
| | gene458 | Enolase | −3.08 | 13.00 |
| | gene924 | Phosphoglycerate mutase 1 | −2.06 | 12.15 |
| | gene4722 | Glyceraldehyde-3-phosphate dehydrogenase 3 | −2.02 | 12.09 |

Table 2 Substitutions of amino acid sequences from key enzymes of glycolysis (GLK1, HK) and gluconeogenesis (PEPCK) pathways

| Enzyme | Gene | Substitutions |
|--------|------|---------------|
| GLK1   | GLK1 | G39A | M13I |
|        |      | T901C | S301P |
|        |      | A1385G | H462R |
| HK     | RAG5 | C255T | Silence |
|        |      | A1141Δ | R381fs (frame-shift) |
| PEPCK  | PCK1 | C75T | Silence |
|        |      | G489A | Silence |
|        |      | T595A | S199T |
|        |      | A921G | Silence |
|        |      | T1101A | Silence |
|        |      | A1156T | T386S |

Fig. 3 Enzymatic activities of a glucokinase-1 (GLK1) and hexokinase (HK), and b phosphoenolpyruvate carboxykinase (PEPCK) from the parental strain and K. marxianus SBK1. Symbols: K. marxianus 17694-DH1 (yellow-filled square), K. marxianus SBK1 (red-filled square)
that there would be no significant change in enzymatic activity of PEPCK. As expected, only slight differences were observed in PEPCK enzymatic activities between the parental strain and \textit{K. marxianus} SBK1 (Fig. 3b).

**Thermotolerant feature of \textit{K. marxianus} SBK1 for simultaneous cofermentation**

For industrial applications of cellulosic biomass utilization using SSF or SSCF, the thermotolerance of \textit{K. marxianus} SBK1 offers various advantages. For evaluation of simultaneous cofermentation of glucose and xylose at high temperature, cofermentation experiments were performed at 40 °C using the parental strain and \textit{K. marxianus} SBK1 (Fig. 4). After consuming 40 g/L glucose and producing 15.89 g/L ethanol within 24 h, the parental strain consumed xylose slowly (Fig. 4a). Glucose and xylose consumption rates were 1.61 g/L h (24 h) and 0.22 g/L h (72 h), respectively. Ethanol yield and productivity were 0.19 g/g and 0.14 g/L h, respectively. These results suggest the parental strain undergoes glucose catabolite repression, even at high cofermentation temperatures. In contrast, \textit{K. marxianus} SBK1 showed cofermentation capability at 40 °C (Fig. 4b). Glucose and xylose consumption rates were 0.55 g/L h and 0.39 g/L h over 72 h, respectively. Maximum ethanol concentration, yield, and productivity were 23.82 g/L, 0.35 g/g, and 0.33 g/L h, respectively, following consumption of 40 g/L glucose and 28 g/L xylose. Ethanol yield and productivity improved by 84% and 129% compared with the parental strain. When cofermentation results from 40 °C were compared with those from 30 °C, ethanol concentrations and yields were similar; however, productivity from the 40 °C fermentation was 57% higher than that from the 30 °C fermentation due to higher glucose and xylose consumption rates. This result clearly demonstrates that thermotolerant \textit{K. marxianus} SBK1 efficiently coferments glucose and xylose to ethanol at 40 °C.

**Conclusions**

Simultaneous co-utilization of glucose and xylose, the two main components in cellulosic biomass, is an excellent strategy for the efficient production of high-value products. To overcome glucose catabolite repression, we developed the \textit{K. marxianus} SBK1 mutant using a directed evolutionary approach mediated by 2-DG. Following alleviation of glucose catabolite repression, \textit{K. marxianus} SBK1 demonstrated simultaneous cofermentation of glucose and xylose up to 40 °C, resulting in 23.82 g/L ethanol production with a yield of 0.35 g/g and productivity of 0.33 g/L h. Simultaneous cofermentation by the thermotolerant \textit{K. marxianus} SBK1 could offer an efficient strategy for the time- and cost-effective utilization of cellulosic biomass. Further research is required to introduce additional biosynthetic pathways for valuable products using \textit{K. marxianus} SBK1.

**Methods**

**Strains and media**

\textit{Kluveromyces marxianus} 17694-DH1, capable of fermenting xylose to ethanol, was used as the parental strain in a directed evolutionary approach [22]. For yeast inoculum preparation, pre-culture was carried out in YP media with 20 g/L glucose (YPD20) or 20 g/L xylose (YPX20). Fermentation experiments were performed using YP media containing YPD40, YPX40, or YPD40X40. To investigate the alleviation of catabolite repression, cofermentation experiments were verified using different concentrations of glucose (20–70 g/L) or xylose (20–50 g/L). \textit{Escherichia coli} TOP10 cells (KCTC 22006) were used for gene cloning and sequencing. Each gene was...
cloned into T-vector using the TOPcloner™ TA core kit (Enzynomics Inc., Korea), which was subsequently used to transform E. coli TOP10 competent cells. Transformed E. coli TOP10 cells were cultured in LB broth with 50 μg/mL ampicillin.

A directed evolutionary approach using 2-DG
A directed evolutionary approach was performed using 2-DG to isolate mutants with alleviated glucose catabolite repression. YP media containing 15 g/L 2-DG and 40 g/L xylose (2-DG/xylose media) was inoculated with K. marxianus 17694-DH1. When the concentration of xylose was almost zero, cultured cells were transferred to fresh 2-DG/xylose media at OD₆₀₀ 0.10. This process was repeated six times over approximately 50 days. Cells from the fourth transfer, which showed the highest consumption rate of xylose, were used for screening 2-DG-resistant mutants. Cells were harvested and plated on YPX₂₀ agar media containing 2-DG at an appropriate dilution. The cofermentation capabilities of ten candidates were evaluated using flask cultivations containing YPD₄₀X₄₀ media.

Fermentation experiments
Pre-cultures of K. marxianus 17694-DH1 and K. marxianus SBK1 were prepared using 5 mL YPD₂₀ and YPX₂₀, respectively, at 30 °C and 200 rpm. The cells were subsequently inoculated in a 250 mL shaking flask containing 50 mL YPD₄₀ YPX₄₀ or YPD₄₀X₄₀ with an initial OD₆₀₀ of 0.00 at 30 °C and 110 rpm. To confirm the effect of catabolite repression by K. marxianus SBK1, cofermentation experiments were performed using various concentrations of glucose (20–70 g/L) or xylose (20–50 g/L) at 30 °C. High-temperature cofermentations were carried out with 10 g/L of CaCO₃ at 40 °C.

Transcriptomic analysis
Transcriptomic analysis was performed using RNA-Seq to compare expression levels of various transcripts from K. marxianus 17694-DH1 and K. marxianus SBK1. K. marxianus 17694-DH1 and K. marxianus SBK1 were cultured in YPD₈₀ and YPD₄₀X₄₀ media, respectively. Cultured cells were sampled during the early-exponential phase and total RNA was extracted using the RNeasy® Mini Kit (Qiagen Inc., Germany) after equal adjustment of cell mass. RNA-Seq analysis was performed by a commercial service (Macrogen Inc., Seoul, Korea) using the Illumina HiSeq 4000 sequencer (Illumina Inc., CA, USA). Sequencing data from K. marxianus NBRC 1777 were used as reference.

Gene cloning and sequencing
Genomic DNA from K. marxianus 17694-DH1 and K. marxianus SBK1 was extracted using the i-genomic BYF DNA Extraction Mini Kit (iNtRON Inc., Seongnam, Korea). Target genes encoding glucokinase-1 (GLK1), hexokinase (RAG5), and phosphoenolpyruvate carboxykinase (PCK1) were amplified using Premix Taq™ (Takara Inc., Japan) and the respective PCR primers (see Additional file 7: Table S2). Each amplified gene was purified and cloned into T-vector using the TOPcloner™ TA core kit (Enzynomics Inc., Korea). Gene sequencing was performed by a commercial service (Macrogen Inc., Korea). Based on the resultant data, Lasergene (DNASTAR Inc., WI, USA) was used for sequence analysis.

Enzymatic assays for hexokinase and phosphoenolpyruvate carboxykinase
For enzymatic assay sample preparation, K. marxianus 17694-DH1 and K. marxianus SBK1 were cultured in YPX₂₀ media. Cells were harvested during the early-exponential growth phase by centrifugation and washed twice with phosphate-buffered saline (PBS) (Noble Bio., Hwaseong, Korea). Washed cells were resuspended in 2 mL assay buffer solution included in each respective enzymatic assay kit, and disrupted using a sonicator (amplitude 40%, pulse on 1 s, and pulse off 1 s for 10 min) on ice. After centrifugation at 4 °C and 18,725×g for 5 min, the resulting supernatant was used as the final cell extract for protein quantification and enzymatic assay.

Protein concentrations were determined by the Bradford method, using Bradford reagent (Biosesang Inc., Seongnam, Korea). Based on the results, enzymatic assays were carried out using equally adjusted protein concentrations of the parental and mutant strains. For enzymatic activity analysis, the Hexokinase Colorimetric Assay Kit (Biovision Inc., CA, USA) and Phosphoenolpyruvate Carboxykinase Activity Kit (Biovision Inc.) were used. Enzymatic assay procedures were as per the manufacturer’s instructions.

Analytical methods
Cell cultures were periodically sampled, and cell densities were measured using optical density (OD) at 600 nm with a GENESYS™ 10S UV–visible spectrophotometer (Thermo Inc., USA). Harvested cells were centrifuged and the concentration of various metabolites (glucose, xylose, xylitol, and ethanol) was analyzed in the resulting supernatant using a high-performance liquid chromatography (HPLC) system (1200 Series, Agilent Inc., USA) with a Rezex ROA-Organic Acid H⁺ column (Phenomenex Inc., USA). The temperature of the column and
refractive index detector was maintained at 50 °C. A solution of 0.005 N H₂SO₄ was used as the mobile phase at a flow rate of 0.6 mL/min.

Additional files

Additional file 1: Fig. S1. Results of a directed evolutionary approach from the parental strain K. marxianus 17694-DH1.

Additional file 2: Fig. S2. Comparisons of 2-DG-resistance through spotting assay onto YPX20 plates with or without 2-DG.

Additional file 3: Fig. S3. Comparisons of xylose consumption rate and ethanol production rate of the selected 2-DG-resistant candidates from glucose and xylose mixture fermentation experiments at 96 h. Symbols: xylose consumption rate (green-filled square) and ethanol production rate (red-filled square).

Additional file 4: Fig. S4. Time profiles of cofermentation in mixed culture of glucose and xylose with various concentrations of glucose from 20 g/L to 70 g/L by the mutant K. marxianus SBK1. Symbols: glucose (blue-filled square), xylose (green-filled circle), OD (open diamond), xylitol (grey-filled upward pointing triangle), and ethanol (red-filled downward pointing triangle).

Additional file 5: Fig. S5. Time profiles of cofermentation in mixed culture of glucose and xylose with various concentrations of xylose from 20 g/L to 50 g/L by the mutant K. marxianus SBK1. Symbols: glucose (blue-filled square), xylose (green-filled circle), OD (open diamond), xyitol (grey-filled upward pointing triangle), and ethanol (red-filled downward pointing triangle).

Additional file 6: Table S1. Substitutions of amino acid sequences from key enzymes of glycolysis (GLK1, HK) and gluconeogenesis (PEPCK) pathways.

Additional file 7: Table S2. Primers used in this study.

Abbreviations

SSF: Simultaneous saccharification and fermentation; SSCF: Simultaneous saccharification and cofermentation; 2-DG: 2-Deoxyglucose; RNA-Seq: RNA-sequencing; YP: Yeast extract-peptone; OD: Optical density; GLK1: Glucokinase-1; HK: Hexokinase; PEPCK: Phosphoenolpyruvate carboxykinase; LB: Luria Broth; PCR: Polymerase chain reaction; PBS: Phosphate-buffered saline; HPLC: High-performance liquid chromatography.

Authors’ contributions

All authors contributed to the conception and planning of the study. SBK conducted most of experiments. SBK, DHK, and JBP analyzed the data. SBK and SJH wrote and revised the manuscript. All authors read and approved the final manuscript.

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Not applicable.

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Consent for publication

All authors consented on the publication of this work.

Ethics approval and consent to participate

Not applicable.

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