Metabolomic and Transcriptomic Analysis for Rate-Limiting Metabolic Steps in Xylose Utilization by Recombinant Candida utilis

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We have reported that a recombinant Candida utilis strain expressing a Candida shehatae xylose reductase K275R/N277D, a C. shehatae xylitol dehydrogenase, and xylulokinase from Pichia stipitis produced ethanol from xylose, but its productivity was low. In the present study, metabolomic (CE-TOF MS) and transcriptomic (microarray) analyses were performed to characterize xylose metabolism by engineered C. utilis and to identify key genetic changes contributing to efficient xylose utilization. The metabolomic analysis revealed that the xylose-fermenting strain accumulated more pentose phosphate pathway intermediates, more NADH, and more glycolytic intermediates upstream of glyceraldehyde 3-phosphate than the wild-type. The resulting strain exhibited a 17% increase in ethanol productivity and a 22% decrease in xylitol accumulation relative to control.

Key words: Candida utilis; xylose; ethanol; metabolomic analysis; transcriptomic analysis

In the past few decades, increasing attention has been paid to methods of improving the production of bioethanol from lignocellulosic biomass. Plant-derived lignocellulosic biomass contains glucose and considerable amounts of pentoses, particularly xylose.1) However, because xylose is not a substrate naturally utilized by the vast majority of fermentative yeasts, including Saccharomyces cerevisiae, effective efforts to redesign metabolic capabilities have been undertaken to obtain strains producing pentose-derived ethanol. For example, to facilitate xylose assimilation, an S. cerevisiae strain was transformed with the genes encoding xylose reductase (XR) and xylitol dehydrogenase (XDH) from the xylose-fermenting yeast Pichia stipitis, and with the S. cerevisiae gene encoding xylulokinase (XK).2) While the engineered S. cerevisiae strain grew on xylose, ethanol productivity was low, as a substantial amount of the xylose was excreted as xylitol.

Overall xylose metabolism in xylose-utilizing recombinant S. cerevisiae strains has been analyzed by transcriptomic, proteomic, and metabolomic analyses.3–7) A moderate increase in the transcript levels of some of the genes encoding the enzymes of the pentose phosphate pathway, as well as in the flux through the pathway, has been observed in xylose-grown cells.3,4) Additionally, the expression of several genes encoding redox-related enzymes was enhanced.5,7) Jin et al. observed upregulation of the genes encoding the enzymes involved in tricarboxylic acid (TCA) cycle and gluconeogenesis, and of respiratory genes under oxygen limitation in cells grown on xylose relative to glucose-repressed cells.7) Furthermore, to prevent higher respiratory activity, they obtained a petite respiration-deficient mutant of the engineered S. cerevisiae strain. This mutant was able to produce more ethanol and accumulated less xylitol from xylose, although the strain did not grow on xylose.

The complete genome of the native xylose-fermenting yeast P. stipitis has been sequenced, and global gene expression has been analyzed.8) Genes encoding certain pentose phosphate pathway enzymes and TCA cycle enzymes were strongly induced on a medium containing xylose. These changes were thought to contribute to the maintenance of redox balance and efficient utilization of xylose under microaerobic conditions. Hence omics technologies are used as a tool to evaluate potential metabolic steps limiting xylose utilization, but no reports have described high-throughput analyses of xylose metabolism by other yeasts.

Candida utilis is an industrially important microorganism currently used to produce a number of valuable chemicals, including glutathione and RNA.9–11) Since the development of an efficient electroporation-based method of transforming it, it has been used for heterologous production.12–17) Because it belongs to the Crabtree-negative group of yeasts, it can grow vigorously under strict aerobic conditions and has been reported to have the highest respiration activity among these yeasts.18) Anaerobic fermentation based on high cell density can enhance production efficiency, and many such results have been reported for C. utilis.19) Hence we speculated that it would produce more ethanol from xylose than other yeasts.

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We reported the construction of a xylose-fermenting strain based on *C. utilis*. Wild-type *C. utilis* grows on xylose but does not ferment it to ethanol. This deficiency might be due to low activity of NADPH-prefering XR and NAD⁺-dependent XDH, which together convert xylose to xylulose, or to an imbalance in coenzyme utilization at the steps catalyzed by XR and XDH, because *C. utilis* can ferment xylulose. Hence we constructed a recombinant *C. utilis* strain overexpressing an NADPH-prefering *Candida shehatae* XR, *C. shehatae* XDH and *P. stipitis* XK. The resulting strain exhibited dramatically higher consumption of xylose than the control strain. Furthermore, the introduction of an NADH-prefering CsheXR enzyme (K275R/N277D) constructed by site-directed mutagenesis improved efficiency of ethanol production, but the improvements in ethanol productivity and yield were modest. Hence the aim of the present study was to characterize xylose metabolism by the engineered *C. utilis* strain by CE-TOF MS and DNA microarray analysis and to identify key genetic changes contributing to efficient xylose utilization.

**Materials and Methods**

**Strains and media.** The wild-type yeast *C. utilis* strain NBRC0988 was obtained from the National Institute of Technology and Evaluation Biological Research Center (Chiba, Japan). The genetic background of TMS174 encompasses genetic changes previously identified to be beneficial for ethanol production from xylose.Cells were cultured at 30°C in YPD2 medium (10 g/L of yeast extract, 20 g/L of peptone, and 20 g/L of glucose). Solid media were made with 2% agar. Hygromycin B (HygB, Wako Pure Chemical Industries, Osaka, Japan) and cycloheximide (Cyh, Wako) were added to YPD2 to final concentrations of 600 and 40 mg/L respectively to select transformants. *Escherichia coli* DH5α was grown in LB (10 g/L of tryptone, 5 g/L of yeast extract, and 5 g/L of NaCl) containing 100 mg/L of ampicillin, and was transformed by standard methods.

**Growth conditions for metabolomic and transcriptomic analyses.** The strains were precultured with shaking at 30°C for 1 d in 100 mL of YPD2, and cells were harvested by centrifugation over 10 min at 4°C. Sakaguchi flasks (500 mL) containing 100 mL ofYPD medium (10 g/L of yeast extract, 20 g/L of peptone, and 50 g/L of glucose) or YPX medium (10 g/L of yeast extract, 20 g/L of peptone, and 50 g/L of xylose) were inoculated to an OD₆₀₀ of 0.1. The cells were then grown semiaerobically at 30°C (100 rpm). Samples collected at OD₆₀₀ of 5–15 were used as sources of RNA and intracellular metabolites for DNA microarray and CE-TOF MS respectively. An OD₆₀₀ of 1 corresponded to approximately 0.46 g of cells (dry weight)/L.

**Extraction of intracellular metabolites and CE-TOF MS analysis.** Intracellular metabolites were extracted using a modification of a previously described procedure. Cells were harvested from culture media by filtration through a filter of 0.45 µm pore size. Internal standard for cations and anions were purchased from Human Metabolome Technologies (Tsunouka, Japan). Lyophilized samples were dissolved in 50 µL of Milli-Q water before CE-TOF MS analysis. All CE-TOF MS experiments were performed by the Agilent Capillary Electrophoresis System, equipped with an air pressure pump, an Agilent 1100 series MSD mass spectrometer, an isotropic HPLC pump, a G1603A Agilent CE-MS adapter kit, and a G1607A Agilent CE-TOF MS sprayer kit (Agilent Technologies, Tokyo, Japan) according to the manufacturer’s instructions.

**Isolation of RNA and DNA microarray analysis.** DNA microarray experiments were carried out using *C. utilis* 8 × 15k oligoarrays (Agilent’s eArray). Each oligoarray consisted of 15,208 spotted probes (60 bp each) designed on the basis of the *C. utilis* genome sequence. In the array, two probes derived from single genes were spotted for each of 6,604 genes. Total RNA was extracted using glass beads, and was purified using an RNAeasy column (QIAGEN, Hilden, Germany). Aliquots of 0.5 µg of RNA were labeled with a Quick Amp Labeling Kit, One-Color (Agilent Technologies) following the manufacturer’s instructions. Hybridization of labeled cRNA to the arrays was performed using the manufacturer’s hybridization protocol. Microarrays were washed, dried, and scanned on a DNA microarray scanner (Agilent Technologies, Model G2565BA). Feature Extraction and Image Analysis software programs were used. Signal intensities were normalized by 75% quantile normalization. Gene ontology (GO) analysis was performed by means of GeneSpring software (Agilent Technologies, version 12.0). Transcriptional data are presented as mean values of normalized signal intensities derived from two probes.

**Other analyses.** Glucose, xylose, xylitol, acetate, and ethanol were analyzed as described previously. Supernatant solutions were subjected to high-pressure liquid chromatographic (HPLC) analysis using an ICSep-ION-300 column (Tokyo Chemical Industry, Tokyo). The column was operated at 60°C at a flow rate of 0.4 mL/min with 0.01 N sulphuric acid as solvent.

**Recombinant DNA techniques.** Standard recombinant DNA techniques were used, or were done as instructed by suppliers. PCR was performed on genomic DNA with KOD plus (Toyobo, Osaka, Japan). Nucleotide sequences were determined with an ABI3310 xl DNA analyzer (Applied Biosystems, Foster City, CA).

**Construction of plasmids.** Expression vectors for *ADH1*, *ADH2* and *TDH1* were constructed in pCU155,12,25 a multi-copy integration terminator, and a cycloheximide resistance marker flanked by two

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**Fig. 1.** Representative Time Course Studies of Semi-Aerobic Fermentation for Wild-Type *C. utilis* Grown on Glucose (A), Xylose (B), and TMS174 Grown on Xylose (C). Arrows indicate the time of quenching of cells. Symbols: Glucose (solid circles), ethanol (hollow circles), biomass (solid triangles), xylose (hollow triangles), and xylitol (solid squares). Data are means of three independent fermentations and error bars indicate SD values (A and B). Several error bars are too small to be seen. Data are means for two independent courses of fermentation (C).
Fig. 2. Metabolites Involved in Central Carbon Metabolism.

Metabolomic data obtained for wild-type *C. utilis* grown on glucose and for wild-type *C. utilis* and TMS174 grown on xylose are shown as bars from left to right respectively. The arbitrary concentration of each metabolite is expressed as the ratio of the internal standard (methionine sulfoxide) to yeast biomass (an arbitrary amount of cells equivalent to an OD$_{600}$ of 10). Three extractions from one sample were performed, and each extract was analyzed by CE-TOF MS. Data are means of 6–9 samples (two or three independent growth experiments were performed, see Fig. 1 legend). Error bars indicate SD values. Abbreviations: 1,3PG, 1,3-bisphosphoglycerate; DHAP, dihydroxyacetonephosphate; E4P, erythrose-4-phosphate; F1,6P, fructose-1,6-bisphosphate; F6P, fructose-6-phosphate; GAP, glyceraldehyde-3-phosphate; G6P, glucose-6-phosphate; PEP, phosphoenolpyruvate; 6PG, 6-phosphogluconate; 2PG, 2-phosphoglycerate; 3PG, 3-phosphoglycerate; R5P, ribose-5-phosphate; Ru5P, ribulose-5-phosphate; S7P, sedoheptulose-7-phosphate; X5P, xylulose-5-phosphate; ND, not detectable.

BglII sites. The *ADH1, ADH2, and TDH1* genes were amplified with TM-411 (CTAGCTAGATGTTGCATGCAAAACCCATC)/TM-414 (CGCGGATCCTCACAACGAATTCATCAGTGTAACC), TM-380 (CTAGACTAGTATGTCTGAGATTCCAAAGACGC)/TM-381 (CGCAGATCTTCACTTGGAAGTGTCAACAACAT) and TM-415 (CTAGTCTAGATGTCAATTCCAAAGACACAGAAGG)/TM-422 (CGCGGATCCTATTGGAAGTGTCAACAACCG) primers respectively, with genomic DNA as template. The PCR products were digested with *XhoI* and *BanHI*, and were ligated to *XhoI*-digested plasmids.

Yeast transformation and strain construction. *C. utilis* was transformed by electroporation as described previously. 26) In order to construct recombinant strains, *C. utilis* was transformed with BglII-digested plasmids.
Fermentation. Cells were cultured aerobically in YPD2 medium for 24 h at 30°C. They were collected by centrifugation for 10 min at 4°C, washed with YPX5 medium, and used to inoculate fresh fermentation medium at a starting OD600 of 1. Fermentation for ethanol production was carried out at 30°C with gentle shaking (100 rpm) in 100 mL spherical flat-bottom flasks containing 30 mL of YPX5 medium.

Measurement of NAD/NADH. Cells growing in YPD5 medium and in YPX5 were collected by centrifugation over 10 min at 4°C and were washed with PBS. Samples were collected at 5 of OD600. Extraction and measurement of intracellular NAD/NADH were done using an NAD/NADH quantification kit (BioVision) according to the manufacturer’s instructions.

Nucleotide sequence and microarray data accession numbers. The nucleotide sequence accession numbers for ADH1, ADH2 and TDIH are AB649224, AB641823, and AB649225 respectively. The microarray data were registered in the Gene Expression Omnibus (GEO) database under accession no. GSE43514.

Results

Cultivation of C. utilis NBRC0988 and TMS174

Wild-type strain NBRC0988 and xylose-fermenting recombinant strain TMS174 exhibited approximately equal performance on YPD5 (e.g., growth rate, glucose consumption rate, ethanol productivity, and final ethanol concentration; data not shown). Hence strains and cultivation conditions were chosen to elicit three different physiological states that are henceforth abbreviated “wild-type strain grown on YPD5” (WG), “wild-type strain grown on YPX5” (WX), and “xylose-fermenting recombinant strain TMS174 grown on YPX5” (RX).

The wild-type strain inoculated into YPD5 consumed glucose completely by 24 h and produced 19.6 g/L of ethanol. After glucose depletion, the wild-type strain continued to grow on ethanol (Fig. 1A). In YPX5 medium, the wild-type strain consumed 12.8 g/L of xylose with a slight accumulation of xylitol (1.9 g/L) by 48 h, while no ethanol was produced (Fig. 2B). TMS174 consumed 40.9 g/L of xylose, and accumulated 4.3 g/L of xylitol and produced 9.4 g/L of ethanol in 48 h. At 48 h, acetic acid was detected only in the RX culture (0.05 g/L).

The concentrations of intracellular metabolites and the gene expression levels in the cultured cells were affected by the culture environment, including concentrations of residual sugars, dissolved oxygen, and sources of stress (e.g., ethanol).5,6 In order to identify the physiological changes associated with carbon sources, we analyzed intracellular metabolites and gene expression for WG, WX, and RX during exponential growth after 10.5, 24, and 24 h, respectively, when the sugar concentrations in each culture were the same, approximately 40 g/L. At sampling, the OD600 values were 5.7, 10.4 and 13.5 respectively (Fig. 1).

Intracellular metabolite profiling

We measured 25 metabolites in central metabolism (the glycolytic pathway, the TCA cycle, and the pentose phosphate pathway), 19 amino acids, and 10 high-energy metabolites in the cultured cells by CE-TOF MS. The overall concentration of glycolytic metabolites was highest in the WG culture (Fig. 2). A greater accumulation of glycolytic intermediates upstream of glyceraldehyde 3-phosphate (GAP), and of pentose phosphate pathway intermediates—except for erythrose 4-phosphate—was observed in the RX culture than in the WX culture. In contrast, the levels of glycolytic intermediates downstream of GAP and of TCA cycle metabolites were lower in the RX culture than in the WX culture.

The intracellular concentrations of high energy metabolites and redox metabolites is pivotal for cell proliferation, because anaerobic growth of engineered S. cerevisiae on xylose is known to be limited by ATP production and the intracellular redox imbalance.3,4 As shown in Fig. 3, even though ATP levels did not change significantly with carbon source or genetic manipulation, differences in redox metabolite levels were observed. For example, the NADH levels in the RX culture increased significantly, 3.2- and 4.0-fold, relative to WG and WX, while the NAD+ levels were almost unchanged, indicating an overall increase in NADH/ NAD+ ratio. Furthermore, the NADPH levels in the RX culture also increased significantly, 1.2- and 2.2-fold, relative to WG and WX.

Gene expression profiling

Scatter plots of the gene expression data for the WG versus the WX culture and for the WG versus the RX culture varied much more than between the WX and the RX culture, indicating that the difference in carbon source had a much greater effect on gene expression than the genetic modifications (Supplemental Fig. S1; See Biosci. Biotechnol. Biochem. Web site). The transcript levels of most glycolytic genes did not change significantly with carbon source or genetic manipulation (Fig. 4). In contrast, expression of the genes involved in the pentose phosphate pathway, gluconeogenesis, and the TCA cycle increased significantly in the WX and the RX culture. Specifically, the expression of FBP1, which encodes fructose-1,6-bisphosphatase, increased 9.1- and 5.8-fold in the WX and the RX culture relative to the WG respectively. Even though the expression of the genes encoding fermentative enzymes (e.g., Adh1p and Pdc1p) involved in ethanol production did not increase during growth on the two carbon sources, the expression of ADH2, which is involved in the oxidation of ethanol, increased significantly during growth on xylose. Fur-
Moreover, a GO analysis of those genes whose expression increased >2-fold relative to the WG culture, indicated a significant enrichment in genes associated with the GO term "aerobic respiration" (accession GO:0009060), \( p < 0.05 \). These results indicate that xylose metabolism in \textit{C. utilis} is oxidative.

We also analyzed the expression of the genes involved in redox homeostasis, because these systems are necessary to maintain a neutral cytoplasmic redox balance during respiratory growth.\(^{27,28}\) In the RX culture, the transcript levels of the genes encoding known NADH/NAD\(^+\) shuttle systems, including \textit{ADH3}, \textit{ALD6-M}, \textit{GPD1}, \textit{GUT2}, and \textit{NDI1}, increased significantly (Fig. 5). Transcripts for several enzymes involved in the malate-oxaloacetate shuttle or malate-pyruvate shuttle, encoded by \textit{MDH1}, \textit{MDH2}, \textit{PYC1}, and \textit{MAE1}, were induced 2.3-, 4.5-, 1.4-, and 5.1-fold respectively. In contrast, the expression of \textit{NDE1}, encoding a
mitochondrial external NADH dehydrogenase, decreased relative to WG. This suggests that NADH/NAD⁺ shuttle systems are responsive to cytosolic and mitochondrial redox balances in xylose metabolism.

Redirection of metabolic flux to ethanol by overexpression of ADH1

Increasing ADH2 transcript levels result in cytoplasmic NADH production, it can also reduce ethanol production. To improve xylose fermentation by the engineered C. utilis strain, we focused on Adh1p, which converts acetaldehyde to ethanol by oxidizing NADH. We tried to maintain the intracellular redox balance, and to increase the metabolic flux towards ethanol production, by overexpressing ADH1 in the engineered C. utilis.

The intracellular NADH/NAD⁺ ratios in the recombinant strain were measured at mid-exponential phase (Table 1). The ADH1-overexpressing strain exhibited a lower NADH/NAD⁺ ratio relative to the control strain in YPX5, while ADH2 overexpression led to an increased NADH/NAD⁺ ratio in both YPD5 and YPX5.

The ADH1-overexpressing strain exhibited a 17% increase in ethanol production and a 22% decrease in xylitol accumulation as compared to TMS174, although the xylose consumption rate was unchanged (Fig. 6 and Table 2). In contrast, the ADH2-overexpressing strain exhibited a 13% decrease in ethanol production and a 33% decrease in xylitol accumulation.

Discussion

As previously reported, metabolomics is a powerful tool to gain information on cell physiology. Analysis of a number of metabolites, and tracking concentration changes under various physiological and genetic conditions, provide direct measures of metabolic phenotypes that complement gene expression analysis. Our results indicated three main points. First, an excess of NADH can limit xylose utilization by the engineered C. utilis.

### Table 1. Intracellular NADH/NAD⁺ Ratio in Recombinant C. utilis

<table>
<thead>
<tr>
<th>Strain</th>
<th>Medium</th>
<th>YPD5</th>
<th>YPX5</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMS174/Empty</td>
<td></td>
<td>0.69 ± 0.05</td>
<td>0.87 ± 0.09</td>
</tr>
<tr>
<td>TMS174/ADH1</td>
<td></td>
<td>0.67 ± 0.04</td>
<td>0.71 ± 0.06</td>
</tr>
<tr>
<td>TMS174/ADH2</td>
<td></td>
<td>0.95 ± 0.07</td>
<td>1.12 ± 0.09</td>
</tr>
<tr>
<td>TMS174/TDH1</td>
<td></td>
<td>0.65 ± 0.06</td>
<td>0.91 ± 0.08</td>
</tr>
</tbody>
</table>

Data are means ± SD for three independent experiments.

Abbreviations: G3P, glycerol-3-phosphate; bc₁, bc₁ complex; cox, cytochrome c oxidase. C. utilis gene names were assigned based on homology to S. cerevisiae and Candida albicans.
While levels of the sense transcript decreased, the antisense transcript of *C. utilis ADH1* indicates that Adh1p is one of the rate-limiting enzymes relative to control (Fig. 6 and Table 1). This itself suggests that the increase from xylose with decreased xylitol formation relative to control (Fig. 6 and Table 1). This itself indicates that Adh1p is one of the rate-limiting enzymes in xylose utilization by the recombinant *C. utilis* strain. The antisense transcript of *C. utilis ADH1* has been reported to be expressed during the stationary phase, while levels of the sense transcript decreased. Thus overproduction of the sense transcript of *ADH1* might have increased the flux from acetaldehyde to ethanol, leading to an improvement in ethanol production.

Another possible cause of this improvement is that the decrease in the NADH/NAD<sup>+</sup> ratio increased the reactivity of glyceraldehyde 3-phosphate dehydrogenase (GAPDH, encoded by *TDH1* in *C. utilis*). Metabolomic analysis of the engineered *C. utilis* grown on xylose indicated an accumulation of glycolytic intermediates upstream of GAP and NADH (Fig. 2). This suggests that GAPDH is the rate-limiting enzyme in xylose utilization. However, overexpression of *TDH1* did not influence xylose fermentability or the NADH/NAD<sup>+</sup> ratio (Fig. 6 and Table 1). In general, mammalian and yeast GAPDH are inhibited by several factors, including ATP, NADH, and reactive oxygen species. Thus the reaction catalyzed by GAPDH might have been limited not by low enzymatic activity but rather by excess NADH.

A significant increase in the expression of the genes involved in gluconeogenesis and the oxidative pentose phosphate pathway, including *ZWF1* and *GND1*, was observed in the engineered *C. utilis* strain grown on xylose (Fig. 4). This suggests the possibility that carbon flux to gluconeogenesis and the oxidative pentose phosphate pathway increased in the engineered *C. utilis* strain grown on xylose. This increase can lead to NADPH accumulation, because glucose-6-phosphate dehydrogenase (encoded by *ZWF1*) and 6-phosphogluconate dehydrogenase (encoded by *GND1*), which are involved in the oxidative pentose phosphate pathway, generate NADPH. In fact, the engineered *C. utilis* strain grown on xylose accumulated not only NADH but also NADPH (Fig. 3). This speculation about carbon flux is consistent with our hypothesis regarding GAPDH inhibition.

The present study identified rate-limiting factors in a metabolic pathway by combined metabolomic and transcriptomic analysis. This was followed by the

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**Table 2. Batch Fermentation of Xylose by Recombinant Strains of *C. utilis***

<table>
<thead>
<tr>
<th>Strain</th>
<th>Ethanol produced (g/L)</th>
<th>Xylitol produced (g/L)</th>
<th>Xylose consumed (g/L)</th>
<th>Yield (g product/g of consumed xylose)</th>
<th>Time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMS174/Empty</td>
<td>13.2 ± 1.1</td>
<td>6.6 ± 0.5</td>
<td>45.4 ± 3.1</td>
<td>0.289 ± 0.012</td>
<td>82</td>
</tr>
<tr>
<td>TMS174/ADH1</td>
<td>15.5 ± 0.9</td>
<td>5.2 ± 0.4</td>
<td>48.7 ± 2.9</td>
<td>0.318 ± 0.015</td>
<td>96</td>
</tr>
<tr>
<td>TMS174/ADH2</td>
<td>11.5 ± 0.8</td>
<td>4.5 ± 0.3</td>
<td>49.6 ± 4.5</td>
<td>0.232 ± 0.021</td>
<td>144</td>
</tr>
<tr>
<td>TMS174/TDH1</td>
<td>12.8 ± 0.9</td>
<td>6.7 ± 0.4</td>
<td>45.1 ± 4.4</td>
<td>0.283 ± 0.011</td>
<td>82</td>
</tr>
</tbody>
</table>

Fermentation parameters are summarized from data shown in Fig. 6. Data are means ± SD for three independent experiments.

<sup>14</sup> "Time" indicates when the ethanol concentration was maximal.
introduction of genetic modifications to overcome these bottlenecks in order to increase ethanol production from xylose in an industrially important yeast, C. utilis. Our results suggest possible routes towards improving the fermentative utilization of hemicellulose-derived sugars.

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References