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Display of cellulases on the cell surface of *Saccharomyces cerevisiae* for high yield ethanol production from high-solid lignocellulosic biomass

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ABSTRACT

Economically feasible processes for industrial cellulosic ethanol production requires increasing the final ethanol titer during fermentation due to the high energy demands of the subsequent ethanol distillation. In the present study, high-yield ethanol production was achieved by short-term liquefaction and fermentation of lignocellulose biomass in a novel drum-type rotary fermentation system using a yeast strain developed for cell-surface display of fungal endoglucanase, cellobiohydrolase, and β-glucosidase. In the presence of 10 FPU/g-biomass cellulase added, the recombinant cellulolytic strain produced 1.4-fold higher ethanol (89% of theoretical yield) from high-solid (200 g-dry weight/L) rice straw within 72 h of fermentation than wild type strain. Cell-surface engineering successfully reduced the amount of commercial enzyme required for the fermentation of cellulose. This study demonstrates that cellulases displayed on the yeast cell surface are capable of hydrolyzing cellulose that was not hydrolyzed by commercial cellulases, leading to increased sugar utilization for improved ethanol production.

1. Introduction

Numerous environmental and social benefits are anticipated from the replacement of petroleum-based transport fuels with bioethanol converted from lignocellulosic materials such as agricultural residues and industrial waste. However, establishing an economically feasible process for industrial cellulosic ethanol production requires markedly increased ethanol titers at the completion of the fermentation process due to the high energy demands of the subsequent ethanol distillation (Galbe and Zacchi, 2007). In addition, increasing the final ethanol titer would have a significant effect on lowering capital and production costs due to the reduced size of the necessary equipment, such as fermentation tanks and distillation columns (Mussatto et al., 2010; Wingren et al., 2003).

Achieving higher ethanol titers inevitably requires higher cellulose-solid loading in the simultaneous saccharification and fermentation (SSF) step, as increasing the solid concentration results in corresponding increases in ethanol production. Pretreatment of raw lignocellulosic biomass with diluted-acid, liquid hot water, or treatments and steam explosions has yielded cellulosic materials in solid form that contain approximately 20–40% (w/w) dry matter (Pérez et al., 2008; Tucker et al., 2003; Zhang et al., 2010). However, operating the SSF at solid concentrations exceeding approximately 10% (w/w) poses a number of technical problems, as the water content of substrates during SSF is directly correlated to the rheology of the fermentation mixture, which is important for the interaction between lignocellulosic materials and cellulolytic enzymes (Pimenova and Hanley, 2004). Upon increasing the solid lignocellulose content in the bioreactor, solids and enzymes become insufficiently mixed. In addition, a low water content increases the viscosity of the substrate material, which also makes mixing within the bioreactor more difficult and leads to decreased ethanol yields.

To achieve sufficient mixing during SSF, several bioreactor configurations with gravimetric (Jørgensen et al., 2006) or helical stirring systems (Zhang et al., 2010) have been developed that successfully maintain the hydrolysis of lignocellulose to ethanol. However, water is not only critical for hydrolysis reactions, but is also crucial for the accessibility of enzymes to the cellulosic substrates. Therefore, a large amount of enzyme is typically required for the efficient hydrolysis of lignocellulose, which potentially decreases the feasibility of industrial bioethanol production due to the high cost associated with enzyme supplementation.

To overcome these limitations, high-solid lignocellulose was hydrolyzed and fermented using a recombinant strain of *Saccharomyces cerevisiae* displaying three types of cellulases (endoglucanase [EG], cellobiohydrolase [CBH], and β-glucosidase [BGL]) on the cell surface. A novel fermentation system was employed in which the fermentation vessel was rotated under controlled temperature. In the vessel, solid lignocellulosic materials were agitated

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by gravity to facilitate their mixing with an added cellulase solution. In this study, the combination of an effective fermentation system and cell-surface engineering of yeast led to improved cellulosic ethanol production.

2. Methods

2.1. Materials

A water insoluble fraction of rice straw obtained by liquid hot water treatments (Alvira et al., 2010) at 130–300 °C under a pressure of less than 10 MPa was purchased from Mitsubishi Heavy Industries, Ltd. (Tokyo, Japan) and separated from the liquid fraction by filtration with a mesh filter and used as cellulosic material. In order to determine its composition, the cellulosic material was hydrolyzed by sulfuric acid as described previously (Sluiter et al., 2008). The composition of the cellulosic material was 43.0% (w/w)glucan, 2.0% (w/w)xylan, 42.3% (w/w)ash and lignin, and 12.7% (w/w) other materials.

2.2. Construction of plasmids

The plasmids used in this study are listed in Table 1. A 2719-bp DNA fragment that included the secretion signal sequence of the *Rhizopus oryzae* glucoamylase gene and the *Trichoderma reesei* endoglucanase gene, *EG2*, fused to the 3′-region encoding 320 amino acids of *S. cerevisiae* α-agglutinin was amplified by PCR using plasmid pEG23u31H6 (Fujita et al., 2002) as template DNA with primers 5′-ctagctagcatgcaactgttcaatttgccattgaaag-3′ and 5′-tccccccgggtttgattatgttctttctatttgaatgagatatg-3′. The amplified fragment was digested with *Nhe*I and *Xma*I and cloned into pGK406 (Ishii et al., 2009) to yield pGK406-EG2. After digestion of pGK406-EG2 with *Apa*I and *Not*I, the resultant EG2 fragment was ligated into the pRS403 and pRS405 vectors (Yamada et al., 2010) to yield pGK403EG2 and pGK405EG2, respectively.

A DNA fragment composed of the *S. cerevisiae TDH3* promoter, secretion signal sequence of the *R. oryzae* glucoamylase gene, *T. reesei CBH2* gene, 3′-region encoding 320 amino acids of *S. cerevisiae* α-agglutinin, and *TDH3* terminator was amplified by PCR using pFCBH2w3 (Fujita et al., 2004) as template DNA with primers 5′-aaggaaaaaagcggccgcaccagttctcacacggaacaccac-3′ and 5′-aaggaaaaaagcggccgctcaatcaatgaatcgaaaatgtcattaaaatag-3′. The amplified fragment was digested with *Not*I and ligated into pGK403EG2, pGK405EG2, pGK406EG2, pRS405, and pRS406 to yield pGK403EG2-CBH2, pGK405EG2-CBH2, pGK406EG2-CBH2, pRS405CBH2, and pRS406CBH2, respectively.

The plasmid used for cell-surface display of *Aspergillus aculeatus* BGL was constructed as follows. A DNA fragment composed of the *TDH3* promoter, secretion signal sequence of the *R. oryzae* glucoamylase gene, *A. aculeatus BGL1*, 3′-region of *S. cerevisiae* α-agglutinin, and *TDH3* terminator was amplified by PCR using pIBG13 (Katahira et al., 2006) as template DNA with primers 5′-aaggaaaaaagcggccgcaccagttctcacacggaacaccac-3′ and 5′-aaggaaaaaagcggccgctc aatcaatgaatcgaaaatgtcattaaaatag-3′. After digestion with *Not*I, the amplified fragment was ligated into pRS404 to yield pIWBGL.

2.3. Yeast transformation

The yeast transformants constructed in this study are summarized in Table 1. NBRC1440ΔHUWL (Yamada et al., 2010), which was generated from NBRC1440 by deleting four auxotrophic genes (*HIS3*, *LEU2*, *TRP1*, and *URA3*), was used as a host strain for cell surface expression. Plasmids were transformed into *S. cerevisiae* NBRC1440ΔHUWL by the lithium acetate method (Hasunuma et al., 2011) using a YEASTMAKER yeast-transformation system (Clontech Laboratories, Inc., Palo Alto, CA). Transformants were selected on synthetic dextrose medium supplemented with appropriate amino acids and nucleotides.

2.4. Enzyme assay

After aerobic cultivation in yeast extract, peptone and dextrose (YPD) medium for 72 h at 30 °C, cells were collected by centrifugation at 6000*g* for 10 min, washed once with distilled water, and then resuspended in 50 mM citrate buffer (pH 5.0) containing 5 g/L phosphoric acid-swollen cellulose (PASC) with the optical density (OD) at 600 nm adjusted to 5.0. The cellulase activity of yeast strains was determined at 50 °C, as described previously (Apiwatanapiwat et al., 2011).After hydrolysis, the mixture was centrifuged at 20,000*g* for 5 min, and the amount of reducing sugar in the supernatant was measured using the Somogyi–Nelson method (Wood et al., 1988). One unit of enzyme activity was defined as the amount of enzyme required to produce 1 μmol reducing sugar per minute.

Table 1
Characteristics of the *S. cerevisiae* strains and plasmids used in this study.

Strain or plasmid	Description	Reference or source
S. cerevisiae strains		
NBRC1440	Wild type	NBRC[a]
NBRC1440ΔHUWL	MATα his3 leu2 trp1 ura3	Yamada et al. (2010)
NBRC1440/B-EC	NBRC1440ΔHUWL (pIWBGL, pGK403EG2-CBH2, pRS405, pRS406)	This study
NBRC1440/B-EC-C	NBRC1440ΔHUWL (pIWBGL, pGK403EG2-CBH2, pRS405CBH2, pRS406)	This study
NBRC1440/B-EC-C2	NBRC1440ΔHUWL (pIWBGL, pGK403EG2-CBH2, pRS405CBH2, pRS406CBH2)	This study
NBRC1440/B-EC2	NBRC1440ΔHUWL (pIWBGL, pGK403EG2-CBH2, pGK405EG2-CBH2, pRS406)	This study
NBRC1440/B-EC3	NBRC1440 ΔHUWL (pIWBGL, pGK403EG2-CBH2, pGK405EG2-CBH2, pGK406EG2-CBH2)	This study
Plasmids		
pRS405	LEU2, no expression (control plasmid)	Yamada et al. (2010)
pRS406	URA3, no expression (control plasmid)	Yamada et al. (2010)
pIWBGL	TRP1, surface expression of BGL1	This study
pGK403EG2	HIS3, surface expression of EG2	This study
pGK403EG2-CBH2	HIS3, surface expression of CBH2 and EG2	This study
pGK405EG2-CBH2	LEU2, surface expression of CBH2 and EG2	This study
pGK406EG2-CBH2	URA3, surface expression of CBH2 and EG2	This study
pRS405CBH2	LEU2, surface expression of CBH2	This study
pRS406CBH2	URA3, surface expression of CBH2	This study

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2.5. Liquefaction and fermentation

Liquefaction of cellulosic material was performed in a 50-mL polypropylene tube (Corning Inc., NY) as a fermentation vessel, which was set in a heat block (Thermo Block Rotator SN-06BN; Nissin, Tokyo, Japan). The vessel was closed with a silicon plug (AS ONE, Osaka, Japan), into which a hole was bored using a disposable needle (φ = 0.6 mm) (Terumo Corp., Tokyo, Japan). In the vessel, cellulosic material (2 g dry weight) was mixed with 8 mL fermentation medium containing 10 g/L yeast extract, 20 g/L peptone, and 50 mM citric acid buffer (pH 5.0), and cellulase (Cellulase SS; Nagase ChemteX Corp., Osaka, Japan) at concentrations of 5–100 FPU/g-biomass by axially rotating the vessel at 35 rpm under a controlled temperature of 50 °C. Fermentation was initiated by the addition of yeast into the vessel.

S. cerevisiae strains used for fermentation were propagated under aerobic conditions at the optimal growth temperature of 30 °C for 48 h in 500 mL YPD medium. The yeast cells were collected by centrifugation at 4000*g* for 10 min at 4 °C, washed twice with distilled water, and 2 mL 500 g-wet cell/L yeast was added into the vessel. During fermentation, the temperature of the fermentor was maintained at 35 °C to increase cellulases activity. Sugars and ethanol were analyzed by HPLC, as described previously (Hasunuma et al., 2011).

2.6. Compositional analysis of biomass

Hydrolysis of cellulosic materials was performed with sulfuric acid according to the NREL 2006 method (Sluiter et al., 2008) with minor modifications. Briefly, 30 mg-dry weight of fermentation residue was transferred into a 1.5-mLEppendorf tube containing 300 μL of 72% sulfuric acid. The sample was hydrolyzed at 30 °C for 60 min, diluted with deionized water to give an acid concentration of 4%, and then autoclaved for 1 h at 121 °C. The residual pellet consisting of ash and Klason lignin was collected by centrifugation at 22,000*g* for 5 min at 4 °C. The resulting supernatant containing sugars, acid-soluble lignin, and lipids was neutralized with Na_2CO_3. The amount of acid-soluble lignin was determined by measuring the OD at 765 nm, as described previously (Julkunen-Tiitto, 1985). Other components, such as lipids and proteins, were estimated by subtracting the amount of sugars and acid-soluble lignin from the dry weight of the liquid fraction measured. Reducing sugars were determined using the 3,5-dinitrosalicylic acid reagent method (Miller, 1959).

3. Results and discussion

3.1. Development of a cellulase-displaying yeast strain

The recombinant yeast strains are shown in Table 1. For cell-surface expression, the *T. reesei* genes *EG2* and *CBH2*, and *A. aculeatus BGL1* were integrated with a sequence encoding the C-terminal of a cell-surface protein, α-agglutinin into the genome of a host strain, NBRC1440ΔHUWL, to yield strain NBRC1440/B-EC. It has been reported that increasing the copy number of transgenes integrated into yeast genomes enhances the activity of the encoded enzyme products such as glucoamylase, α-amylase and endoglucanase (Yamada et al., 2010, 2011). Therefore, to improve cellulase activity on the cell surface of the recombinant strain, additional integrations of cellulase genes (*CBH2* or both *CBH2* and *EG2*) were performed. As shown in Fig. 1, PASC hydrolysis activity of NBRC1440/B-EC-C2 was1.7-times higher than that of NBRC/B-EC, indicating that increasing the copy numbers of *CBH2* led to higher PASC hydrolysis activity. NBRC1440/B-EC3 demonstrated a 2.5-fold higher saccharification efficiency than

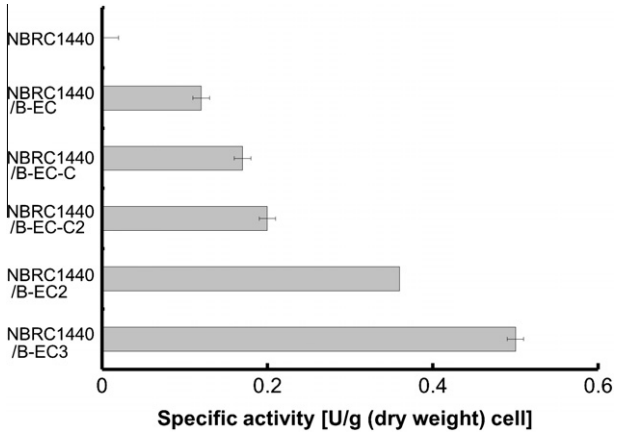

Fig. 1. Cellulase activities of wild-type and recombinant yeast of *S. cerevisiae* strains displaying cellulases on the cell surface. Data are expressed as the mean ± SD (error bars) of three independent experiments.

NBRC1440/B-EC-C2. These results indicate that increasing the copy numbers of both *EG2* and *CBH2* led to higher saccharification efficiency. Thus, strains NBRC1440 and NBRC1440/B-EC3 were used for the following experiments.

3.2. Ethanol production from rice straw using a rotary fermentation system

The cellulosic material obtained by liquid hot water treatments of rice straw was used for ethanol production with the wild yeast strain, NBRC1440.To produce high-titer ethanol by fermentation, 200 g-DW/L cellulosic material was initially mixed with cellulase and cells of *S. cerevisiae* strain NBRC1440. However, the mixing of 200 g-DW/L cellulosic material with cellulase and yeast cells was not sufficient as a result of the low water content in the fermentation vessel. In an attempt to improve the mixing efficiency, a rotary fermentation system, consisting of a fermentation vessel positioned horizontally in a drum-type heat block and which axially rotates under controlled temperature was developed. In the vessel, the high-solid material is moved by the force of gravity, which improves the mixing efficiency of the lignocellulosic biomass, cellulase, and yeast cells.

Using the developed rotary fermentation system, the relationship between the amount of added cellulase and final ethanol concentration was investigated. After 72 h, the concentration of ethanol produced from 200 g-DW/L cellulosic material with 5, 10, and 100 FPU/g-biomass of cellulase was 25.6, 27.3, and 38.8 g/L after 72 h fermentation, respectively (Fig. 2A), indicating that the final ethanol titer increased with increasing cellulase activity, as described in previous studies (Lau et al., 2010; Wang et al., 2011). Ethanol produced in the fermentor supplemented with 100 FPU/g-biomass cellulase reached 80% of the theoretical yield, and the high-solid cellulosic material was liquefied after 72 h fermentation (Fig. 2A).

Previous studies have suggested that ethanol yields decrease with increasing concentrations of solid materials used as substrates (Roche et al., 2009; Wang et al., 2011). For example, the yield of ethanol produced from 200 g-DW/L biomass in a jar fermentor was only 20% of the theoretical yield (Jin et al., 2010). Thus, Jørgensen et al. (2006) developed a rotation chamber fermentation process, in which high-solid materials were mixed with enzymes using paddles set in a drum-type fermentation vessel. Using this system, ethanol production from 200 g-DW/L wheat straw, which was obtained as a water-insoluble fraction after liquid hot water treatments, was markedly improved; however, the yield of ethanol

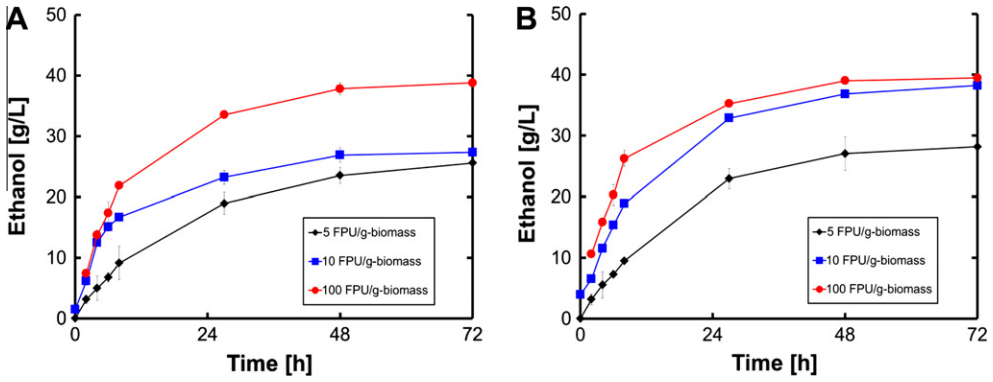

Fig. 2. Simultaneous saccharification and fermentation of a cellulosic material in the presence of commercial cellulase in a rotary fermentation system. Fermentation was performed using strains NBRC1440 (A) and NBRC1440/B-EC3 (B). Data are expressed as the mean ± SD (error bars) of three independent experiments.

reached only 60% of the theoretical yield. Therefore, the rotary fermentation system used in the present study was effective for the fermentation of high-solid materials, likely due to the improved mixing efficiency.

3.3. Reduction of cellulase addition through cell-surface engineering

To develop a cost-effective ethanol production process, the addition of commercial enzymes should be minimized, because the cost of cellulases presently represents a high proportion of the total bioethanol production cost (Gregg and Saddler, 1996; Sun and Cheng, 2002; Zhu et al., 2009). Cell-surface engineering, which enables the display of various types of functional proteins on microbial cell surfaces without loss of their functions (Kondo and Ueda, 2004), is a promising technology for reducing the requirement for cellulase addition as cellulases can be displayed on the yeast cell surface. Fujita et al. (2004) successfully produced ethanol from pure cellulose sources, such as PASC, without the addition of cellulases using a recombinant yeast strains displaying *T. reesei* EG and CBH2, and *A. aculeatus* BGLon the cell surface.

In the presence of 10 FPU/g-biomass cellulase, the cellulase-displaying strain NBRC1440/B-EC3 produced 38.2 ± 0.5 g/L ethanol from 200 g-DW/L cellulosic material, which was similar to the value (39.4 ± 2.3 g/L) obtained in the fermentation performed in the presence of 100 FPU/g-biomass cellulase after 72 h (Fig. 2B). As the yield of ethanol reached 79% of the theoretical yield in the fermentation with 10 FPU/g-biomass cellulase, the display of cellulases on the yeast cell surface enables a reduction in the amount of added commercial cellulase. Notably, strain NBRC1440/B-EC3 produced 1.4-fold more ethanol than the control strain, NBRC1440, in the presence of 10 FPU/g-biomass cellulase. Furthermore, the combined application of cell-surface engineering and the novel rotary fermentation system permitted complete ethanol production from 200 g-DW/L cellulosic material within 72 h, which is shorter than the length required in previous studies (Jin et al., 2010; Jørgensen et al., 2006).

3.4. Improvement in ethanol production by liquefaction treatment

In the present study, ethanol was produced with high yield from a high concentration of lignocellulosic material (Fig. 2), which would be partly due to the improved mixing efficiency of the fermentation system, resulting in a high fluidity of the fermentation mixture. Accordingly, in an attempt to further increase the fluidity of the highly concentrated lignocellulosic materials, liquefaction treatment was performed prior to the fermentation. The liquefaction of 200 g-DW/L biomass was performed with 10 FPU/g-biomass cellulase at 50 °C, which is the optimum temperature of the cellulase reagent used in this study (data not shown), directly in the vessel of the rotary fermentation system. After liquefaction treatment, 100 g of wet cells of *S. cerevisiae* per Liter of liquefied biomass were added into the vessel to initiate the fermentation.

In the fermentation using strain NBRC1440, the addition of 10 FPU/g-biomass cellulase and a 24-h liquefaction treatment led to a maximum ethanol titer of 37.5 g/L, corresponding to a yield of 77% (Fig. 3A). The obtained titer was markedly higher than that observed without liquefaction treatment using the identical amount of cellulase (Fig. 2A). The ethanol yield was nearly unchanged with cellulase concentrations up to 100 FPU/g-biomass and 24 h liquefaction treatment (data not shown).

When strain NBRC1440/B-EC3 was used for the fermentation of pretreated rice straw pretreated, a maximum ethanol concentration of 43.1 g/L was obtained by a 2 h liquefaction treatment, corresponding to 89% of the theoretical ethanol yield (Fig. 3B). The ethanol titer produced by strain NBRC1440/B-EC3 was 12% higher than that produced by strain NBRC1440. When liquefaction treatment was performed for 2 h, a cellulase concentration of 10 FPU/g-biomass resulted in the highest ethanol production (data not shown). Interestingly, although the highest ethanol titer was obtained by a 2 h liquefaction treatment, longer liquefaction treatment resulted in reduced ethanol production (Fig. 2B). The cell concentration of NBRC1440 and NBRC1440/B-EC3 remained constant during the fermentation (data not shown).

In previous studies, a 24-h liquefaction treatment was used for the acceleration of saccharification of high-solid concentration substrates (Jørgensen et al., 2006; Oberoi et al., 2011). Although optimization of liquefaction time has not been reported, a longer liquefaction treatment is disadvantageous for cost-effective ethanol production due to lengthening of the operation time. Here, maximum ethanol production was achieved with a 2 h liquefaction pretreatment step and 72 h fermentation using a cellulase-displaying yeast strain.

3.5. Analysis of residual sugars after ethanol production

Residual sugars in the fermentation medium were measured after the fermentation of cellulosic material by strains NBRC1440 and NBRC1440/B-EC3 (Table 2). The sulfuric acid hydrolysis of 200 g-DW/L cellulosic material released 95.4 g/L glucose. After 2 h liquefaction in the presence of 10 FPU/g-biomass cellulase and subsequent fermentation with strain NBRC1440, the residual glucose in the fermentation vessel was reduced to 16.0 g/L (Table 2). In contrast, the concentration of residual glucose in the fermentation medium was only 1.6 g/L for cellulase-displaying strain NBRC1440/B-EC3 (Table 2). These results indicate that the cellulases displayed on the yeast cell surface hydrolyzed cellulose that

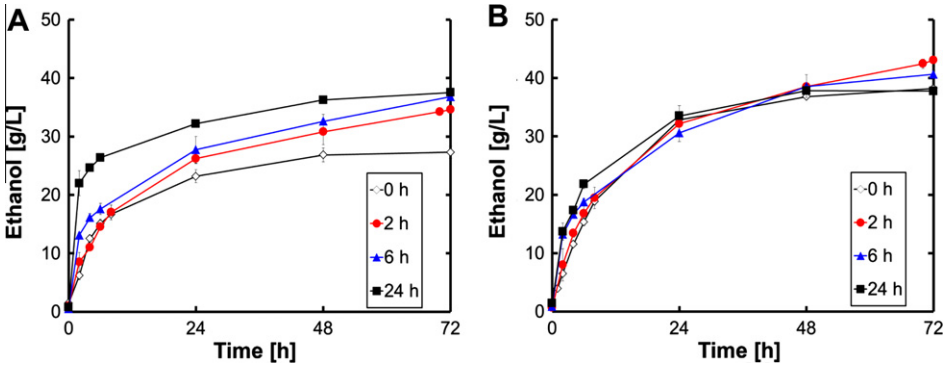

Fig. 3. Effect of liquefaction time on ethanol production from cellulosic material. Fermentation was performed with strains NBRC1440 (A) and NBRC1440/B-EC3 (B). Cellulase was loaded at a concentration of 10 FPU/g-biomass. Data are expressed as the mean ± SD (error bars) of three independent experiments.

Table 2
Residual sugar after liquefaction of 200 g-DW/L cellulosic materials in the presence of 10 FPU/g-biomass cellulase and subsequent 72 h fermentation with yeast strains NBRC1440 and NBRC1440/B-EC3.

Strain	Liquefaction time [h]	Residual sugar [g/L ± SD]	Ethanol yield (g-ethanol/g-biomass ± SD)	Initial ethanol production rate (g/L/h ± SD)
NBRC1440	2	16.0 ± 1.1	0.173 ± 0.004	2.43 ± 0.02
NBRC1440	24	15.5 ± 1.5	0.188 ± 0.002	4.40 ± 0.09
NBRC1440/B-EC3	2	1.6 ± 0.9	0.216 ± 0.004	2.81 ± 0.27

SD; standard deviation (n = 3).

was not hydrolyzed by commercial cellulases. The close proximity of multiple enzymes on the cell surface might enable synergistic hydrolysis of the lignocellulosic materials. Thus, cell-surface engineering of yeast led to increased sugar availability for ethanol production, thereby increasing the yield of bioethanol.

Although most lignocellulosic materials are agricultural byproducts or industrial residues and show great potential for the production of ethanol as a renewable fuel, the bioconversion processes are more complicated than those necessary to produce ethanol from sources such as cornstarch and cane juice. Therefore, consolidated bioprocessing (CBP), which combines enzyme production, saccharification of lignocellulosic materials, and fermentation into a single process, is increasingly recognized as having potential for the low-cost production of ethanol (Hasunuma and Kondo, 2011; Lynd et al., 2005). In particular, apart from the production of high-titer ethanol, the avoidance of enzyme addition would greatly contribute to the cost reduction of cellulosic ethanol production (Lynd et al., 2005). However, as natural microorganisms with the capability for CBP have not been identified to date, the heterologous expression of cellulases has been studied in both bacterial hosts, including *Escherichia coli* and *Zymomonas mobilis*, and the yeasts *S. cerevisiae*, *Hansenula polymorpha*, and *Kluyveromyces marxianus* (la Grange et al., 2010). To our knowledge, the present study is the first to report the fermentation of high-solid lignocellulosic materials using a cellulase-expressing microorganism.

4. Conclusion

High-yield ethanol production in a novel rotary fermentation system was achieved using a cell-surface engineered strain *S. cerevisiae* NBRC1440/B-EC3, which displayed cellulases EG, CBH, and BGL. In addition, by performinga2-h liquefaction treatment with a small amount of enzyme, high-titer ethanol production from high-solid lignocellulosic materials was achieved using strain NBRC1440/B-EC3 with an ethanol yield corresponding to 89% of the theoretical yield. Notably, the recombinant strain was able to hydrolyze a portion of the cellulosic material that was not hydrolyzed by commercial cellulase. Thus, cellulase displaying yeast is effective for high-titer ethanol production from lignocellulosic material.

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