Direct Ethanol Production from Steam-Exploded Corn Stover using a Synthetic Diploid Cellulase-displaying Yeast Consortium

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The assembly of functional cellulolytic enzymes was displayed using a synthetic, cell-surface engineered diploid yeast consortium. *Trichoderma reesei* endoglucanase II (EGII), cellobiohydrolase II (CBHII), and *Aspergillus aculeatus* β-glucosidase I (BGLI) were displayed as fusion proteins with the AGA2p C-terminus of a-agglutinin on the cell surface of the diploid yeast strain *Saccharomyces cerevisiae* Y5. The cell-surface immobilization of each enzyme was confirmed by immunofluorescence microscopy. This type of yeast consortium allowed convenient optimization of ethanol production by adjusting the combination ratios of each cell type for inducing synergy in cellulose hydrolysis. Next, the direct ethanol fermentation from steam-exploded corn stover was investigated. The optimized cellulase-displaying consortium produced 20.4 g/L ethanol from 48.4 g cellulose per liter after 72 h in the presence of a small amount of cellulase reagent (0.9 FPU/mL). These results suggested the feasibility of the cellulase-displaying yeast consortium for simultaneous saccharification and fermentation from insoluble cellulosic materials.

Keywords: Cellulosic ethanol; Cellulase-displaying yeast consortium; Diploid Saccharomyces cerevisiae Y5; Steam-pretreated corn stover

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INTRODUCTION

Currently, many technological barriers exist with respect to the economical production of ethanol from lignocellulosic biomasses (Himmel *et al.* 2007). In the process of hydrolyzing cellulose into soluble sugars, multiple cellulases including endoglucanase (EG), cellobiohydrolase (CBH), and β-glucosidase (BGL) are required (Zhang *et al.* 2006). Consolidated bioprocessing (CBP), featuring enzyme production, saccharification of cellulose, and fermentation in one step, is an alternative approach for the cost-effective production of bioethanol from biomass.

Saccharomyces cerevisiae is the traditional microorganism used for ethanol production, but it is unable to utilize cellulosic materials and requires a saccharification process prior to fermentation to produce glucose (Ito *et al.* 2004; Nevoigt 2008). Numerous attempts have been made to construct engineered *S. cerevisiae* strains to express cellulases by cell-surface display for direct ethanol production from cellulose, and although bi-functional or tri-functional cellulose-degrading strains have been constructed, the ethanol yield has not been sufficiently improved (theoretical yield less than 50% in general) (Apiwatanapiwat *et al*. 2011; Yamada *et al*. 2011; Nakatani *et al*. 2013; Yamada *et al*. 2013; Yang *et al*. 2013b; Fitzpatrick *et al*. 2014). It would appear

that co-expression of all cellulolytic enzymes in a single cell resulted in relatively low expression levels of cellulases, which may have been due to the additional burden of metabolic flux and protein secretion (Van Rensburg *et al*. 2012). Therefore, the assembly of functional cellulolytic enzymes by using a synthetic engineered yeast consortium has the potential to have the desired properties of cellulolytic ability and ethanol production (Tsai *et al*. 2010; Fan *et al*. 2012; Goyal *et al*. 2012; Kim *et al*. 2013; Zuroff *et al*. 2013).

In view of the importance of fermentation properties and inhibitor tolerance in the further industrial application, the development of a diploid yeast strain to enhance the expression and secretion of heterologous protein and improve the fermentation performance would be a highly attractive option (Wan *et al*. 2012). Previously, our group reported on the construction of an a-agglutinin expression system for genetic immobilization of β-glucosidase I on the cell surface of *S. cerevisiae* Y5 (Yang 2008). This diploid robust yeast strain possessed many advantages, such as higher ethanol yield, higher resistance to ethanol, and higher physiological tolerance to inhibitors present in lignocellulosic hydrolysates (Tian *et al*. 2010, 2011, 2013).

The objective of the present study was to demonstrate the feasibility of constructing a novel cell surface engineered diploid yeast consortium for bioethanol production from phosphoric acid swollen cellulose (PASC) and steam-exploded corn stover (CS), a basic step toward construction CBP stain for simultaneous saccharification and fermentation from insoluble cellulosic materials.

EXPERIMENTAL

Materials

Strains, media, and plasmids

The strains and plasmids used in this work are provided in Table 1. *Saccharomyces cerevisiae* Y5 preserved No. CGMCC2660 (China General Microbiological Culture Collection Center) was obtained from Capital Normal University (Beijing, China). *E. coli* Top 10 was used for recombinant DNA manipulation. *T. reesei* was purchased from CICC (China Center of Industrial Culture Collection). *E. coli* transformants were grown in Luria-Bertani (LB) medium (1% tryptone, 0.5% yeast extract, and 1% NaCl, pH 7.0) supplemented with 100 μg/mL of ampicillin. *S. cerevisiae* Y5 transformants were selected and maintained on Geneticin (G418, Beijing Biotopped Science & Technology Co. Ltd.) plates (1% yeast extract, 2% peptone, and 2% glucose supplemented with 600 μg/mL Geneticin) at 30 °C. They were induced in yeast extract peptone galactose (YPG) medium (1% yeast extract, 2% peptone, and 2% galactose) at 20 °C. The fermentation medium was composed of 10 g/L yeast extract, 20 g/L polypeptone, and 10 g/L PASC as the sole carbon source. The filamentous fungus *T. reesei* was cultured in potato dextrose agar (PDA) medium (2% potato extract, 2% glucose) at 27 °C. Its cDNA was synthesized from mRNA by using the First-Strand cDNA synthesis kit (Fermentas) which was purchased from Thermo Fisher Scientific Ltd. (Beijing).

Unless otherwise indicated, all chemicals, media components, and supplements were of analytical grade standard and obtained from Sigma-Aldrich (St. Louis, MO, USA). All restriction enzymes used in this study were purchased from New England BioLabs Ltd. (Beijing).

Table 1. Characteristics of Strains and Plasmids Used in This Study

Strain or plasmid	Relevant feature	Reference
Strains		
S. cerevisiae strains		
Y5	Wild type, diploid, strong flocculation ability	Tian *et al*. 2010
Y5/AGA1	No display, contained plasmid pAGA1	Yang *et al*. 2013a
Y5/pYD1	No display ,contained plasmids pAGA1 and pYD1	This study
Y5/BGLI	Display of BGLI of *A. aculeatus*	Yang *et al*. 2013a
Y5/EGII	Display of EGII of *T. reesei*	This study
Y5/CBHII	Display of CBHII of *T. reesei*	This study
Bacterial strain		
E. coli Top 10	F- mcrA△(mrr-hsd RMS-mcr BC) 80lacZ△M15△lac X74 recA1 ara△139△(ara-leu) 7697 galU galK rpsL (Strr) endA1 nupG	Tiangen Biotech (Ltd., Beijing)
Plasmids		
pYD1	Yeast display vector	Invitrogen
YIP5-KanR	Integrative plasmid	Jin *et al.* 2014
pAGA1	AGA1, No expression	Yang *et al.* 2013a
pBGLI	Surface expression β-glucosidase gene (BGLI)	Yang *et al*. 2013a
pEGII	Surface expression endoglucanase gene (EGII)	This study
pCBHII	Surface expression cellobiohydrolase gene (CBHII)	This study

Methods

Construction of plasmids

Primers used for plasmid construction are summarized in Table 2. Plasmid pAGA1 for over-expression of the AGA1 gene and plasmid pBGLI for cell surface display BGLI were constructed previously (Yang *et al.* 2013a).

Plasmid pEGII for cell surface expression of the EGII (*egl2*) was constructed as follows. The 1194 bp DNA fragment encoding the *egl2* gene without its native secretion signal was amplified with the first-strand cDNA prepared from *T. reesei* as the template using primer pairs *egl2*-For/Rev. This DNA fragment was introduced into the yeast display vector pYD1 (Invitrogen, Life Technologies Ltd., Beijing) with *Kpn* I/*BamH* I. *MAT* terminator was amplified from pYD1 by using primer pairs *MAT*-For/Rev and then digested with *BamH* I/*EcoR* I to create plasmid pYD1-*egl2*-*MAT*.

The *KanR* fragment was obtained from plasmid YIP5-KanR by two-step cloning (Jin *et al*. 2014). First, the DNA fragment containing *ADH* promoter and *KanR* ORF was amplified from YIP5-KanR by PCR using the *KanR*-For/Rev primers and inserted into *EcoR* I/*Apa* I site of plasmid pYD1-*egl2*-*MAT*; next, the *ADH* terminator digested with *Bgl* II/*Nde* I was also introduced into pYD1-*egl2*-*MAT*. The resulting plasmid was named pEGII.

For displaying the *T. reesei* CBHII gene (*cbh2*) in *S. cerevisiae* Y5, plasmid pCBHII was created. A 1344 bp gene fragment coding for the mature region of the CBHII was amplified using primers *cbh2*-For/Rev-KT and introduced into plasmid pEGII digested with *Kpn* I/*BamH* I for replacing *egl2* to form pCBHII (Fig. 1).

Table 2. Primers Used in This Study

Primer name	Sequence (5'-3'). Restriction sites are underlined
egl2-For/Rev	GGGGTACCCAGCAGACTGTCTGGGGCCA
	CGGGATCCGCTACTTTCTTGCGAGACACGAG
MAT-For/Rev	CGCGGATCCATGTAACAAAATCGAC
	CGCGCGAATTCAATTCTCTTAGGATTCG
KanR-For/Rev	GCGAATTCGAAACGCGCGAGGCAGCTGTAATC
	CTGGGCCCGGCATATGCCAGATCTGCTTAGAAAAACTCATCGAGC
ADH-For/Rev	CGCATATGTTTACCGCAGCTGTAATC
	CGCGAGATCTCGTCAGAATTGGTTAATTGG
cbh2-For/Rev-KT	GCGGTACCCAAGCTTGCTCAAGCGTCT
	GCGGATCCTTACAGGAACGATGGGTTTGCG
cbh2-For-N/Rev	GCGCTAGCCAAGCTTGCTCAAGCGTCT
	GCGGATCCCAGGAACGATGGGTTTGCG

Yeast transformation

Transformation of *S. cerevisiae* Y5 was carried out using the lithium acetate method (Ito *et al*. 1983). The plasmid pAGA1 was linearized by *Apa* I for chromosome integration. The plasmid pYD1 was transformed into *S. cerevisiae* Y5 as a negative control. *S. cerevisiae* Y5 clones transformed with different plasmids (strain Y5/pYD1 contained plasmids pAGA1 and pYD1, strain Y5/EGII contained plasmids pAGA1 and pEGII, and strain Y5/CBHII contained plasmids pAGA1 and pCBHII) were selected and maintained on Geneticin plates.

Immunofluorescence microcopy

Immunofluorescence microscopy was performed according to a previously described method (Kobori *et al*. 1992). Immunostaining was carried out as follows: Induced recombinant yeast cells expressing cellulases were harvested by centrifugation at 3341 ×g for 5 min and washed with phosphate-buffered saline (PBS). As the primary antibody, mouse anti-Xpress tag antibody (Invitrogen, R910-25) for EGII and CBHII was used at dilution rates of 1:1000. As the second antibody, fluorescein (FITC)-conjugated goat anti-mouse IgG(H+L) (Jackson ImmunoResearch Ltd., Beijing) was used at dilution rate 1:200. Cells and the antibody were incubated at room temperature. After washing the cell-antibody complex with PBS twice, cellular localizations of the cellulases were observed under a fluorescence microscope (Carl Zeiss LSM 5 LIVE, Germany). Yeast strains Y5 and Y5/pYD1were used as control.

Enzyme assay

Yeast cells were induced in YPG medium for 48 h at 20 °C and harvested by centrifugation for 5 min at 3341 ×g, washed with distilled water. The BGLI activity of strain Y5/BGLI was measured using ρ-nitrophenyl-β-D-glucopyranoside as described previously (Walseth 1952).

The enzyme activities of endoglucanase and cellobiohydrolase were determined by hydrolysis of carboxymethyl cellulose (CMC) and phosphoric acid swollen cellulose (PASC), respectively. Amorphous cellulose PASC was prepared from Avicel PH-101. The cell pellet was re-suspended in a reaction mixture of 1% CMC or 1% PASC in 50 mM sodium acetate buffer (pH 5.0) with the optical density at 600 nm adjusted to 1.0. After a reaction at 50 °C for 30 min, the activities were determined using the 3,5-Dinitrosalicylic acid (DNS) method (Wood and Bhat 1988). The amount of enzyme

releasing 1 μmol reducing sugar from the substrate per minute was defined as one unit of enzyme activity.

Ethanol fermentation

The abilities of the engineered yeast consortium (Y5/EGII + Y5/CBHII + Y5/BGLI) to ferment ethanol from PASC and steam-exploded corn stover were investigated. The steam-exploded corn stover used in this study was treated as described previously (Wang *et al.* 2014). The composition of materials is shown in Table 3. An enzyme mixture composed of equal amounts of cellulase (Sigma-Aldrich, St. Louis, MO) and β-glucosidase (Sigma-Aldrich) was used. The reaction system composed of EGII: CBHII: BGLI, with the optimized ratio of 2:1:1 in the presence of commercial cellulase (Sigma-Aldrich, St. Louis, MO) with different enzyme loadings (0, 0.3, 0.6, 0.9, 1.2, and 1.5 FPU/mL). Cells were collected by centrifugation at 2320 ×g for 5 min at 4 °C, washed with distilled water twice, and mixed in the adjustable ratio to a total initial cell concentration of 30 g/L (wet weight) to form the functional consortium. A mixture of cells was incubated in 100 mL of YP medium (20 g/L peptone, 10 g/L yeast extract) for 1 h to remove residual carbon source, and then resuspended in YP-CS medium (YP medium containing 100 g/L steam-exploded corn stover, corresponding to 48.4 g of cellulose per liter).

Ethanol fermentation proceeded at 30 °C with 90 rpm in 250-mL Erlenmeyer flasks. Then, 1-mL samples of the fermentation broth were taken periodically and stored at -4 °C until they were analyzed to determine sugar and ethanol content. The total sugar was analyzed using phenol-sulfuric acid method (DuBois *et al.* 1956). Glucose was measured by high performance liquid chromatography (HPLC; model 1260, Agilent Technologies, Santa Clara, CA, USA) equipped with a Hi-Plex H column (300 mm × 7.7 mm) and a refractory index (RI) detector. Samples were run at a temperature of 60 °C with a mobile phase of 5 mM sulfuric acid at a flow rate of 0.6 mL/min. Ethanol analysis was carried out using gas chromatography (GC; model 7890A, Agilent Technologies) equipped with the flame ionization detector and a HJ-PEG column. Samples were run under the following conditions: column oven at 120 °C, front injection port at 200 °C, with N_2 as the carrier gas at a flow rate of 4 mL/min.

Table 3. Main Chemical Compositions of Untreated and Treated Corn Stover (% of DM)

Materials	Cellulose	Hemicelluloses	Lignin[b]	Ash	Extractives[c]
Untreated CS[a]	37.3 ± 0.3	24.4 ± 0.4	19.1 ± 0.5	6.5 ± 0.3	12.7 ± 0.3
Steam explored CS[a]	48.5- ± 0.4	11.3 ± 0.3	24.1 ± 0.3	5.7 ± 0.2	13.6 ± 0.2

[a] Data shown as percentage of dry matter
[b] Acid-soluble lignin included
[c] Non-structural material from biomass

RESULTS

Construction of Yeast Strains Displaying Cellulolytic Enzymes

The expression plasmids pEGII and pCBHII (Fig. 1) were transformed into the yeast *S. cerevisiae* Y5 strains. All of recombinant yeast strains had a pAGA1 plasmid for integrating AGA1 into the chromosome, and the resultant transformants were designated

strains Y5/EGII and Y5/CBHII (Table 1). Upon galactose induction, the proteins were expected to be expressed and interact with the Aga1p and Aga2p anchor system by using the glycosylphosphatidylinositol (GPI) anchor linked to the cell surface.

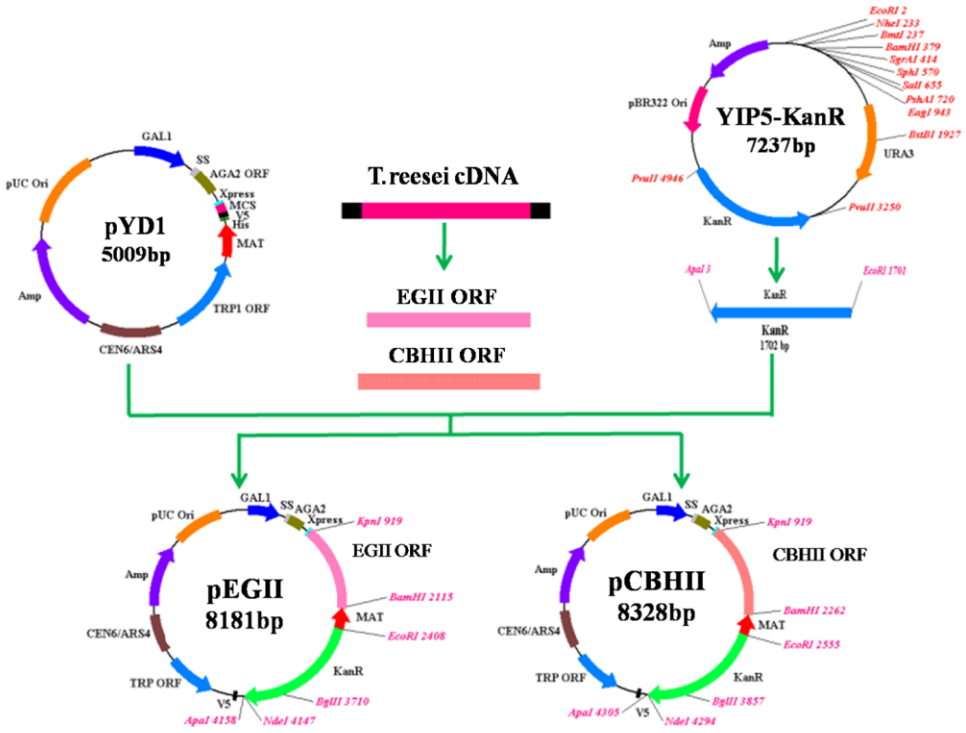

Fig. 1. Expression plasmids for displaying *T. reesei* EGII and CBHII on the yeast cell surface

Immunofluorescence Microcopy

To confirm the yeast cells surface-displaying of EGII and CBHII, immunofluorescence labeling of the cells was carried out using mouse anti-Xpress IgG antibody as the primary antibody. The green fluorescence of fluorescein (FITC)-conjugated goat anti-mouse IgG was observed for strains Y5/EGII and Y5/CBHII, indicating that EGII and CBHII were displayed on the cell surface, respectively; the cells harboring the control plasmids were hardly labeled with mouse anti-Xpress IgG (Fig. 2). These results suggested that two types of cellulase were successfully expressed on the cell surface of *S. cerevisiae* Y5 strain.

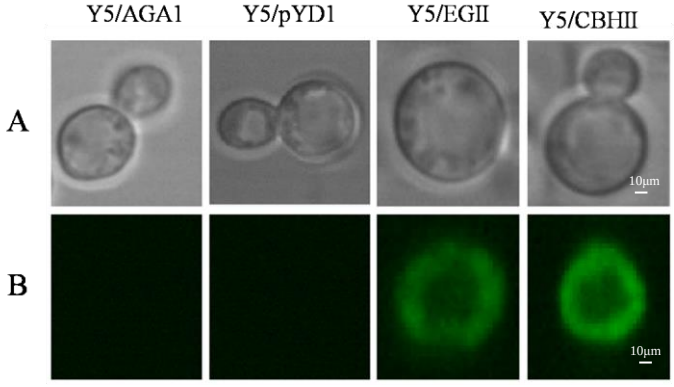

Fig. 2. Immunofluorescence labeling of transformants: ZEISS laser scan confocal micrographs (line A) and immunofluorescence micrographs (line B); cells were labeled with mouse anti-Xpress IgG antibody conjugated with FITC

Enzyme Assay

As shown in Table 4, EGII, CBHII, and BGLI activities were detected in the pellet fraction of strain Y5/EGII, Y5/CBHII, and Y5/BGLI, respectively. The strain Y5/CBHII and strain Y5/EGII showed moderate CBHII and EGII activity (1.14 U/mL and 1.27 U/ mL, respectively). The BGLI activity of strain Y5/BGLI cells was relatively low, which was only 0.72 U/ mL. No enzyme activity was detected in the culture supernatant (data not shown), and the control strain without displayed enzymes exhibited less than 0.1 U/mL of enzyme activity. These results clearly indicated that active enzymes were immobilized on the cell surface without leakage into the culture medium.

Table 4. Enzyme Activities Displayed on the Cell Surface of Yeast Strain Y5

Strains	Cellulase	Substrate(w/v)	Enzyme activity cells (U/mL)[a]
Y5/pYD1	ND	1% PASC	0.06 ± 0.01
Y5/EGII	EGII	1% CMC	1.14 ± 0.06
Y5/CBHII	CBHII	1% PASC	1.27 ± 0.13
Y5/BGLI	BGLI	0.8% pNPG	0.72 ± 0.01

[a] Values are averages ± standard deviation of three independent experiments
ND: not detected

Ethanol Fermentation from PASC

Ethanol fermentation from 10 g amorphous cellulose per liter was performed using a cell combination system consisted of three cellulase-displaying yeast populations. Cells displaying EGII, CBHII, and BGLI were mixed in various ratios and the produced ethanol from PASC were measured. *S. cerevisiae* Y5 without displayed enzymes was the control strain. A mixture of cells composed of EGII: CBHII: BGLI, with the population ratio of 2:1:1 produced the highest amount of ethanol (1.76 g/L) after 84 h, a 1.6-fold improvement of ethanol production compared to cells composed of an equal amount of each cell type. The ethanol yield (based on grams of ethanol produced per gram of consumed reducing sugar) reached 0.42 g/g (Fig. 3).

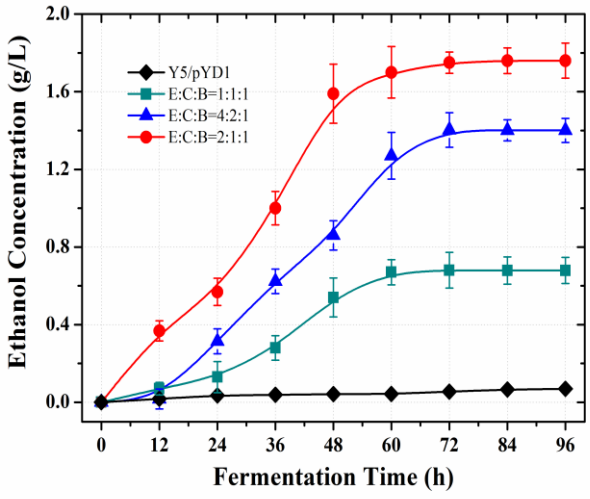

Fig. 3. Time course of ethanol production from 10 g/L PASC by negative control Y5/pYD1 (black diamond), EGII:CBHII:BGLI ratio of 1:1:1 (green square),4:2:1 (blue triangle), and the optimized ratio 2:1:1 (red circle). The data points represent the averages ± standard deviation of three independent experiments.

However, a large portion of the substrate (the amount of residual sugar after 84 h hydrolysis of 10 g/L PASC was 5.5 g/L, and the sugar consumption rate was 43.3%) remained after 96 h without being hydrolyzed because the cellulase activities displaying on the cell surface were not enough for complete cellulose digestion.

Cellulosic Ethanol Fermentation from Steam-Exploded Corn Stover

The simultaneous saccharification and fermentation performance of cellulose-displaying consortium by using steam-exploded corn stover as a sole carbon source was further investigated.

As shown in Fig. 4, in the presence of 0, 3, 6, 9, 12, and 15 FPU/ g stover cellulase, 34.49, 18.71, 7.03, 2.11, 1.98, and 1.23 g/L of residual cellulose remained after 84 h, respectively. Addition of 9 FPU/ g stover cellulase enabled utilization of 92.3% of the initial cellulose (Fig. 4).

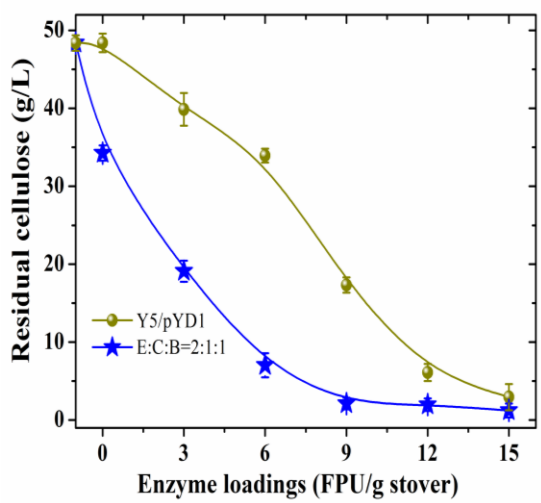

Fig. 4. The residual cellulose concentration of simultaneous saccharification and fermentation from steam-exploded corn stover. The data points represent the averages ± standard deviation of three independent experiments.

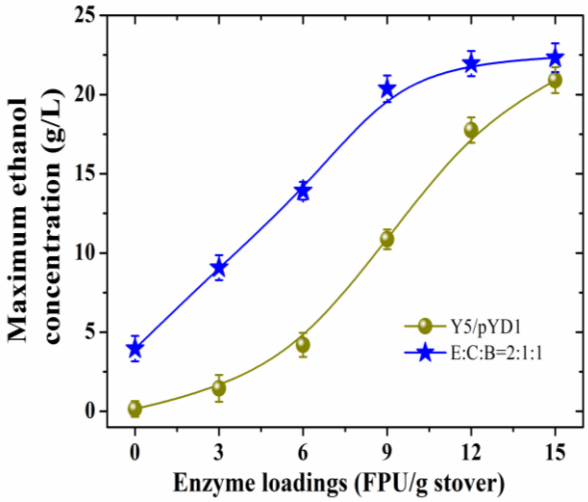

Fig. 5. The maximum ethanol concentration of simultaneous saccharification and fermentation from steam-exploded corn stover. The data points represent the averages ± standard deviation of three independent experiments.

The cellulose hydrolyzed by cellulase-displaying yeast consortium with an additional 9 FPU/ g stover cellulase was nearly the same as that by control strain *S. cerevisiae* Y5 with an additional 15 FPU/ g stover. These results indicate that cellulases displayed on the yeast cell surface can improve hydrolysis of cellulose, although their activities were lower than commercial enzymes.

Furthermore, using the optimized cell combination system, the relationship between the amount of added cellulase and final ethanol concentration was investigated. As shown in Fig. 5, in the presence of 9 FPU/ g stover cellulase, the cellulase-displaying consortium produced 20.4 g/L ethanol after 84 h, which was similar to the value (20.9 g/L) obtained by control strain in the presence of 15 FPU/ g stover cellulase (Table 5). Notably, as the ethanol yield reached 86% of the theoretical yield with 9 FPU/ g stover cellulase, the cell-surface engineered system enabled a reduction in the amount of added commercial cellulase.

Table 5. Results of Steam-exploded Corn Stover Fermentation

	Optimized consortium			Y5/pYD1		
Enzymatic loadings (FPU/ g stover)	Ethanol Concentration (g/L)	Cellulose consumption (g/L)	Ethanol Yield[a] (g/g)	Ethanol Concentration (g/L)	Cellulose consumption (g/L)	Ethanol Yield[a] (g/g)
0	3.96±0.84	13.91±1.15	0.28±0.06	0.12±0.74	3.13±0.51	0.04±0.24
3	9.08±1.21	29.69±0.95	0.30±0.04	1.45±0.78	8.52±0.83	0.17±0.09
6	13.92±0.88	41.37±1.43	0.33±0.02	4.19±0.66	13.97±0.74	0.30±0.05
9	20.37±1.46	46.29±1.57	0.44±0.04	10.87±0.98	31.05±0.62	0.35±0.03
12	21.96±1.14	46.42±0.77	0.47±0.03	17.76±0.83	42.28±0.98	0.42±0.02
15	22.33±0.93	47.17±0.94	0.47±0.02	20.91±1.14	45.45±0.91	0.46±0.03

Values are averages ± standard deviation of three independent experiments
[a] Ethanol yield (g/g) =(Maximum Ethanol Concentration)/ Cellulose consumption

DISCUSSION

The key challenge of CBP lies in choosing the optimal host strain to directly convert cellulose to ethanol. In previous studies, several researchers had made attempt to co-display three types of cellulases in a single cell for cellulose degradation and ethanol production. In that case, expression levels of cellulases can be limited due to the overburden of physiological metabolism. Furthermore, it is difficult to control the expression levels of each cellulase for optimal ethanol production. Apiwatanapiwat *et al*. (2011) constructed a haploid engineered yeast strain NBRC-5Es, co-displaying two types of amylolytic enzymes, as well as three types of cellulolytic enzymes on the cell surface. The recombinant strain produced 1.04 g/L ethanol from 8.44 g/L of the acid-treated Avicel after 48 h of fermentation and resulted in a large portion of the substrate remained without being hydrolyzed by the enzymes and resulted in a relatively low ethanol yield. In recent years, other researchers turned to engage in establishing a synthetic engineered yeast consortium for reducing the metabolic burden and improving the efficiency of substrate hydrolysis (Fig. 6). However, haploid *S. cerevisiae* strains were commonly employed in most of the reported studies, and the PASC was generally utilized as a sole carbon source. Few reports of direct ethanol fermentation from pretreated lignocellulosic material have been published.

Table 6. Summaries of Similar Published Reports of Cellulosic Ethanol Fermentation by Using Yeast Consortium

Materials	Substrate conc.(g/L)	Consortium ratio	Ethanol conc.(g/L)	Overall ethanol yield (%)	Reference
PASC	10	EG:CBH:BGL 6:2:1	2.12	41.5	Baek et al. 2013
PASC	10	EG:CBH:BGL 3:1:1	1.78	34.9	Baek et al. 2013
PASC	10	SC:AT:CB:BF 7:2:4:2	1.87	93	Tsai et al. 2010
PASC	10	CipA:CelA:CBHII:BGLI 2:3:3:0.53	1.80	35	Kim et al. 2013
PASC	10	EG:CBH:BGL 1:1:1	0.77	68.6	Mo et al. 2014
PASC	10	EG:CBH:BGL 2:1:1	1.76	82.3	This study

In this study, a cellulase-displaying consortium system consisting of three types of cells, each immobilizing different enzyme for inducing a synergy in cellulose hydrolysis, was built on a robust diploid yeast strain Y5. The potential advantages of this yeast consortium of using steam-exploded corn stover as a sole substrate for direct hydrolysis and fermentation was then investigated. The ethanol yield (based on grams of ethanol produced per gram of consumed cellulose) reached 86% of the theoretical yield in the presence of a small amount of cellulase reagent (9 FPU/ g stover). Although cellulase activities displaying on cell surface were not enough for complete cellulose digestion, further improvements of cellulase activity and expression levels are ongoing studies in our laboratory.

CONCLUSIONS

1. The diploid robust strain *S. cerevisiae* Y5 was employed to develop a yeast consortium in which yeast cells displaying different types of cellulases were combined in an optimized ratio.
2. A mixture of cells composed of EGII: CBHII: BGLI, with the optimized population ratio of 2:1:1 produced 1.76 g/L ethanol from 10 g/L PASC after 84 h, which the yield was 0.42g/g which corresponds to 82.3% of the theoretical yield.
3. Cellulosic ethanol fermentation from steam-exploded corn stover was carried out using the optimized co-display yeast consortium. Without any cellulase addition, the cellulase-displaying yeast consortium produced 3.96 g/L ethanol after 84 h, while the negative control strain Y5/pYD1 could only produce a similar amount of ethanol in the presence of 0.6 FPU/mL; addition of 0.9 FPU/ml cellulase enabled utilization of 92.3% of the initial cellulose and the ethanol production reached 20.37 g/L, which was similar to the value obtained by control strain in the presence of 1.5 FPU/mL cellulase.
4. Our results suggests that the combined cell system described here which clearly reduced the costs of commercial enzyme sets up a promising development platform for the cellulosic ethanol production using cell surface engineered yeast.

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