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High β-Glucosidase Secretion in *Saccharomyces cerevisiae* Improves the Efficiency of Cellulase Hydrolysis and Ethanol Production in Simultaneous Saccharification and Fermentation

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Bioethanol production from lignocellulose is considered as a sustainable biofuel supply. However, the low cellulose hydrolysis efficiency limits the cellulosic ethanol production. The cellulase is strongly inhibited by the major end product cellobiose, which can be relieved by the addition of β-glucosidase. In this study, three β-glucosidases from different organisms were respectively expressed in *Saccharomyces cerevisiae* and the β-glucosidase from *Saccharomycopsis fibuligera* showed the best activity (5.2 U/ml). The recombinant strain with *S. fibuligera* β-glucosidase could metabolize cellobiose with a specific growth rate similar to the control strain in glucose. This recombinant strain showed higher hydrolysis efficiency in the cellulose simultaneous saccharification and fermentation, when using the *Trichoderma reesei* cellulase, which is short of the β-glucosidase activity. The final ethanol concentration was 110% (using Avicel) and 89% (using acid-pretreated corncob) higher than the control strain. These results demonstrated the effect of β-glucosidase secretion in the recombinant *S. cerevisiae* for enhancing cellulosic ethanol conversion.

Keywords: β-Glucosidase, *Saccharomyces cerevisiae*, cellulase, simultaneous saccharification, fermentation, ethanol

Introduction

Cellulose, as the most abundant renewable biomass on earth, is an unexploited potential source for the production of biofuel such as bioethanol [19, 26]. The cellulosic bioethanol fermentation process contains the enzymatic hydrolysis of cellulosic biomass into reducing sugars, and the fermentation of the sugars to ethanol. Converting cellulose into fermentable sugars is considered as the major obstacle of industrial bioethanol production, because of the low efficiency and high cost of the enzymatic hydrolysis process [8, 33]. Simultaneous saccharification and fermentation (SSF) of lignocellulose to ethanol would greatly enhance the efficiency of bioethanol production. In SSF, the continuous consumption of sugars released during lignocellulose hydrolysis can prevent the feedback-inhibition of the hydrolytic enzymes and decrease the risk of contamination because of the presence of ethanol [31].

Cellulose hydrolysis requires the synergistic use of cellulase, primarily including endoglucanases (hydrolyzing the internal bonds of cellulose to yield cellobiose and cellooligosaccharides; EG), cellobiohydrolases (cleaving cellulose from reducing or nonreducing ends to release cellobiose; CBH) and β-glucosidases (hydrolyzing cellobiose to glucose; BGL) [11]. Cellulase from different filamentous fungi such as *Trichoderma reesei* that currently dominates the industrial applications of cellulase is short of the β-glucosidase activity [21, 22]. Cellobiose, one of the major end products of *T. reesei* cellulase, acts as a strong inhibitor, especially for cellobiohydrolase, which plays a key role in cellulase [1]. Therefore, β-glucosidase is not only critical to catalyze glucose generation, but is also required to remove cellobiose inhibition of other enzymatic hydrolysis [3, 6]. In the SSF process, removal of the inhibitory cellobiose can be resolved by the addition of exogenous β-glucosidase or the construction of a fermentation strain harboring high β-

glucosidase activity [15, 30, 31].

The yeast *Saccharomyces cerevisiae* is an efficient fermentation microorganism for ethanol production, due to its high ethanol productivity, high tolerance to toxic compounds, and robust growth [14, 17]. However, it cannot assimilate and metabolize cellobiose owing to the lack of cellobiose transporter and β-glucosidase. Therefore, cellobiose utilization of *S. cerevisiae* was needed to improve the SSF efficiency.

In previous studies, β-glucosidase or cellobiose phosphorylase combined with cellodextrin transporter, which can transport cellobiose across the plasma membrane, was introduced to *S. cerevisiae* to enable yeast to transport and metabolize cellobiose. Other studies have also shown that the expression of β-glucosidase genes with signal sequence in *S. cerevisiae* can also confer the recombinant strain to secrete β-glucosidase and utilize cellobiose [13, 27]. Wilde *et al*. compared the activity of 35 β-glucosidases from 12 filamentous fungi such as *Aspergillus niger*, and *Aspergillus oryzae* expressed in *S. cerevisiae*, and found the β-glucosidase from *A. niger* had the highest cellobiase activity [34]. The *BGL1* gene from *S. fibuligera* is also frequently expressed in *S. cerevisiae* owing to its high activity [4, 11, 28, 36]. In addition, BGL from bacteria such as *Cellulomonase biazotea* was also expressed in *S. cerevisiae* [26]. The β-glucosidase in previous studies was mainly expressed by the yeast expression vectors using auxotroph markers such as pYES2, Yeplac195, and ySFI, which need to grow on selective synthetic complete (SC) medium to maintain plasmid stability. However, to obtain the strains that can be used in SSF, a constitutive and high expression of the β-glucosidase in complex unselective medium was mandatory.

In this study, β-glucosidase genes from different origins were respectively expressed in *S. cerevisiae* [18] and the signal peptides were also compared. The strain expressing *S. fibuligera* BGL with its native signal peptides showed best activity, and it has 30% higher β-glucosidase activity than previous reported levels [32]. This recombinant strain can metabolize cellobiose with a similar specific growth rate in glucose. The ethanol production from SSF of Avicel or acid-pretreated corncob hydrolysis was also improved significantly by the strain expressing *S. fibuligera* β-glucosidase when using *T. reesei* T1 fermentation broth for the hydrolysis.

Materials and Methods

Strains, Media, and Enzymes

The strains and plasmids used in the present study are summarized in Table 1. *S. cerevisiae* CEN.PK102-3A and *S. fibuligera* were cultivated

Table 1. Microbial strains and plasmids used in the present study.

Strain or plasmid	Genotype	Source of reference
S. cerevisiae		
CEN.PK102-3A	*MATa ura3-52 leu2-112*	[7]
102-ΔTPI	CEN.PK102-3A derivative; *TPIΔ*	This work
102CT	102-ΔTPI derivative; CPOT	This work
102SB	102-ΔTPI derivative; CPOTSB	This work
102AB	102-ΔTPI derivative; CPOTAB	This work
102CB	102-ΔTPI derivative; CPOTCB	This work
102SMB	102-ΔTPI derivative; CPOTSMB	This work
102SIB	102-ΔTPI derivative; CPOTSIB	This work
102SSB	102-ΔTPI derivative; CPOTSSB	This work
102SPB	102-ΔTPI derivative; CPOTSPB	This work
Plasmids		
CPOT	*TPI1* promoter, 2 μm plasmid with *POT1* gene from *S. pombe* as a selection marker	[18]
CPOTSB	CPOT with BGL from *S. fibuligera;* native signal peptide	This work
CPOTAB	CPOT with BGL from *A. niger;* native signal peptide	This work
CPOTCB	CPOT with BGL from *T. reesei* QM9414	This work
CPOTSMB	CPOT with BGL from *S. fibuligera*, *MFα* signal peptide	This work
CPOTSIB	CPOT with BGL from *S. fibuligera*, signal peptide of *Kluyveromyces INU*	This work
CPOTSSB	CPOT with BGL from *S. fibuligera*; *SUC2* signal peptide	This work
CPOTSPB	CPOT with BGL from *S. fibuligera*; *PHO5* signal peptide	This work

in YPD medium (10 g/l yeast extract, 20 g/l peptone, 20 g/l glucose), and *S. cerevisiae* CEN.PK102-ΔTPI with deletion of the *TPI1* gene was cultivated on YPE (10 g/l yeast extract, 20 g/l peptone, 20 g/l ethanol, 0.5 g/l glucose). Mycelial growth of *T. reesei* QM9414 and *A. niger* Nip35 was cultivated with 20% wheat bran medium. *Escherichia coli* trans5α was used for recombinant plasmids amplification in Luria−Bertani medium with 100 μg/ml of ampicillin.

The commercial cellulase with a filter paper activity (FPA) of 82.40 IU/ml and β-glucosidase activity of 602.00 IU/ml was purchased from Genencor (E-072095; USA). *T. reesei* T1 cellulase was directly from the fermentation broth with a FPA of 6.34 IU/ml and β-glucosidase activity of 3.38 IU/ml.

Plasmid Construction

The PCR primers used in this study are listed in Table 2. Based on previous study, the disruption cassette of the *kanMX4* module, conferring the geneticin resistance in yeast, was amplified from CEN.PK530-1D [16] and transformed into *S. cerevisiae* CEN.PK102-3A resulting in 102-ΔTPI [10, 23]. β-Glucosidase genes with or without the signal peptide were amplified from *S. fibuligera* genomic DNA (SF-BGL1), *A. niger* Nip35 cDNA (AN-BGL1), and *T. reesei* QM 9414 cDNA (CEL1B). The signal peptides of *S. cerevisiae MFα*, *SUC2*, *PHO5*, and *INU* of *Kluyveromyces* were also used to replace their native signal peptides. Plasmids CPOT [18], which contains the *TPI* promoter and *TPI* terminator and the *POT1* gene from *Schizosaccharomyces pombe* as the marker, was used to insert the β-glucosidase gene by one-step ISO assembly of overlapping dsDNA of the Gibson method [9]. The plasmids were verified by sequencing and then transformed into 102-ΔTPI. The resulting recombinant strains are listed in Table 1.

Enzymatic Assays

β-Glucosidase activity was assayed using the substrate *p*-nitrophenyl-β-D-glucopyranoside (pNPG) [2]. Cells were centrifuged at 13,000 rpm for 4 min and the supernatant was incubated in 50 mM citrate buffer with 5 mM pNPG at pH 5.0, 50°C for 30 min. The *p*-nitrophenol released from pNPG was detected at 405 nm after adding 10% sodium carbonate to stop the reaction. One unit of enzyme activity was defined as the amount of enzyme required to release 1 μmol of *p*-nitrophenol per minute.

Fermentation of Glucose and Cellobiose

The yeast was precultured in YPD medium (10 g/l yeast extract, 20 g/l peptone, and 20 g/l glucose) at 30°C, 300 rpm. The glucose and cellobiose fermentations were performed in YP medium with 20 g/l glucose or cellobiose as carbon source in 100 ml shake flasks with a 40 ml working volume. The initial OD600 was controlled as 0.2. The shake flasks were plugged with rubber plugs to maintain the oxygen-limited conditions or cotton plugs to maintain the aerobic condition in batch fermentations.

Simultaneous Saccharication and Fermentation

SSF of Avicel was performed in the medium with 30 g/l Avicel, 10 g/l yeast extract, and 20 g/l peptone at 30°C, 300 rpm in 100 ml flasks with a 40 ml working volume. The corncob was pretreated by acid to remove xylose and lignin [28], and the substrate

Table 2. The PCR primers used for the amplification.

Name	Sequence (5′-3′)	Template
SF-F	TATAACTACAAAAAACACATACATAAACTAAAAGGTACCATGTTGATGATAGTACAGC	*S. fibuligera BGL1*
SF-R	TTTTATATAATTATATTAATCTTAGTTTCTAGACTCGAGTCAAATAGTAAACAGGACAG	
M-F	TATAACTACAAAAAACACATACATAAACTAAAAGGTACCATGAGATTTCCTTCAATTTTT	*MFα* leader
M-R	GGTATAGTTTTGAATTGGGACAGCTTCAGCCTCTCTTTT	
MS-F	AAAAGAGAGGCTGAAGCTGTCCCAATTCAAAACTATACC	
INU-F	TATAACTACAAAAAACACATACATAAACTAAAAGGTACCATGAAGTTCGCATACTCCC	*INU* leader
INU-R	TCTCTGGGATGGAGACTGGGTATAGTTTTGAATTGGGACTCTCTTGTAATTGATCACTG	
SUC2-F	TATAACTACAAAAAACACATACATAAACTAAAAGGTACCATGCTTTTGCAAGCTTTCC	*SUC2* leader
SUC2-R	TCTCTGGGATGGAGACTGGGTATAGTTTTGAATTGGGACTGCAGATATTTTGGCTGC	
PHO5-F	TATAACTACAAAAAACACATACATAAACTAAAAGGTACCATGTTTAAATCTGTTGTTTAT	*PHO5* leader
PHO5-R	TCTCTGGGATGGAGACTGGGTATAGTTTTGAATTGGGACTGCATTGGCCAAAGAAGCG	
AN-F	TATAACTACAAAAAACACATACATAAACTAAAAGGTACCATGAGGTTCACTTTGATC	*A. niger BGL1*
AN-R	TTTTATATAATTATATTAATCTTAGTTTCTAGACTCGAGTTAGTGAACAGTAGGCAG	
TR-F	CGGGGTACCATGCCCGAGTCGCTAGCTCTG	*T. reesei* QM9414 cDNA
TR-R	CCGCTCGAGTTATGCCGCCACTTTAACCCTCT	
TPI-F	ACCCATCAGGTTGGTGGAAG	CEN.PK530-1D genomic DNA
TPI-R	CAACGCGAAAATGACGCCTC	

contained 70.5% cellulose. SSF of acid-pretreated corncob was performed with the medium of 80 g/l acid-pretreated corncob (dry weight/volume), 2 g/l $(NH4)_2SO_4$, 5 g/l KH_2PO_4, 2 g/l $MgSO_4$•$7H_2O$, 0.2 g/l $CaCl_2$, and pH 4.8 at 30°C, 300 rpm in 100 ml flasks with a 40 ml working volume. Cellulase (30 IU of FPA/g of Avicel or 25 IU of FPA/g of acid-pretreated corncob) was added to the medium, incubated at 45°C for 2 h, and then the yeast cells were inoculated to the medium with initial OD600 of 1.0. The shake flasks were plugged with rubber plugs to maintain the oxygen-limited conditions or cotton plugs to maintain the aerobic condition in batch fermentations.

Metabolite Analysis

Samples from the fermentation were centrifuged and filtered by 0.45 μm filters, and the cellobiose, glucose, and ethanol concentrations were determined by HPLC using an Aminex HPX-87 H column (Bio-Rad, Richmond, CA, USA) with 5 mmol/l H_2SO_4 at a flow rate of 0.6 ml/min, at 45°C as a mobile phase. The peaks were detected by a RID-10A refractive index detector (Shimadzu, Kyoto, Japan).

The sugar concentration in the biomass was determined according to Chinese Standard Methods [29]. Briefly, the dry biomass was extracted by pure alcohol and hydrolyzed for 2.5 h at 20°C using 72% H_2SO_4 (15 ml/g biomass) and then for another 1 h at 121°C using 3% H_2SO_4. The hydrolysate was then filtered and the supernatant was neutralized with powder $Ba(OH)_2$ and centrifuged at 8,000 rpm for 15 min. The glucose concentration in the supernatant was measured using an SBA-40C biological sensor analyzer (Biological Institute of Shandong Academy of Science, Shandong Province, China).

Results

Recombinant β-Glucosidase Secretion in *S. cerevisiae*

The β-glucosidase genes from *S. fibuligera* (SF-BGL1), *A. niger* Nip35 (AN-BGL1), and *T. reesei* QM9414 (CEL1B) with their own signal peptide were expressed respectively under the control of the *TPI1* promoter. The activity of β-glucosidases showed that 102SB (SF-BGL1) had the highest activity, up to 5.22 U/ml fermentation broth at 72 h, and the activity of 102AB (AN-BGL1) was 0.85 U/ml, but β-glucosidase activity was not detected in 102CB (CEL1B) (Fig. 1A). We also compared the activity of SF-BGL1 with different signal peptides. The signal peptides from *S. cerevisiae MFα*, *SUC2*, *PHO5*, and *Kluyveromyces INU*, which has been successfully used to secrete heterologous proteins in *S. cerevisiae*, were used to secrete SF-BGL1. As shown in Fig. 1B, the protein with INU and its native peptide, but not the frequently used MFα peptide, showed the highest activity. Consequently, we chose the strain expressing SF-BGL1 (102SB) with its native signal peptide for further analysis.

Glucose and Cellobiose Fermentation of the Recombinant Strains

To investigate the physiological characteristics of recombinant strain 102SB, glucose or cellobiose cultivations were performed

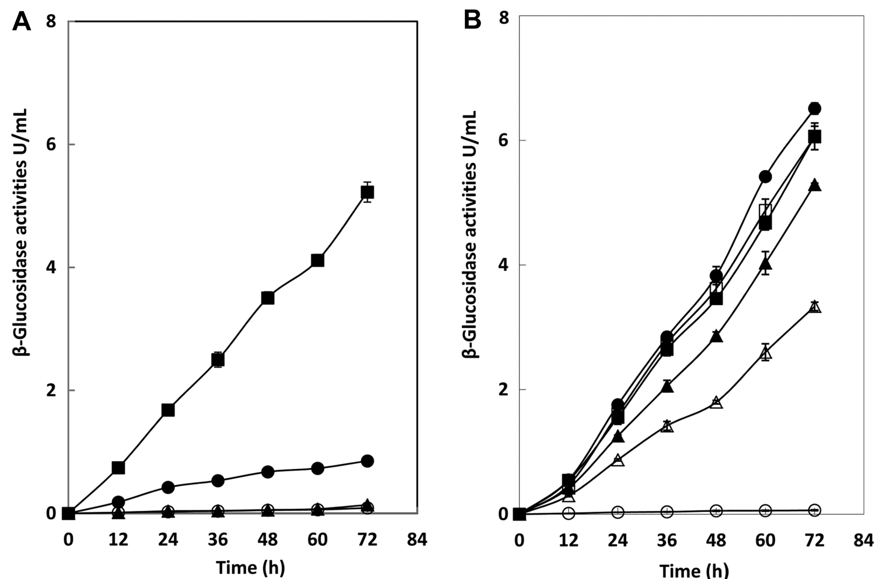

Fig. 1. Activity of different β-glucosidases on pNPG in recombinant yeasts.
(**A**) Activity of different β-glucosidases with native peptide. The symbols: ■, 102SB; ●, 102AB; ▲, 102CB; ○, Control. (**B**) Activity of β-glucosidase with the different signal peptides. The symbols: □ , native peptide; ■, SUC2; ▲, PHO5; △, MF alpha ●, INU; ○, Control. Values shown are the mean ± standard error.

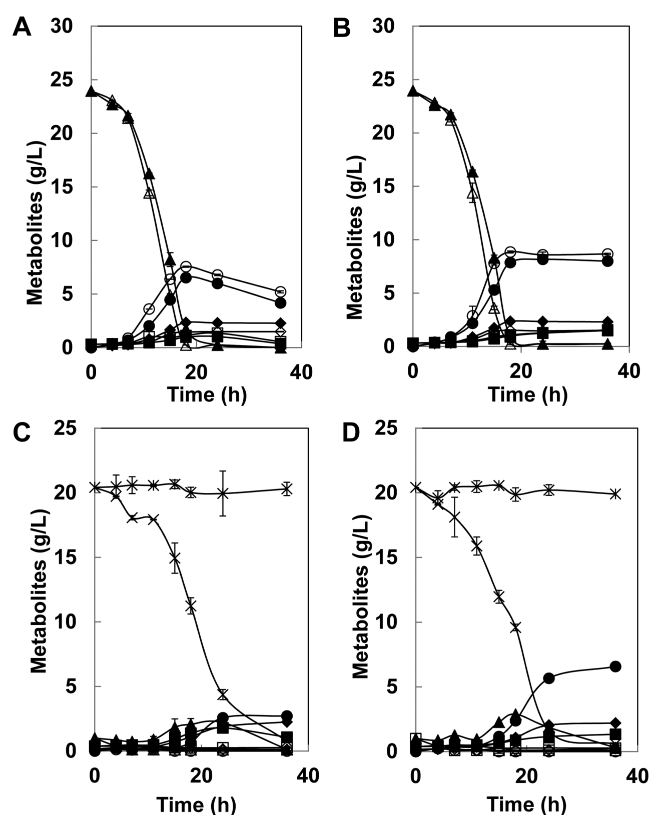

Fig. 2. Fermentations of glucose and cellobiose by control strain 102CT (hollow symbols) and recombinant yeast 102SB (solid symbols).

(**A**) Aerobic fermentation of glucose. (**B**) Oxygen-limited fermentation of glucose. (**C**) Aerobic fermentation of cellobiose. (**D**) Oxygen-limited fermentation of cellobiose. The symbols: ⁎ , cellobiose (control); ×, cellobiose (102SB); ▲ or △, glucose; ● or ○, ethanol; ■ or □, acetate; ◆ or ◇, glyceol.

in aerobic or oxygen-limited conditions, and the strain 102CT with the empty plasmid was used as a control. The 102SB had similar growth characteristics in glucose fermentation, compared with the control strain 102CT, despite of a slight decrease of the specific growth rate and ethanol yield, perhaps resulting from the metabolic burden of BGL expression (Figs. 2A, 2B, and Table 3). As shown in Figs. 2C and 2D, 102SB could metabolize cellobiose, and the specific growth rate was 0.29 h^{-1} in aerobic condition and 0.26 h^{-1} in oxygen-limited condition, similar to the growth in glucose fermentation (Table 3). The ethanol yield was 0.33 g/g, and the specific cellobiose consumption rate was 2.16 g/g DCW/h in oxygen-limited cultivation (Table 3). Strain 102CT could not utilize cellobiose, as expected.

Simultaneous Saccharification and Fermentation of Avicel

The SSF characteristics of the recombinant strain were assessed using either the commercial cellulase from Genencor or the cellulase directly from *T. reesei* T1 fermentation broth. We found that the ratio of β-glucosidase activity (BGL) and filter paper activity (FPA) in the commercial cellulase was 7.3 and the major hydrolysis end product of Avicel was glucose. However, the ratio of BGL and FPA in cellulase directly from *T. reesei* T1 fermentation broth was only 0.53, and the main hydrolysis end product of Avicel was glucose and cellobiose.

SSF of 102SB and 102CT was performed using Avicel as the sole carbon source and supplementing with 30 IU/g Avicel cellulase [31]. As observed in Fig. 3A, no difference of the final ethanol concentration was found (~8.2 g/l) in the two strains in the presence of commercial cellulase, indicating that the activity of β-glucosidase in commercial cellulase is abundant for Avicel hydrolysis. However, for T1 cellulase, 102CT showed low cellulosic ethanol production (~4 g/l) and had obvious cellobiose accumulation. In contrast, the ethanol concentration of 102SB increased to 2.1-folds (~8.2 g/l). The cellobiose generated from Avicel hydrolysis in 102SB was consumed much faster than the control strain, indicating that the conversion of cellobiose to glucose is a

Table 3. Fermentation profiles from batch cultivations of the *S. cerevisiae* recombinant strains on glucose and cellobiose.

Carbon source	Culture condition	Strain	μ_{max} (h^{-1})[a]	Product yields (g/g sugar)				r_s[b]
				Biomass	Ethanol	Glycerol	Acetate	
Glucose	Aerobic	Control	0.33	0.13	0.31	0.06	0.03	2.54
		102SB	0.29	0.12	0.28	0.10	0.03	2.42
	Oxygen-limited	Control	0.29	0.12	0.39	0.06	0.03	2.41
		102SB	0.27	0.11	0.34	0.10	0.03	2.38
Cellobiose	Aerobic	102SB	0.29	0.13	0.22	0.11	0.09	2.23
	Oxygen-limited	102SB	0.26	0.12	0.33	0.12	0.05	2.16

[a]Maximum specific growth rate.

[b]The specific sugar consumption rate (g/g DCW/h).

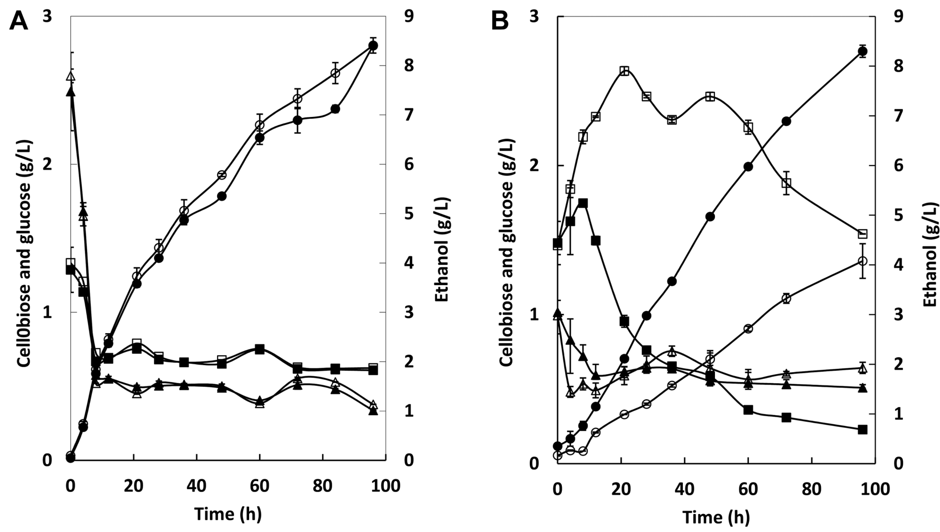

Fig. 3. SSF of control strain 102CT (hollow symbol) and recombinant yeast 102SB (solid symbol) with Avicel under oxygen-limited condition.

(**A**) Genencor cellulase; (**B**) T1 cellulase. The symbols: ■ or □, cellobiose; ▲ or △, glucose; ● or ○, ethanol.

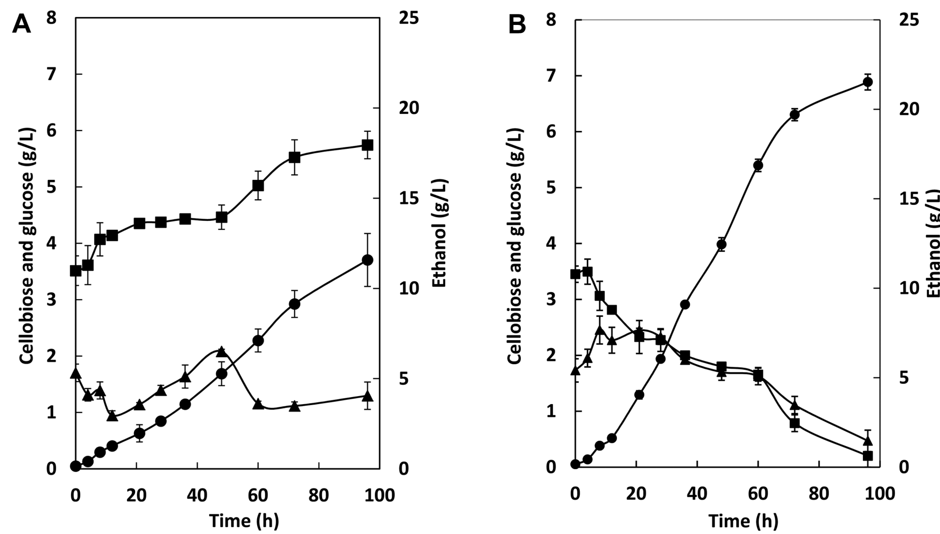

Fig. 4. SSF of lignocellulose by T1 cellulase under oxygen-limited condition.

(**A**) Control strain; (**B**) Recombinant yeast 102SB. The symbols: ■, cellobiose; ▲, glucose; ●, ethanol.

limiting step in cellulosic SSF and the cellobiose fermentation capability of the yeast strains could enhance the efficiency of the cellulose hydrolysis (Fig. 3B). We also found that the ethanol production of 102SB by using two kinds of cellulase was similar, showing that in the recombinant strain, the hydrolysis of cellobiose was not a bottleneck for the production of relatively high ethanol concentrations from Avicel in the SSF process.

Simultaneous Saccharification and Fermentation of Acid-Pretreated Corncob

To evaluate the fermentative performance of 102SB, the lignocellulose SSF was carried out in oxygen-limited condition using 80 g/l acid-pretreated corncob and 25 IU T1 cellulase per gram substrate. As shown in Fig. 4, 102CT showed obvious cellobiose accumulation, while 102SB showed much faster consumption of cellobiose and glucose produced

from the lignocellulose. The ethanol titer of 102SB reached to 21.5 g/l within the 96 h, 89% higher than 102CT. The residue sugar in the biomass was 9.4 g/l and the ethanol yield of 102SB was 0.40 g/g consumed sugar. However, in the SSF of 102CT, the residue sugar in the biomass was 33.1 g/l, which demonstrated *S. cerevisiae* with high β-glucosidase secretion can significantly improve the efficiency of simultaneous saccharification and cellulosic ethanol conversion.

Discussion

SSF has been identified as an effective process for lignocellulose ethanol conversion, because it combines the cellulose hydrolysis and ethanol production by *S. cerevisiae* in a single process. However, the accumulation of cellobiose as a strong inhibitor of cellulase, especially exoglucanases, affects the degradation of the cellulose and ethanol production [5, 12, 24, 25]. Therefore, recombinant yeast expressing β-glucosidase was used to relieve cellobiose inhibition. In our study, to obtain the best β-glucosidases secretion capability, β-glucosidases from *S. fibuligera, A. niger* Nip35, and *T. reesei* QM9414 were expressed and compared in the *S. cerevisiae*. Importantly, β-glucosidase from *S. fibuligera* in 102SB could be successfully secreted extracellularly and the activity reached to 5.22 U/ml (equal to 1005.3 U/g DW) in fermentation broth at 72 h, much higher than the previous reported activity. For example, Gurgu *et al.* [11] only obtained 250 mU/ml β-glucosidase activity in an industry strain and Zhang *et al.* [36] demonstrated a strain with 450 mU/ml activity. 102SB could produce β-glucosidase with activity of 706 U/g DW at 36 h, and increased to 1,005.3 U/g DW at 72 h, whereas Y294[SFI] produced β-glucosidase with activity of 770 U/g DW at 36 h, but started to decrease after 36 h [4].

The factors affecting heterologous protein secretion in *S. cerevisiae* include plasmid copy number, the stability of the plasmids, the promoter strength, the signal peptide, the folding and secretion of the proteins, and so on. In our study, the plasmid using gene *POT1* (encoding triose-phosphate isomerase from *S. pombe*) as the marker and the strong *TPI1* promoter was chosen for recombinant expression. The endogenous gene encoding triosephosphate isomerase (*TPI1*) in the reference strain was deleted, and when the plasmid carrying the β-glucosidase gene was transformed into the strain, the corresponding gene (*POT1*) could recover its growth on glucose as the sole carbon source. If the plasmid is lost, the cells lack a key glycolytic enzyme, resulting in impaired growth. Therefore, the recombinant strains have an inherent growth advantage to ensure high plasmid stability, and thereby high protein production even in the complex medium. Different protein properties require different secretion mechanisms, and therefore, the signal peptides was also compared in order to obtain the best secretion capability. We found that the protein with the *S. fibuligera* BGL native signal peptide and INU signal peptide had the highest activity, thus, the native signal peptide was chosen for further study.

Although high ethanol yield in SSF of lignocellulosic materials can be obtained when using commercial cellulase, the commercial cellulase generally also required the optimization of the cellulase composition such as the addition of β-glucosidase from *A. nige*r [15, 30, 31]. In not only the cellulolytic enzyme system from *T. reesei* but also in the other species such as *Neurospora crassa, Thielauia terrestris,* and *Sporotrichum cellulophilum*, the ratio of β-glucosidase was also quiet low [35]. The cost of cellulase production is considered as an important factor in the commercialization of lignocellulosic biomass-to-ethanol processes, and the addition of β-glucosidase, produced by the cultivation of another microorganism will increase the cost significantly [20]. In this study, we chose the cellulase of *T. reesei* T1 directly from the fermentation broth for cellulose hydrolysis. When recombinant *S. cerevisiae* with high β-glucosidase secretion was used, SSF of Avicel by T1 cellulase could produce ethanol to the same level of the process by commercial cellulase.

Cellobiose-induced inhibition can be relieved by the addition of exogenous β-glucosidase. Thus, β-glucosidase expression in a recombinant yeast needs to be optimized to overcome the rate-limiting step of cellulose hydrolysis**.** The construction of 102SB largely increased the efficiency of simultaneous saccharification and cellulosic ethanol fermentation. It also provided a potential system for the construction of a consolidated bioprocessing yeast strain, which can combine the cellulose hydrolysis and ethanol production in a single organism. Although the SSF has been improved significantly, further optimization is still necessary to reduce the cost of cellulosic ethanol production.

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