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ORIGINAL PAPER

Ethanol production from acid- and alkali-pretreated corncob by endoglucanase and β-glucosidase co-expressing *Saccharomyces cerevisiae* subject to the expression of heterologous genes and nutrition added

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Abstract Low-cost technologies to overcome the recalcitrance of cellulose are the key to widespread utilization of lignocellulosic biomass for ethanol production. Efficient enzymatic hydrolysis of cellulose requires the synergism of various cellulases, and the ratios of each cellulase are required to be regulated to achieve the maximum hydrolysis. On the other hand, engineering of cellulolytic *Saccharomyces cerevisiae* strains is a promising strategy for lignocellulosic ethanol production. The expression of cellulase-encoding genes in yeast would affect the synergism of cellulases and thus the fermentation ability of strains with exogenous enzyme addition. However, such researches are rarely reported. In this study, ten endoglucanase and β-glucosidase co-expressing *S. cerevisiae* strains were constructed and evaluated by enzyme assay and fermentation performance measurement. The results showed that: (1) maximum ethanol titers of recombinant strains exhibited high variability in YPSC medium (20 g/l peptone, 10 g/l yeast extract, 100 g/l acid- and alkali-pretreated corncob) within 10 days. However, they had relatively little difference in USC medium (100 g/l acid- and alkali-pretreated corncob, 0.33 g/l urea, pH 5.0). (2) Strains 17# and 19#, with ratio (CMCase to β-glucosidase) of 7.04 ± 0.61 and 7.40 ± 0.71 respectively, had the highest fermentation performance in YPSC. However, strains 11# and 3# with the highest titers in USC medium had a higher ratio of CMCase to β-glucosidase, and CMCase activities. These results indicated that nutrition, enzyme activities and the ratio of heterologous enzymes had notable influence on the fermentation ability of cellulase-expressing yeast.

Keywords *Saccharomyces cerevisiae* · Consolidated bioprocessing · Endoglucanase · β-Glucosidase

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Introduction

Lignocellulosic biomass is an abundant and potentially sustainable resource for bioethanol production (Stephanopoulos 2007). Currently, the main technological barrier to more widespread utilization of this resource is the lack of low-cost technologies to overcome the recalcitrance of its major component cellulose (Himmel et al. 2007). Efficient enzymatic hydrolysis of cellulose requires synergistic action of three major types of enzymes, endoglucanase (EG), exoglucanase (including cellodextrinase and cellobiohydrolase (CBH)), and β-glucosidase (BGL) (Zhang and Lynd 2004). EGs act randomly on the amorphous

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regions of cellulose to produce reducing and non-reducing ends for CBHs, which produce cellobiose from reducing or non-reducing ends of crystalline cellulose. Cellulose chains are thus degraded to cellobiose and cello-oligosaccharides, which are converted to glucose by BGLs (la Grange et al. 2010; Fujita et al. 2004). The various cellulases synergistically act on the substrates, any changes of cellulase components will affect the synergism, and the ratios of each cellulase are required to be regulated to achieve the maximum hydrolysis. On the other hand, consolidated bioprocessing (CBP), which could reduce the cost of hydrolysis and fermentation, has gained increasing recognition as a promising technology for bioethanol production from lignocellulosic biomass (Lynd et al. 2005). The key to CBP is the engineering of a microorganism that can efficiently utilize biomass polysaccharides to produce ethanol (Hahn-Hägerdal et al. 2006). Development of cellulolytic *Saccharomyces cerevisiae* strains is one of strategies due to its many superior traits, including GRAS organism; high ethanol productivity, yield, and tolerance; robustness in industrial process; and easiness of genetic manipulation (van Zyl et al. 2007).

There are a number of reports on cellulolytic *S. cerevisiae* strains construction (reviewed by Hasunuma and Kondo 2012; la Grange et al. 2010; van Zyl et al. 2007). Some studies showed that the recombinant strains could decrease the loading of exogenous cellulase or improve the efficiency of cellulase hydrolysis and ethanol production in simultaneous saccharification and fermentation (SSF) process. Our previous researches also indicated that *S. cerevisiae* strains with or without exo- and endo-cellulase activity, expressing *Aspergillus aculeatus* β-glucosidase encoding gene, could enhance the performance of commercial cellulase in SSF process (Hong et al. 2014; Wang et al. 2013). Efficient degradation of lignocellulosic biomass requires an appropriate expression ratio of cellulolytic enzymes in recombinant *S. cerevisiae*. However, currently it is impossible to efficient utilize cellulose to ethanol without exogenous enzyme addition. From a practical point of view, the construction of cellulase expressing yeast is aimed at reducing the amount of externally added enzymes that are needed to convert cellulose to ethanol. With exogenous cellulase, the ratio and activities of cellulase components in cellulolytic *S. cerevisiae* undoubtedly will affect the synergism of cellulases in medium, thus affecting the fermentation performance of recombinant strains. However, such researches are rarely reported.

In our previous research, a recombinant strain *S. cerevisiae* W2 expressing endoglucanase and β-glucosidase, was constructed by sequential integratation of *Trichoderma reesei eg*2 and *Aspergillus aculeatus bgl*1. Compared with the parent strain W303-1A, W2 could efficiently utilize acid- and alkali-pretreated corncob to produce ethanol with exogenous cellulase addition (Hong et al. 2014). The results indicated that the cellulolytic *S. cerevisiae* strain co-expressing EG and BGL could reduce the loading of commercial cellulase. As described above, the activity and ratio of EG and BGL in cellulases expressing yeast affect the fermentation performance of recombinant strains from cellulose. To study this influence, ten cellulases expressing *S. cerevisiae* strains with different expression levels of EG and BGL were constructed in this study. And these strains were evaluated by enzyme activity measurement and fermentation ability determination in pretreated corncob containing media. To our knowledge, this is the first study on the effect of the activity and ratio of heterologous cellulases in EG and BGL co-expressing *S. cerevisiae* strains on the fermentation performance in cellulose containing media with exogenous cellulase addition.

Materials and methods

Strains, plasmids, media, and growth conditions

All strains and plasmids used in this study are listed in Table 1. *Escherichia coli* TOP10 was used for recombinant DNA manipulation. *S. cerevisiae* W1 expressing *T. reesei eg*2, constructed by Hong et al. (2014), was used as the host for BGL expression. And *S. cerevisiae* W303-1A was used as the control for the evaluation of fermentation performance. The δ-integrative plasmid H-AaBGLI was constructed in our previous research (Hong et al. 2014).

Escherichia coli transformants were grown at 37 °C in Luria–Bertani (LB) medium (10 g/l peptone, 5 g/l yeast extract, and 10 g/l NaCl) with 100 μg/ml ampicillin when necessary. *S. cerevisiae* strains were cultured at 30 °C in CMG medium (6.7 g/l yeast nitrogen base without amino acids (Bio Basic Inc.), 20 g/l glucose, supplemented with appropriate amino acids and nucleic acids), YPD medium (20 g/l peptone, 10 g/l yeast extract, and 20 g/l glucose), YPSC (20 g/l peptone, 10 g/l yeast extract, and 100 g/l acid- and alkali-pretreated corncob), USC (100 g/l acid- and alkali-pretreated corncob, 0.33 g/l urea, pH 5.0).

Yeast transformation

10 μg of the δ-integrative plasmid H-AaBGLI was digested with the appropriate restriction enzymes, purified, and transformed into W1 according to the lithium acetate method (Gietz et al. 1995). The transformants were screened on CMG plates with L-histidine and L-tryptophan default. Ten recombinant strains with higher β-glucosidase activity were selected from 20 colonies and used in the subsequent experiments.

Table 1 Characteristics of bacterial, yeast strains and plasmid used in this study

Strain or plasmid	Relevant features	References
Bacterial strain		
E. coli TOP10	F^- *mcr*A Δ(*mrr-hsd* RMS-*mcr*BC) φ80 *lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*Δ139 Δ(*ara-leu*)7697 *gal*U *gal*K *rps*L (Str^R) *end*A1 *nup*G	Invitrogen
S. cerevisiae strain		
W303-1A	*MAT*a *leu2-3, 112 ura3-1 trp1-92 his3-11,15 ade2-1 can1-100*	In our lab
W1	*MAT*a *leu2-3, 112 ura3-1 his3-11,15 ade2-1 can1-100* δ::P*tpi-xyn*2 *s*-TEGII-T*adh*I	Hong et al. (2014)
3#	*MAT*a *leu2-3, 112 ura3-1 ade2-1 can1-100* δ::P*tpi-xyn*2 *s*-TEGII-T*adh*I, P*tpi-xyn*2 *s*-ABGLI-T*adh*I	This study
4#	*MAT*a *leu2-3, 112 ura3-1 ade2-1 can1-100* δ::P*tpi-xyn*2 *s*-TEGII-T*adh*I, P*tpi-xyn*2 *s*-ABGLI-T*adh*I	This study
6#	*MAT*a *leu2-3, 112 ura3-1 ade2-1 can1-100* δ::P*tpi-xyn*2 *s*-TEGII-T*adh*I, P*tpi-xyn*2 *s*-ABGLI-T*adh*I	This study
7#	*MAT*a *leu2-3, 112 ura3-1 ade2-1 can1-100* δ::P*tpi-xyn*2 *s*-TEGII-T*adh*I, P*tpi-xyn*2 *s*-ABGLI-T*adh*I	This study
9#	*MAT*a *leu2-3, 112 ura3-1 ade2-1 can1-100* δ::P*tpi-xyn*2 *s*-TEGII-T*adh*I, P*tpi-xyn*2 *s*-ABGLI-T*adh*I	This study
11#	*MAT*a *leu2-3, 112 ura3-1 ade2-1 can1-100* δ::P*tpi-xyn*2 *s*-TEGII-T*adh*I, P*tpi-xyn*2 *s*-ABGLI-T*adh*I	This study
15#	*MAT*a *leu2-3, 112 ura3-1 ade2-1 can1-100* δ::P*tpi-xyn*2 *s*-TEGII-T*adh*I, P*tpi-xyn*2 *s*-ABGLI-T*adh*I	This study
16#	*MAT*a *leu2-3, 112 ura3-1 ade2-1 can1-100* δ::P*tpi-xyn*2 *s*-TEGII-T*adh*I, P*tpi-xyn*2 *s*-ABGLI-T*adh*I	This study
17#	*MAT*a *leu2-3, 112 ura3-1 ade2-1 can1-100* δ::P*tpi-xyn*2 *s*-TEGII-T*adh*I, P*tpi-xyn*2 *s*-ABGLI-T*adh*I	This study
19#	*MAT*a *leu2-3, 112 ura3-1 ade2-1 can1-100* δ::P*tpi-xyn*2 *s*-TEGII-T*adh*I, P*tpi-xyn*2 *s*-ABGLI-T*adh*I	This study
Plasmid		
H-AaBGLI	Amp^R HIS3 δ sequence β-Glucosidase I gene of *Aspergillus aculeatus*	Hong et al. (2014)

Enzyme assay

CMCase and β-glucosidase activity of *S. cerevisiae* recombinant strains were determined as described as in Hong et al. (2014).

Fermentation of the pretreated corn

The fermentation was performed in 100 ml screw flask containing 40 g YPSC or USC medium with 5 FPU IJT (Imperial Jade Bio-Technology Company, China) cellulase/g biomass. *S. cerevisiae* strains were grown aerobically in YPD medium for 24 h at 30 °C. The resultant cells were collected by centrifugation and then inoculated. The yeast loading was 1 g dry weight/kg slurry. The samples were drawn at 24-h intervals for ethanol analysis by HPLC (Agilent 1200) with RI-detection after separation on a guard column (Cation-H Refill Cartridges) and an Aminex HPX-87H column (Bio-Rad), using 4 mM H_2SO_4 as fluent with 0.6 ml/min flow at 40 °C.

Results

δ-Integration and enzyme assay

β-Glucosidase activity of commercial cellulase is commonly insufficient. In our previous research, β-glucosidase was shown to be an important component synergizing with IJT cellulase in the hydrolysis of acid- and alkali-pretreated corncob (Wang et al. 2013). Thus, the recombinant strains with higher β-glucosidase activity were desired to be constructed.

The linearized plasmid H-AaBGLI was transformed into W1, and the recombinants were screened via auxotrophic markers. Twenty colonies were randomly selected and

tested by enzyme assay, the results showed that all strains exhibited CMCase and β-glucosidase activities (data not shown). The results of 10 strains with higher β-glucosidase activity are shown in Table 2. The ratio of the two enzyme activities was also presented. The results showed that the activity values, and ratio of strains varied greatly, which made it possible to study the effect of heterologous cellulases expression on the fermentation performance of cellulolytic yeast.

Fermentation performance in cellulose-containing media

In previous reports, the cellulase dose used was usually 5–20 FPU/g biomass. In this study 5 FPU IJT cellulase/g biomass was added into two media with different nutrients in order to better compare the difference in ethanol production between strains.

The results in YPSC medium are shown in Fig. 1a and Table 3. Compared with the control strain W303-1A, 9 strains except strain 9# could efficiently utilize pretreated corncob to produce ethanol with exogenous cellulase addition. Based on the highest ethanol yielding, the 9 strains could be divided into three groups: (1) Strains 17# and 19# exhibited the highest fermentation performance within 10 days; (2) Strains 15#, 4#, 7#, and 11# had higher concentration; (3) Strains 3#, 6#, and 16# had the lowest titer.

However, the aforementioned *S. cerevisiae* strains showed different fermentation performance in USC medium (Fig. 1b; Table 3). All the recombinant strains had higher ethanol productivity than W303-1A within 6 days. On the other hand, the highest ethanol titer of only two strains 3# and 11# was notably higher than that of W303-1A, and that of four strains 7#, 9#, 15#, and 17# was notably lower than that of the control.

To compare the cellulolytic strains, the values of cellulase activities in YPD medium and maximum ethanol titer in cellulose containing medium were divided by that of strains with the lowest ethanol concentration, and the results are shown in Fig. 2. According to Fig. 2, it showed that: (1) nutrition had significant influence on the fermentation performance. The cellulases expressing strains exhibited highly variable maximum ethanol titers in YPSC medium (Fig. 2a). However, they had relatively little difference in USC medium (Fig. 2b). The control strain W303-1A exhibited much higher fermentation performance in USC medium than that in YPSC medium (Fig. 2). These results revealed notable effects and significance of nutrition added into media; (2) heterologous genes expression also affected fermentation ability. Based on comparison of enzyme activities and ratio, it seemed that recombinant strains with ratio of 7.00–7.40 had the highest fermentation ability in YPSC medium (Tables 2, 3). However, strains 11# and 3# with the highest titer in USC medium had higher ratio of CMCase to β-glucosidase, and CMCase activities (Figs. 1, 2; Tables 2, 3). The results indicated that the enzyme activities and the ratio of heterologous enzymes influenced the fermentation ability of cellulases-expressing yeast.

Discussion

As previously described, CBP has the potential to provide the lowest cost route for biological conversion of lignocellulosic biomass to bioethanol in processes featuring hydrolysis by enzymes and/or microorganisms (Lynd et al. 2005). Although there are a number of reports on cellulolytic *S. cerevisiae* development, the recombinant strains with the capability of efficient enzyme production, cellulose saccharification, and ethanol production are not available nowadays. On the other hand, enzyme-microbe synergy has a central role in microbial cellulose hydrolysis by anaerobic microbes, which impacts the potential feasibility and performance of CBP (Lynd et al. 2002, 2005). In addition, *T. reesei*, a well-known cellulolytic microbe, is able to secrete a cocktail of cellulases, which

Table 2 Enzyme activity of the recombinant strains

Strain	CMCase (U/ml)	β-Glucosidase (U/ml)	CMCase/β-Glucosidase
3#	31.41 ± 1.62	4.18 ± 0.11	7.51 ± 0.61
4#	28.49 ± 1.12	5.00 ± 0.13	5.70 ± 0.38
6#	26.16 ± 1.01	5.51 ± 0.22	4.75 ± 0.39
7#	39.70 ± 0.88	4.95 ± 0.21	8.02 ± 0.54
9#	27.36 ± 1.21	3.41 ± 0.13	8.02 ± 0.69
11#	39.61 ± 1.32	3.19 ± 0.12	12.41 ± 0.92
15#	24.35 ± 0.98	3.31 ± 0.04	7.35 ± 0.40
16#	27.89 ± 1.12	4.66 ± 0.21	5.98 ± 0.54
17#	36.40 ± 1.76	5.17 ± 0.18	7.04 ± 0.61
19#	26.02 ± 1.32	3.52 ± 0.15	7.40 ± 0.71

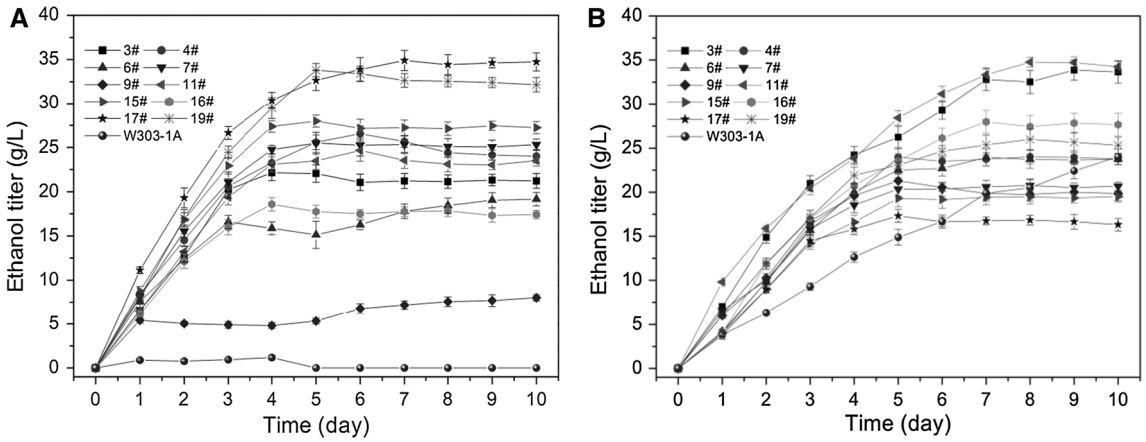

Fig. 1 Fermentation of acid- and alkali-pretreated corncob by the *S. cerevisiae* strains in YPSC medium (**a**) and USC medium (**b**). The cellulase IJT and the dose of 5 FPU/g biomass were used

Table 3 Maximum ethanol titer (g/l) of *S. cerevisiae* strains in pretreated corncob containing media

Strains	YPSC medium	USC medium
3#	22.14 ± 0.88	33.87 ± 1.14
4#	26.56 ± 0.87	24.02 ± 0.82
6#	19.16 ± 0.76	23.95 ± 0.99
7#	25.49 ± 1.22	20.75 ± 0.66
9#	8.01 ± 0.35	21.30 ± 0.75
11#	24.67 ± 1.24	34.76 ± 0.57
15#	27.98 ± 0.69	19.52 ± 0.62
16#	18.58 ± 0.75	27.85 ± 1.14
17#	34.90 ± 1.14	17.31 ± 0.73
19#	33.81 ± 0.74	25.99 ± 1.17
W303-1A	1.17 ± 0.23	24.02 ± 0.87

synergistically act on lignocellulosic biomass, and the ratios of each cellulase are fine-tuned via regulation the expression of cellulase-encoding genes in *T. reesei* to achieve the maximum hydrolysis in response to the environment (Hasunuma and Kondo 2012; Sticker et al. 2008). So the traits of CBP strains are subjected to the feedstock, pretreatment methods, and selection approaches (Hong et al. 2014; la Grange et al. 2010).

In this study, ten endoglucanase and β-glucosidase co-expressing *S. cerevisiae* strains were constructed and evaluated by enzyme assay and fermentation performance determination in cellulose containing media. The results indicated that the fermentation performance of strains was influenced by nutrition added into the media and the expression of the two heterologous enzymes. We assume that one of the causes is that the two aforementioned

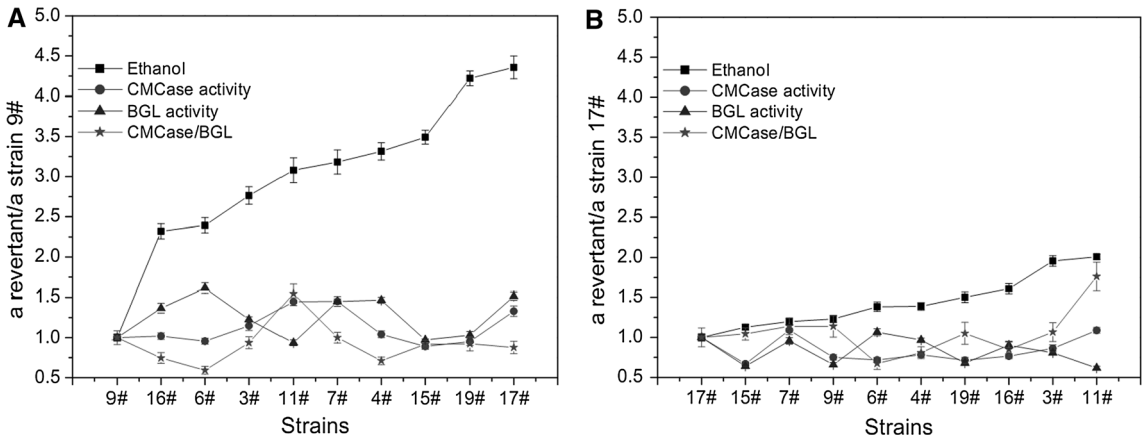

Fig. 2 Cellulase activities in YPD medium and maximum ethanol titer of cellulolytic *S. cerevisiae* strains in YPSC medium (**a**) and USC medium (**b**)

factors would affect whether and how the initial pH value of fermentation broth could rapidly be changed into one that was preferable for yeast cell growth and fermentation. For cellulolytic yeast strains, the pH values of broth were usually observed to be changed and decreased to variable extent during the fermentation course. But for W303-1A, the pH value of YPSC broth almost kept constant, while it decreased as expected for USC fermentation broth. On the other hand, both the pH change of fermentation broth and ethanol yield were the comprehensive reflection of enzymatic hydrolysis, nutrition addition, cell growth, cellulase expression, and yeast fermentation. Thus the relationship between the pH value curve and the ethanol curve as well as the fundamental mechanism behind warrant further investigation.

Compared with the control strain W303-1A, some strains exhibited higher ethanol production, which indicated that the expression of the two foreign enzymes in these strains could improve the synergism among various cellulases existing in media. On the other hand, IJT cellulase was analyzed by enzyme assay, the results showed that CMCase and β-glucosidase activities were 891,864.05 ± 21,060.31 and 458.79 ± 22.96 U/g, respectively, with the ratio (CMCase to β-glucosidase) of 1943.95 which was much higher than that of the recombinant strains constructed in this study (Table 2). The use of the cellulolytic strains would change cellulase components in media, thus affecting the synergism of various cellulases, the efficiency of cellulose hydrolysis, and the fermentation performance, which was testified by the performance of recombinant strains in YPSC and USC media. Realtime cellulase activities in the two cellulose-containing media would better reflect the effects of heterologous cellulases expression on the different fermentation performance of recombinant strains, however, the CMCase and BGL activities were difficult to measure because of the insoluble pretreated corncob. Furthermore, the nutrition added into media notably affected the fermentation performance, the most probable reason was that the expression of heterologous cellulases in recombinant strains was notably influenced by nutrition. And this warrants further research.

The results presented in this study indicated that the ethanol production from pretreated corncob by endoglucanase and β-glucosidase co-expressing *S. cerevisiae* was subject to the activity and ratio of two heterologous enzymes. Thus, any attempts to improve the expression and optimize the ratio of enzyme components are worth trying. To achieve this goal, several approaches could be employed: (1) cocktail δ-integration. Cocktail δ-integration is an efficient method to optimize the expression of multiple cellulase genes in *S. cerevisiae* (Yamada et al. 2010a); (2) changing the number of chromosomes. Compared with haploid strains, diploid and polyploidy yeast strains might have superiority in cellulolytic yeast construction (Hong et al. 2014; Yamada et al. 2010b, 2011; Ekino et al. 2002). On the other hand, combining δ-integration and meiosis in an isogenic triploid is a promising approach for improving the expression of exogenous proteins in *S. cerevisiae* (Yang et al. 2014); (3) crossing. The crossing of strains with different level of cellulase expression might result in strains with preferable traits. Furthermore, it is worthwhile to note that the construction and screen of cellulolytic *S. cerevisiae* strains should rely on the feedstock, pretreatment methods, and selection procedure.

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References

Ekino K, Hayashi H, Moriyama M, Matsuda M, Goto M, Yoshino S, Furukawa K (2002) Engineering of polyploid *Saccharomyces cerevisiae* for secretion of large amounts of fungal glucoamylase. Appl Environ Microbiol 68:5693–5697

Fujita Y, Ito J, Ueda M, Fukuda H, Kondo A (2004) Synergistic saccharification, and directfermentation to ethanol, of amorphous cellulose by use of an engineered yeast strain codisplaying three types of cellulolytic enzyme. Appl Environ Microbiol 70(2):1207–1212

Gietz RD, Schiestl RH, Willems AR, Woods RA (1995) Studies on the transformation of intact yeast cells by the LiAc/SS-DNA/PEG procedure. Yeast 11(4):355–360

Hahn-Hägerdal B, Galbe M, Gorwa-Grauslund M-F, Lidén G, Zacchi G (2006) Bio-ethanol—the fuel of tomorrow from the residues of today. Trends Biotechnol 24:549–556

Hasunuma T, Kondo A (2012) Development of yeast cell factories for consolidated bioprocessing of lignocellulose to bioethanol through cell surface engineering. Biotechnol Adv 30:1207–1218

Himmel ME, Ding SY, Johnson DK, Adney WS, Nimlos MR, Brady JW, Foust TD (2007) Biomass recalcitrance: engineering plants and enzymes for biofuels production. Science 315:804–807

Hong JF, Yang HJ, Zhang K, Liu C, Zou SL, Zhang MH (2014) Development of a cellulolytic *Saccharomyces cerevisiae* strain with enhanced cellobiohydrolase activity. World J Microbiol Biotechnol 30(11):2985–2993

la Grange DC, de Haan R, van Zyl WH (2010) Engineering cellulolytic ability into bioprocessing organisms. Appl Microbiol Biotechnol 87:1195–1208

Lynd LR, Weimer PJ, van Zyl WH, Pretorius IS (2002) Microbial cellulose utilization: fundamentals and biotechnology. Microbiol Mol Biol Rev 66:506–577

Lynd LR, van Zyl WH, McBride JE, Laser M (2005) Consolidated bioprocessing of cellulosic biomass: an update. Curr Opin Biotechnol 16:577–583

Stephanopoulos G (2007) Challenges in engineering microbes for biofuels production. Science 315:801–804

Sticker AR, Mach RL, de Graaff LH (2008) Regulation of transcription of cellulases- and hemicellulases-encoding genes in *Aspergillus niger* and *Hypocrea jecorina* (*Trichoderma reesei*). Appl Microbiol Biotechnol 78:211–220

van Zyl WH, Lynd LR, den Haan R, McBride JE (2007) Consolidated bioprocessing for bioethanol production using *Saccharomyces cerevisiae*. Adv Biochem Eng Biotechnol 108:205–235

Wang G, Liu C, Hong J, Ma Y, Zhang K, Huang X, Zou S, Zhang M (2013) Comparison of process configurations for ethanol production from acid- and alkali-pretreated corncob by *Saccharomyces cerevisiae* strains with and without β-glucosidase expression. Bioresour Technol 142:154–161

Yamada R, Taniguchi N, Tanaka T, Ogino C, Fukuda H, Kondo A (2010a) Cocktail delta-integration: a novel method to construct cellulolytic enzyme expression ratio-optimized yeast strains. Microb Cell Fact 9:32

Yamada R, Tanaka T, Ogino C, Fukuda H, Kondo A (2010b) Novel strategy for yeast construction using delta-integration and cell fusion to efficiently produce ethanol from raw starch. Appl Microbiol Biotechnol 85(5):1491–1498

Yamada R, Taniguchi N, Tanaka T, Ogino C, Fukuda H, Kondo A (2011) Direct ethanol production from cellulosic materials using a diploid strain of *Saccharomyces cerevisiae* with optimized cellulase expression. Biotechnol Biofuels 4:8

Yang H, Liu C, Zou S, Ma Y, Hong J, Zhang M (2014) Improving *bgl*1 gene expression in *Saccharomyces cerevisiae* through meiosis in an isogenic triploid. Biotechnol Lett 36:1279–1285

Zhang YH, Lynd LR (2004) Toward an aggregated understanding of enzymatic hydrolysis of cellulose: noncomplexed cellulase systems. Biotechnol Bioeng 88:797–824