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Repeated fermentation from raw starch using *Saccharomyces cerevisiae* displaying both glucoamylase and α-amylase

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ABSTRACT

A diploid yeast strain displaying both α-amylase and glucoamylase was developed for repeated fermentation from raw starch. First, the construct of α-amylase was optimized for cell surface display, as there have been no reports of α-amylase-displaying yeast. The modified yeast displaying both glucoamylase and α-amylase produced 46.5 g/l of ethanol from 200 g/l of raw corn starch after 120 h of fermentation, and this was 1.5-fold higher when compared to native α-amylase-displaying yeast. Using the glucoamylase and modified α-amylase co-displaying diploid strain, we repeated fermentation from 100 g/l of raw starch for 23 cycles without the loss of α-amylase or glucoamylase activity. The average ethanol productivity and yield during repeated fermentation were 1.61 g/l/h and 76.6% of the theoretical yield, respectively. This novel yeast may be useful for reducing the cost of bio-ethanol production and may be suitable for industrial-scale bio-ethanol production.

1. Introduction

Bio-ethanol is an important agro-based fuel that can be produced from inexpensive, renewable, and abundantly available biomass resources. These resources have advantages over limited fossil fuel-based sources, as they do not result in any net carbon dioxide release into the atmosphere [1]. However, to realize the numerous environmental and social benefits resulting from the replacement of petroleum-based automotive fuels, it will be necessary to produce bio-ethanol efficiently from starchy materials using microorganisms [2].

The yeast *Saccharomyces cerevisiae* has traditionally been used for industrial-scale ethanol production. However, *S. cerevisiae* cannot utilize starchy materials without liquefaction and saccharification processes, which are both costly and time intensive. To reduce the cost of ethanol production from starch, researchers have developed direct ethanol-fermenting recombinant *S. cerevisiae* capable of expressing amylolytic enzymes [3–5]. However, the ethanol productivity has not been sufficiently improved and production costs remain high. Therefore, the development of an efficient and cost-effective process for ethanol production from starchy materials is still needed.

One of the promising strategies for reducing the cost of ethanol production is repeated batch fermentation without the loss of amylolytic activity or fermentation ability. We previously reported direct ethanol production from raw starch with *Streptococcus bovis* α-amylase-secreting and *Rhizopus oryzae* glucoamylase/α-agglutinin fusion protein-displaying diploid *S. cerevisiae* strain [6]. Although ethanol productivity was significantly improved by diploidization, α-amylase activity and ethanol productivity decreased gradually on repeated batch fermentation. The additive $CaCl_2$, which has been reported to stabilize α-amylase and improve α-amylase activity [7,8], was significantly useful for maintaining α-amylase activity after 10-cycle repeated batch fermentation [9]. However, such additives increase ethanol production costs and their activity is lost during repeated batch fermentation.

Cell surface display is another promising strategy to maintain amylase activity during repeated fermentation, and this approach is supported by the observation that glucoamylase activity on the yeast cell surface is maintained after 10-cycle repeated fermentation [6]. Previously, yeast strains displaying three cellulolytic enzymes showed high cellulose degradation activity when compared to secreting strains [10], while a lipase-displaying yeast strain showed higher hydrolysis and esterification activity than commercial enzyme [11]. However, there have been no reports on the production of a yeast strain displaying both α-amylase and glucoamylase on the cell surface.

In present study, we aimed to develop an efficient and stable repeated fermentation process from raw starch. First, α-amylase was displayed on the cell surface and optimized to improve amylase activity. Subsequently, a diploid yeast strain displaying both glucoamylase and α-amylase was produced and 23-cylce repeated fermentation from raw starch was performed without loss of ethanol fermentation ability.

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Table 1
Characteristics of strains and plasmids used in this study.

Strains of plasmids	Relevant features	Reference of source
Bacterial strain		
E. coli Novablue	*endA1 hsdR17*($r_{K12}^{-}m_{K12}^{+}$) *supE44 thi-I gyrA96 relA1 lac recA1/F'* [*proAB*$^{+}$ *lacI*^{q} ZΔM15::Tn*10*(Tet^{r})]	Novagen
S. cerevisiae yeast strain		
MT8-1	*MAT*a *ade leu2 his3 ura3 trp1*	[12]
NBRC1440ΔHUWL	*MAT*α leu2 *his3 ura3 trp1*	
MT8-1/pδUPGGluRAG	*MAT*a *ade leu2 his3 trp1* δ-integration of glucoamylase gene	[6]
MNII/δGS/405	*MAT*a/α *his3* δ-integration of glucoamylase gene and α-amylase gene	[6]
MNII/δGS/405/pIUPGAGT1	*MAT*a/α δ-integration of glucoamylase gene and α-amylase gene and integration of maltose transporter gene AGT1	[9]
MT8-1/pIUPGSBAAG	*MAT*a *ade leu2 his3 trp1* integration of α-amylase gene SBA	This study
MT8-1/pIUPGSBAIAG	*MAT*a *ade leu2 his3 trp1* integration of modified α-amylase gene SBAI	This study
MT8-1/pIUPGSBAIIAG	*MAT*a *ade leu2 his3 trp1* integration of modified α-amylase gene SBAII	This study
MT8-1/pIUPGSBAIIIAG	*MAT*a *ade leu2 his3 trp1* integration of modified α-amylase gene SBAIII	This study
MT8-1/δGSBAAG	*MAT*a *ade leu2 his3* δ-integration of glucoamylase gene and α-amylase gene SBA	This study
MT8-1/δGSBAIAG	*MAT*a *ade leu2 his3* δ-integration of glucoamylase gene and modified α-amylase gene SBAI	This study
MT8-1/δGSBAIIAG	*MAT*a *ade leu2 his3* δ-integration of glucoamylase gene and modified α-amylase gene SBAII	This study
MT8-1/δGSBAIIIAG	*MAT*a *ade leu2 his3* δ-integration of glucoamylase gene and modified α-amylase gene SBAIII	This study
1440/δGSBAIAG	*MAT*α *ade leu2 his3* δ-integration of glucoamylase gene and modified α-amylase gene SBAI	This study
MNII/δGSBAIAG/405	*MAT*a/α *his3* δ-integration of glucoamylase gene and α-amylase gene SBAI	This study
MNII/δGSBAIAG/405/pIHPGAGT1	*MAT*a/α δ-integration of glucoamylase gene and α-amylase gene SBAI and integration of maltose transporter gene AGT1	This study
Plasmids		
pIHPGAGT1	HIS3 Expression of *S. cerevisiae* maltose transporter gene AGT1 with PGK promoter and terminator	[9]
pGK406AG	URA3 Emzyme displaying vector on cell surface	This study
pIUPGSBAAG	URA3 Displaying of α-amylase gene SBA with PGK promoter and terminator	This study
pIUPGSBAIAG	URA3 Displaying of modified α-amylase gene SBAI with PGK promoter and terminator	This study
pIUPGSBAIIAG	URA3 Displaying of modified α-amylase gene SBAII with PGK promoter and terminator	This study
pIUPGSBAIIIAG	URA3 Displaying of modified α-amylase gene SBAIII with PGK promoter and terminator	This study
pδWPGSBAAG	TRP1 Displaying of α-amylase gene SBA by δ-integration with PGK promoter and terminator	This study
pδWPGSBAIAG	TRP1 Displaying of modified α-amylase gene SBAI by δ-integration with PGK promoter and terminator	This study
pδWPGSBAIIAG	TRP1 Displaying of modified α-amylase gene SBAII by δ-integration with PGK promoter and terminator	This study
pδWPGSBAIIIAG	TRP1 Displaying of modified α-amylase gene SBAIII by δ-integration with PGK promoter and terminator	This study
pδUPGGluRAG	URA3 Displaying of glucoamylase gene by δ-integration with PGK promoter and terminator	[13]
pδWGPSBAAG	TRP1 Secreting of α-amylase gene by δ-integration SBA with GAP promoter and terminator	[13]

2. Materials and methods

2.1. Strains and medium

The genetic properties of all strains and plasmids used in this study are summarized in Table 1. Briefly, recombinant DNA was amplified in *Escherichia coli strain* NovaBlue (Novagen, WI, USA). *E. coli* transformants were grown in Luria–Bertani medium (10 g/l of tryptone (Nacalai Tesque, Inc., Kyoto, Japan), 5 g/l of yeast extract (Nacalai Tesque) and 5 g/l of sodium chloride (Nacalai Tesque)) containing 100 μg/ml of ampicillin. α-Amylase from *S. bovis* and glucoamylase from *R. oryzae* expressing yeast strains derived from haploid strains MT8-1 [12] and NBRC1440ΔHUWL [13], respectively, were used for ethanol fermentation. Recombinant yeast strains were screened with SD medium (6.7 g/l of yeast nitrogen base without amino acids (Difco Laboratories, Detroit, MI) and 20 g/l of glucose (Nacalai Tesque)) containing appropriate amino acids.

2.2. Construction of modified *S. bovis* α-amylase for cell surface expression

S. bovis α-amylase (SBA) consists of three domains; a catalytic domain, a substrate-binding domain and a linker domain [14]. Fig. 1 shows the three types of amylase constructed for cell surface display in this study. SBAI has an additional substrate-binding domain at its N-terminus. In the case of SBAII, the C-terminus of the catalytic domain of SBAI was deleted. SBAIII has an additional catalytic domain and a substrate-binding domain at its N-terminus. All amylases were displayed on the yeast cell surface using α-agglutinin anchor protein [15].

2.3. Construction of plasmids

The primers used in this study are summarized in Table 2. The integrative plasmid for cell surface expression was constructed as follows. The DNA fragment encoding *S. cerevisiae PGK1* promoter and prepro-α-factor was prepared by digestion of pFGK426 [16] with *XhoI*/*SalI* sites, followed by ligation at the same sites into yeast integrating shuttle vector pRS406 (Stratagene, CA, USA). The resulting plasmid was designated pRS406-PGK5′-prepro. The DNA fragment containing the 3′-half of the α-agglutinin region and the SAG1 terminator was also amplified from BY4741 [17] genomic DNA using MCSFw-SalI and SAG1Rv-NotI primers. The fragment was digested with *SalI*/*NotI*, and was inserted into the plasmid pRS406-PGK5′-prepro. The resultant plasmid was designated PGK406AG. The DNA fragment encoding *S. bovis* α-amylase was amplified from pδWGPSBA [13] using CatFw-EcoRI and SBDRv-SphI primers. The amplified fragment was digested with *EcoRI*/*SphI*, and was inserted into the plasmid PGK406AG. The resulting plasmid was designated pIUPGSBAAG. The DNA fragment encoding the catalytic domain was amplified from pδWGPSBA using CatFw-OLLink and SBDRv-SphI primers. Similarly, the DNA fragments encoding the substrate-binding domain or linker domain were amplified using SBDFw-EcoRI and SBDRv-OLLink or LinkFw-OLSBD and LinkRv-OLCat primers, respectively. These three types of fragment were fused using the overlapping PCR method with SBDFw-EcoRI and SBDRv-SphI primers. The extended fragment was digested with *EcoRI*/*SphI*, and was inserted into the plasmid PGK406AG. The resulting plasmid was designated pIUPGSBAIAG. The DNA fragments encoding each catalytic domain, linker domain or substrate-binding domain were amplified from pδWGPSBA using CatFw-OLLink and CatRv-SphI, LinkFw-OLSBD and LinkRv-OLCat or SBDFw-EcoRI and SBDRv-OLLink primers, respectively. These fragments were

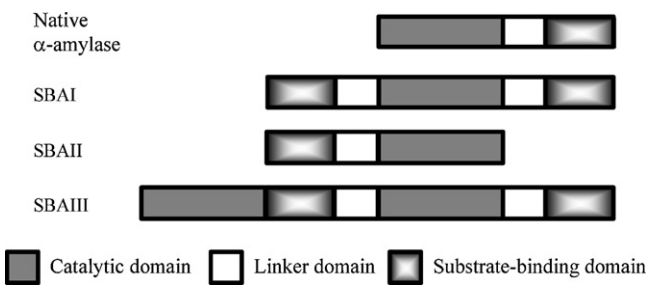

Fig. 1. Construction of novel α-amylases derived from *S. bovis* α-amylase.

Table 2
Polymerase chain reaction primers used in this study.

Primes	Sequence
MCSFw-SalI	aaaagtcgacgaattcgcatgcagatcttccggaggcggaggttccga ctacaaggatgacgatgacaagagcgccaaaagctcttttatctcaaccact
SAG1Rv-NotI	aaaagcggccgctttgattatgttctttctatttgaatgagatatgagagag
SBDFw-EcoRI	atcggaattcatttacttccaaaacccagataattggtca
SBDFw-MluI	atcgacgcgtatttacttccaaaacccagataattggtca
SBDRv-SphI	tctggaaactataataaagatgggctaaaagcatgcatcg
SBDRv-OLLink	ggctagcttgttttagcccatctttattatagtttccaga
CatFw-OLLink	tgagactaacgatgaacaagtgtcaatgaaagatggtacg
CatFw-EcoRI	atgcgaattcgatgaacaagtgtcaatgaaagatggtacg
CatRv-SphI	tcacgctcagccgttgttttgtatgatgacgcatgcatcg
CatRv-MluI-Sph I	tcacgctcagccgttgttttgtatgatgacacgcgtgcatgcatcg
LinkFw-OLSBD	tgggctaaaagctagccaagcagctcaagt
LinkRv-OLCat	ctttcattgaaatgttagtctcagcacttgggtctttcat

fused by overlap PCR using SBDFw-EcoRI and CatRv-SphI primers. The extended fragment was digested with *EcoRI*/*SphI* and inserted into plasmid PGK406AG. The resulting plasmid was designated pIUPGSBAIIAG. The DNA fragment encoding the catalytic domain was amplified from pIUPGSBAAG using CatFw-EcoRI and CatRv-MluI-SphI primers. The amplified fragment was digested with *EcoRI*/*SphI* and inserted into plasmid PGK406AG. The resulting plasmid was designated pIUPGCatAG. The DNA fragment encoding SBAI was amplified from pIUPGSBAIAG using SBDFw-MluI and SBDRv-SphI primers. The amplified fragment was digested with *MluI*/*SphI* and inserted into plasmid pIUPGSLAG. The resulting plasmid was designated pIUPGSBAIIIAG.

The δ-integrative plasmids for surface expression of native α-amylase, SBAI, SBAII, and SBAIII were constructed as follows. The DNA fragments encoding *S. cerevisiae* PGK promoter, a secretion signal sequence, each α-amylase, the 3′-half of α-agglutinin and SAG1 terminator were obtained by *NotI*/*XhoI* digestion of plasmids pIUPGSBAAG, pIUPGSBAIAG, pIUPGSBAIIAG and pIUPGSBAIIIAG, respectively. These DNA fragments were inserted into plasmid pδW [13]. The resultant plasmids were designated pδWPGSBAAG, pδWPGSBAIAG, pδWPGSBAIIAG and pδWPGSBAIIIAG, respectively.

2.4. Yeast transformation

Yeast transformation was performed by the lithium acetate method, using the YEASTMAKER transformation system (Clontech Laboratories Inc., CA, USA). All transformants and their features are summarized in Table 1.

2.5. Mating

The diploid MNII/δGSBAIAG/405 strain was constructed by mating the haploid strains MT8-1/δGSBAIAG and 1440/δGSBAIAG. Both strains were grown on SD liquid medium for 24 h, harvested, spread together on SD plates, and incubated for 72 h at 30 °C. The resulting diploid strain formed single colonies on SD plates.

2.6. Analysis of amylase activities

α-Amylase and glucoamylase activities were determined using 24 h aerobic cultivated broth or anaerobic fermentation medium with yeast cells. Analysis of α-amylase and glucoamylase activities was carried out using α-amylase and saccharification assay kits (Kikkoman Corp., Chiba, Japan), respectively, with 2-chloro-4-nitrophenyl 6^5-azide-6^5-deoxy-β -maltopentaoside and 4-nitrophenyl β-maltoside as the substrates. α-Amylase and glucoamylase activities were determined according to the manufacturer's instruction by measuring the absorbance of the liberated 2-chloro-4-nitrophenol (CNP) and 4-nitrophenol (PNP) at 400 nm using spectrophotometer (Model U-2000A, Hitachi, Tokyo, Japan). One unit (U) of activity was defined as the amount of enzyme required to release 1 μmol of CNP and PNP per minute at 37 °C.

2.7. Ethanol fermentation from raw starch

For ethanol fermentation, yeast cells were aerobically grown in YPD medium (10 g/l of yeast extract, 20 g/l of bacto-peptone (Difco Laboratories) and 20 g/l of glucose) (1000 ml flask with a working volume of 500 ml) at 30 °C for 72 h, harvested by centrifugation at 3000 × *g* for 5 min, washed twice with water and re-suspended in fermentation medium (10 g/l of yeast extract, 20 g/l of bacto-peptone, and 200 g/l of raw corn starch (Wako Pure Chemical Industries, Ltd., Osaka, Japan)) containing 0.5 g/l of potassium pyrosulfite (Nacalai Tesque) to prevent contamination by anaerobic bacteria. Raw corn starch contained 10.0% water. The initial cell density was adjusted to 50 g-wet cells/l. Ethanol fermentation was carried out at 30 °C with mild agitation in 100 ml bottles with a working volume of 50 ml equipped with a bubbling CO_2 outlet line.

Repeated batch fermentation from 100 g/l of raw corn starch was carried out as follows. After 24 h of fermentation, fermentation medium was centrifuged at 3000 × *g* for 5 min in order to separate the soluble and insoluble fractions. The insoluble fraction contains yeast cells with immobilized amylases and undigested starch. Fresh medium and new raw starch were added to the insoluble fraction, and the next batch fermentation was carried out. Repeated batch fermentation was carried out every 24 h for a total of 23 cycles.

Table 3
α-Amylase activity and ethanol productivity from 200 g/l of raw starch by α-amylase displaying strains.

α-Amylase	α-Amylase activity of 24 h aerobic cultivated broth (U/g-wet cells)	Anaerobic ethanol production in 120 h (g/l)
SBA	0.24 ± 0.02	30.9 ± 2.6
SBAI	0.26 ± 0.02	46.5 ± 2.1
SBAII	0.11 ± 0.03	36.8 ± 1.2
SBAIII	0.05 ± 0.00	44.6 ± 1.1

Average ± standard error
Data are averages of three independent experiments.

2.8. Other analytical methods

Ethanol and glucose concentration were simultaneously measured using an HPLC (Shimadzu, Kyoto, Japan) equipped with a Shim-pack SPR-Pb column (Shimadzu). Analysis was carried out at 80 °C, with water as the mobile phase at a flow rate of 0.6 ml/min, and detection was performed with a refractive index detector (Shimadzu RID-10A). Fermentation broth were withdrawn from fermentation bottle every 24 h with mild agitation, and the supernatant after separating fermentation broth by centrifugation at 14,000 × *g* for 10 min was applied for HPLC analysis.

3. Results and discussion

3.1. Alpha-amylase activity of α-amylase-displaying strains

In order to confirm the functional expression of the three types of α-amylase, α-amylase integrative plasmids were introduced into *S. cerevisiae* MT8-1, and the activity was measured after 24 h aerobic cultivation in YPD medium. The SBAI-displaying strain showed slightly higher activity when compared with the SBA-displaying strain, while SBAII-displaying strain and SBAIII-displaying strain showed lower activity than the control strain (Table 3).

3.2. Batch fermentation from raw starch

In order to evaluate the ethanol productivity of modified α-amylase-displaying yeast, we produced recombinant starch-assimilating strains that displayed both glucoamylase and α-amylase. Glucoamylase and α-amylase δ-integrative plasmids were introduced into MT8-1. Batch fermentation from 200 g/l of raw starch was carried out using these strains (Table 3). Modified α-amylase-displaying strains produced ethanol more efficiently than the native α-amylase-displaying strain, with ethanol production of SBAI-displaying strain being 1.5-fold higher than that of SBA-displaying strain after 120 h of fermentation. The theoretical final ethanol concentration will be about 101 g/l (based on 200 g-raw starch/l × 0.90 g-starch/g-raw starch × 1.1 g-glucose/g-starch × 0.51 g-ethanol/g-glucose) [18]. Thus the ethanol yield of the SBAI-displaying strain in 120 h was approximately 46.0% of the theoretical yield.

As shown in Table 3, α-amylase activity of SBAI was improved when compared to that of native *S. bovis* α-amylase. On the other hand, α-amylase activities of SBAII and SBAIII were considerably lower than that of native α-amylase. However, ethanol productivity of native α-amylase-displaying strain was lower than those of SBAII-and SBAIII-displaying strains. These results indicate that substrate binding to the N-terminus can result in effective degradation of raw starch by surface displayed amylase. These results also suggest that the substrate-binding domain of α-amylase is critically important for degradation of non-soluble raw starch, as compared

Table 4
Ethanol production from 200 g/l of raw starch by glucoamylase and modified α-amylase co-displaying strains.

Strain	Ethanol production in 120 h (g/l)	Ethanol yield of the theoretical yield (%)	Maximum α-amylase activity (U/l)	Maximum glucoamylase activity (U/l)
MT8-1/δGSBAIAG	46.5 ± 2.1	46.0	624 ± 100	412 ± 9
MNII/δGSBAIAG/405	77.6 ± 4.1	76.8	1306 ± 30	790 ± 32
MNII/δGSBAIAG/405/pIHPHAGT1	88.9 ± 0.8	88.0	2486 ± 72	749 ± 18

Average ± standard error
Data are averages of three independent experiments.

with soluble substrate, which corresponds with previous reports [14,19].

In order to improve ethanol yield, the haploid strain 1440/δGSBAIAG was produced, and the diploid strain MNII/δGSBAIAG/405 was produced from the resultant strain and MT8-1/δGSBAIAG. The *AGT1* expression plasmid pIHPGAGT1 [9] was then introduced into MNII/δGSBAIAG/405, and the resulting strain was designated MNII/δGSBAIAG/405/pIHPGAGT1. As shown in Table 4, in batch fermentation, ethanol productivity was significantly enhanced by diploidization. In addition, introduction of AGT1 transporter further improved ethanol productivity. Ethanol production, maximum α-amylase activity, and glucoamylase activity of the AGT1 expressing diploid strain were approximately 1.9-fold, 4.0-fold and 1.8-fold higher than those of MT8-1/δGSBAIAG, respectively. After 120 h of fermentation, ethanol production by MNII/δGSBAIAG/405 and MNII/δGBAIAG/405/pIHPGAGT1 was 77.6 g/l and 88.9 g/l. Ethanol yields were 76.8% and 88.0% of the theoretical yield, respectively.

We produced an SBAI- and AGT1-expressing diploid strain. As expected, ethanol productivity and maximum α-amylase activity were improved significantly (Table 4), which agrees with a previous report [9]. α-Amylase activity is the rate-limiting step in fermentation from raw starch [20]; therefore, in the case of cell surface displayed α-amylase, improving α-amylase activity in the diploid strain may be responsible for the improvement in ethanol productivity.

We succeeded in co-displaying both α-amylase and glucoamylase at the same time. Co-cultivation of α-amylase displaying strain and glucoamylase displaying strain is one of the strategies to produce ethanol from starch. However, it would be difficult to control the ratio of α-amylase displaying strain and glucoamylase displaying strain in the fermentation broth. If one strain washed out, the ethanol production would be stopped. Moreover, it has many advantages to display the enzymes which catalyze sequential reaction on the cell surface at the same time. For example, sequential reaction occurs in smooth because the enzymes are located near each other on cell surface [10]. Besides, glucose concentration is maintained low because the produced glucose at cell surface is consumed immediately, and it will reduce the contamination risk [21]. Thus, the strain displaying both α-amylase and glucoamylase at the same time should be useful for long term fermentation process.

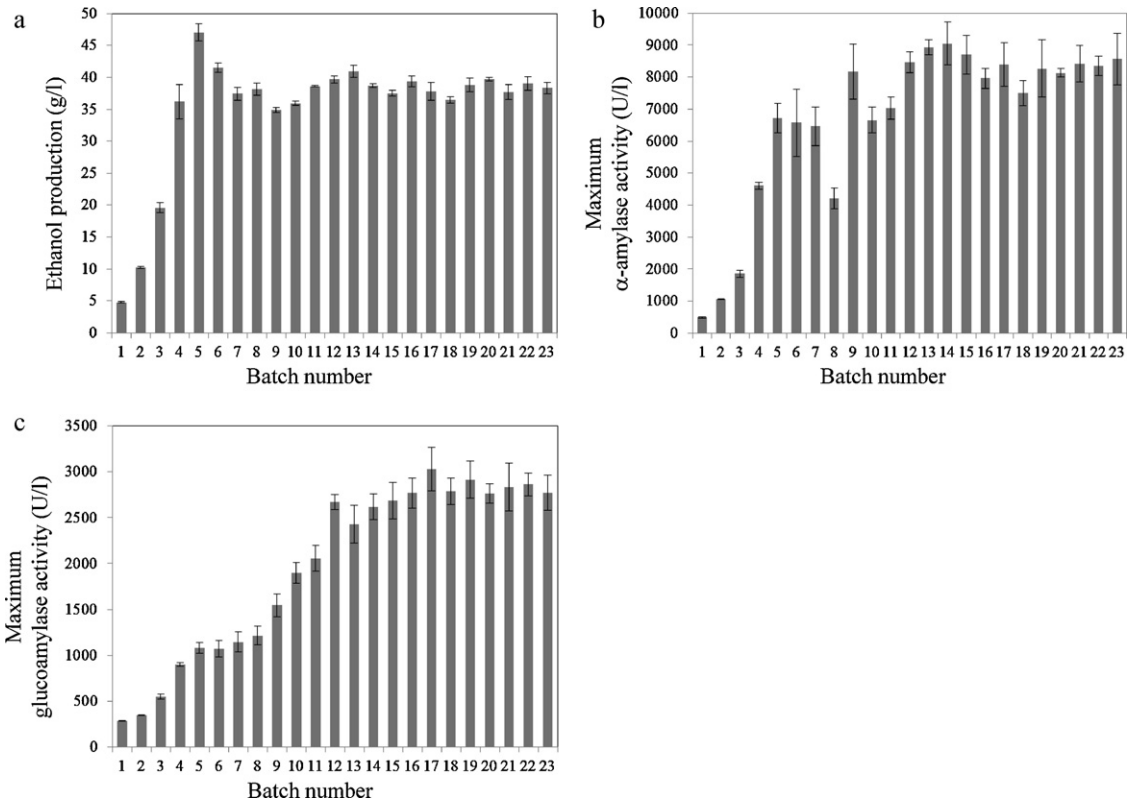

Fig. 2. Ethanol production (a), maximum α-amylase activity (b), maximum glucoamylase activity (c) through 23-cycle repeated batch fermentation of 100 g/l of raw starch using MNII/δGSBAIAG/405/pIHPGAGT1. Fermentation cycles were 24 h. Data are averages of three independent experiments.

3.3. Repeated batch fermentation

Twenty three-times of repeated batch fermentations were carried out using MNII/δGSBAIAG/405/pIHPGAGT1. As shown in Fig. 2, α-amylase and glucoamylase activity increased gradually and raw starch was efficiently converted to ethanol during repeated batch fermentation. After 4-cycle batch fermentation, ethanol productivity and amylase activity were increased enough to be ferment ethanol efficiently. The average values of ethanol productivity (ethanol production in 24 h fermentation (g/l)/24 (h)) and yield from 4th to 23rd batch were 1.61 g/l/h and 76.6% of the theoretical yield, respectively. The total yield of ethanol from input starch for the whole batches was 69.6%.

We succeeded in maintaining α-amylase and glucoamylase activity and achieved an 23-cycle efficient ethanol fermentation, which is, to our knowledge, the most repeated cycles of fermentation reported to date [22–24]. α-Amylase and glucoamylase activity increased gradually during early batch of repeated fermentation. In previous study, the α-amylase and glucoamylase activity expressed by yeast cells are increasing during fermentation from starch [6]. Besides, yeast *S. cerevisiae* can grow under oxygen limited fermentation condition [25]. Thus amylase activities in fermentation broth would be enhanced due to both increasing the amount of expressed amylase and increasing the number of yeast cells. Because the displayed α-amylase and glucoamylase activities were highly maintained after 23-cycle repetition, the recombinant strain may be useful for greater numbers of fermentation cycles.

4. Conclusions

In conclusion, we produced a modified α-amylase and glucoamylase co-displaying recombinant yeast strain MNII/δGSBAIAG/405/pIHPGAGT1 suitable for repeated fermentation of raw starch. In repeated batch fermentation, cell surface displayed amylase was easily collected and reused with yeast cells with no loss amylase activity during fermentation. Moreover we succeeded in producing ethanol from ground whole rice grains with same amylases expressing yeast in previous study [26]. Thus this novel yeast may be useful for reducing the cost of bio-ethanol production, and may be suitable for industrial-scale bio-ethanol production from whole grains.

Acknowledgments

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