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Ethanol production from inulin and unsterilized meal of Jerusalem artichoke tubers by *Saccharomyces* sp. W0 expressing the endo-inulinase gene from *Arthrobacter* sp.

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HIGHLIGHTS

- The endo-inulinase gene was expressed *Saccharomyces* sp. W0.
- Inulinase and ethanol production in the recombinant yeast were enhanced.
- The unsterilized meal of Jerusalem artichoke tubers was converted into ethanol.

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ABSTRACT

After the endo-inulinase gene from *Arthrobacter* sp. was ligated the expression vectors pMIDSC31 and pMIRSC31, the endo-inulinase gene was inserted into the chromosomal DNA of *Saccharomyces* sp. W0. It was found that the inulinase activity of the recombinant yeast D5 in which the endo-inulinase gene was inserted into the delta sequence was higher than that of the recombinant yeast R1 in which the endo-inulinase gene was inserted into 18S rDNA sequence. More ethanol from inulin was produced by the recombinant yeast D5 than by the recombinant yeast R1. But *Saccharomyces* sp. W0 produced the lowest inulinase activity and concentration of ethanol. During the 3-l fermentation, the recombinant yeast D5 could produce 13.6 ml of ethanol per 100 ml of the fermented medium from 30% inulin. The recombinant yeast D5 could actively convert the unsterilized meal of Jerusalem artichoke tubers, yielding 10.1 ml of ethanol per 100 ml of the fermented medium.

1. Introduction

In recent years, inulin, inulin-containing materials and inulinases have received more and more attention as inulin, inulin-containing materials can be converted into different kinds of bioproducts, such as ethanol, biodiesel, 2,3-Butanediol, single cell protein, citrate, ultra-high-fructose syrup, inulooligosaccharide, lactic acid, sugar alcohols and so on (Chi et al., 2011; Li et al., 2013). Inulin as an important material for biofuel production has many advantages over starch which is a common material for biofuel production (Chi et al., 2011). Therefore, *Kluyveromyces marxianus* which can produce both active inulinase and ethanol, was used to ferment inulin directly into ethanol (Bajpai and Maregartis, 1995). *Saccharomyces diastaticus* that can use sucrose and small polymers of inulin, but does not easily ferment large polymers of inulin was also utilized to produce ethanol (Schorr-Galindo et al., 1995). At the same time, after *K. marxianus* was co-cultured with a distillery yeast *Saccharomyces cerevisiae* or the bacterium, *Zymomonas mobilis*, inulin was converted into ethanol (Szambelan et al., 2004). *Zygosaccharomyces bailii* that produced 8.67 U/ml of inulinase activity was also co-cultured with *S. cerevisiae* CCMI 885 for ethanol production from inulin (Paixao et al., 2013).

Inulinases are classified among the hydrolases and target on the β-2,1 linkage of inulin and hydrolyze it into fructose and glucose. They can be divided into exo-inulinase and endo-inulinase. The exo-inulinase catalyzes removal of the terminal fructose residues from the non-reducing end of the inulin molecule while the endo-inulinase hydrolyzes the internal linkages in inulin to yield inulotriose, inulotetraose, and inulopentaose as the main products but lack invertase activity (Chi et al., 2009a). The exo-inulinase was used to hydrolyze inulin into fructose and glucose, then, the produced fructose and glucose were fermented into ethanol by *S. cerevisiae* (Nakamura et al., 1996; Zhang et al., 2010a). In order to develop one step ethanol fermentation, the exo-inulinase gene was expressed in *Saccharomyces* sp. and the recombinant yeasts with inulinase activity were used to directly ferment inulin and inulin-containing materials to produce

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ethanol (Zhang et al., 2010b; Wang et al., 2011). In fact, many native *Saccharomyces* spp. were found to be able to ferment inulin into ethanol (Flor and Hayashida, 1983). A newly isolated inulinase-producing *Saccharomyces* sp. from spontaneously fermented sugar cane was capable of producing inulinase in solid state fermentation (Flor and Hayashida, 1983). It was found that *S. cerevisiae* KCCM50549 strain could utilize the fructo-oligosaccharides of >DP15 (Choi et al., 2010), in contrast to the other *S. cerevisiae* strains known to ferment the fructo-oligosaccharides of DP of up to around 6 (Onilude et al., 2012).

The invertase encoded by *SUC2* gene in *S. cerevisiae* has low inulinase activity and hydrolyzes inulin with low molecular weight (Wang and Li, 2013). In fact, thirty years ago, it was found that invertase from *S. cerevisiae* preferentially cleaved sucrose, but could also hydrolyze inulin at low efficiency (Laloux et al., 1991). It has been reported that the conserved motif SVEVF in endo-inulinase, which does not appear in yeast exo-inulinase and invertase, plays an important role in binding of the high-Mr. fructans (Ohta et al., 1993; Liu et al., 2013). Therefore, if the endo-inulinase gene is actively expressed in the invertase-producing *Saccharomyces* spp., the inulin in the medium can be hydrolyzed into fructo-oligosaccharides. Then, the invertase convert the fructo-oligosaccharides into fructose which is fermented into ethanol by *Saccharomyces* spp. However, little is still known about the role of the endo-inulinase in hydrolysis of inulin and ethanol production in *S. cerevisiae.* Many results have shown that the invertase-producing *Saccharomyces* sp. W0 can produce high concentration of ethanol from glucose and sucrose and has high ethanol tolerance (Chi and Liu, 1993; Chi et al., 1999). In our previous study (Li et al., 2012), the endo-inulinase gene (*EnIA*) from *Arthrobacter* sp. S37 could be over-expressed in *Yarrowia lipolytica* Po1h and the recombinant endo-inulinase activity produced by the transformant 1317-EnIA were 16.7 U/ml. Therefore, in this study, the *EnIA* gene was expressed in *Saccharomyces* sp. W0 and the recombinant yeast was used to ferment inulin and meal of Jerusalem artichoke tubers into ethanol in order to know the role of the endo-inulinase in hydrolysis of inulin and ethanol production in *S. cerevisiae.*

2. Methods

2.1. Strains, plasmids and media

Escherichia coli DH5α [*supE44 DlacU169* (*B80lacZDM15*) *hsdR17 recA1 endA1 gyrA96 thi-1 relA1*] was used to amplify the plasmids carrying the cloned endo-inulinase gene. The *E. coli* transformants were grown in LB medium with 100.0 μg/ml of ampicillin. *Saccharomyces* sp. W0 which is a high ethanol-producing yeast (Chi and Liu, 1993) was used as a control in this study. The uracil mutant W12d of *Saccharomyces* sp. W0 was obtained in the previous study (Zhang et al., 2010a). Yeast growth medium was YPD medium containing 2.0% glucose, 2.0% peptone, 1.0% yeast extract. The endo-inulinase production medium contained 2.0% inulin, 1.0% yeast extract and 2.0% polypeptone. The medium used for ethanol production from inulin was composed of 30.0% or 25.0% inulin and 2.0% malt extract. The medium used for ethanol production from the tuber meal of Jerusalem artichoke contained 30.0% of the tuber meal of Jerusalem artichoke and 0.75% malt extract. The homologous integration expression vectors pMIRSC31 which carries 18S rDNA and pMIDSC31 which carries delta-sequence in *S. cerevisiae* were constructed by Wang et al. (2011). pMD19-T simple vector (TaKaRa, Japan) was used to transform *E. coli* DH5α. The plasmid K1 carrying endo-inulinase gene (*EnIA*) cloned from *Arthrobacter* sp. S37 was kindly supplied by Dr. Su-Il Kang from Seoul National University, Korea.

2.2. The tuber meal of Jerusalem artichoke

The tuber meal of Jerusalem artichoke was supplied by Professor Zhao-Pu Liu from Nanjing Agricultural University, China.

2.3. Isolation of DNA, restriction digestions, and transformation

DNA manipulations were performed by using standard methods (Sambrook et al., 1989). Bacterial plasmid DNA was purified by using Perfect-prep plasmid mini kits (Eppendorf). Restriction endonuclease digestions and DNA ligations were performed according to the manufacturer's recommendations. *E. coli* was transformed with plasmid DNA and according to Sambrook et al. (1989).

2.4. Expression of the EnIA gene in Saccharomyces sp. W0

To insert the *EnIA* gene into the chromosomal DNA of *Saccharomyces* sp. W0 and highly express the *EnIA* gene in it, the primers LY3 (5′-**AGATCT**GCCACCGGTGACCCCGTCCTGCGCCT-3', the bold bases encode *Bgl*II site) and LY4 (5'-**AAGCTT**CTA***GTGATGGTG ATGGTGATG***GAAGGCCGGCGTCGGCCAG-3', the bold bases encode *Hin*dIII site and both the bold and italic bases encode 6 × His) for amplification of the gene encoding endo-inulinase were designed according to the sequence of the gene (accession number: AJ131562). The plasmid K1 was used as the template for PCR. The PCR reaction system (50.0 μl) was composed of 25.0 μl of 2 × GC buffer I (5.0 M Mg^{2+} Plus), 8.0 μl of dNTP mixture (2.5 mM each), 1.0 μl of the plasmid (plasmid K1), 1.0 μl of the primer LY3, 1.0 μl of the primer LY4, 0.5 μl of TaKaRa LA Taq (5.0 U/μl), and 13.5 μl of distilled water. The conditions for the PCR amplification were as follows: initial denaturation of 94 °C for 8 min, followed by 30 cycles of 94 °C for 0.5 min, 68 °C for 30 s and 72 °C for 2.5 min, with a final extension at 72 °C for 10 min. The PCR cycle was a GeneAmp PCR System 2400 (PerkinElmer, Waltham, MA, USA). The PCR products were separated by agarose gel electrophoresis and ligated into the plasmid pMD19-T simple vector. The recombinant vector was transformed into *E. coli* DH5α. The recombinant vectors carrying the PCR products were extracted from the *E. coli* transformants and purified. The purified recombinant vectors carrying the PCR products were digested with *Bgl*II and *Hind*III, and the digests were ligated into pMIRSC31 or pMIDSC31 digested with the same enzymes, and transformed into *E. coli* DH5α. The resulting plasmids carrying the *EnIA* gene were designated as pMIRSC31-*EnIA* or pMIDSC31-*EnIA* (Fig. 1). The linear DNA fragments carrying the *EnIA* gene were obtained by digestion of pMIRSC31-*EnIA* or pMIDSC31-*EnIA* using *Not*I (Fig. 1). The reaction system and the conditions for PCR amplification were the same as described above. The linear DNA fragments carrying the *EnIA* gene were separated by agarose gel electrophoresis and recovered using TaKaRa Agarose Gel DNA Purification Kit Ver.2.0. The recovered linear DNA fragments carrying the *EnIA* gene were transformed into the uracil mutant W12d of *Saccharomyces* sp. W0 by lithium acetate methods (Zhang et al., 2010a). The transformants were spread on YNB-N_{5000} plates without uracil. The positive transformants carrying the *EnIA* gene were grown in the endo-inulinase production medium for 72 h and endo-inulinase activity in the cultures of different positive transformants was determined as described below. The uracil mutant W12d of *Saccharomyces* sp. W0 cells only carrying the linear DNA fragments without the *EnIA* gene and *Saccharomyces* sp. W0 were used as the controls. After determination of endo-inulinase activity in the cultures from over 100 transformants, the recombinant yeast D5 in which the endo-inulinase gene was inserted into the delta sequence and the recombinant yeast R1 in which the endo-inulinase

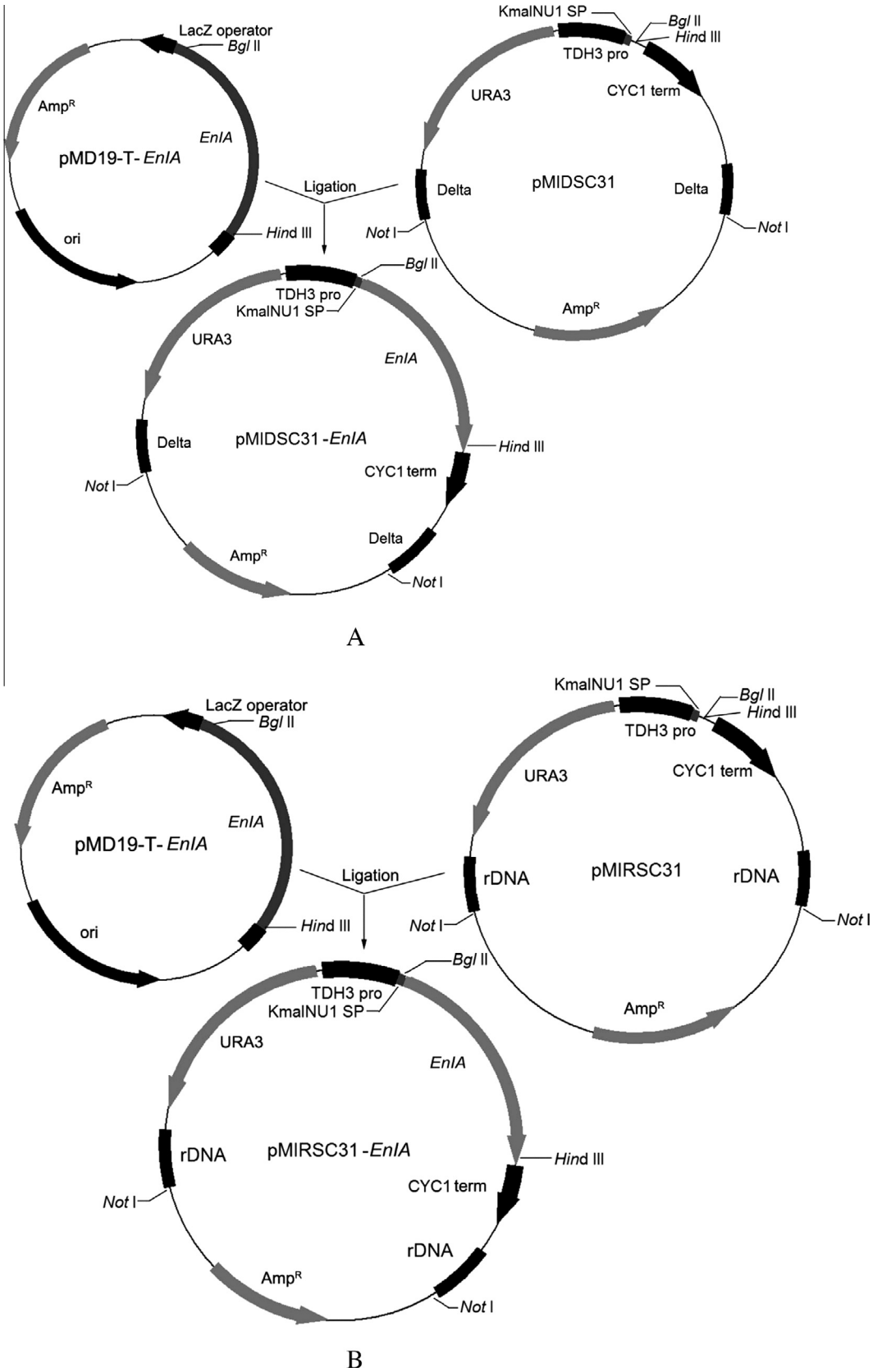

Fig. 1. Construction of the integration vector carrying both the *EnlA* gene and the homologous recombination sequence delta-sequence (A) or the integration vector carrying both the *EnlA* gene and the homologous recombination sequence 18S rDNA (B). The promoter in these two integration vectors came from the gene encoding glyceraldehyde-3-phosphate dehydrogenase isozyme 3 (TDH3); The terminator was CYC1; The DNA fragment (KmalNU1 SP) encoding the signal sequence came from the exo-inulinase gene in *Kluyveromyces marxianus*.

gene was inserted into 18S rDNA sequence were used as the recombinant endo-inulinase producers subsequently.

2.5. Confirmation of integration of the target gene into Saccharomyces sp. W0 genome

Confirmation of integration of the target gene into the *Saccharomyces* sp. W0 genome was performed according to the method described by Wang et al. (2011).

2.6. Preparation of the crude recombinant endo-inulinase

After the recombinant yeast D5 and the recombinant yeast R1 were aerobically grown in the endo-inulinase production medium for 72 h, the cultures were used as the crude endo-inulinase preparation.

2.7. Determination of endo-inulinase activity

After 100.0 μl of the crude endo-inulinase preparation obtained above was mixed with 900.0 μl of 2.0% inulin in 50.0 mM citrate–Na_2HPO_4 buffer (pH 4.5), the mixture was incubated at 50 °C for 15 min. After that, the mixture was immediately inactivated in the boiling water for 10 min. The reducing sugar released from inulin was measured using Nelson–Somogyi method (Spiro, 1966). The same mixture with 100.0 μl of the crude endo-inulinase preparation heated at 100 °C for 10 min was used as the control. One unit of endo-inulinase activity was defined as the amount of enzyme causing release of reducing sugars equivalent to 1.0 μM from inulin in 1 min under the assay conditions.

2.8. The small scale ethanol fermentation

During the small scale ethanol fermentation, the lost weight (CO_2 release) was used to estimate the weight of ethanol produced in the closed fermentation system (Zhang et al., 2010a). *Saccharomyces* sp. W0 and the recombinant yeast D5 mentioned above were grown at 28 °C and shaking speed of 180 rpm in the endo-inulinase production medium for 72 h. Fifteen milliliters of the culture were transferred into the 250-ml bottle with the final volume of 150 ml of the ethanol production medium containing 30.0% or 25% inulin. The bottles were fitted with rubber bungs perforated by a needle and incubated statically at 30 °C. The loss of weight by CO_2 liberation during the fermentation was monitored daily until fermentation ceased. The final ethanol concentration, residual reducing sugar and residual total sugar were measured as described below.

2.9. Optimization of ethanol production from the tuber meal of Jerusalem artichoke

The different volumes of the culture (1×10^8 cells/ml) were transferred to the 250-ml bottle with the final volume of 100 ml of the ethanol production medium (at different pHs from 4.0 to 8.0) containing different concentrations of the tuber meal of Jerusalem artichoke and different nitrogen sources: $(NH_4)_2SO_4$, urea, yeast extract, polypeptone and malt extract. The loss of weight by CO_2 liberation during the fermentation was monitored daily until fermentation ceased (Zhang et al., 2010a). The final ethanol concentration, residual reducing sugar and residual total sugar in the fermented media were determined as described below.

2.10. Ethanol fermentation

The seed cultures of *Saccharomyces* sp. W0 and the recombinant yeast D5 were prepared as described above. Three-liter fermentation was carried out in an SY-3005 B fermentor (SHIYUAN Bioengineering Equipment, CO. LTD). Four hundred and fifty milliliters of the seed culture obtained above were transferred into 3000 ml of the ethanol production medium containing 30% of the tuber meal of Jerusalem artichoke. The fermentation was performed under the conditions of 30 °C for 120 h without aeration and agitation. The final ethanol concentration, residual reducing sugar and residual total sugar in the fermented media were determined as described below.

2.11. Ethanol assay

Ethanol concentration in the fermented medium was determined according to the methods described by Zhang et al. (2010a).

2.12. Determination of reducing sugar and total sugar in the fermented media

Reducing sugar in the initial media and fermented media was determined by the Nelson-Somogyi method (Spiro, 1966). Total sugar was measured as reduction of sugar after hydrolysis of the initial and fermented media (Chi et al., 2001).

3. Results and discussion

3.1. Expression of the EnIA gene in Saccharomyces sp. W0

In the previous studies (Zhang et al., 2010a; Wang et al., 2011; Chi and Liu, 1993; Chi et al., 1999), many results have shown that *Saccharomyces* sp. W0 can produce more than 14 ml of ethanol per 100 ml of the fermented medium within short period. As indicated in Table 1, *Saccharomyces* sp. W0 could ferment inulin into ethanol as the invertase produced by this high ethanol producing yeast had low inulinase activity. In order to enhance its ability to convert inulin into ethanol, the endo-inulinase gene (*EnIA* gene) from *Arthrobacter* sp. was ligated the expression vectors pMIDSC31 and pMIRSC31, and inserted into the delta-sequence and 18S rDNA of the chromosomal DNA of *Saccharomyces* sp. W0, respectively (Fig. 1). After expression of the *EnIA* gene, the results in Table 1 indicated that the inulinase activity of the recombinant yeast D5 in which the endo-inulinase gene was inserted into the delta sequence was 8.6 U/ml and the inulinase activity of the recombinant yeast R1 in which the endo-inulinase gene was inserted into 18S rDNA sequence was 7.6 U/ml. However, the inulinase activity of the native *Saccharomyces* sp. W0 was only 3.2 U/ml. This meant that after the endo-inulinase gene was inserted into delta-sequence, the inulinase activity produced by the recombinant yeast D5 was higher than that produced by the recombinant yeast R1 in which the endo-inulinase gene was integrated into 18S rDNA. It has been reported that rDNA exists in about 200 copies in the haploid yeast genome as tandemly repeated sequences and it is

Table 1
The inulinase activities of the recombinant yeast D5, the recombinant R1 and the native yeast W0.

Strains	Type of recombination	The recombinant inulinase activity (U/ml)
D5	Delta-sequence	8.6 ± 0.13
R1	18S rDNA	7.6 ± 0.15
W0	None	3.2 ± 0.03

Data are given as means ± SD, $n = 3$.

possible to integrate heterologous genes at up to 100 copies (Cho et al., 1999). The delta-sequences are flanking regions of the *S. cerevisiae* Ty1 retrotransposon. It has been estimated that there exist about 425 copies of solo delta-sequences in the haploid yeast genome. So this delta-integration system makes it possible to integrate more copies of heterologous genes into yeast chromosomes than any other conventional integration systems (Cho et al., 1999). That may be why the recombinant yeast D5 had more inulinase activity than the recombinant yeast R1 (Table 1). However, after the endo-inulinase gene *inuA* from *Aspergillus niger* was transformed into *S. cerevisiae* strain JZ1C, the inulinase activity of the recombinant yeast JZ1C-I obtained was only 3.1 U/ml while that of the wild type *S. cerevisiae* strain JZ1C was 2.1 U/ml (Yuan et al., 2013). Therefore, the recombinant yeast D5 obtained in this study had higher inulinase activity (8.6 U/ml) than the recombinant yeast JZ1C-I.

The results indicated that the endo-inulinase gene had been inserted into the chromosomal DNAs of the recombinant yeast D5 and the recombinant yeast R1. However, the chromosomal DNAs of *Saccharomyces* sp. W0 did not contain any endo-inulinase gene (data not shown).

3.2. Ethanol production from inulin in the closed systems

After ethanol fermentation from different concentrations (25% and 30%) of inulin using the recombinant yeast D5, the recombinant yeast R1 and native yeast strain *Saccharomyces* sp. W0, the lost weight caused by the recombinant yeast D5 because of CO_2 release in the closed systems was higher than that caused by the recombinant yeast R1 (data not shown). However, the lost weight caused by the recombinant yeast R1 because of CO_2 release in the closed systems was higher than that caused by the native yeast strain *Saccharomyces* sp. W0 (data not shown). The results also showed that when the yeast strains were statically grown in the closed system, more weight loss appeared in the medium containing 30% inulin than in the medium containing 25% inulin. During the ethanol fermentation, the weight loss caused by the recombinant yeast D5 because of CO_2 release in the closed systems was also higher than that caused by the native yeast W0 (data not shown). Therefore, the recombinant yeast D5 was used in the subsequent investigation. After determination of ethanol concentrations, reducing sugar and total sugar in the fermented medium, it can be seen from the data in Table 2 that more ethanol (13.5 ml of ethanol per 100 ml of the fermented medium) in the medium containing 30% inulin was produced by the recombinant yeast D5 than that (12.8 ml of ethanol per 100 ml of the fermented medium) in the medium containing 25% inulin. It also can be observed from the data in Table 2 that more ethanol (12.4 ml of ethanol per 100 ml of the fermented medium) in the medium containing 30% inulin was produced by the native *Saccharomyces* sp. W0 than that (11.1 ml of ethanol per 100 ml of the fermented medium) in the medium containing 25% inulin, suggesting that *Saccharomyces* sp. W0 could indeed produce ethanol from inulin directly. However, the results in Table 2 indicated more reducing sugar and total sugar were left in the fermented medium containing 30% inulin than in that containing 25% inulin.

Table 2
The concentrations of ethanol and reducing sugar and total sugar in the fermented media.

Strain	Inulin concentration (w/v%)	Weight loss (g/150 ml)	Ethanol concentration (v/v)	Utilization of total sugar (%)	Utilization of reducing sugar (%)
W0	25	13.5 ± 0.06	11.1 ± 0.06	92.0 ± 0.06	99.1 ± 0.09
D5	25	15.4 ± 0.10	12.8 ± 0.02	95.1 ± 0.10	99.4 ± 0.07
W0	30	14.4 ± 0.06	12.4 ± 0.10	88.4 ± 0.02	97.5 ± 0.11
D5	30	16.6 ± 0.06	13.5 ± 0.05	91.5 ± 0.06	98.2 ± 0.16

Fermentation time was 120 h and the nitrogen source in the medium was 0.75% (w/v) malt extract. Data are given as means ± SD, $n = 3$.

3.3. Ethanol production from inulin in 3-l fermentor

To scale up for ethanol production from inulin, 3-l fermentation was carried out as described in Methods and ethanol concentration, and changes in reducing sugar and total sugar during the fermentation were monitored. The results showed that during the 3-l fermentation the recombinant yeast D5 produced 13.6 ml of ethanol per 100 ml of the fermented medium from 30% inulin within 120 h, and 91% of total sugar and 98.8% reducing sugar were utilized by the recombinant yeast D5 (data not shown). In the previous studies (Zhang et al., 2010a,b), after the *INU1* gene encoding exo-inulinase cloned from the marine-derived *Pichia guilliermondii* was transformed into the same uracil mutant of *Saccharomyces* sp. W0, during the small-scale fermentation, 13.7 ml of ethanol in 100 ml of medium was produced and 99.1% of the added inulin was utilized by the transformant. During the 2-l fermentation, 14.9 ml of ethanol per 100 ml of the fermented medium was produced from inulin and 99.5% of the added inulin was converted into ethanol, CO_2 and cell mass. After *Saccharomyces* sp. W0 was statically grown in the mixture of 4.0% hydrolysate of soybean meal and 20.0% of the enzymatic hydrolysate of inulin, over 14.6 ml of ethanol per 100 ml of the fermented medium was produced, and over 98.8% of total sugar was utilized. In another study (Wang et al., 2011), after the *INU1* gene encoding exo-inulinase was ligated into the 18S rDNA of the same uracil mutant of *Saccharomyces* sp. W0, the transformant MguINU1-04 obtained could produce 14.7 ml of ethanol per 100 ml of the fermented medium during 5-l fermentation. This meant that the yeast strain expressing exo-inuinase gene produced more ethanol than the yeast strain expressing endo-inuinase gene.

3.4. Ethanol production from the unsterilized tuber meal of Jerusalem artichoke in 3-l fermentor

In the conventional ethanol production from starch, a slurry containing 15% starch is gelatinized at a temperature of 105 °C and high pressure in order to sterilize the slurry and open the crystalline structure of starch. The gelatinized starch is then liquefied with high temperature α-amylase at the same time, followed by the saccharification with glucoamylase at a much lower temperature of 50–60 °C. The whole process requires a high-energy input, thus increasing the ethanol production cost from starch (Chi et al., 2009b). Therefore, in recent years, the process for enzymatic saccharification of raw starch without heating has become well recognized, mainly from the viewpoints of energy savings and effective utilization of the biomass thereby reducing the cost of starch processing for ethanol production (Chi et al., 2009b). In ethanol production from inulin, the similar problems exist. In order to reduce the cost of inulin-containing material processing for ethanol production, the unsterilized inulin-containing material is also expected to be utilized directly by the recombinant yeast expressing inulinase. Therefore, the conditions for ethanol production from the unsterilized tuber meal of Jerusalem artichoke were optimized. The results showed that the optimal conditions for ethanol production were ratios of the solid to liquid was 1:3.5, initial pH was native pH, nitrogen source was 0.75% malt extract, tuber meal of Jerusalem artichoke was unsterilized (data not shown). Under the optimal conditions, 10.1 ml of ethanol per 100 ml of the fermented medium was produced by the recombinant yeast D5, leaving 4.8% of total sugar and 0.4% reducing sugar in the fermented media (data not shown). In the previous studies (Zhang et al., 2010b), after the *INU1* gene encoding exo-inulinase cloned from

the marine-derived *Pichia guilliermondii* was transformed into the same uracil mutant of *Saccharomyces* sp. W0, during the fermentation, *Saccharomyces* sp. W0 carrying the inulinase gene produced 12.1 ± 0.35 ml of ethanol per 100 ml in the medium containing 50 g of the sterilized tuber meal of Jerusalem artichoke per 100 ml for 144 h, leaving 3.7% of total sugar and 0.5% of reducing sugar in the fermented media. In another study (Wang et al., 2011), when the sterilized tuber meal of Jerusalem artichoke (50.0%) was fermented into ethanol by the transformant MguI-NU1-04, 12.6 ml of ethanol per 100 ml of the fermented medium was produced within 120 h and the ethanol productivity was over 0.331 g of ethanol per g of sugar. Therefore, this is the first time to report that the unsterilized tuber meal of Jerusalem artichoke can be converted into ethanol by the recombinant yeast D5 expressing the recombinant endo-inulinase. However, ethanol concentration produced by *S. cereviaise* JZ1C-I expressing the endo-inulinase gene from *A. niger* was only 7.0% (v/v) ethanol while that produced by its wild type *S. cereviaise* JZ1C was 6.3% (v/v) ethanol when sterilized Jerusalem artichoke tuber flour was used as the material (Yuan et al., 2013). This meant that the recombinant yeast D5 obtained in this study could produce more ethanol than the recombinant yeast JZ1C-I. Therefore, the process developed for ethanol production from the unsterilized tuber meal of Jerusalem artichoke in this study will have highly potential application in bioenergy industries.

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