Direct production of bioethanol from Jerusalem artichoke inulin by gene-engineering *Saccharomyces cerevisiae* 6525 with exoinulinase gene

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**Abstract**

Jerusalem artichoke (*Helianthus tuberosus* L.), an important crop, containing over 50% inulin in its tubers on a dry weight basis is an agricultural and industrial crop with a great potential for production of ethanol and industrial products. Inulin is a good substrate for bioethanol production. *Saccharomyces cerevisiae* 6525 can produce high concentrations of ethanol, but it cannot synthesize inulinase. In this study, a new integration vector carrying *inuA1* gene encoding exoinulinase was constructed and transformed into 18SrDNA site of industrial strain *S. cerevisiae* 6525. The obtained transformant, BR8, produced 1.1 U mL⁻¹ inulinase activity within 72 h and the dry cell weight reached 12.3 g L⁻¹ within 48 h. In a small-scale fermentation, BR8 produced 9.5% (v/v) ethanol, with a productivity rate of 0.385 g ethanol per gram inulin, while wild-type *S. cerevisiae* 6525 produced only 3.3% (v/v) ethanol in the same conditions. In a 5-L fermentation, BR8 produced 14.0% (v/v) ethanol in fermentation medium containing inulin and 1% (w/v) (NH₄)₂SO₄. The engineered *S. cerevisiae* 6525 carrying *inuA1* converted pure nonhydrolyzed inulin directly into high concentrations of ethanol.

**Keywords:** Bioethanol, Jerusalem artichoke, inulinase, *Saccharomyces cerevisiae*, gene engineering

**Introduction**

Bioethanol represents an alternative to petroleum, and it is receiving increasing attention because of oil price hikes, greenhouse gas emissions from fossil fuels, and gradual depletion of crude oil resources. At present, the main raw materials for bioethanol production include sugarcane, cassava starch, sweet potato, and grain-based feedstocks (Li & Chan-Halbrendt 2009; Kabir & El-Shehawy 2012). This drives significant increases in grain prices, and consequently, there is much debate about food security and affordability for the world's poorest populations (Manchanda & Garg 2011; Kalavrouziotis & Koukoulakis 2012; Zhang et al. 2012; Radhakrishnan & Kumari 2013). Therefore, non-food grain feedstocks are being sought for bioethanol production, especially in developing countries such as China.

Inulin and inulin-containing plants have received increasing attention as a renewable raw material for ethanol production (Chi et al. 2009; Liu et al. 2011; Mahesh & Jeyachandran 2011). Inulin consists of linear chains of β-2 → 1-D-fructosfuranose terminated by a glucose residue linked to fructose by an α (1 → 2) bond (Martellos 2012). Exo-inulinase catalyzes the one-step removal of the terminal fructose residue from the nonreducing end of the inulin molecule, producing fructose as the main product which can be easily converted into ethanol by *S. cerevisiae*. Furthermore, inulin dissolves readily in water, and solutions containing high concentrations of inulin have low viscosity. Inulin is naturally present in a wide variety of plants. For example, Jerusalem artichoke (*Helianthus tuberosus* L.) containing over 50% inulin in its tubers on a dry weight basis is an agricultural and industrial crop with a great potential for production of ethanol and...
industrial products (Zhang et al. 2010; Yuan et al. 2012).

*S. cerevisiae* is traditionally used for production of bioethanol because of its ability to tolerate low pH and high concentrations of ethanol and inhibitors, and its ability to grow anaerobically (Hirasawa et al. 2007; Nevoigt 2008; Stanley et al. 2010). However, *S. cerevisiae* cannot synthesize and secrete inulinase, the enzyme that hydrolyzes inulin into fructose. Therefore, it would be useful to clone and actively express the inulinase gene in this yeast. In previous studies, the exoinulinase gene *inuA1* (GenBank ID: JF961344.1) was cloned from *Penicillium janthinellum* strain B01 and expressed at high levels in *Pichia pastoris*. In this study, to produce a strain of *S. cerevisiae* capable of direct production of ethanol from inulin, we constructed an integration expression vector carrying the exoinulinase gene (*inuA1*), and transformed it into *S. cerevisiae* 6525, an industrial ethanol production strain. We selected the transformant with the highest inulinase activity and used it to produce ethanol from inulin in a one-step process (Ow & Sim 2012).

Materials and methods
Gene, plasmids, and yeast strains

The *inuA1* gene encoding exo-inulinase was amplified from *Penicillium janthinellum* strain B01 (Wang et al. 2011; Sandionigi et al. 2012). We used *Saccharomyces cerevisiae* 6525 (kindly supplied by Dr. Bai, Dalian, China), a strain with good fermentation ability, as the host for harboring the inulinase gene. The plasmid pUG6 was used to construct the integration expression vector.

### Media

The yeast growth medium was yeast peptone dilution (YPD) medium containing 2.0% (w/v) glucose, 2.0% (w/v) peptone, and 1.0% (w/v) yeast extract. The yeast transformants were selected on YPD medium with 200 mg mL⁻¹ G418 (Genview, Carlsbad, CA, USA). The inulinase production medium (fermentation medium) contained 2.0% (w/v) inulin, 1.0% (w/v) yeast extract, and 2.0% (w/v) peptone. The medium used for small-scale ethanol production in flask cultures consisted of 20% (w/v) pure nonhydrolyzed inulin and 1.0% (w/v) (NH₄)₂SO₄. The pH of the suspension (5.5–6.0) was not adjusted. We used 30% (w/v) inulin for the 5-L fermentation study and the pH of the suspension was adjusted 5.0. The suspension was autoclaved for 15 min and used for fermentation without any addition of other nutrients except for 1% (w/v) (NH₄)₂SO₄.

Construction of the 18SrDNA integration vector

DNA was manipulated according to standard procedures (Skornik et al. 2010). First, the PGK1 promoter (approx. 780 bp) was polymerase chain reaction (PCR) amplified from genomic DNA of *S. cerevisiae* 6525, using the primers PGK1-F and PGK1-R (Table I), which were designed according to the PGK1 promoter sequence (GenBank ID: X59720.2). The PCR product was extracted and digested with *Sac* II and *Spe* I, and then inserted into pUG6 that had been pre-linearized with the same enzymes, yielding pUG-PGK. Then, the CYC1 terminator (approx. 250 bp) was PCR-amplified from DNA of the pYES2.0 plasmid, using the following primers: CYC1-F, which shared the same sequence as the *inuA1*-specific primer *inuA1*-R used in subsequent PCR amplifications, and CYC1-R, which contained an *Eco* RV site. The *inuA1* gene fragment was PCR-amplified using primers *inuA1*-F with a *Spe* I site and *inuA1*-R. The PCR products from the two PCR amplifications were mixed, denatured, allowed to anneal using the shared sequence of the primers, and subjected to a fusion PCR, using primers *inuA1*-F and CYC1-R as described earlier. A fusion fragment including the *inuA1* gene and the CYC1 terminator was generated by overlap extension PCR, then digested by *Eco* RV and *Spe* I, and inserted into pUG-PGK digested with same enzymes, generating pUG-PIC. The PCR reaction and conditions for the construction of the recombinant vector.

| Primers name | Sequence (5'–3') |
|--------------|-----------------|
| PGK1-F       | TCCCCCGGCGGACTCTTCAACTCAAGACGACACAG (SacII site italicized) |
| PGK1-R       | CGGACTAGTGTGGTTATATTGTTGTTGA (SpeI site italicized) |
| *inuA1*-F    | GGACTAGTATGGTGATTCTTCTCCTCAAAAACCCCT (SpeI site italicized) |
| *inuA2*-R    | GTGACATACTAAATTACATGATCTATCATCACCTCAGGTCCAA |
| CYC1-F       | TTCCGACGTGGGATATGGAATATGATATATGATATATGATASGCACAG (shared bases shown in bold type) | |
| CYC2-R       | CCCGATATCCGCAAATTTAAAGGCTTCTGA (EcoRV site italicized) |
| 18SrDNA-F    | GCTCTAGATGAGACGGCTACCACAT (XhoI site italicized) |
| 18SrDNA-R    | GGTCACCCTCCTGCCCTGGAGTTT (BstEII site italicized) |
PCR amplification were as recommended in the manual of the PrimeSTAR® HS DNA polymerase.

To integrate the *inuA1* gene into the chromosomes of *S. cerevisiae* 6525, a partial 18SrDNA fragment of approximately 840 bp was PCR-amplified from the genomic DNA of strain 6525, using the primers 18SrDNA-F and 18SrDNA-R, which were designed according to the 18S rDNA gene sequence (GenBank ID: AB628065.1). The partial 18S rDNA fragment was extracted, digested with *Xba*I and *Eco*RI, and then inserted into pUG-PIC which was digested with same enzymes, resulting in pUG-PICS which contained the PGK1 promoter, the *inuA1* open-reading frame, the CYC1 terminator, and 18S rDNA. The recombinant pUG-PICS was linearized with *Rsr*II, and then transformed into *S. cerevisiae* 6525 by electroporation. A 200 μL aliquot of cells was plated on YPD medium containing 200 mg mL−1 G418 and grown at 30°C until the transformants became visible. The different positive transformants were grown in inulinase production medium at 30°C for 3 days. Inulinase activity was monitored in the supernatant of each sample by the method that will be described further. The transformant with the highest inulinase activity in the culture supernatant was used for subsequent investigations.

To confirm that the linear DNA fragments carrying the inulinase gene had been integrated into the chromosomes of *S. cerevisiae* 6525, we carried out PCR amplifications using genomic DNAs from the transformant and the wild-type *S. cerevisiae* 6525 as templates. The forward primer was *inuA1*-F and the reverse primer was CYC1-R (see Table 1 for sequences). The sizes of the PCR products were as expected, approximately 2400 bp.

**Determination of recombinant inulinase activity**

The recombinant inulinase activity in the supernatants of the transformants obtained earlier was determined according to Wang et al. (2011). We determined the concentration of reducing sugars in the mixture using the 3,5-dinitrosalicylic (DNS) acid assay with fructose as the standard (Miller 1959). One unit of exoinulinase activity (U) was defined as the amount of enzyme that produces 1 μmol reducing sugars per minute under the assay conditions used in this study.

**Ethanol fermentation**

For flask cultures, seed cultures were prepared by inoculating *S. cerevisiae* 6525 and the transformant BR8 grown on YPD overnight was transferred into a 250-mL Erlenmeyer flask containing 50 mL yeastpectone liquid medium. The cultures were then incubated at 30°C for 3 days with shaking at 200 rpm. Then, 10 mL of the seed culture was transferred into a 250-mL flask containing 100 mL ethanol production liquid medium. The fermentation was carried out at 30°C for 120 h. The final concentrations of ethanol, residual reducing sugars, and residual total sugars in the fermented media were determined as will be described further.

The 5-L fermentation was carried out in a GUCS-5 bioreactor (DongFang BioTech Zheng Jiang Co., Ltd, Hangzhou, China). A 300 mL aliquot of the seed culture obtained earlier was transferred into 3000 mL medium containing 30% inulin with the initial pH adjusted to 5.0. The fermentation was carried at 30°C for 192h without aeration and agitation.

**Analytical methods**

After the system reached equilibrium, samples were taken every 24 h. The cell dry weight was measured according to the method of Chi et al. (2001) and Verma et al. (2012). Reducing sugars were assayed by the DNS method (Wang et al. 2012) and are expressed as fructose equivalents. Total reducing sugars were estimated by the same method after acid hydrolysis (pH adjusted to 2 with H2SO4; 60 min at 100°C). The ethanol concentration was determined by gas chromatography (Agilent Company, Chicago, USA). Chromatography column: HP-PFAP (30 m × 0.25 mm i.d., 0.25 m film thickness); column temperature: 80°C; injector temperature 160°C; detector temperature: 230°C and isopropanol were used as the internal standard. The ethanol concentration was expressed as a percentage (v/v), the unit used by distillers; 1% (v/v) ethanol corresponds to 7.92 g ethanol per liter.

**Results and discussion**

**Construction of the 18SrDNA integration vector**

To create a strain of *S. cerevisiae* that could directly ferment inulin into ethanol, we constructed the expression vector pUG-PICS (Figure 1) to integrate *inuA1* into the genome of *S. cerevisiae* 6525 using 18SrDNA as the target site for homologous recombination. Previously, it was reported that the inulinase activity of a yeast transformant with an inulinase gene integrated into its genome remained very stable during five sequential batch cultivations in a nonselective medium. In contrast, the inulinase activity of a transformant carrying the same gene in a plasmid (Ycplacc33) decreased gradually (Zhao et al. 2010; Wang et al. 2011). The integration strategy used in the present study not only facilitates stable recombination of the heterologous gene, but also increases the possibility of integrating multiple copies.
of the gene into the yeast genome. Since there are approximately 200 copies of rDNA present as tandemly repeated sequences in the yeast genome, up to 100 copies of the heterologous gene could be integrated. The recombinant plasmid (Figure 1) contained the constitutive promoter \( PGK1 \) (782 bp) and the effective terminator \( CYC1 \) (249 bp). Therefore, the cloned inulinase gene could be expressed constitutively in \( S. cerevisiae \) and gene expression could be effectively terminated.

The recombinant plasmid was linearized and transformed into \( S. cerevisiae \) 6525. We measured the inulinase activity in the supernatant of cultures of the transformants, and selected the one with highest activity, BR8, for further experiments. The wild-type \( S. cerevisiae \) 6525 had no inulinase activity (data not shown). As shown in Figure 2, the PCR products obtained from the transformant were of the expected size, 2381 bp. However, no such PCR products were amplified from the genomic DNA of the wild-type \( S. cerevisiae \) 6525. This indicated that the linear DNA fragments containing the inulinase gene were indeed integrated into the genome of \( S. cerevisiae \) 6525.

**Time-course of cell growth and inulinase production by the transformant BR8**

We measured inulinase activity in the supernatant and cell mass during cell growth in the inulinase production medium. As shown in Figure 3, the transformant BR8 showed the highest inulinase activity (10.9 U mL\(^{-1}\)) within 72 h of culture, when cell growth reached the stable phase. The maximum dry cell weight was 12.3 g L\(^{-1}\) at 48 h of culture. In another study, the \( INU2 \) gene encoding an endoinulinase of \( Aspergillus ficuum \) was expressed under the control of the \( Kluyveromyces marxianus \) \( INU1 \) promoter in a SUC2-deleted strain of \( S. cerevisiae \), and the endo-inulinase activity of the recombinant was 4.04 U mL\(^{-1}\). Optimization of the culture conditions could increase the inulinase activity of the transformant BR8. Further experiments are required to study the expression and secretion of inulinase in yeast in greater detail. Such experiments, which are currently in progress, will include replacement of the \( P. jathinellum \) signal sequence by homologous signal sequences (e.g. \( \alpha \)-factors) as well as more detailed studies on the cellular localization of the inulinase protein in yeast. Surprisingly, although the expression level of inulinase in \( S. cerevisiae \) was less than that in \( P. pastoris \) reported earlier (Wang et al. 2011), it greatly enhanced the production of ethanol from inulin, as described further in the text.

**Ethanol fermentation**

To determine whether the transformant BR8 constructed earlier could directly convert inulin into ethanol, we carried out fermentation experiments using BR8 and the wild-type \( S. cerevisiae \) 6525 in flask. As shown in Table II, the transformant BR8 produced 9.5% (v/v) ethanol and the ethanol productivity rate was 0.385 g ethanol per gram inulin. Most of the added sugar (inulin) was used for ethanol production and cell growth by the transformant, with only 2.7% (w/v) total sugars remaining in the medium at the end of the fermentation. In the same conditions, the wild-type \( S. cerevisiae \) 6525 produced only 3.3% (v/v) ethanol, and used only some of the inulin with 8.0% (w/v) residual total sugars remaining in the culture medium at the end of the fermentation. It was reported that the wild-type \( S. cerevisiae \) cannot secret inulinase to directly convert inulin to ethanol (Zhang et al. 2010). In our study, however, the wild-type \( S. cerevisiae \) 6525 was able to use some of the inulin because it contained the invertase
gene encoding invertase (EC.3.2.1.26), which catalyzes the hydrolysis of sucrose. *S. cerevisiae* KCCM50549 strain produced 4.6% (v/v) concentrations of ethanol at 34 h in a 5-L jar fermentor without addition of inulinase. Our results indicated that direct production of ethanol from inulin was significantly increased in the *S. cerevisiae* 6525 transformant harboring the inulinase gene.

Next, we conducted an experiment in which the transformant BR8 was grown and its ethanol production assessed in a 5-L fermentor over a 192-h period. The ethanol concentration reached 12.1% (v/v) after 120 h fermentation and increased to 14.0% (v/v) at 192h (Figure 4). The ethanol productivity rate was 0.483 g ethanol per gram total consumed sugars. The conversion efficiency of inulin-type sugars to ethanol was 94.8% of the theoretical ethanol yield. The cell growth reached the stationary phase after 120 h cultivation and the maximum dry cell weight was 12.1 g L$^{-1}$. Yeast cells can be recycled and utilized to produce single-cell protein. At the end of the fermentation period, 1.8% (w/v) reducing sugars and 2.5% (w/v) total sugars remained in the fermented medium (Figure 4), indicating that most of the added inulin was

![Figure 3. Inulinase production and cell growth during growth of the transformant BR8.](image)

![Figure 4. Fermentation of inulin with transformant BR8 in a 5-L jar fermentor. The medium containing 300 g L$^{-1}$ of nonhydrolyzed inulin was used with the initial pH adjusted to 4.6. The culture was carried out at 30°C without agitation and aeration.](image)

| *S. cerevisiae* strains | Ethanol % (v/v) | Initial inulin % (w/v) | Residual total sugars % (w/v) | Residual reducing sugars % (w/v) |
|------------------------|----------------|------------------------|-----------------------------|-------------------------------|
| 6525                   | 3.3 ± 0.2      | 19.5                   | 8.0 ± 0.3                   | 1.0 ± 0.01                    |
| BR8                    | 9.5 ± 0.4      | 19.5                   | 2.7 ± 0.2                   | 1.4 ± 0.02                    |

Table II. Fermentation of inulin by transformant BR8 and wild-type *S. cerevisiae* 6525.
converted into ethanol. Our study indicated that the engineered *S. cerevisiae* 6525 carrying the *inuA1* gene cloned from *P. janthinellum* could directly convert inulin extracted from a nonfood material into ethanol. Such consolidated bioprocessing integrating inulinase production, saccharification of inulin and ethanol production from sugars released from inulin by the enzyme is a potential breakthrough for low-cost production of ethanol from inulin biomass. Ethanol fermentations from Jerusalem artichoke tubers were carried out with two trains from the inulinase-producing species *K. marxianus* Y179 and ATCC8554, and the experimental results indicated that *K. marxianus* Y179 with improved inulinase production is more efficient than *K. marxianus* ATCC8554 (Yuan et al. 2012). Therefore, improving inulinase activity facilitates the direct conversion of inulin into ethanol (Bogani et al. 2012). At present, we are conducting experiments to increase the amount and activity of the secreted inulinase, which could increase the rate of ethanol production and reduce the duration of the fermentation period (Kabir & El-Shehawy, 2012).

**Conclusion**

In summary, we have successfully constructed an integrated expression vector and transformed it into the chromosomes of *S. cerevisiae*. The inulin derived from Jerusalem artichoke could be converted efficiently to ethanol by transformant BR8 without acidic or enzymatic hydrolysis prior to fermentation. The transformant BR8 produced approximately three times more ethanol compared with the wild-type *S. cerevisiae* 6525. Direct fermentation of inulin at 30% (w/v) yielded 14.0% (v/v) of ethanol in a 5-L fermentor, thus it is a very promising strain for industrial applications.

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**References**

Bogani P, Intrieri MC, Buiatti M. 2012. Genetic and epigenetic factors in the control of dedifferentiation/tumourisation in *Nicotiana* species and hybrids. Plant Biosyst 146(3): 511–521.

Chi Z, Chi Z, Zhang T, Liu G, Yue L. 2009. Inulinase-expressing microorganisms and applications of inulinases. Appl Microbiol Biotechnol 82(2): 211–220.  

Chi Z, Liu J, Zhang W. 2001. Trehalose accumulation from soluble starch by Saccharomycopsis fibuligera sdu. Enzyme Micr Technol 28(2): 240–245.

Hirasawa T, Yoshikawa K, Nakakura Y, Nagahisa K, Furosawa C, Katakura Y, et al. 2007. Identification of target genes conferring ethanol stress tolerance to *Saccharomyces cerevisiae* based on DNA microarray data analysis. J Biotechnol 131: 34–44.

Kabir AH, El-Shehawy R. 2012. Expression of *nifH, hetR*, and *nda* genes in *Nodularia spumigena* under nitrogen sources. Plant Biosyst 146(4): 992–1000.

Kalavrouziotis IK, Koukoulakis PH. 2012. Contribution of elemental interactions in total essential nutrient and heavy metal content in cabbage under treated wastewater irrigation. Plant Biosyst 146(3): 491–499.

Li SZ, Chan-Halbrendt C. 2009. Ethanol production in (the) People’s Republic of China: potential and technologies. Appl Energy 86: S162–S169.

Liu CG, Lin YH, Bai FW. 2011. A kinetic growth model for *Saccharomyces cerevisiae* grown under redox potential-controlled very-high-gravity environment. Biochem Eng J 56(1–2): 63–68.

Mahesh A, Jeyachandran R. 2011. *Agrobacterium rhizogenes*-mediated hairy root induction in *Taraxacum officinale* and analysis of sesquiterpene lactones. Plant Biosyst 145(3): 620–626.

Manchanda G, Garg N. 2011. Alleviation of salt-induced ionic, osmotic and oxidative stresses in *Cajanus cajan* nodules by AM inoculation. Plant Biosyst 145: 88–97.

Martell S. 2012. From a textual checklist to an information system: The case study of ITALIC; the Information System on Italian Lichens. Plant Biosyst 146(4): 764–770.

Miller GL. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 31(3): 426–428.

Nevoigt E. 2008. Progress in metabolic engineering of *Saccharomyces cerevisiae*. Microbiol Mol Biol R 72: 379–412.

Ow LF, Sim EK. 2012. Detection of urban tree roots with the ground penetrating radar. Plant Biosyst 146: 288–297.

Radhakrishnan R, Kumari BDR. 2013. Protective role of pulsed magnetic field against salt stress effects in soybean organ culture. Plant Biosyst 147: 135–140.

Sandionigi A, Galimberti A, Labra M, Ferri E, Panunzi E, De Mattia F, et al. 2012. Analytical approaches for DNA barcoding data – how to find a way for plants? Plant Biosyst 146(4): 805–813.

Skornik S, Vidrih M, Kaligaric M. 2010. The effect of grazing pressure on species richness, composition and productivity in North Adriatic Karst pastures. Plant Biosyst 144(2): 355–364.

Stanley D, Bandara A, Fraser S, Chambers PJ, Stanley GA. 2010. The ethanol stress response and ethanol tolerance of *Saccharomyces cerevisiae*. J Appl Microbiol 109: 13–24.

Verma P, Mathur AK, Shanker K. 2012. Growth, alkaloid production, rol genes integration, bioreactor up-scaling and plant regeneration studies in hairy root lines of *Catharanthus roseus*. Plant Biosyst 146: 27–40.

Wang L, Huang Y, Long X, Meng X, Liu Z. 2011. Cloning of exoinulinase gene from *Penicillium janthinellum* strain B01 and
its high-level expression in *Pichia pastoris*. J Appl Microbiol 111 (6): 1371–1380.

Wang L, Wang SP, Shao HB, Wu YJ, Wang QJ. 2012. Simulated water balance of forest and farmland in the hill and gully region of the Loess Plateau in China. Plant Biosyst 146: 226–243.

Yuan WJ, Chang BL, Ren JG, Liu JP, Bai FW, Li YY. 2012. Consolidated bioprocessing strategy for ethanol production from Jerusalem artichoke tubers by *Kluyveromyces marxianus* under high gravity conditions. J Appl Microbiol 112: 38–44.

Zhang T, Chi Z, Zhao CH, Chi ZM, Gong F. 2010. Bioethanol production from hydrolysates of inulin and the tuber meal of Jerusalem artichoke by *Saccharomyces* sp. W0. Bioresource Technol 101: 8166–8170.

Zhang ZJ, Li HZ, Qiao SJ, Zhang X, Liu XL. 2012. Effect of salinity on seed germination, seedling growth, and physiological characteristics of *Perilla frutescens*. Plant Biosyst 146: 245–251.

Zhao CH, Zhang T, Li M, Chi ZM. 2010. Single cell oil production from hydrolysates of inulin and extract of tubers of Jerusalem artichoke by *Rhodotorula mucilaginosa* TJY15a. Process Biochem 45(7): 1121–1126.