Characterization of an exo-inulinase from *Arthrobacter*: A novel NaCl-tolerant exo-inulinase with high molecular mass

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A glycoside hydrolase family 32 exo-inulinase gene was cloned from *Arthrobacter* sp. HJ7 isolated from saline soil located in Heijing town. The gene encodes an 892-residue polypeptide with a calculated mass of 95.1 kDa and a high total frequency of amino acid residues G, A, and V (30.0%). *Escherichia coli* BL21 (DE3) cells were used as hosts to express the exo-inulinase gene. The recombinant exo-inulinase (rInuAHJ7) showed an apparently maximal activity at pH 5.0–5.5 and 40–45°C. The addition of 1.0 and 10.0 mM Zn\(^{2+}\) and Pb\(^{2+}\) had little or no effect on the enzyme activity. rInuAHJ7 exhibited good salt tolerance, retaining more than 98% inulinase activity at a concentration of 3.0%–20.0% (w/v) NaCl. Fructose was the main product of inulin, levan, and Jerusalem artichoke tubers hydrolyzed by the enzyme. The present study is the first to report the identification and characterization of an *Arthrobacter* sp exo-inulinase showing a high molecular mass of 95.1 kDa and NaCl tolerance. These results suggest that the exo-inulinase might be an alternative material for potential applications in processing seafood and other foods with high saline contents, such as marine algae, pickles, and sauces.

Introduction

Inulin, a reserve polysaccharide of plant origin, is a linear chain composed of multiple D-fructofuranose units linked primarily by \(\beta-(2,1)\)-glycosidic bonds and a terminal glucose residue linked by \(\alpha-(1,2)\)-glycosidic bond.\(^1\) Inulin is an important agricultural product planted in China, and it is found in the roots and tubers of many plants such as Jerusalem artichoke, *Cichorium endivia*, *Dahlia pinnata*, and *Arctium lappa*.\(^1\) Inulin has the potential to be a renewable raw material for ultra-high-fructose syrup or fructo-oligosaccharide production by inulinase hydrolysis.\(^1,2\) These hydrolysis products can be further converted into products with higher margins by fermentation, such as butanediol, lactic acid, sugar alcohols, bioethanol, single-cell oil, single-cell protein, and citric acid.\(^3\)

Exo-inulinases (EC 3.2.1.80; fructan \(\beta\)-fructosidases, exo-\(\beta\)-D-fructosidases) hydrolyze terminal, non-reducing D-fructofuranose residues linked by \(\beta-(2,1)\)-glycosidic bonds. As such, exo-inulinases are capable of efficiently hydrolyzing inulin to produce fructose yields as high as 90%–95%.\(^4\) In addition, many exo-inulinases from bacteria also hydrolyze levans, sucrose, raffinose, stachyose, and starch.\(^1,2,5\) Based on amino acid sequence similarities, exo-inulinases are classified into the glycoside hydrolase (GH) family 32.\(^6\)

Enzymes obtained from extremophilic sources have numerous potential applications. We previously sampled the saline soil from an abandoned salt mine located in Heijing town, aka the “town of salt”, in Yunnan Province, China.\(^7\) The saline soil provides novel genetic resources and salt-tolerant xylanases that are active and stable at high concentrations of NaCl and could be used in harsh industrial processes.\(^7,8\) In addition, food processing, biosynthesis, or fermentation under high-salt conditions can reduce total costs by removing the sterilization process.\(^9\) In this study, an exo-inulinase gene from *Arthrobacter* sp. HJ7 was cloned and expressed in *Escherichia coli* BL21 (DE3). The recombinant enzyme showed a molecular mass of approximately 91.5 kDa and salt tolerance.

Results

Strain identification

Comparison of the partial 16S rDNA sequence from strain HJ7 (710 bp; JQ863112) with that in GenBank showed
nucleotide identity of 99% with Arthrobacter globiformis (Accession No. NR_112192), Arthrobacter humicola (NR_041546), Arthrobacter pascens (NR_026191), and Arthrobacter oxydans (NR_026236). Thus, strain HJ7 was classified under the genus Arthrobacter.

Gene cloning and sequence analysis

A fragment of inuAHJ7 (360 bp) was amplified by PCR using the degenerate primers elnu32F and elnu32R. DNA fragments amplified by TAIL-PCR were assembled with the inuAHJ7 fragment. The results showed that the open reading frame of inuAHJ7 (JQ863113, 2,679 bp) starts with the initiation codon ATG, ends with the termination codon TAG, and encodes an 892-residue polypeptide with a calculated mass of 95.1 kDa.

The signal peptide was predicted from M1 to A41 followed by a catalytic domain of GH 32 from H55 to A835 of InuAHJ7. The consensus pattern H-x(2)-[PV]-x(4)-[LIVMA]-N-D-P-[GA] (http://prosite.expasy.org/PS00609) of GH 32 was also detected in InuAHJ7 (HYTPQQNWMNDPNG in Fig. 1). The two amino acids D65 and E248 (Fig. 1), corresponding to D41 and E241 of the exo-inulinase from Aspergillus awamori var. 2250 (1Y4W),10 are the putative nucleophile and catalytic acid/base, respectively.

BLASTP results revealed that InuAHJ7 was most similar (57%–92% identity) to a number of putative GH 32 glycosidases with high molecular masses obtained by genome sequencing of Arthrobacter spp., such as the putative glycosidases from Arthrobacter globiformis NBRC 12137 (Accession Number WP_003799354; 825 residues), Arthrobacter sp 162MFSha1.1 (WP_018769218; 902 residues), and Arthrobacter sp. M2012083 (WP_020372846; 867 residues). Aligned with the characterized GH 32 enzymes, such as the GH 32 levansase from Actinomyces naeslundii T14V (AAA67876), InuAHJ7 showed less than 45% identity.

Expression, purification, and identification of rInuAHJ7

rInuAHJ7 was effectively expressed in E. coli BL21 (DE3). SDS-PAGE indicated that the recombinant enzyme could not be purified to electrophoretic homogeneity (Fig. 2). However, elution with 500 mM imidazole led to the reduction of most non-specific proteins and a distinct band with a molecular mass of approximately 91.5 kDa (Fig. 2), which is close to the calculated value of rInuAHJ7 (93.1 kDa). The MALDI-TOF MS spectrum of the specific band matched the molecular mass of the internal peptides of rInuAHJ7 (Fig. S1), confirming that the protein in the band was indeed rInuAHJ7. Therefore, the impure rInuAHJ7 eluted by 500 mM imidazole was used for further studies.

Enzyme characterization

The activities of the impure rInuAHJ7 (determined at pH 5.3 and 45°C) toward substrates of 0.5% (w/v) inulin, levan, sucrose,
Several studies have been published on salt-tolerant enzymes,\(^5,7,8\) including the salt-tolerant exo-inulinase InuAMN8 from *Arthrobacter* sp MN8, which shows 127.9%–88.4% activity at concentrations between 3.5% and 15.0% (w/v) NaCl and 49.8% activity at 20.0% (w/v) NaCl.\(^5\) However, among the published studies to date, salt-tolerant inulinases have rarely been reported.\(^5\) The inulinase from *Aspergillus fumigatus* MTCC 3009 retains 77% activity in the presence of approximately 0.006% (w/v) NaCl,\(^12\) and the inulinase from *Kluyveromyces marxianus* var *bulgaricus* retains 50% activity in the presence of approximately 0.3% (w/v) NaCl.\(^13\) In this study, rInuAHJ7 retained even higher relative activity (98.8%) at high NaCl concentration of 20.0% (w/v) than the salt-tolerant exo-inulinase InuAMN8.

The results indicate that rInuAHJ7 is a novel salt-tolerant exo-inulinase.

**Discussion**

Compared with their homologs, salt-tolerant enzymes have a higher proportion of amino acid residues G, A, and V.\(^14\) The total frequencies of amino acid residues G, A, and V of 15 characterized inulinases are over the range of 15.4%–26.6% (Accession Numbers BAC45010,\(^15\) AAF44125,\(^16\) CAA04518,\(^17\) AI003624,\(^18\) AAD27873,\(^19\) AAL82575,\(^20\) AHN08014,\(^21\) BAC16218,\(^22\) ACC61059,\(^23\) CAC44220,\(^10\) AEO12135,\(^24\) CAB14645,\(^25\) BAD88632,\(^26\) BAL70276,\(^27\) and AAK0076828).\(^28\)

| Primer name | Primer sequence (5’→3’)\(^a\) | \(T_m^b\) (° C) |
|-------------|---------------------------------|----------------|
| 27F         | AGAGTTGATGCCTGCGTATGCA         | 52             |
| 1492R       | GCTGACCTCGGCTGGCAACGG            | 44–55          |
| eInu32F     | CACGACGGGAGCGGAGGA              | 60             |
| eInu32R     | TCTGTCGTCGCCAAGAAGAC             | 60             |
| inuAHJ7sp1 | CACGACGGGAGCGGAGGA              | 60             |
| inuAHJ7sp2 | GGCAGCTGCTGCTCCTTCA             | 60             |
| inuAHJ7sp3 | TGCGTACGCGGAGTATGAC             | 70             |
| inuAHJ7sp4 | GGCAGACGGGAGCGGAGGA             | 68             |

Table 1 Primers used in this study

\(^a\)IUPAC/IUB symbols are used.

\(^b\)\(T_m\): annealing temperature. The \(T_m\) values of specific (SP) primers are used for the high-stringency step in TAIL-PCR.

**Hydrolysis products**

The hydrolysis products of 0.5% (w/v) inulin, 0.5% (w/v) levan, and 10.0% (w/v) Jerusalem artichoke tuber were analyzed by TLC (Fig. 5). Fructose was the primary product released from fructans and fructo-oligosaccharides in these substrates. The results revealed the exo-acting mode of rInuAHJ7.

**Table 2**

**Fig. 2.** SDS-PAGE analysis of rInuAHJ7. Lanes: 1, cell extract from an induced transformant harboring the empty plasmid pEasy-E1; M, low-molecular weight marker; 2, crude rInuAHJ7; 3, 4, 5, and 6, rInuAHJ7 purified by Ni\(^{2+}\)-NTA chelating affinity chromatography with imidazole gradient of 100, 200, 300, and 500 mM, respectively. The arrow indicates the band cut for MALDI-TOF MS analysis.

**Fig. 3A**

**Fig. 3B**

**Fig. 3C**

**Fig. 3D**

**Fig. 4**

**Fig. 5**

**Fig. 6**

**Result**

rInuAHJ7

**Discussion**

Several studies have been published on salt-tolerant enzymes,\(^5,7,8\) including the salt-tolerant exo-inulinase InuAMN8 from *Arthrobacter* sp MN8, which shows 127.9%–88.4% activity at concentrations between 3.5% and 15.0% (w/v) NaCl and 49.8% activity at 20.0% (w/v) NaCl.\(^5\) However, among the published studies to date, salt-tolerant inulinases have rarely been reported.\(^5\) The inulinase from *Aspergillus fumigatus* MTCC 3009 retains 77% activity in the presence of approximately 0.006% (w/v) NaCl,\(^12\) and the inulinase from *Kluyveromyces marxianus* var *bulgaricus* retains 50% activity in the presence of approximately 0.3% (w/v) NaCl.\(^13\) In this study, rInuAHJ7 retained even higher relative activity (98.8%) at high NaCl concentration of 20.0% (w/v) than the salt-tolerant exo-inulinase InuAMN8.

The results indicate that rInuAHJ7 is a novel salt-tolerant exo-inulinase.

Compared with their homologs, salt-tolerant enzymes have a higher proportion of amino acid residues G, A, and V.\(^14\) The total frequencies of amino acid residues G, A, and V of 15 characterized inulinases are over the range of 15.4%–26.6% (Accession Numbers BAC45010,\(^15\) AAF44125,\(^16\) CAA04518,\(^17\) AI003624,\(^18\) AAD27873,\(^19\) AAL82575,\(^20\) AHN08014,\(^21\) BAC16218,\(^22\) ACC61059,\(^23\) CAC44220,\(^10\) AEO12135,\(^24\) CAB14645,\(^25\) BAD88632,\(^26\) BAL70276,\(^27\) and AAK0076828).\(^28\)

| Primer name | Primer sequence (5’→3’)\(^a\) | \(T_m^b\) (° C) |
|-------------|---------------------------------|----------------|
| 27F         | AGAGTTGATGCCTGCGTATGCA         | 52             |
| 1492R       | GCTGACCTCGGCTGGCAACGG            | 44–55          |
| eInu32F     | CACGACGGGAGCGGAGGA              | 60             |
| eInu32R     | TCTGTCGTCGCCAAGAAGAC             | 60             |
| inuAHJ7sp1 | CACGACGGGAGCGGAGGA              | 60             |
| inuAHJ7sp2 | GGCAGCTGCTGCTCCTTCA             | 60             |
| inuAHJ7sp3 | TGCGTACGCGGAGTATGAC             | 70             |
| inuAHJ7sp4 | GGCAGACGGGAGCGGAGGA             | 68             |

Table 1 Primers used in this study

\(^a\)IUPAC/IUB symbols are used.

\(^b\)\(T_m\): annealing temperature. The \(T_m\) values of specific (SP) primers are used for the high-stringency step in TAIL-PCR.
Table 2  Effect of metal ions and organic reagents on the activity of rInuAHJ7.

| Reagent          | Relative activity (%)<sup>a</sup> |
|------------------|-----------------------------------|
|                  | 10.0 mM                           | 1.0 mM                          |
| None             | 100.0 ± 1.5                       | 100.0 ± 3.0                     |
| Ca<sup>2+</sup>  | 107.6 ± 6.1                       | 111.9 ± 8.1                     |
| Ni<sup>2+</sup>  | 105.6 ± 6.6                       | 103.4 ± 4.2                     |
| Co<sup>2+</sup>  | 103.4 ± 2.1                       | 102.3 ± 6.3                     |
| β-Mercaptoethanol| 101.9 ± 4.9                       | 105.3 ± 3.5                     |
| Mg<sup>2+</sup>  | 100.9 ± 1.8                       | 100.6 ± 3.6                     |
| K<sup>+</sup>    | 99.9 ± 3.8                        | 92.2 ± 9.0                      |
| Zn<sup>2+</sup>  | 99.6 ± 2.3                        | 89.1 ± 5.2                      |
| Fe<sup>3+</sup>  | 98.0 ± 4.6                        | 107.6 ± 7.0                     |
| Pb<sup>2+</sup>  | 90.8 ± 3.0                        | 94.9 ± 4.4                      |
| Mn<sup>2+</sup>  | 89.3 ± 5.5                        | 112.2 ± 4.1                     |
| Fe<sup>2+</sup>  | 75.0 ± 2.3                        | 103.9 ± 2.0                     |
| EDTA             | 22.9 ± 5.7                        | 63.8 ± 2.9                      |
| SDS              | 0.0                               | 48.6 ± 5.0                      |
| Hg<sup>2+</sup>  | 0.0                               | 0.0                             |

<sup>a</sup>Values represent the mean ± SD (n = 3) relative to the untreated control samples.

Figure 3. Characterization of rInuAHJ7. (A) Effect of pH on rInuAHJ7. (B) pH stability of rInuAHJ7. (C) Thermoactivity of rInuAHJ7. (D) Thermostability assay. The error bars represent the mean ± SD (n = 3).

Figure 4. Effect of NaCl on the activity of rInuAHJ7. The error bars represent the mean ± SD (n = 3).
To our knowledge, most characterized exo-inulinases have a molecular mass lower than 60 kDa. For example, the molecular masses of 9 characterized inulinases are over the range of 49.8–59.1 kDa (Accession Numbers CAA04518,17 BAL70276,27 BAC45010,15 AAK00768,28 AHN08014,21 ACC61059,23 AAF44125,16 AIO03624,18 and CAC4422010). Two reports are available on exo-inulinases from *Penicillium janthinellum* B0124 and *Lactobacillus casei* IAM 104526 with molecular masses of 74.8 and 138.8 kDa, respectively. Characterization of the 2 exo-inulinases reveals that they are inhibited by 1 mM Zn$^{2+}$,24,26 and the exo-inulinase from *P. janthinellum* B01 is also inhibited by 1 mM Na$^+$.24 Compared with the above exo-inulinases, InuAHJ7 is different: the exo-inulinase has a molecular mass of 95.1 kDa and shows tolerance to 3.0%–20.0% (w/v) Na$^+$ and 1.0 and 10.0 mM Zn$^{2+}$ and Pb$^{2+}$.24

**Conclusion**

The present study is the first to report the identification and characterization of an *Arthrobacter* sp. exo-inulinase that has a high molecular mass of 95.1 kDa and shows tolerance to 3.0%–20.0% (w/v) Na$^+$ and 1.0 and 10.0 mM Zn$^{2+}$ and Pb$^{2+}$. Considering its NaCl tolerance, the exo-inulinase has potential uses in processing seafood and other foods with high saline contents, such as marine algae, pickles, and sauces.

**Materials and Methods**

**Bacterial, vectors, and reagents**

The strain HJ7 was isolated from the saline soil located in Heijing town. The method of strain isolation has been described in our previous study.7 The taxon of HJ7 was identified by 16S rDNA sequence PCR-amplified using primers 27F and 1492R (Table 1).

The pMD 18-T vector from TaKaRa (6011) and the *E. coli* Trans1-T1 from TransGen (CD501) were used for gene cloning. The pEASY-E1 Expression Kit (CE101) and *E. coli* BL21 (DE3) (CD601) from TransGen were used for gene expression. Genomic DNA isolation (DP302), DNA purification (DP209), and plasmid isolation kits (DP103) were purchased from Tiangen. Nickel-NTA agarose from Qiagen (30210) was used for Histagged protein purification. DNA polymerases Taq (R500A) and LA Taq (RR52A) and dNTPs (4030) were purchased from TaKaRa. Inulin was purchased from J&K (543181), levan from Advanced Technology & Industrial (1151239), stachyose from TCI (50397), raffinose from Sigma (R0514), fructo-oligosaccharides set including kestose, nystose, and fructofuranosynystose from Wako Pure Chemical (062-02381), and TLC Silica gel 60 plates from Merck KGA (HX394049). All other reagents are analytical grade and commercially available.

**Gene cloning**

The genomic DNA of strain HJ7 was extracted using the Tiangen genomic DNA isolation kit following the manufacturer’s instructions. The degenerate primer set eInu32F and eInu32R (Table 1),5 corresponding to the conserved blocks—H-N-W-M-N-D-P-N-G and R-D-P-K-V-F-W-H-E-Q-S of GH32 exo-inulinases (Fig. 1), respectively, was used to clone a partial exo-inulinase gene. The full-length exo-inulinase gene (*inuAHJ7*) was amplified by improved TAIL-PCR (thermal asymmetric interlaced-PCR) method described in a previous study.30 The nested insertion-specific (SP) primers (*inuAHJ7*uSP1, *inuAHJ7*uSP2, *inuAHJ7*dSP1, *inuAHJ7*dSP2, *inuAHJ7*dSP3, and *inuAHJ7*dSP4) and annealing temperatures used for high-stringency step in TAIL-PCR are shown in Table 1. The PCR product was gel purified, ligated to pMD 18-T vector, transformed into *E. coli* Trans1-T1, and sequenced by Beijing Genomics Institute (Guangzhou, China).

**Sequence analysis**

The amino acid sequence (*InuAHJ7*) deduced from *inuAHJ7* was loaded to SignalP (http://www.cbs.dtu.dk/services/SignalP/) for signal peptide analysis. The identity values of DNA and protein sequences were calculated with BLASTN and BLASTP programs, respectively (http://blast.ncbi.nlm.nih.gov/Blast/). The classification of the glycoside hydrolase family of protein was determined with the dbcan online tool (http://csbl.bmb.uga.edu/dbCAN/annotate.php). Other sequential analyses were

![TLC chromatograms of the hydrolysis products of 0.5% (w/v) inulin, 0.5% (w/v) levan, and 10.0% (w/v) Jerusalem artichoke tuber powder. Lanes: 1, glucose; 2, fructose; 3, fructo-oligosaccharide mixture (kestose, nystose, and fructofuranosynystose); 4, 6, and 8, inulin, levan, and artichoke powder with the inactivated (90°C for 5 min) rInuAHJ7, respectively; 5, 7, and 9, inulin levan, and artichoke powder hydrolyzed by rInuAHJ7 for 3 h, respectively.](image-url)
performed using Vector NTI 10.3 software (InforMax, Gaithersburg, MD, USA).

**Expression of inuAHJ7 in E. coli**

The CDS of inuAHJ7 was amplified by PCR using LA Taq DNA polymerase and the primers inuAHJ7/EF and inuAHJ7/ER (Table 1). The resulting PCR product was directly cloned into the vector pEASY-E1 by T-A ligation. The recombinant plasmid (pEASY-E1-inuAHJ7) was transformed into E. coli BL21 (DE3) competent cells. Then, the positive transformants were identified by PCR analysis and confirmed by DNA sequencing. The transformant harboring pEASY-E1-inuAHJ7 was picked up from a single colony and grown overnight at 37°C in LB medium containing 0.1 mg/mL ampicillin. The culture was then inoculated at 1:100 dilution into culture-complex auto-inducing media for 16 h, and the vector harboring inuAHJ7C in different buffers, with pH ranging from 3.0 to 9.0. The optimal pH for the inulinase activity was determined at 37°C in McIlvaine buffer (pH 5.3). The thermostability of the enzyme was determined under standard assay conditions following pre-incubation of the enzyme for 60 min at 30°C, 37°C, 50°C, or 60°C, with the untreated enzyme defining 100% activity.

**Purification and identification of recombinant inulinase**

To purify recombinant InuAHJ7 (rInuAHJ7) tagged with His6, the cells were harvested by centrifugation at 5,000 x g at room temperature for 5 min and resuspended in Mcllvaine buffer (pH 7.0). The cells were disrupted by sonication (7 s, 150 W) on ice several times and centrifuged at 13,000 x g for 10 min at 4°C. The supernatant was applied to a Ni2+-NTA agarose gel column for purification with a linear imidazole gradient of 20–500 mM in buffer A (20 mM Tris–HCl, 0.5 M NaCl, 10% (v/v) glycerol, pH 7.2).

The purified protein was detected by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE; 10% running gel), and the in-gel band was cut and verified using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) performed by Tianjin Biochip (Tianjin, China).

**Enzyme assay**

Exo-inulinase activity was determined by measuring the release of reducing sugars from the substrate. The standard reaction consisted of 900 μL of 0.5% (w/v) substrate in Mcllvaine buffer (pH 5.3) and 100 μL of appropriately diluted enzyme. After incubation at 37°C for 10 min, the reaction was stopped with 1.5 mL of 3,5-dinitrosalicylic acid (DNS) reagent and the mixture was boiled at 100°C for 5 min to produce a measurable reddish brown (540 nm) product. One unit (U) of exo-inulinase activity was defined as the amount of enzyme that catalyzed the formation of 1.0 μmol of reducing sugars equivalent to D-fructose per minute. The enzyme activity was assayed by following this standard procedure unless otherwise noted.

**Biochemical characterization**

To identify the substrate specificity of rInuAHJ7, 0.5% (w/v) inulin, levan, sucrose, raffinose, or stachyose (determined at pH 5.3 and 45°C) was added to each reaction solution. Any further biochemical characterizations were determined using inulin as the substrate.

The optimal pH for the inulinase activity was determined at 37°C in different buffers, with pH ranging from 3.0 to 9.0. The pH stability of the enzyme were determined by incubating the enzyme solution in various buffers at 37°C for 1 h, and the residual enzyme activity was measured under the standard assay conditions. The buffers used were McIlvaine buffer for pH 3.0–8.0 and 0.1 M glycine–NaOH for pH 9.0–11.0.

The thermostability of rInuAHJ7 was determined over the range of 10–70°C in McIlvaine buffer (pH 5.3). Thermostability of the enzyme was determined under standard assay conditions following pre-incubation of the enzyme for 60 min at 30°C, 37°C, 50°C, or 60°C, with the untreated enzyme defining 100% activity.

**Hydrolysis products**

Jerusalem artichoke tubers (purchased from a local market) were sliced, sun-dried, and then ground into powder form. The hydrolysis of inulin (0.5%, w/v; pH 5.3), levan (0.5%, w/v; pH 5.3), and artichoke powder (10.0%, w/v; pH 5.3) was performed with the reaction system of 1.0 U/mL rInuAHJ7 at 45°C for 3 h. The hydrolysis products were analyzed by thin layer chromatography (TLC) as previously described.5 Glucose, fructose, and fructo-oligosaccharide mixture (kestose, nystose, and fructofuranosylnystose) were used as standards. Inulin, levan, or artichoke powder with the inactivated rInuAJB13 (90°C for 5 min) was used as a control.

**Nucleotide sequence accession numbers**

The nucleotide sequences for the *Arthrobacter* sp HJ7 16S rDNA and exo-inulinase gene (*inuAHJ7*) were deposited in GenBank under the accession numbers JQ863112 and JQ863113, respectively.

**Disclosure of Potential Conflicts of Interest**

No potential conflict of interest were disclosed.

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**Supplemental Material**

Supplemental data for this article can be accessed on the publisher’s website.
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