A Novel Endo-Polygalacturonase from *Penicillium oxalicum*: Gene Cloning, Heterologous Expression and Its Use in Acidic Fruit Juice Extraction

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Introduction

Pectin is a biologically active macromolecule with a main chain constructed of polygalacturonic acid molecules connected by α-1,4 linkages [1]. Pectin mainly exists in the mesoglea of plant cells, and is important for the rigidity, flexibility and shape of the plant [2]. The group of enzymes that can degrade pectin is named pectinases [3], and pectinase reagent is widely used in fruit and vegetable juices production, animal feed, textiles, tea and coffee production, as well as in pulping and waste treatment [4]. In the fruit juice industry, the degradation of pectin results in higher yields, shorter production times, better impressions of the juice, lower production costs and higher profits. In the pectinase family, endo-polygalacturonase (endo-PGase), which randomly hydrolyzes the inner α-1,4 linkages of pectin, exhibits the highest rate of depolymerization of pectin [3, 5]. Thus, endo-PGase is highly important for the fruit juice industry [6].

The pH of most fruit juices is mildly acidic at 4.5–6.0, and such fruits are widely used in the production of fruit juices [7]. However, some fruits with lower pH (<4.5) are also rich in nutrients, have a unique smell and taste and even show probiotic function [8]. Most reported endo-PGases function well during mildly acidic juice production [9], but endo-PGases that function well in fruit juices with pH <4.5 have rarely been reported. Thus, the discovery of an endo-PGase that functions well during fruit juice production at low pH would be highly attractive to the fruit juice industry.

In previous reports, two endo-PGase encoding genes were found in the genome of *Penicillium oxalicum* 114-2 [10], and the recombinant endo-PGases (genes cloned from *P. oxalicum* CZ1028) produced by *Pichia pastoris* showed excellent performance in the production of fruit juice at pH >4.5 [9]. In this study, another gene from *P. oxalicum* CZ1028 encoding a putative endo-polygalacturonase was cloned and expressed in *P. pastoris*. The recombinant protein was purified and its enzyme characteristics were determined. Interestingly, this enzyme functioned well in fruit juice production at pH <4.5.
**Materials and Methods**

**Chemicals and Reagents**
LA Taq DNA polymerase, DNA restriction endonuclease, T4 DNA ligase and RNA purification reagent RNAliso Plus were obtained from TaKaRa Bio Co. Ltd. (China). D-galacturonic acid, trigalacturonic acid, polygalacturonic acid (PGA, de-esterified), citrus pectin (<26% esterified) and apple pectin (50–75% esterified) were from Sigma-Aldrich Co. Ltd. (USA). Unless otherwise stated, all other chemicals were of analytical grade.

**Strains and Plasmid**
The cultivation of *P. oxalicum* CZ1028 (CGMCC 3.15505) was conducted as described in a previous report [11]. Vector pPIC9K was used for gene cloning and expression in *P. pastoris* GS115, and *Escherichia coli* DH5α was used for construction of the recombinant plasmid pPIC9K-*PoxaEnPG28C*. Both *E. coli* and *P. pastoris* strains were cultivated using methods reported previously [12].

**Gene Cloning and Heterologous Expression**
According to the genomic information of *P. oxalicum* 114-2, a putative protein EPS32977 was annotated as putative polygalacturonase [10]. Because the genomic information of different strains belonging to the same species is highly similar [5], a set of specific primers [5´-CGTACGTAGCACCCGCTCCCTCGCAGGT-3´ (SnaBI) and 5´-CCCCCTAGGTCAACACGAGGCCCCCGTAG-3´ (AvrII)] was designed according to the gene sequence encoding the putative protein EPS32977 to amplify the cDNA fragment without the signal peptide-encoding region of the corresponding gene. The amplification conditions were 94°C for 5 min; followed by 30 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 1 min; and a final step of 72°C for 10 min. The amplified cDNA was inserted into the vector pPIK9K, and plasmid transformation and gene expression were conducted according to a previous report [12].

**Protein Purification and Determination**
The recombinant protein *PoxaEnPG28C* in the culture broth was concentrated and applied to size exclusion chromatography using a HiLoad 16/600 Superdex 75 column (GE Co., Ltd., Sweden). Sodium dodecyl sulphate polyacrylamide gel electrophoresis [SDS-PAGE, 5% (w/v) stacking gel and 10% (w/v) separating gel] was used to determine the purity and molecular weight of the enzyme [13], and the Bradford method with bovine serum albumin protein as the standard was used to determine the protein concentration [14]. The purified enzyme was desalted by dialyzing against ultrapure water, then biochemically characterized and used for fruit juice extraction.

**Enzyme Assay**
The enzyme activity of *PoxaEnPG28C* was determined by the same method as used for the previously reported enzymes *PoxaEnPG28A* [12] and *PoxaEnPG28B* [12], and the concentration of the released reducing sugar was calculated using a standard curve constructed with D-galacturonic acid as the standard. One unit of enzyme activity was defined as the liberation of 1 μmol of reducing sugar from the enzyme-catalyzed reaction system in 1 min.

**Mode of Action of PoxaEnPG28C**
The purified and desalted *PoxaEnPG28C* was incubated with 0.5% PGA at 45°C for 0.25, 0.5, 1, 2, 8, 12, 18, and 24 h, and then the liberated products were analyzed by thin-layer chromatography according to the previously reported method [11].

**Effects of pH and Temperature on the Enzyme Activity and Stability**
To determine the optimal pH, the enzyme activity of *PoxaEnPG28C* was determined at different pH values and at 50°C; for the optimal temperature, the enzyme activity of *PoxaEnPG28C* was determined at different temperatures (at the optimal pH). The highest enzyme activity was defined as 100%, and other enzyme activities were calculated as relative values.

For pH stability, 0.1 mL of *PoxaEnPG28C* was mixed with 0.9 mL of buffers with different pH values (0.1 M citric acid-Na₂HPO₄ for pH 3.0–7.0 and 0.1 M Tris-HCl for pH 7.0–9.0) at 4°C for 24 h, and then the residual activities were determined. For the thermal stability, *PoxaEnPG28C* was incubated at 45, 50, 55, and 60°C for 15, 30, 45, and 60 min, and the residual enzyme activities were measured. The enzyme activity of un-incubated enzyme was defined as 100%, and the residual enzyme activities were calculated as relative values.

**Substrate Specificity and Michaelis-Menten Constant of the Enzyme**
For substrate specificity, the enzyme activities of *PoxaEnPG28C* against 0.5% (w/v) PGA, citrus pectin and apple pectin were determined under optimal reaction conditions (pH 4.5 and 45°C). The enzyme activity against PGA was defined as 100%, and other enzyme activities against other substrates were calculated as relative values. For the Michaelis-Menten constant, enzyme activities of *PoxaEnPG28C* against 0.2–2.0 g/l PGA were determined under the optimal reaction conditions (pH 4.5 and 45°C). The *Kₘ* and *Vₘₐₓ* values were then obtained by calculation using the Lineweaver-Burk plotting method.

**Effects of Metal Ions on Enzyme Activity**
The enzyme activities of *PoxaEnPG28C* were determined in the presence of each of the following metal ions: 2 mM Na⁺, K⁺, Mg²⁺, Ca²⁺, Mn²⁺, Ba²⁺, Co²⁺, Zn²⁺, Fe²⁺, or Cu²⁺. The enzyme activity without extra additive was...
defined as 100%, and other enzyme activities were calculated as relative values.

Use of PoxaEnPG28C for Acidic Fruit Juice Extraction

Fresh oranges, lemons, strawberries and hawthorn were purchased from the local market. Oranges and lemons were peeled, and the hawthorns were cored. Fresh fruits were then mixed with ultrapure water and pulped. Twenty-five grams of pulp were treated with 0 or 100 U of PoxaEnPG28C per gram of pulp (at pH 4.5 and 45°C) for 2.5 h, and then centrifuged in a tubular filter (400 mesh) at 500 rpm for 2 min. The filtered juice was centrifuged at 5,000 × g for 10 min, and the parameters of the supernatant were determined according to previously reported methods [12]. The parameters of the juices without addition of PoxaEnPG28C were set as the control values (100%).

Sequence Analysis and Nucleotide Sequence Accession Number

The SignalP 4.1 online server (http://www.cbs.dtu.dk/services/SignalP/) and SMART online server (http://smart.embl-heidelberg.de/) were used to predict the signal peptide and catalytic domain, respectively. ExPaSy online server (http://web.expasy.org/protparam/) was used to calculate the hypothetical molecular weight of the protein. DNAMAN 6.0 software was used to carry out the multiple sequence alignment. The nucleotide sequence of the gene encoding PoxaEnPG28C has been deposited in the GenBank database under the Accession No. MZ614864.

Results and Discussion

Gene Cloning, Heterologous Expression and Identification of the Recombinant Protein

After prediction using the SMART online tool, a putative signal peptide including the initial 20 amino acids and a GH 28 catalytic domain were found in the putative protein EPS32977 of P. oxalicum 114-2 [10]. The amino acid sequence encoded by the amplified cDNA from P. oxalicum CZ1028 showed 100% identity with the putative protein EPS32977 of P. oxalicum 114-2, and showed 78.35% identity with a functional identified endo-PGase from Penicillium janthinellum IF0 7719 [15]. In comparison with a functionally mutated endo-PGase [16], Asp169, Asp170, Asp171, and His71 were expected to be the catalytic residues of the amplified putative endo-PGase, and Arg174 and Lys27 of the putative endo-PGase were expected to be involved in the binding of substrate. Four putative disulfide bonds were formed by Cys28 and Cys31, Cys192 and Cys208, Cys318 and Cys323, and Cys342 and Cys346 of the amplified putative endo-PGase (Fig. 1). Compared with two previously reported endo-PGases from P. oxalicum CZ1028 (PoxaEnPG28A [12] and PoxaEnPG28B-Pp [9]), PoxaEnPG28C had a closer phylogenetic relationship with endo-PGases from Aspergillus niger (RePgaA from A. niger JL-15 [17] and endo-PgaA from A. niger SC323 [18]), emphasizing the diversity of the genome of P. oxalicum (Fig. 2).

After 4 days of cultivation, the enzymatic activity of the pectinase in the culture broth of the recombinant P. pastoris reached 1057.98 ± 28.31 U/ml, which was higher than the activities of endo-PGI produced by P. pastoris (6.2 U/ml) [19], endo-PG1 produced by P. pastoris (50 U/ml) [20], PG1 produced by Saccharomyces cerevisiae (50 U/ml) [21], and PG7fn produced by P. pastoris (678.1 U/ml) [22]. After purification, a protein showing a sharp band at about 36 kDa on the SDS-PAGE gel was obtained (Fig. 3), and the apparent molecular weight matched the theoretical molecular weight of 35.87 kDa.

At the early stage of hydrolysis, galacturonic acid, digalacturonic acid and trigalacturonic acid appeared among the products (Fig. 4). Tetramers and pentamers of galacturonic acid appeared later, but as the hydrolysis time became prolonged, the amounts of tetramers and pentamers of galacturonic acid decreased. Meanwhile, the amount of galacturonic acid did not increase during the hydrolysis process, indicating the bond connecting the terminal galacturonic acid was not the prime target of the recombinant enzyme. The variations in the amounts of mono- and oligo-galacturonic acid in the hydrolysis product confirmed an endo-acting pattern of the recombinant enzyme [3]. Because the enzyme was the third reported endo-PGase from P. oxalicum [12], it was named as PoxaEnPG28C.

Effects of pH and Temperature on the Enzyme Activity and Stability

PoxaEnPG28C exhibited its highest enzyme activity at pH 4.5 and greater than 90% enzyme activity at pH 4.0 and 5.0 (Fig. 5A); it was also stable within the acidic pH range of 3.0–6.5 (Fig. 5B). The range of conditions under which PoxaEnPG28C exhibited high relative activity (pH 4.0 to 5.0) covered some acidic fruit juices such as lemon (pH 3.5) [23], apple (pH 3.8) [19], grape (pH 3.4 to 3.9) [24], mango (pH 4.52) and pitaya (pH 4.56) [12]. PoxaEnPG28C was a mesophilic endo-PGase that exhibited its highest enzyme activity at 45°C and greater than 90% enzyme activity at 40–50°C (Fig. 5C), and its optimal temperature was similar to that of PgaB (40°C) [25], endo-PGI (45°C) [26] and endo-PG1A (50°C) [20]. PoxaEnPG28C was quite stable at its optimal temperature (45°C), and lost less than 20% of its enzyme activity after incubation for 1 h at 50°C (Fig. 5D).

Endo-PGases (previously reported as PoxaEnPG28A [12] and PoxaEnPG28B-Pp [9] and herein PoxaEnPG28C) from P. oxalicum CZ1028 have different enzymatic characteristics. Similarly, several endo-PGases have been reported in A. niger with different enzymatic characteristics [17, 18, 27-32]. Compared with PoxaEnPG28A (optimal pH of 5.5) [12] and PoxaEnPG28B-Pp (optimal pH of 5.0) [9] and herein PoxaEnPG28C had a lower optimal pH (pH 4.5) and might be more suitable in the production of acidic fruit juices.

Substrate Specificity and Michaelis-Menten Constant of the Enzyme

PoxaEnPG28C showed higher enzyme activity towards PGA (4.377.65 ± 55.37 U/mg, 100.00 ± 1.26%) than towards citrus pectin (1,321.32 ± 43.13 U/mg, 30.18 ± 0.99%) and apple pectin (704.39 ± 51.61 U/mg, 16.09 ± 1.87 U/mg) at pH 4.5.
Fig. 1. Multiple homology alignment of PoxaEnPG28C with other GH 28 endo-PGases. The amino acid residue sequences of PoxaEnPG28C and 11 functionally characterized endo-PGases of PoxaEnPG28B (from *P. oxalicum* CZ1028, GenBank Accession No. APZ75903), PoxaEnPG28A (from *P. oxalicum* CZ1028, GenBank Accession No. ANS70886), RePgaA (from *A. niger* JL-15, GenBank Accession No. AGV40780), PG7fn (from *T. arenaria*, GenBank Accession No. AJZ95162), endo-PgaA (from *A. niger* SC323, GenBank Accession No. AJD09825), Epg1-2p (from *Kluyveromyces marxianus* CECT1043, GenBank Accession No. AAR841999), PGA-ZJ5A (from *A. niger* ZJ5, GenBank Accession No. AQF01640), endo-PG I (from *Achaetomium* sp. Xz8, GenBank Accession No. AGR51994), endo-PGase (from *Pectobacterium carotovorum*, GenBank Accession No. WP_039543807), ThPG1 (from *Trichoderma harzianum* T34, GenBank Accession No. CAM07141) and PG1 (from *Penicillium occitanis*, GenBank Accession No. AB550231) were used for multiple homology alignment. Amino acid residues showing identities of higher than 80% and 40% are shaded in light red and light green, respectively. The Asp169, Asp190, Asp191 and His212 of PoxaEnPG28C, which were expected to be the catalytic residues, are marked by solid circles. The Arg245 and Lys247 of PoxaEnPG28C, which were expected to be involved into the binding of substrate, are marked by solid stars. The Cys18 and Cys33, Cys192 and Cys208, Cys318 and Cys323, and Cys342 and Cys351 of PoxaEnPG28C, which were expected to form disulfide bonds, are marked by solid regular triangles, solid downward triangles, solid upward triangles and solid squares, respectively.
indicating that its hydrolysis activity was hindered by the methylation of the backbone [12]. As the concentration of PGA increased, the specific enzyme activity of PoxaEnPG28C increased and then remained constant (Fig. S1A). Moreover, the $K_m$ and $V_{max}$ values were calculated to be 1.64 g/l and 6127.45 U/mg, respectively (Fig. S1B). The $K_m$ of PoxaEnPG28C was in the range of previously reported $K_m$ values of endo-PGases (from 0.32 g/l to 19.5 g/l) [12]. The $V_{max}$ value of PoxaEnPG28C was higher than those of some reported endo-PGases (from fungi, yeast and bacteria).

![Fig. 2. Phylogenetic tree of amino acids of PoxaEnPG28C with reported endo-PGases.](image)

The phylogenetic tree of the amino acids of PoxaEnPG28C with other reported endo-PGases (from fungi, yeast and bacteria) was constructed by using MEGA software. PoxaEnPG28C is boldfaced.

![Fig. 3. SDS-PAGE analysis of PoxaEnPG28C.](image)

Lane 1, protein molecular weight ladder; lane 2, purified recombinant protein PoxaEnPG28C.

![Fig. 4. Thin-layer chromatography analysis of liberated products from the hydrolysis of polygalacturonic acid by PoxaEnPG28C.](image)

Lane 1, galacturonic acid; lane 2, trigalacturonic acid; lanes 3 to 11, liberated products of PGA by PoxaEnPG28C for 0, 0.25, 0.5, 1, 2, 8, 12, 18, and 24 h.
endo-PGases, 55.55 U/mg [33], 103.58 U/mg [25] and 240 U/mg [34], but lower than that of PoxaEnPG28B-Pp (77,882.2 U/mg) [12].

Effects of Metal Ions on Enzyme Activity

Ba²⁺ reduced the enzyme activity of PoxaEnPG28C to 80.88 ± 1.76 (Table S1), which was higher than that of PG2 (0%) [23] and lower than that of PoxaEnPG28A (93.7 ± 0.7%) [12]. Similar to that of PoxaEnPG28A [12], the enzyme activity of PoxaEnPG28C was also strongly inhibited by Mn²⁺. Different from PG2 (2.48%) [23], endo-PGA1 (22.2±2.9%) [20] and PoxaEnPG28A (19.7 ± 4.6%) [12], PoxaEnPG28C exhibited 88.78 ± 1.59% enzyme activity in the presence of 2 mM Cu²⁺. The enzyme activity of PoxaEnPG28C was partially inhibited by Ca²⁺, K⁺, Na⁺, Ni²⁺, and Zn²⁺. The slight inhibitory effects of Co²⁺ and Mg²⁺ were similar to those of PoxaEnPG28A [12]; however, the inhibitory effect of Co²⁺ was obvious in some other cases such as PoxaEnPG28-Pp (67.7 ± 0.4%) [12] and PG2 (0.31%) [23].

Use of PoxaEnPG28C for Acidic Fruit Juice Extraction

The pH of oranges (Citrus reticulata Blanco) used in this study (pH 4.44) was different from that of a previous report of pH 3.5 [23]. PoxaEnPG28C reduced the viscosity by 25.7 ± 1.6%, increased the light transmittance by 13.9 ± 0.3% and increased the yield by 24.5 ± 0.7% (Table 1). In a previous report, a purified endo-PGase PG1 also increased the yield of citrus juice from 2.5 ml to 3.75 ml [35]. Lemon (Citrus limon (L.) Burm. F.) belongs to the same genus as orange, but it is more acidic than orange. The pH of lemons used in this study (pH 2.58) was lower than that of a previous report of 3.50 [23]. PoxaEnPG28C reduced the viscosity by 52.0 ± 4.5%, increased the light transmittance by 29.4 ± 3.8% and increased the yield by 12.7 ± 2.2% (Table 1). In previous reports, endo-PGases PG2 and PgaB increased the light transmittance of lemon juice by 43% [23] and about by 40% [25], respectively. However, some other important improvements in the juice such as a reduction in viscosity and an incremental yield of the juice were not mentioned in those reports [9].

Table 1. Application of PoxaEnPG28C in fruit juice production.

| Fruits      | Initial pH of juice | Reduction of viscosity (%) | Increment of light transmittance (%) | Increment of yield (%) |
|-------------|---------------------|-----------------------------|--------------------------------------|------------------------|
| Orange      | 4.44                | 25.7 ± 1.6                  | 13.9 ± 0.3                           | 24.5 ± 0.7             |
| Lemon       | 2.58                | 52.0 ± 4.5                  | 29.4 ± 3.8                           | 12.7 ± 2.2             |
| Strawberry  | 3.41                | 48.2 ± 0.7                  | 95.7 ± 10.2                          | 48.5 ± 4.2             |
| Hawthorn    | 2.92                | 80.5 ± 2.3                  | 79.8 ± 1.7                           | 104.5 ± 6.4            |
The enzymatic characteristics of PoxaEnPG28C were determined, and it was found to be an acidic enzyme. P. pasteurii PoxaEnPG28C could be considered an excellent candidate enzyme for acidic fruit juice production. In future studies, a recombinant enzyme production of PoxaEnPG28C. In industrial enzyme production [41], which allow the utilization of inexpensive agricultural residues (e.g., wheat bran, corn core, and peanut cake) for fine processes (e.g., inducement, and high cell density) [26] and high-cost materials (e.g., yeast extract, peptone, and glycerol) are required for the production of recombinant endo-Pgases by host because fine processes (e.g., O2, inducement, and high cell density) [26] and high-cost materials (e.g., yeast extract, peptone, and glycerol) are required for the production of recombinant endo-Pgases by strain will be used for the low-cost production of PoxaEnPG28C. In this study, a gene encoding a putative endo-polygalacturonase was cloned from Penicillium oxalicum CZ1028 and expressed in Penicillium oxalicum JCM 12802. This study was financially supported by the Middle-aged and Young Teachers’ Basic Ability Promotion Project of Guangxi (2019KY0924), the Doctoral Scientific Research Fund of Nanning University (2021DSRF01), and the Science Foundation of Nanning University (2018XJ42).

Table 2. Reported application of endo-Pgases in acidic fruit juice production.

| Enzyme name | Source strain | Optimal pH of enzyme | Fruit | Initial pH of juice | Performance of enzyme | Reference |
|-------------|---------------|----------------------|-------|--------------------|-----------------------|-----------|
| PoxaEnPG28C | Penicillium oxalicum CZ1028 | 4.5 | Orange | 4.44 | RVa: 25.7±1.6 %, ILTb: 13.9±0.3 %, IYc: 24.5±5.7 %, | This study |
| | | | Lemon | 2.58 | RVa: 50.4±5.5 %, ILTb: 29.4±3.8 %, IYc: 12.7±2.2 %, | |
| | | | Strawberry | 3.41 | RVa: 48.2±0.7 %, ILTb: 95.7±10.2 %, IYc: 48.5±4.2 %, | |
| | | | Hawthorn | 2.92 | RVa: 80.5±2.3 %, ILTb: 79.8±1.7 %, IYc: 104.5±6.4 %, | |
| NM1 | Penicillium occidentans CT1 | 6.0 | Lemon | 3.50 | ILTb: 43.00 %, increased the reducing sugars by 17%, reduced color by 34%, | [23] |
| endo-PG I | Penicillium sp. CGMCC 1669 | 3.5 | Apple | 3.8 | RVa: 4.5 %, ILTb: 71.8 %, | [19] |
| endo-PGA1 | Bispora sp. MEY-1 | 3.5 | Apple | 4.0 | RVa: 7.56 %, ILTb: 84.46 %, | [20] |
| TePG28b | Talaromyces leycettanus CTM 12802 | 3.5 | Grape | 3.4-3.9 | ILTb: 69 % | [24] |

RVa: Reduction of viscosity (%); ILT: Increment of light transmittance (%); IY: Increment of yield (%). NM1: Not mentioned in the reference.

The pH of strawberries (Fragaria vesca) used in this study (pH 3.41) was close to that of a report of 3.9 [36]. PoxaEnPG28C reduced the viscosity by 48.2 ± 0.7%, increased the light transmittance by 95.7 ± 10.2% and increased the yield by 48.5 ± 4.2% (Table 1). The performance of PoxaEnPG28C was better than those of other endo-Pgases including NfPGI (reduction of viscosity: 32.4%; increment of light transmittance: 28.72%; increment of yield: 6%) and NfPGII (reduction of viscosity: 20.6%; increment of light transmittance: 46.41%; increment of yield: 9%) [36]. Moreover, the enzyme dosage quantity of PoxaEnPG28C was 100 U/g juice pulp, which was lower than the dosages of 7,500 U/ml (for NfPG I) and 3,000 U/ml (for NfPGII) /g juice pulp (the density of strawberry pulp was 1.02 g/ml) [36].

Hawthorn fruit (Crataegus pinnatifida Bunge) is high in nutritional value; it contains (100 g fresh weight basis) 23.688 g citric acid, 18.378 g fructose, 13.893 g glucose and 9.418 mg vitamin C, and has a unique taste [37]. However, it is seldom used to produce juice. PoxaEnPG28C reduced the viscosity of hawthorn juice by 80.5 ± 2.3%, increased the light transmittance by 95.7 ± 10.2% and increased the yield by 48.5 ± 4.2% (Table 1). The excellent performance of PoxaEnPG28C might aid the development of hawthorn juice as a commercial product.

Endo-Pgase should function well in several types of fruit juice since the varied hydrolysis environment of pectin is a challenge for the enzyme [7]. The pH values of fruit pulp used in this study were different from previous reports, which might be due to the differences in the cultivars and the maturity of the fruits [7]. However, the differences in catalytic environment in the different fruit juices go well beyond pH and include different cell-wall compositions and polymer structures, which can have a major influence on the catalytic efficiency of endo-Pgase. The degradation of natural pectin in fruits is much more difficult than the degradation of purified pectin because natural pectin takes part in and is embedded in the network of the plant cell wall [2]. The structure of plant cell wall is a challenge for the enzyme [7]. The pH values of fruit pulp used in this study (pH 3.41) was close to that of a report of 3.9 [36].

In this study, a gene encoding a putative endo-polygalacturonase was cloned from Penicillium oxalicum and expressed in Penicillium oxalicum and Penicillium pasteurii, and the recombinant protein PoxaEnPG28C was purified and identified as an endo-polygalacturonase. The enzymatic characteristics of PoxaEnPG28C were determined, and it was found to be an acidic enzyme. Remarkably, PoxaEnPG28C functioned well in orange, lemon, strawberry and hawthorn juice production. Thus, PoxaEnPG28C could be considered an excellent candidate enzyme for acidic fruit juice production.

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Conflict of Interest
The authors have no financial conflicts of interest to declare.

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