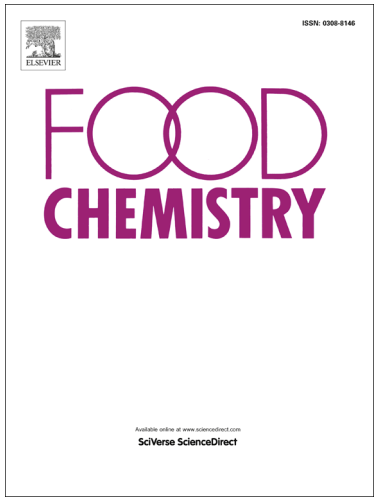

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Purification and characterization of an intracellular α-L-rhamnosidase from a newly isolated strain, *Alternaria alternata* SK37.001

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Abstract

A strain, *Alternaria alternata* SK37.001, which produces an intracellular α-L-rhamnosidase, was newly isolated from citrus orchard soil. The molecular mass of the enzyme was 66 kDa, as evaluated by SDS-PAGE and 135 kDa, as determined by gel filtration, which indicated that the enzyme is a dimer. The enzyme had a specific activity of 21.7 U mg^{-1} after step-by-step purification. The optimal pH and temperature were 5.5 and 60 °C, respectively. The enzyme was relatively stable at a pH of 4.0-8.0 and a temperature between 30 and 50 °C compared with other pH levels and temperatures investigated. The enzyme activity was accelerated by Ba^{2+} and Al^{3+} but inhibited by Ni^{2+}, Cu^{2+} and Co^{2+}, especially Ni^{2+}. The kinetic parameters of K_m and V_{max} were 4.84 mM and 53.1 μmol mg^{-1} min^{-1}, respectively. The α-L-rhamnosidase could hydrolyze quercitrin, naringin and neohesperidin, hesperidin and rutin rhamnose-containing glycosides but could not hydrolyze ginsenoside Rg2 or saiko-saponin C.

Key words: α-L-Rhamnosidase; Glycosides; *Alternaria Alternarta*; L-rhamnose; Flavonoid; Saponin

1. Introduction

Citrus juice is a popular fruit juice. However, excessive bitterness is a major problem in citrus juice processing. Naringin, a bitter flavanone glycoside which is responsible for the bitterness in citrus fruits (Radhakrishnan, Sampath, & Kumar, 2013), can be hydrolyzed by naringinase into prunin and then into naringenin, which is non-bitter and tasteless. Hence this enzyme has two different enzyme activities on naringin. One is α-L-rhamonosidase (E.C.3.2.1.40) which can act on naringin to release prunin and α-L-rhamnose; the second is β-D-glucosidase (E.C.3.2.1.21) which acts on prunin to release the aglycone naringenin and β-D-glucose (Radhakrishnan et al., 2013). Some methods using naringinase for citrus juice debittering are patented (Ito & Takiguchi, 1970; Krasnobaev, 1973) and many immobilization investigations of the enzyme for debittering the citrus juices have also been reported (Busto, Meza, Ortega, & Perez-Mateos, 2008). However, usually α-L-rhamnosidase is the limiting enzyme of the naringinase reaction (Vila-Real, Alfaia, Calado, & Ribeiro, 2007).

Alpha-L-rhamnosidase acts on terminal α-L-rhamnose, which is found in many natural compounds, in which α-L-rhamnose units are linked by α-1,2, α-1,3, α-1,4 or α-1,6 glycosidic bonds (Amaro et al., 2009). So, in addition to being used to make citrus juices less bitter, α-L-rhamnosidase can also be used to enhance wine aromas (Manzanares, Orejas, Ibañze, Vallés, & Ramón, 2000; Prakash, Singhal, & Kulkarni, 2002) by action on terpenyl glycosides and to obtain hesperetin glucoside by the enzymatic hydrolysis of hesperidin (Vila-Real, Alfaia, Bronze, Calado, & Ribeiro, 2011), which is an important precursor of many drugs. Due to these special applications of the enzyme, many studies have focussed on the screening and characterization of α-L-rhamnosidase from different sources. Alpha-L-rhamnosidase is present in many plants and

animals, as well as in microorganisms (Yadav, Yadav, Yadav, & Yadav, 2010). Some microorganisms can produce an extracellular α-L-rhamnosidase, e.g. *Penicillium* (Yadav, Yadav, Yadav, & Yadav, 2012a; Yadav, Yadav, & Yadav, 2013), *Aspergillus* (Manzanares et al., 2000; Puri & Kalra, 2005), *Pseudomonas* (Miake, Satho, Takesue, Yanagida, Kashige, & Watanabe, 2000) and *Bacteroides* (Jang & Kim, 1996).

An intracellular α-L-rhamnosidase-producing strain was screened and identified as *Alternaria alternata* SK37.001 (*A. alternata* SK37.001). The target of the research was to purify and characterize the intracellular enzyme from *A. alternata* SK37.001 and to investigate whether the enzyme has potential applications in L-rhamnose hydrolysis.

2. Materials and methods

2.1. Materials and chemicals

The soil samples used in this study to isolate the strain were collected from different citrus orchards in Wuxi, China. Naringin was purchased from Xian Yuensun Biological Technology Co., Ltd. (Xian, China). L-rhamnose and naringenin were bought from Sigma (St. Louis, USA). Quercetrin, p-nitrophenyl-α-L-rhamnopyranoside (pNPR), neohesperidin, ginsenoside, saikosaponin, hesperidin and rutin were obtained from Nanjing Jingzhu Bio-technology Co., Ltd. (Nanjing, China). Other chemicals were of analytical grade and were bought from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Strain screening and identification

A. alternata SK37.001, for use in producing large amounts of α-L-rhamnosidase, was isolated from citrus orchard soil. The soil sample (5 g) was suspended in 100 ml of the sterilized enrichment medium composed of (g l^{-1}): naringin 10, $NaNO_3$ 2, $K_2HPO_4 \cdot 3H_2O$ 1, KCl 0.5,

$MgSO_4•7H_2O$ 0.5, $FeSO_4$ 0.1 (natural pH). Incubation was carried out at 30 °C and 200 rpm with shaking. After three serial passages in the above medium, the final enrichment was plated out on the solid medium for its isolation (g l^{-1}): sucrose 5, naringin 5, $NaNO_3$ 2, $K_2HPO_4•3H_2O$ 1, KCl 0.5, $MgSO_4•7H_2O$ 0.5, $FeSO_4$ 0.1, agar 20 (natural pH). Then the colonies were picked up and seeded into the fermentation medium, shaking for 48 h at 30 ºC, which contained (g l^{-1}): sucrose 15, naringin 5, $NaNO_3$ 5, $K_2HPO_4•3H_2O$ 1, KCl 0.5, $MgSO_4•7H_2O$ 0.5, $FeSO_4$ 0.01 (natural pH). The enzyme activity was determined by the following method. The genotypic identification of the strain with the highest α-L-rhamnosidase activity was performed according to the 5.8S rDNA sequence analysis by the China Center for Type Culture Collection (CCTCC, Wuhan, China), using the BLAST program (NCBI, Bethesda, MD, USA).

2.3. α-L-rhamnosidase preparation

The strain culture medium was composed of (in g l^{-1}) sucrose 15, naringin 5, $NaNO_3$ 5, $K_2HPO_4•3H_2O$ 1, KCl 0.5, $MgSO_4•7H_2O$ 0.5, $FeSO_4$ 0.01 (natural pH). The culture conditions were 30 °C and 200 rpm. The mycelia at the highest enzyme activity from 160 ml of culture medium were collected and resuspended in a sodium phosphate buffer (100 mM, pH 6.0). The mycelia were disrupted, first for 3 min at 4 °C with a semi-homogenizer (Janke & Kunkel Ultra-Turrax T25) at 24,000 rpm and then for 10 min by ultrasonication at 4 °C. The supernatant with the unbroken cells and cellular debris removed was considered the crude enzyme.

2.4. Purification of the enzyme

All the following purification steps were completed at 4 °C. First, ammonium sulfate was added to the crude enzyme solution with 60-90% saturation to get rid of protein impurities. Then, the precipitate was resuspended and dialyzed with 20 mM of sodium phosphate buffer at a pH of

6.0. The enzyme solution was separated on a HiTrap TM DEAE-FF anion-exchange column. The column was eluted with a NaCl successive gradient elution (0-1.0 M) in a sodium phosphate buffer (20 mM, pH 6.0) at a flow rate of 0.5 ml min^{-1}. Fractions with α-L-rhamnosidase activity were collected, dialyzed and purified further with a Superdex 200 10/300 GL column from Pharmacia Amersham Biotech (Uppsala, Sweden). The elution was completed with the sodium phosphate buffer (20 mM, pH 6.0) at 0.5 ml min^{-1}. The fractions that exhibited α-L-rhamnosidase activity were concentrated and saved for later use. The molecular weight standards were also eluted in the same chromatography to determine the molecular mass of the enzyme. The standards used were thyroglobulin (bovine, 670,000), γ-globulin (bovine, 158,000), ovalbumin (chicken, 44,000), myoglobin (horse, 17,000), and vitamin B12 (1,350).

2.5. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

The homogeneity and molecular weight of the purified enzyme were estimated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli, 1970). The separating and stacking gels were 12% and 4% acrylamide, respectively. Proteins were marked by Coomassie Brilliant Blue R-250. The molecular weight standards were lysozyme (14.4 kDa) from hen egg white, carbonic anhydrase (31 kDa) from bovine erythrocytes, ovalbumin (42.7 kDa) from chicken, bovine serum albumin (66.2 kDa) from bovine serum and phosphorylase B (97.4 kDa) from rabbit muscle.

2.6. Enzyme assay and protein determination

The activity of α-L-rhamnosidase was evaluated by mixing 400 µl of a 1 mM preheated solution of pNPR (in 50 mM of sodium acetate buffer, pH 5.5) with 100 µl of the enzyme sample. The mixture was incubated at 50 °C for 10 min with 2.5 ml of 1 M Na_2CO_3 to stop the reaction.

The absorbance was measured at 405 nm. One unit was defined as the amount of enzyme needed to liberate 1 μmol of p-nitrophenol per min at 50 °C. The protein concentration was evaluated by the Braford (1976) method with bovine serum albumin as the standard protein.

2.7. Enzyme characteristics

2.7.1. Optimal pH and pH stability

To analyze the optimum pH, the enzyme activity was determined as in section 2.6, except that the pH varied within the range of 3.5-9.0. To evaluate the pH stability, the enzyme was kept in buffers with different pH levels for 12 h at 4 °C; then the enzyme activity was assayed as described in section 2.6. The buffers used were 50 mM sodium acetate buffer (pH 3.5-6.0), sodium phosphate (pH 6.0-8.0) and Tris-HCl (pH 8.0-10.0). The highest enzyme activity was set as 100%.

2.7.2. Optimal temperature and temperature stability

To evaluate the optimum temperature, the enzyme activity was determined as in section 2.6. at different temperatures (30-70 °C). The highest activity was assumed to be 100%. The thermal stability was measured with the residual activities after being incubated at different temperatures (30, 40, 50, 55, 60 and 65 °C) for 30, 60, 120, 180 and 240 min. For thermal denaturation evaluation of the enzyme, the activation energy Ea was determined by drawing an Arrhenius plot between log k and 1/T, by which the slope gave the value of Ea/2.303R, where k is the thermal denaturation rate constant calculated from the half life time of the denaturation curves using the equation $k = 0.693/t_{1/2}$.

2.7.3. Effects of metal ions on the enzyme activity

The enzyme activity was evaluated as in section 2.6. with 2 mM concentration of different metal ions (Cu^{2+}, Fe^{2+}, Zn^{2+}, Mg^{2+}, Mn^{2+}, Ni^{2+}, Ba^{2+}, Al^{3+} and Co^{2+}). The relative activity without

chemicals was set as 100%.

2.7.4. Determination of the kinetic parameters

The kinetic parameters of the purified enzyme were determined by assaying the enzyme activity at different concentrations of pNPR (2.0-6.0 mM), using the method in section 2.6. The Michaelis-Menten constant (*K*m) and maximum velocity (*V*max) of the enzyme were calculated by double-reciprocal plot.

2.8. Substrate specificity

The substrate specificity of the enzyme was assayed by enzyme reactions in the presence of 20 mg ml^{-1} of quercetin, neohesperidin, ginsenoside-Rg2, saikosaponin C, hesperidin or rutin and 100 μl enzyme solution under the optimum conditions for 12 h. After the reaction was terminated by raising the temperature to 100 °C for 10 min, the released L-rhamnose was detected by thin-layer chromatography (TLC), using Merck silica gel 60 F254. The mobile phase was ethyl acetate:methanol:water 8:3:0.5 (v/v/v) (Monti, Pišvejcová, Křen, Lama, & Riva, 2004). The colour of the plate was formed by spraying with sulfuric acid:methanol 20:80 (v/v) and dried at 105 °C for 5 min (Robyt, 2000).

2.9. Naringin hydrolysis with the enzyme

Two millilitres of 0.1% naringin in 0.5 M sodium acetate buffer (pH 5.5) was incubated with the crude α-L-rhamnosidase at 40 °C for 10 min. The crude enzyme dosage was 15 U g^{-1} of naringin. After the reaction was stopped by placement in a boiling water bath for 10 min, the reaction solution was mixed with 1.9 ml of methanol and centrifuged at 8,000 *g* for 5 min. The supernatant was analyzed using high performance liquid chromatography (HPLC) equipped with Sepax C18 column at 35 °C. The mobile phase was methanol:water 50:50 (v:v) at a flow rate of 1

ml min^{-1}. The products were detected using an ultraviolet detector at 280 nm.

2.10. Statistical analysis

The data are presented as means ± SD (n = 3). Statistical calculation was performed using the GraphPad Prism® 5 package (GraphPad Software Inc., San Diego, CA).

3. Results and discussion

3.1. Strain screening and identification

In order to screen the naringin-hydrolyzing strain, naringin was used as the only carbon source in the strain enrichment medium. Among all of the strains screened from the citrus orchard soil samples, 5 strains exhibited higher α-L-rhamnosidase activity as shown in Table. 1. The strain of number 7 exhibiting the highest α-L-rhamnosidase activity (106 U ml^{-1} of culture medium) was isolated and identified. The strain was approximately 99% phylogenetically homologous (gene accession number: KU055607) with *A. alternata* based on ITS-5.8S rDNA sequencing analysis and a BLAST search. It was named *A. alternate* SK 37.001 and kept at CCTCC (Wuhan, China) with the accession number CCTCC M 2015309.

3.2. Purification of α-L-rhamnosidase

α-L-rhamnosidase from the culture of *A. alternate* SK 37.001 was purified step-by-step, as summarized in Table 2. The cells were collected at the highest α-L-rhamnosidase activity. The cells were then disrupted by homogenization and sonication. After centrifugation, the crude enzyme was obtained, in which the α-L-rhamnosidase activity was 0.34 U mg^{-1} protein. The specific activity increased 1.5-fold with ammonium sulfate precipitation (Table 2). After that, the activity increased to 16.7 and 21.7 U mg^{-1} protein after purification by ion-exchange chromatography and gel filtration, respectively. By the above purification steps, the enzyme was

purified approximately 64-fold with a 13% enzyme activity recovery. The specific activity of α-L-rhamnosidase was higher than that of 12.3 U mg^{-1} of protein from *Curvularia lunata* (Feng et al., 2007) and similar to the activity of 23.3 U mg^{-1} of protein from *Bifidobacterium dentium* (*B. dentium*) (Bang, Hyun, Shim, Hong, & Kim, 2015). The fractions with enzyme activity from each purification step were separated by SDS-PAGE, as shown in Fig. 1(a). The enzyme was purified step-by-step, and the protein with electrophoretic homogeneity was obtained after purification by gel filtration, as shown in lane 4.

3.3. Molecular weight determination

From the SDS-PAGE analysis (Fig. 1(a)), the α-L-rhamnosidase subunit molecular weight was estimated to be 66.0 kDa, and the molecular mass of the enzyme was calculated to be 135 kDa, based on the gel filtration (Fig. 1(b)). The subunit size and gel filtration molecular weight indicate that the α-L-rhamnosidase is a dimer. Different molecular masses of the enzyme have been reported, which ranged from 53.0 to 240 kDa with different oligomeric forms (Yadav, Yadav, Yadav, & Yadav, 2010).

3.4. Enzyme properties

3.4.1. Optimal pH and pH stability

The variation of pH values in a reaction system will affect the substrate and the enzyme dissociation state with which the enzyme activity changes. The optimal pH of the α-L-rhamnosidase was determined, from Fig. 2a, to be 5.5, using pNPR as the substrate. Most of its activity was retained when the enzyme was kept at different pH levels (pH 4.0-8.0) for 12 h at 4 °C, which means that it was stable over a wide pH range. The optimum pH of this enzyme ranged from acid to basic and varied with microorganisms; some are acidic (Puri et al., 2005;

Miake et al., 2000; Yadav, Yadav, Yadav, & Yadav, 2011), some are basic (Manzanares, van den Broeck, de Graaff, & Visser, 2001; Rojas, Voget, Hours, & Cavalitto, 2011), and some are in the neutral pH range (Yadav et al., 2012a).

3.4.2. Optimum temperature and thermal stability

To evaluate the optimum temperature of the enzyme, the activity of the purified enzyme at different temperatures was analyzed, as shown in Fig. 2b. The highest activity occurred at a temperature of 60 ℃, which was higher than the optimum temperature of α-L-rhamnosidase from *P. citrinum* MTCC3565 (Yadav, Yadav, Yadava, & Yadav, 2012b) but was the same as that of *A. nidulans* (Manzanares et al., 2000) and *P. citrinum* MTCC-8897 (Yadav et al., 2012a). To evaluate the enzyme thermostability, the enzyme was incubated at different temperatures varying from 30 to 65 ℃ for different times in phosphate buffer solution (pH 5.5). Fig. 2c indicates that the enzyme was stable within the range 30 to 50 °C, and the residual enzyme activity was still more than 60%, even after incubation for 240 min. The enzyme was inactivated rapidly at temperatures higher than 60 °C. Based on the first-order deactivation kinetic model, the half-life ($t_{1/2}$) values were determined. When the enzyme was incubated at 50, 55, 60 and 65 ℃, the $t_{1/2}$ values were 630, 240, 59.5 and 45.9 min, respectively. Puri et al. (2005) reported that the half-life values of the enzyme from *Aspergillus niger* 1344 at 50 and 60 ºC were 15 and 90 min, respectively. The enzyme from *Penicillium citrinum* MTCC-3565 was relatively stable up to 40 ºC, but lost the activity very rapidly above 50 ºC (Yadav et al., 2012b). The activation energy of 164 kJ mol^{-1} K^{-1} was needed for the thermal denaturation of the pure enzyme, which was determined from the Arrhenius plot. It was higher than that of the enzyme from *P. corylophilum* MTCC-2011 (Yadav et al., 2013).

3.4.3. Effects of metal ions on enzyme activity

The effects of metal ions on the enzyme activity were assayed by incubating the enzyme and pNPR in 2 mM concentrations of various metal ions at 50 °C for 10 min. As shown in Fig. 2d, the activities varied as the metal ion concentrations changed. Fe^{2+} had a negligible effect on the α-L-rhamnosidase activity. Zn^{2+}, Mg^{2+} and Mn^{2+} inhibited the activity slightly. However, the relative activity dramatically decreased to 46.7% and 64.2% with the addition of Cu^{2+} and Co^{2+}, respectively; particularly, Ni^{2+} inactivated the enzyme completely. By contrast, the same concentration of Ba^{2+} and Al^{3+} stimulated the activity to a higher level, which is different from other α-L-rhamnosidases. Puri et al. (2005) reported that Cu^{2+} and Mn^{2+} could completely deactivate the α-L-rhamnosidases from *A. niger*, while Ca^{2+}, Co^{2+} and Mg^{2+} had almost no effects on the enzyme. Yanai and Sato (2000) thought divalent cations had no effect on the enzyme activity. All these observations illustrated that the effects of different metal ions on the enzyme activity varied with the source.

3.4.4. Kinetic parameters

The kinetic parameters were investigated using pNPR as the substrate at 55 °C and pH 5.5. The values of *K*m and *V*max were 4.16 mM and 49.0 U mg^{-1}, respectively (Fig. 2e), as calculated from the double-reciprocal plot. The *K*m value was similar to that of *Acrostalagmus luteo albus* (Rojas et al., 2011). However, it was higher than those of the enzymes from some other microorganisms, which means the affinity of the enzyme for the substrate was not as good as that of other enzymes (Puri et al., 2005; Manzanares et al., 1997; Yadav et al., 2011; Manzanares et al., 2000; Yadav et al., 2012b; Miake et al., 2000). However, the *V*max was higher than those of most of the other enzymes, which range from 10.7 to 24.3 U mg^{-1} (Manzanares, de Graaff, & Visser,

1997; Manzanares et al., 2000).

3.4.5. Substrate specificity

α-L-Rhamnosidase hydrolyzes flavonoids, polysaccharides and steroids to liberate L-rhamnose, in which the L-rhamnose residue is linked to the terminus of β-D-glucoside by α-1,2, α-1,4 or α-1,6 linkages. The substrate specificity of the enzyme from *A. alternata* SK37.001 was evaluated with several plant L-rhamnose-containing flavonoids and saponins. The L-rhamnose released was detected by TLC, as shown in Fig. 3. The enzyme could hydrolyze the α-1,2 linkages of neohesperidin and naringin or α-1,6 rhamnosidic linkages of hesperidin and rutin. Moreover, the enzyme hydrolyzed L-rhamnose linked to the aglycone directly at the C-1 position of quercetin. However, there were no effects on the α-1,2 rhamnosidic linkage of ginsenoside-Rg2 or the α-1,4 linkage of saikosaponin C. α-L-Rhamnosidase from *P. paucimobilis* FP2001 hydrolyzed quercitrin (rhamnose linked to the aglycone directly at the C-1 position), naringin (α-1,2 linkage), hesperidin (α-1,6 linkage) and saikosaponin C (α-1,4 linkage) (Miake et al., 2000). However, *A. niger* can act on naringin and rutin, but not on hesperidini and quercitrin although a-1,6 linkage existed in both rutin and hesperidin (Kurosawa, Ikeda, & Egami, 1973). The enzyme from *Turbo cornutus* has no activity on naringin (Kurosawa et al., 1973). The above results indicated that the substrate specificities of the α-L-rhamnosidases vary with their sources, which may be affected by the substrate structure, the enzyme's three-dimensional structure and the binding manner between the substrate and the enzyme. More experiments are necessary to understand the molecular mechanism underlying the degradation of substrates.

3.5. The enzymatic hydrolysis of naringin

To confirm the L-rhamnoside release ability of the enzyme, the hydrolysis of naringin with

the crude enzyme was investigated. Fig. 4(b) shows that the substrate naringin was hydrolyzed to prunin and naringenin after the reaction with the crude enzyme for 10 min compared to the inactivated enzyme (Fig. 4(a)). This finding indicated that the crude enzyme contained not only α-L-rhamnosidase but also glucosidase. In microorganisms, the enzyme is normally combined with glucosidase, allowing some compounds to be hydrolyzed in succession to release L-rhamnose and D-glucose as shown in Fig. 4(c).

4. Conclusions

In food processing, α-L-rhamnosidase is a hydrolytic enzyme used to enhance wine aromas or to debitter citrus juices by releasing L-rhamnose. An intracellular α-L-rhamnosidase from *A. alternata* SK37.001 was purified and characterized. The enzyme displayed several characteristics different from those of other microorganisms, and it has the ability to hydrolyze L-rhamnose-containing compounds. For all the above reasons, this enzyme is applicable in a variety of biotechnological processes.

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Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Figure captions

Fig. 1 Molecular mass (Mw) of the α-L-rhamnosidase estimated by SDS-PAGE (a) and gel filtration on Superdex 200 10/300 GL (b). (a) SDS-PAGE results of α-L-rhamnosidase purification analysis (lane 1, crude enzyme; lane 2, enzyme purified with $(NH_4)_2SO_4$ precipitation; lane 3, enzyme purified with DEAE-FF; lane 4, enzyme purified with Superdex G-200; lane M, molecular weight markers (from top to bottom: rabbit phosphorylase b (97,400 Da), BSA (66,200 Da), rabbit actin (43,000 Da), trypsin inhibitor (20,100 Da) and hen egg-white lysozyme (14,400 Da)); (b) Gel filtration on Superdex 200 10/300 GL. The standards were thyroglobulin (bovine, 670,000 Da), γ-globulin (bovine, 158,000 Da), ovalbumin (chicken, 44,000 Da), myoglobin (horse, 17,000 Da), and vitamin B12 (1,350 Da). Ve, volume of the elution buffer; V0, void volume of the column.

Fig. 2 Properties of the enzyme. (a) Optimum pH (●) and pH stability (■), (b) optimum temperature (●), (c) thermal stability (■), (d) effects of metal ions and (e) kinetic parameters.

Fig. 3 Thin-layer chromatography analysis of the products obtained from the enzyme hydrolysis (lanes: 1, L-rhamnose; 2, quercitrin hydrolysis; 3, neohesperidin hydrolysis; 4, naringin hydrolysis; 5, ginsenoside-Rg2 hydrolysis; 6, saikosaponin C hydrolysis; 7, hesperidin hydrolysis; 8, rutin hydrolysis).

Fig. 4 Chromatography of naringin hydrolytes by HPLC (a) reaction with inactivated enzyme, (b) reaction with active enzyme, and (c) stepwise degradation of naringin by the action of naringinase expressing α-L-rhamnosidase and β-glucosidase activities.

Figures

Fig. 1

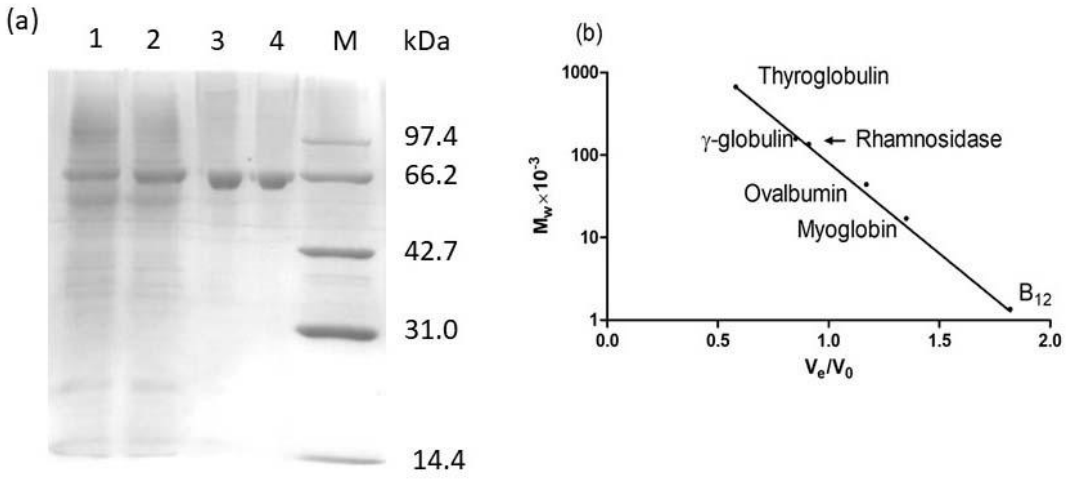


Fig. 2

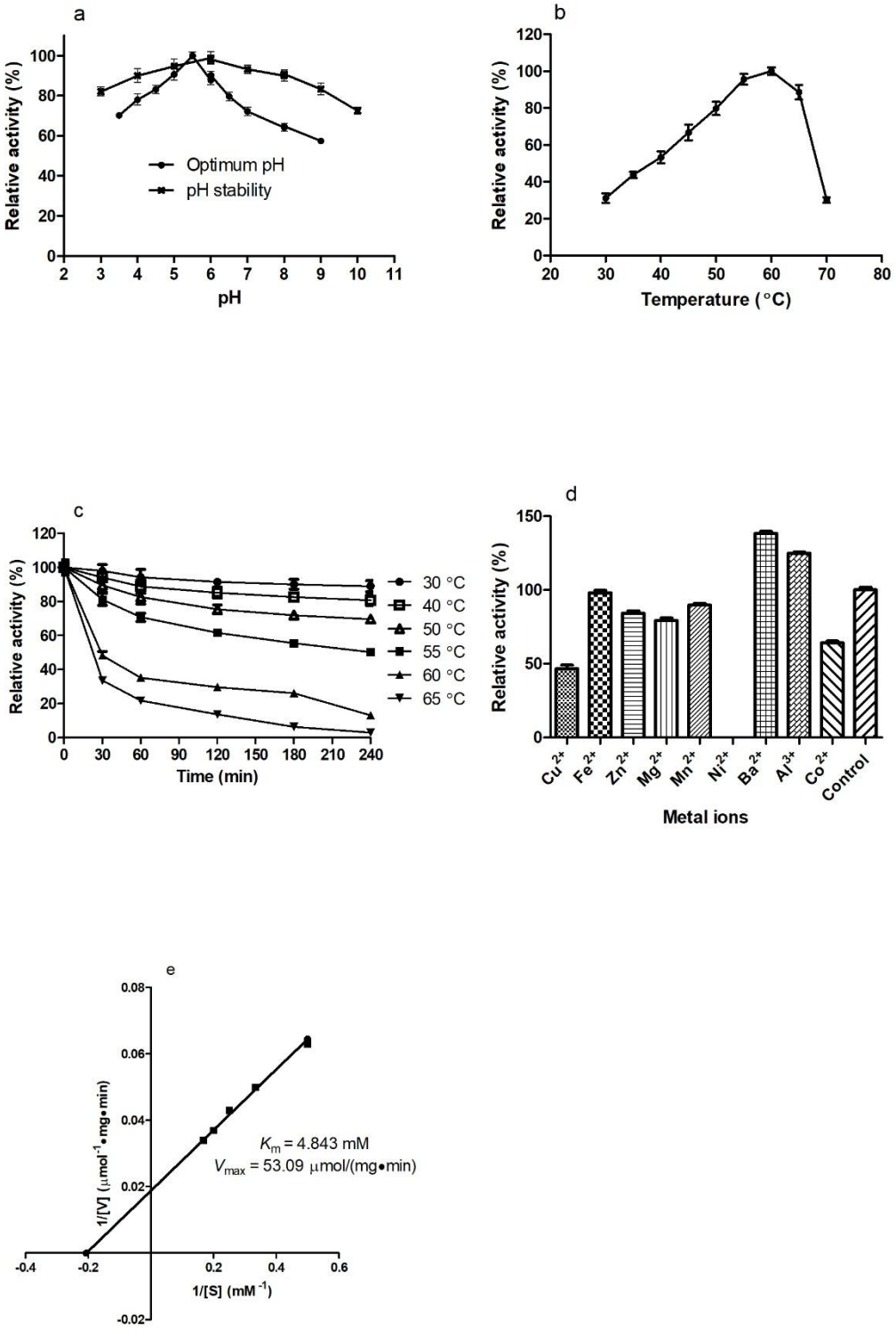


Fig. 3

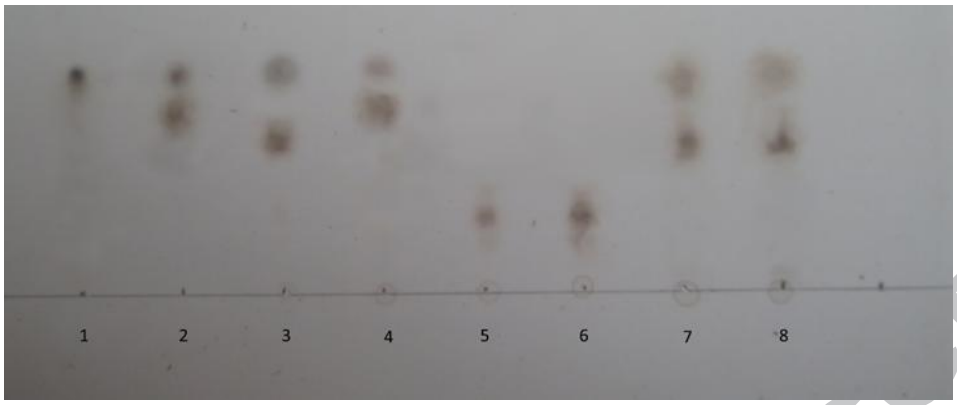
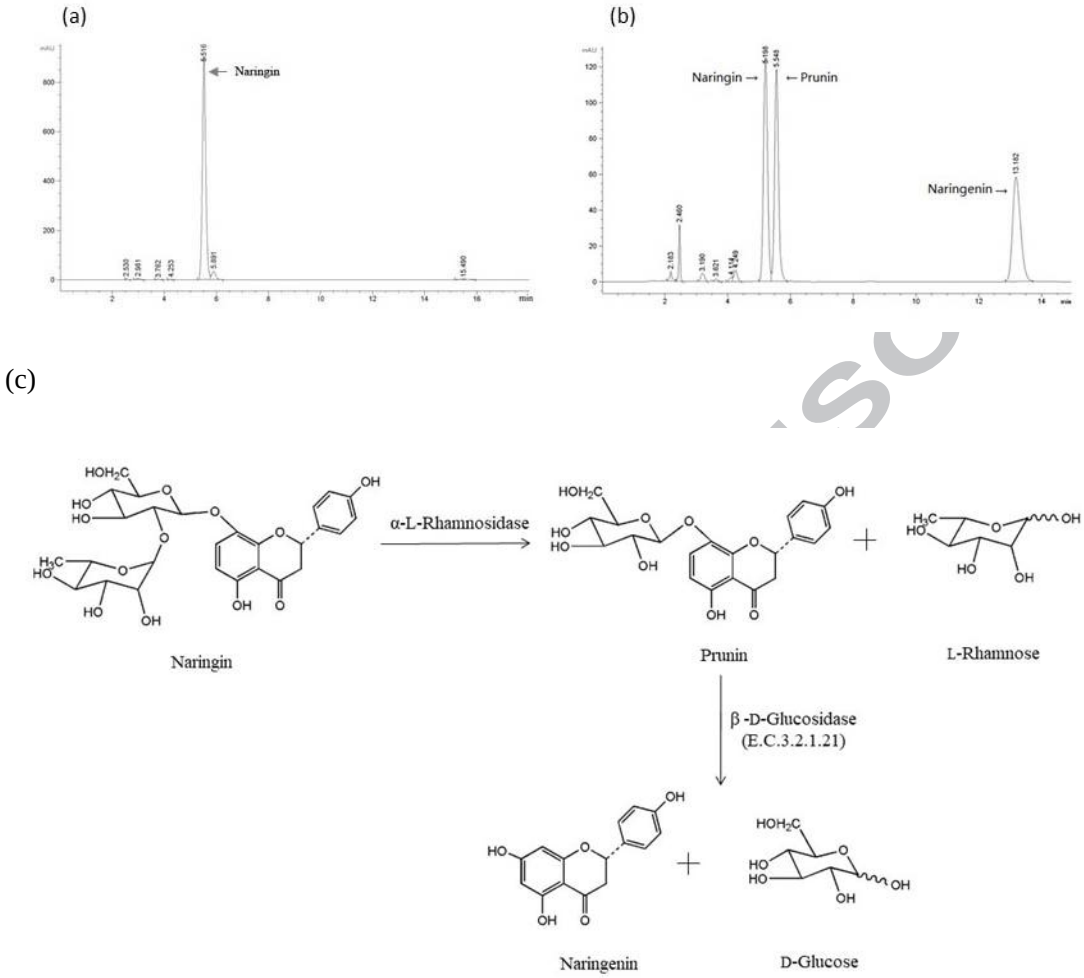


Fig. 4



Tables

Table 1 Alpha-L-rhamnosidase-producing strain screening

Strain number	2	7	8	15	19
α-L-rhamnosidase（U ml^{-1}）	72.3±3.3	106±4.4	91.8±2.9	84.9±3.3	86.6±3.2

Table 2 Purification of α-L-rhamnosidase from *A. alternata* SK37.002

Purification steps	Total protein (mg)	Total activity (U)	Specific activity (U mg^{-1})	Purification fold	Recovery (%)
Crude enzyme	126	43.1	0.34	0	100
Ammonium sulfate precipitation	64.9	32.9	0.51	1.5	76.4
HiTrap TM DEAE-FF	0.79	13.2	16.7	49.2	30.7
Superdex 200 gel filtration	0.26	5.71	21.7	63.8	13.3

Highlights

An intracellular α-L-rhamnosidase-producing strain was screened and identified.

The enzyme activity was accelerated by Ba^{2+} and Al^{3+}.

The enzyme displayed several characteristics different from those of other sources.

It has potential to be used in rhamnose hydrolysis fields.