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An alkali tolerant α-L-rhamnosidase from *Fusarium moniliforme* MTCC-2088 used in de-rhamnosylation of natural glycosides

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ABSTRACT

Analkali tolerant α-L-rhamnosidase has been purified to homogeneity from the culture filtrate of a new fungal strain, *Fusarium moniliforme* MTCC-2088, using concentration by ultrafiltration and cation exchange chromatography on CM cellulose column. The molecular mass of the purified enzyme has been found to be 36.0 kDa using SDS-PAGE analysis. The K_m value using *p-nitrophenyl-α-L-rhamnopyranoside* as the variable substrate in 0.2 M sodium phosphate buffer *pH*10.5 at50 °C was 0.50 mM. The catalytic rate constant was15.6 s^{-1}giving the values of k_{cat}/K_m is 3.12 × $10^4 M^{-1} s^{-1}$. The *pH* and temperature optima of the enzyme were 10.5 and 50 °C, respectively. The purified enzyme had better stability at 10 °C in basic *pH* medium. The enzyme derhamnosylated natural glycosides like naringin to prunin, rutin to isoquercitrin and hesperidin to hesperetin glucoside. The purified α-L-rhamnosidase has potential for enhancement of wine aroma.

1. Introduction

α-L-rhamnosidase [E.C.3.1.1.40] selectively derhamnosylates natural glycosides containing terminalα-L-rhamnose [1,2]. The derhamnosylated products are rare compounds of pharmaceutical importance [3–11]. α-L-rhamnosidase transforms naringin (4′,5,7trihydroxy **flavanone-7**-rhamnoglucoside) to prunin (4′5,7trihydroxy flavanone-7-glucoside), which has enhanced bioavailability compared to naringin and its aglycon naringenin (4′5,7,trihydroxy flavanone) while maintaining its bioactivity [3]. The derhamnosylation of hesperidin (hesperetin-7-O-rhamnoglucoside) by α-L-rhamnosidase gives hesperetin-*7-O*-glucoside, which is a rare pharmaceutically important compound [5,6]. α-L-rhamnosidase transforms rutin (quercetin-*3-O*-**rutinoside)** to isoquercitrin (quercetin-*3-O*-glucoside) which has been reported as a drug for a number of diseases due to its non-oxidisable, anti-inflammatory, anti-mutagenetic, anti viral properties and other pharmacological effects [7,8]. Moreover, isoquercitrin is a precursor for the enzymatic biosynthesis of enzymatically modified isoquercitrin (EMIQ) which has been approved as a multiple food additive [12,13]. An **alkali**tolerant **thermostable** α-L-rhamnosidase from *Aspergillus terreus* have been reported, its recombinant enzyme has been prepared and used for selective conversion of rutin to isoquercitrin [14–16]. α-L-rhamnosidases in combination with other glycosidases has been found to improve the quality of wine [17,18]. These biotechnological applications of α-L-rhamnosidases have prompted the authors to purify and characterize α-L-rhamnosidases from new sources with novel properties and to demonstrate their applications for different bio transformations. In this communication, the authors report an alkali resistant α-L-rhamnosidase from *Fusarium moniliforme* MTCC-2088 which transforms naringin to prunin, hesperidin to hesperetin glucoside and rutin to isoquercitrin and releases L-rhamnose from a wine sample indicating that it has potential for aroma enhancement of wine.

2. Materials and methods

2.1. Materials

Naringin, rutin, hesperidin, L-rhamnose, *p-nitrophenyl-α-L*-rhamnopyranoside, and CM cellulose were purchased from Sigma Chemical Company, St. Louis, (USA). The chemicals for gel-electrophoresis including the protein molecular weight markers used in the SDS-PAGE and native-PAGE analysis were procured from Bangalore GENEI Pvt. Limited Bangalore (India). All other chemicals were either from Merck Ltd., Mumbai (India) or from s.d. fine chem. Ltd., Mumbai (India) and were used without further purifications.The wine sample was of **Macleods** distillers Ltd. Broxbum (UK) make.

2.2. The fungal strain

Five fungal strains namely *Fusarium graminearum* MTCC-2093, *F.*

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pallidorosaum MTCC-2083, *F. sabucinum* MTCC-2085, *F. moniliforme* MTCC-2088 and F. *oxysporum* MTCC-3075 were procured from the Microbial Type Culture Collection Center and Gene Bank, Institute of Microbial Technology, Chandigarh (India) and were maintained on agar strains of the media reported for them [19]. All these five fungal strains were tested for the secretion of α-L-rhamnosidase in the liquid culture growth medium using the method reported in the literature [20]. Out of the above five fungal strains, only *F. moniliforme* MTCC-2088 secreted α-L-rhamnosidase in the liquid culture growth medium. Hence further studies on the α-L-rhamnosidase of only *F. moniliforme* MTCC-2088 were carried out.

2.3. α-L-Rhamnosidase secretion

The secretion of α-L-rhamnosidase by the fungal strain in the liquid culture growth medium was studied using the method reported by Yadav et al. [20]. The liquid culture growth medium consisted of $CaCl_2$ 1 g, $MgSO_4{\cdot}7H_2O$ 3 g, KH_2PO_4 20 g, $N(CH_2COONa)_3$ 1.5 g, $MnSO_4$ 1 g, $ZnSO_4{\cdot}7H_2O$ 0.1 g, $CuSO_4{\cdot}5H_2O$ 0.1 g, $FeSO_4{\cdot}7H_2O$ 0.1 g, H_3BO_3 10.0 mg, sucrose 40.0 g, ammonium tartrat8.0 g in 1L MilliQ Water. One mL of spore suspension (spore density 5×10^6 spore/mL) from the agar slants of the fungal strain was inoculated aseptically into the sterilized liquid culture growth medium (20 mL) kept in 100 mL culture flasks. The flasks were incubated in a B.O.D incubator at 25 °C under stationary culture condition. Aliquots of one mL of the fungal growing cultures were withdrawn at the regular intervals of 24 h, filtered through millex syringe filters (0.22 mm) and analyzed for the presence of α-L-rhamnosidase activity by the method reported by Romero et al. [21] as described below. The experiments were done in triplicates and the average of enzyme unit/mL present in the growth medium was plotted against the fungus growth time in days. In order to enhance the secretion of the enzyme in the above liquid growth medium by *F. moniliforme* MTCC-2088, the liquid culture growth medium was amended by adding 0.5 g of each of naringin, rutin, hesperidin, and L-rhamnose separately in four sets of experiments and the above experiment was repeated. The maximum activity of α-L-rhamnosidase was observed in the medium amended with hesperidin. For the purification of the enzyme, the fungal culture was grown in the medium amended with hesperidin.

2.4. α-L-Rhamnosidase assay

The activity of α-L-rhamnosidase was assayed using *p-nitrophenyl-α-L*-rhamnopyranoside as the substrate and monitoring the liberation of *p*-nitrophenol spectrophotometrically at $\lambda = 400$ nm using molar extinction coefficient value of 21.44 $mM^{-1}\,cm^{-1}$ by the reported method [21]. 400 μL of 1 mM solution of *p-nitrophenyl- α-L*-rhamnopyranoside in a 0.5 M sodium phosphate buffer pH 10.5 was mixed with 500 μL of the same buffer in an Eppendoff tube. The tube was incubated in a thermostat maintained at 50 °C and was allowed to maintain thermal equilibrium. 100 μL of the enzyme sample was added to the Eppendoff tube and mixed.100 μL reaction mixture was withdrawn immediately and added to 3.0 mL of 0.1 M NaOH solution. The aliquots of 100 μL of reaction mixture were withdrawn at regular intervals of 2 min and were added to 3.0 mL of 0.1 M NaOH solutions kept in test tubes. The solutions were allowed to stand for 10 min after which absorptions of solutions at $\lambda = 400$ nm were measured. All spectrophotometric measurements were made with UV/Visible spectrophotometer Hitachi (Japan) model U-2000 which was connected to an electronic temperature control unit. The least count of the absorbance measurement was 0.001 absorbance unit. One enzyme unit was the amount of the enzyme which liberated 1 μmol of *p*-nitrophenol/min at 50 °C temperature under the assay conditions specified above.

2.5. α-L-Rhamnosidase purification

For the purification of the α-L-rhamnosidase from *F. moniliforme* MTCC-2088, the fungal strain was grown in 20 sterilized 250 mL culture flasks each containing 25 mL liquid culture medium amended with the 2.0% hesperidin and 4.0% sucrose. The α-L-rhamnosidase activity reached the maximum value on 4th day from inoculation of the fungal spores. On that day, the cultures of all the flasks were pooled, mycelia were removed by filtration through four layers of cheese cloth, and the culture filtrate was centrifuged using Sigma refrigerated centrifuge model 3K30 at 8000 rpm for 20 min at 4 °C to remove the particles. The centrifuged culture filtrate was concentrated by ultrafiltration using Amicon concentration cell model 8200 with PM10 membrane. The concentrated enzyme sample was dialyzed against 0.01 M of sodium phosphate buffer *pH* 7.0. The dialyzed enzyme was loaded on CM cellulose column equilibrated with the same buffer. The adsorbed enzyme was washed with three times bed volume of the column and was eluted by applying the linear NaCl gradient of 0–1 M in the same buffer. The 3.5 mL fractions were collected and were analyzed for the activity of α-L-rhamnosidase [21] and for protein concentration using Lowry's method [22]. The α-L-rhamnosidase active fractions were pooled and concentrated by putting the solution in a dialysis bag which was kept in solid powderedsucrose. The purified, concentrated enzyme sample was stored in the refrigerator at 4 °C and was used whenever required. The enzyme did not loseits activity for two months under these conditions.

2.6. SDS-polyacrylamide and native-polyacrylamide gel electrophoresis

The homogeneity of the enzyme preparation was checked by SDS-PAGE analysis and the molecular mass was determined using the method reported by Weber and Osborn [23]. These parating gel was 12% acrylamide in 0.375 M Tris–HCl buffer of pH 8.8 and stackinggel was 5% acrylamide in 0.5 M Tris–HCl buffer of pH 6.8. Proteins were visualized by silver staining. The molecular weight markers used were phosphorylase B (97.4 kDa), bovine serum albumin (66.0 kDa), ovalbumin (43.0 kDa), carbonic anhydrase (29.0) and soyabean trypsin inhibitor (20.1 kDa). The gel was run at the constant current of 20 mA using Electro gel 50 equipment of Technosource, Mumbai, (India). The native polyacrylamide gel electrophoresis was done using the reagent kitsupplied by Bangalore GENEI Pvt. Limited Bangalore (India). The resolving gel was 10% acrylamide in 0.5 M Tris–HCl buffer of pH 8.8 and the stacking gel was 5% acrylamide in 0.5 M Tris–HCl buffer of pH 6.8.

2.7. Steady-state kinetics of the enzyme

The values of K_m and V_{max} of the purified enzyme for the substrate *p-nitrophenyl-α-L*-rhamnopyranoside were determined by measuring the steady state velocity of the enzyme catalyzed reaction at different concentrations of *p-nitrophenyl-α-L*-rhamnopyranoside (0.1–1.2 mM) using the method reported by Romero et al. [21]. The K_m and V_{max} values were calculated by linear regression analysis of the data points of the double reciprocal plot. The *pH* optimum of the purified enzyme was determined by using *p-nitrophenyl-α-L*-rhamnopyranoside as the substrate and measuring the steady state velocity of the enzyme catalyzed reaction in solutions of varying *pH* in the range5.0–13.0. The buffers used were 0.2 M sodium acetate/ acetic acid buffer solution (*pH*5–7), and sodium phosphate buffer solution (*pH*8.0–13.0). The steady state velocity was plotted against the *pH* of the reaction solutions and the *pH* optimum was determined from the graph. The *pH* stability of the enzyme was determined by incubating theenzyme in the buffers of different *pHs* for 24 h at 25 °C. The remaining activities were assayed and plotted against pH to which the enzyme was exposed for 24 h. The temperature optimum was determined by measuring the steady statevelocity of the enzyme catalyzed reaction in solutions of varying temperatures (30–70 °C) using *p-nitrophenyl-α-L*-rhamnopyranoside as the

substrate. The steadystate velocity of the enzyme catalyzed reaction was plotted against the temperature of the reaction solution and the temperature optimum was determined from the graph. The thermal stability of the enzyme was determined by incubating the aliquots of theenzyme at different temperatures (viz. 10, 20, 30, 35, 40, 45, 50, 55, 60, 65 and 70 °C) and assaying the remaining activity at the intervals of 10 min for 2 h. The remaining activity was plotted against the time for which the enzymes were exposed at that temperature.

2.8. Studies on the enzymatic hydrolysis of naringin, hesperidin and rutin

This experiment was performed in 1.0 mL solution of 1.0 mM naringin in 0.5 M sodium phosphate buffer *pH* 10.5 at 30 °C. 25 µL purified enzyme stock (0.30 U/mL) was added. The reaction solution was left overnight and the release of prunin was detected by thin layer chromatography using silica gel on glass plates and using butanol:acetic acid:water (40:11:29) (v/v) as mobile phase. The detection was made using the iodine chamber. Similar experiments were performed with rutin and hesperidin. Preparative TLC in silica gel on glass plates were used for purifying the enzymatic hydrolysis products of naringin, rutin and hesperidin for HPLC-Mass spectrometric analysis. The HPLC-Mass Spectrometric analysis was done at Sophisticated Analysis Instrument Centre, CDRI, Lucknow, U.P. (India). The release of L-rhamnose was also detected by the thin layerchromatography using silica gel on glass plate and chloroform: methanol (70:30) (v/v) as the mobile phase. The L-rhamnose released as the results of enzymatic hydrolysis of naringin, rutin and hesperidin also was purified by preparative TLC and was analyzed by HPLC-Mass Spectrophotometric studies.

2.9. Studies on the enzymatic release of L-rhamnose from wine sample

In 1.0 mL of the wine sample, 0.30 U of the purified enzyme from *F. moniliforme* MTCC-2088 was added. The reaction solution was left overnight, and the release of L-rhamnose was detected by thin-layer chromatography using silica gel on glass plates. The mobile phase used was chloroform: methanol mixture 70:30 (v/v). The detection was made using the iodine chamber. Again the identity of L-rhamnose released by the enzymatic hydrolysis was confirmed by HPLC-Mass Spectrophotometric studies of the product which was purified by preparative TLC.

3. Results and discussion

3.1. Secretion and purification of the α-L-rhamnosidase

In order to enhance the secretion of α-L-rhamnosidase in the liquid culture growth medium, the effects of presence of naringin, rutin, hesperidin and L-rhamnose in the growth medium on the secretion of α-L-rhamnosidase were studied. The results are shown in Fig. 1. All the three substrate, naringin, rutin, hesperidin and one product L-rhamnose, enhance the secretion of α-L-rhamnosidase in the growth medium. However, the enhancement of α-L-rhamnosidase secretion in the growth medium containing hesperidin was maximum. Since the structure and regulation of α-L-rhamnosidasegenes is not fully understood [24], the reason for enhancement of α-L-rhamnosidase secretion in the presence of the above substrates and the products cannot be discussed further. The purification procedure of α-L-rhamnosidase from the culture filtrate of *F. moniliforme* MTCC-2088is summarized in Table 1 and the elution profile of the enzyme from the cation exchanger carboxymethyl cellulose is shown in Fig. 2. The enzyme bound to CM cellulose in 10 mM sodium phosphate buffer *pH*7.0 and was eluted by the linear gradient of NaCl in the range 400–660 mM. About6 fold purification with 19% yield was achieved. The specific activity of the purified enzyme using *p*-nitrophenylα-L-rhamnopyranoside is 26 U/mg is greater than the specific activity of α-L-rhamnosidases reported from *A. clavato-nanicus* MTCC-9611 [20] and *P. corylopholum* MTCC-2011 [25], the value being

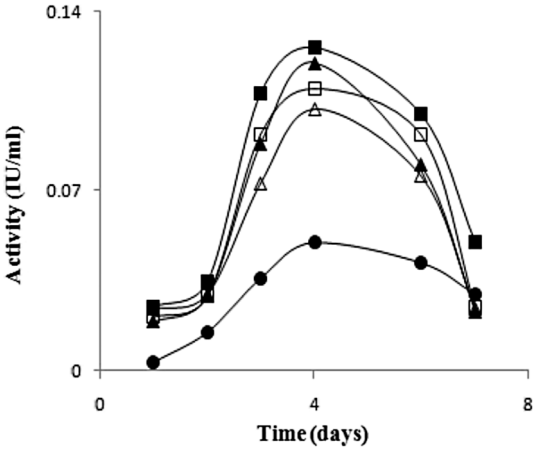


Fig. 1. Secretion of α-L-rhamnosidase in the culture medium of *Fusarium moniliforme* MTCC-2088. Hesperidin (■), naringin (▲), control (●) bagasse (△) and corncob (□).

6.0 U/mg and 13.0 U/mg, respectively. The value of specific activity of this enzyme is comparable to the specific activity of α-L-rhamnosidases reported from *A. awamori* MTCC-2879 [26] and *P. citrinum* MTCC-3565 [27] both of which have specific activity 27 U/mg. However, the specific activity of this enzyme is less than specific activity of α-L-rhamnosidase reported from P. citrinum MTCC-8897 [28] 42.7 U/mg. The purification procedure is relatively simpler than the procedures reported for the purifications of α-L-rhamnosidases from other sources [2].

The results of SDS-PAGE and native PAGE analysis are shown in Fig. 3A and B, respectively. The presence of a single protein band in lane 2 of Fig. 3A in which the purified enzyme was applied showed that the enzyme was pure. The purity of the enzyme was further confirmed by the presence of a single protein band in lane 1 of the native-PAGE analysis result shown in Fig. 3B in which the purified enzyme was applied in lane 1 and the reference protein, bovin serum albumin, which was applied in lane 2. The molecular mass of the purified α-L-rhamnosidase calculated from the SDS-PAGE analysis was 36.0 kDa. The molecular mass of the purified α-L-rhamnosidases reported in the literature [2] are in the range 41.0–240 kDa. Thus the purified α-L-rhamnosidase is one of the α-L-rhamnosidases having lower molecular mass.

3.2. Kinetics characteristics of the enzyme

The kinetic characteristics of the purified enzyme were studied using *p*-nitrophenyl-α-L-rhamnopyranoside as the substrate. The enzyme followed the Michaelis-Menten kinetics (data not shown here) the values of K_m and k_{cat} calculated from the double reciprocal plots were 0.5 mM and 15.6 s^{-1}at 50 °C in 0.2 M sodium phosphate buffer pH 10.5 using *p*-nitrophenyl-α-L-rhamnopyranoside as the substrate. The k_{cat}/K_m value of the purified enzyme under the above condition is $3.12 \times 10^4\ M^{-1}\ s^{-1}$ which is lower by a factor 10^4 in comparison to a perfectly evolved enzyme [29]. Thus there is scope for improving the catalytic efficiency of this enzyme using the molecular biology techniques of directed evolution [30,31].

3.3. pH and temperature optima and stabilities

The results of studies on the variation in the activity of the enzyme with the variation in the *pH* of the reaction solution are shown in Fig. 4A. The *pH* optimum of the enzyme was 10.5 at pH 50 °C. Thus it is one of the α-L-rhamnosidase which has *pH* optimum in very alkaline range. There are a few reports on α-L-rhamnosidases having *pH* optima in alkaline range i.e. α-L-rhamnosidase from *Clostridium stercorarium*

Table 1
Purification chart *α-L*-rhamnosidase from *Fusarium moniliforme* MTCC-2088.

S. no.	Steps	Total volume mL	Activity U/mL	Protein mg/mL	Total activity U	Total protein mg	Specific activity U/mg	Protein fold	% Yield
1	Culture filtrate of the fungal strain	600	0.095	0.023	57	13.8	4.13	1	100
2	After Amicon concentration	10	4.82	0.81	48.2	8.1	5.95	1.14	84
3	After Dialysis	12	4.01	0.60	48.12	7.2	6.68	1.16	84
4	After CM cellulose column concentration	10.5	1.04	0.04	10.92	0.42	26	6.3	19

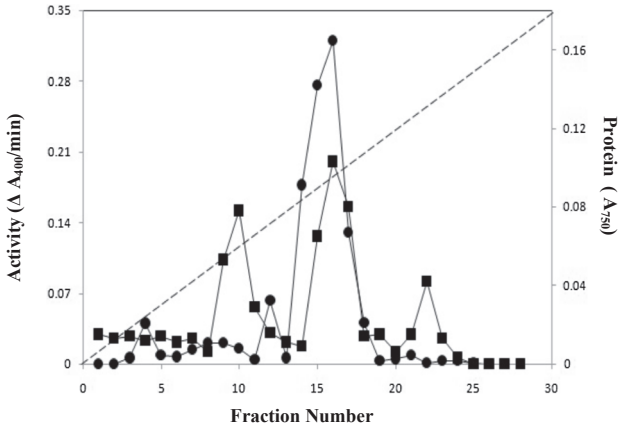


Fig. 2. Elution profile of *α-L*-rhamnosidase from CM-cellulose column. Protein concentration (A_{750}) (■); enzyme activity (A_{400}/min) (●); NaCl gradient (– – – –).

(*pH*7.5), *α-L*-rhamnosidase from PRI-1686 RhmA (*pH*7.9), *α-L*-rhamnosidase of *Pseudomonas paucimobilis* FP 2001 (*pH* 7.8) [2] and *α-L*-rhamnosidases from *A. terreus* (*pH* 8.0) [12], but all these *α-L*-rhamnosidases have *pH* optima 8.0 or less than. It is also essential to mention that *pH* and temperature optima are not always independent of each other as is assumed in traditional enzymology. To check this point, the *pH* optima of the purified enzyme have been determined at two additional temperature 40 °C and 60 °C using naringin as the substrate [2]. The results are shown as insert (B) in Fig. 4(a). The *pH* optima of the enzyme at 40 °C is 9.5 *pH* unit were as at 60 °C it is 10.5 *pH* unit. The *pH* stability of the purified enzyme was also studied for the point of view of determination of pH at which the enzyme could be preserved. The results are shown in Fig. 4B in which residual activity of the enzyme exposed for 24 h at a particular *pH* has been plotted against the *pH* at which the enzyme has been exposed for 24 h. The enzyme is most stable between pH 9.0–10.0. Thus the most suitable *pH* for preserving the enzyme is between 9.0 and 10.0. The data on the pH stabilities of *α-L*-rhamnosidases are not available in literature [2] for comparison.

The results of the studies on the variation of the activity of the enzyme with variation of temperature of the reaction solution are shown in Fig. 5A. It can be seen that the temperature optimum of the enzyme is 50 °C which is in the range of temperature optima (40–80 °C) reported in the literature [2] for other *α-L*-rhamnosidases. The thermal stability of the enzyme has been studied with the objective of determining the suitable temperature at which the enzyme could be preserved. The results are also shown in Fig. 5B, in which the remaining activity of the enzyme exposed for 1 h at a particular temperature has

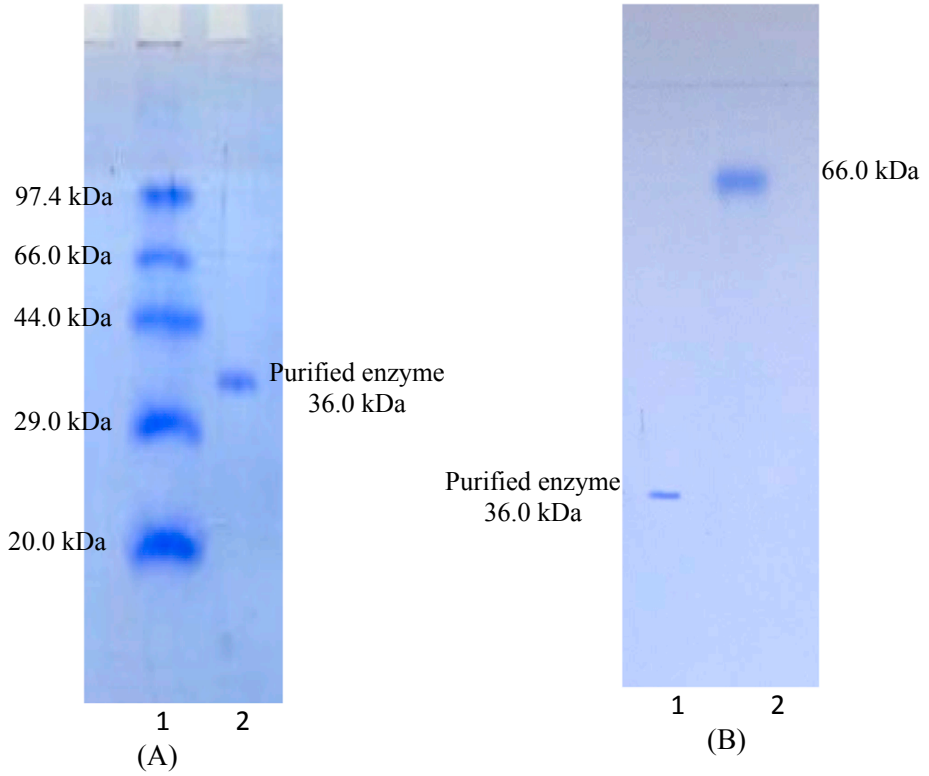


Fig. 3. Results of gel electrophoresis (A) SDS-PAGE and (B) Native-PAGE: (A) Lane 1 molecular weight markers and lane 2 purified enzyme. (B) Lane 1 purified enzyme and lane 2 bovine serum albumin.

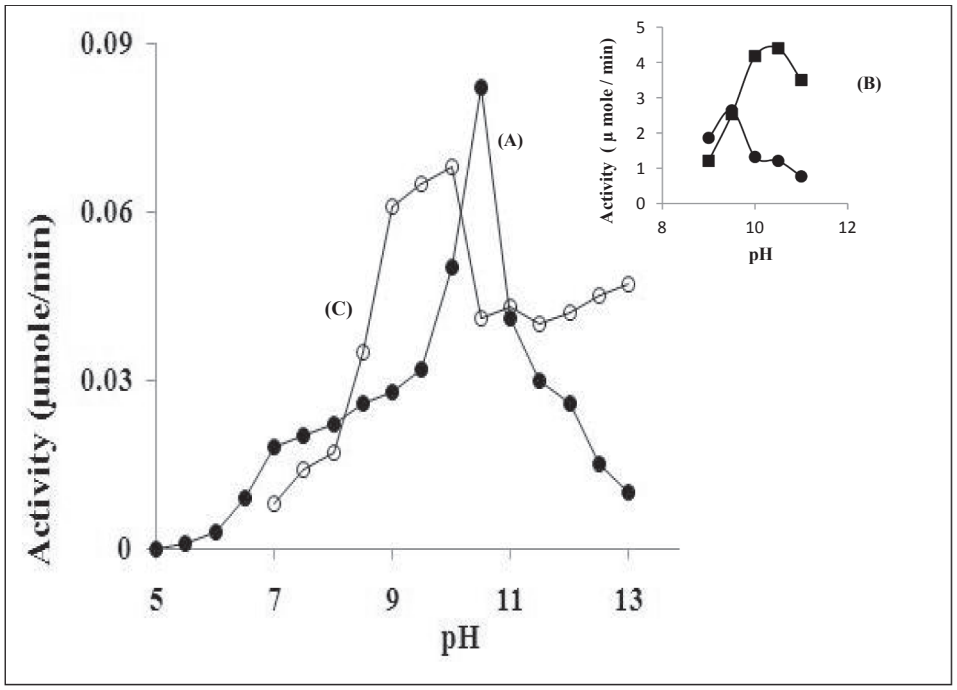


Fig. 4. Effect of pH on the activity of the enzyme. (A) *pH* optimum at 50 °C (●). (B) *pH* optimum at 40 °C and 60 °C (C) *pH* stability of enzyme (○). (A) The assay solution 1.0 mL contained 0.4 mM substrate, 1.50 µg of the pure enzyme in sod. Phosphate buffer of varying in the range 5–13 at 50 °C. (B) The assay solution of varring *pH* in the range 7–13 left overnight at 25 °C and analyzed for the activity after 24 h.

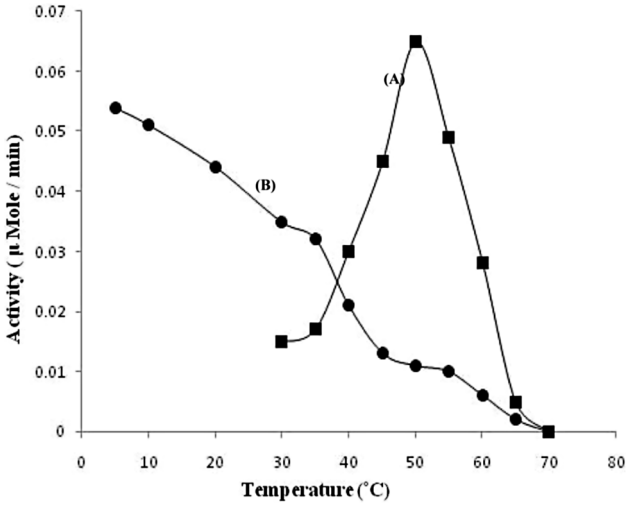


Fig. 5. Effect of temperature on the activity of enzyme. (A) Temperature Optima (■). The assay solution 1.0 mL continued 0.4 mM substrate, 1.50 µg of pure enzyme in 0.5 M sodium phosphate buffer pH 10.5 at varying temperature (30–70 °C). (B) Thermal stability (●). The assay solution at varying temperature (10–70 °C) left for one hour and analyzed for the remaining activity.

been plotted against the temperature at which the enzyme was exposed for 1 h. The results show that the enzyme starts loosing activity even at 10 °C but it is more pronounced above 30 °C. Thus a suitable temperature for preserving the enzyme is below 10 °C. The energy of activation for thermal denaturation of the enzyme was also determined and it was 22.09 kJ/mol. On the basis of the above studies, the purified enzyme was preserved in a solution of *pH*10 at 4 °C in the refrigerator.

3.4. Derhamnosylation of naringin, rutin and hesperidin

The results of studies on the enzymatic de-rhamnosylation of naringin, rutin and hesperidin are shown in Fig. 6A. The spots in lane 1 and lane 2 are due to the standard sample of naringin and its aglycon naringenin, respectively, whereas the spot in lane 3 is due to the enzymatic de-rhamnosylated product of naringin. The R_f value of the derhamnosylated product of naringin neither coincides with the R_f value of naringin nor with the value of aglycon naringenin. It has been inferred that the enzymatically derhamnosylated product of naringin is prunin. Further confirmation of the identity of the product prunin was done by purifying it by preparative TLC and analyzing by HPLC-Mass spectrometry (Fig. 6B). The hydrolysis products of rutin and hesperidin were also analyzed in the same way as the enzymatic hydrolytic product of naringin. The results are given in Fig. 6C and D respectively. Rutin is converted to isoquercitrin and hesperidin is converted to hesperetin glucoside.

3.5. Enzymatic release of L-rhamnose from the wine sample

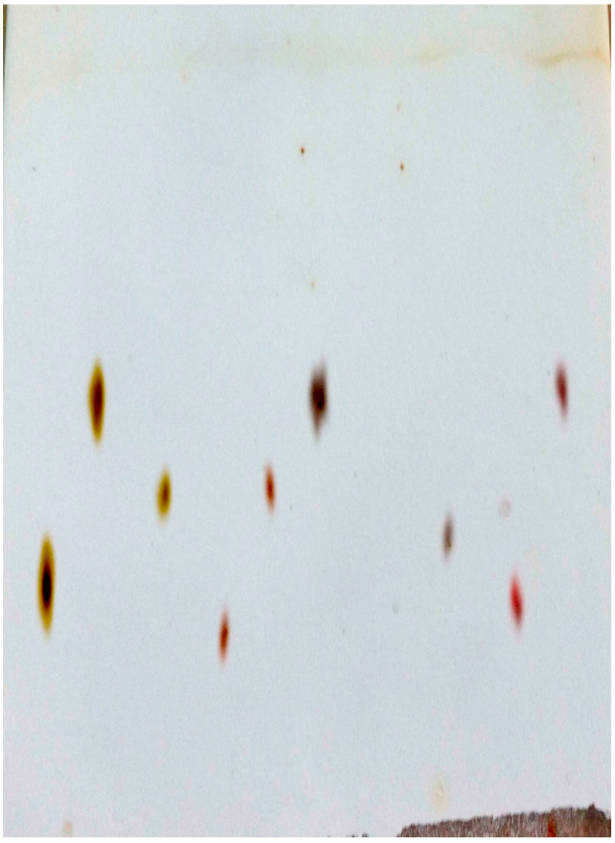
The enzymatically released L-rhamnose from the wine sample was analyzed in the same way as L-rhamnose released in the enzymatic hydrolysis of naringin, rutin and hesperidin and the result is given in Fig. 7(A and B). Fig. 7B shows the results of TLC experiment on wine sample (lane 1), wine sample treated with enzyme (lane 2) and standard sample of L-rhamnose. There is no free L-rhamnose in wine sample but when wine sample is treated with enzyme L-rhamnose is released. The result of HPLC-Mass analysis of the purified L-rhamnose by preparative TLC is shown in fig.B. The presence of molecular ion peak at m/z = 164.2 confirms the identity of L-rhamnose.

The derhamnosylation of wine ingredients is related to aroma enhancement of wine. The volatile components such as linalool, geraniol, nerol, citronellol and terpinol are responsible for the aroma of wine (2,17,18). Most of these components are present in grape skin as odourlessdiglycosides of terpenes, viz. α-L-arabinofuranosyl-β-D-glucopyranosidases and α-L-rhamnopyranosyl-β-D-glucopyranosides which on two sequential hydrolysis release volatile terpinol. The immobilized α-L-rhamnosidase along with β-glucosidase and α-arabinosidase has been used for the aroma enhancement of wine [32]. Thus the purified α-L-rhamnosidase has also a potential in the aroma enhancement of wine.

4. Conclusions

This communication reports the purification of an α-L-rhamnosidase from the culture filtrate of a novel fungal strain *F. moniliforme* MTCC-2088, using a simpler procedure. The enzyme has pH optima of 10.5

(A)



1 2 3 4 5 6 7 8 9

(B)

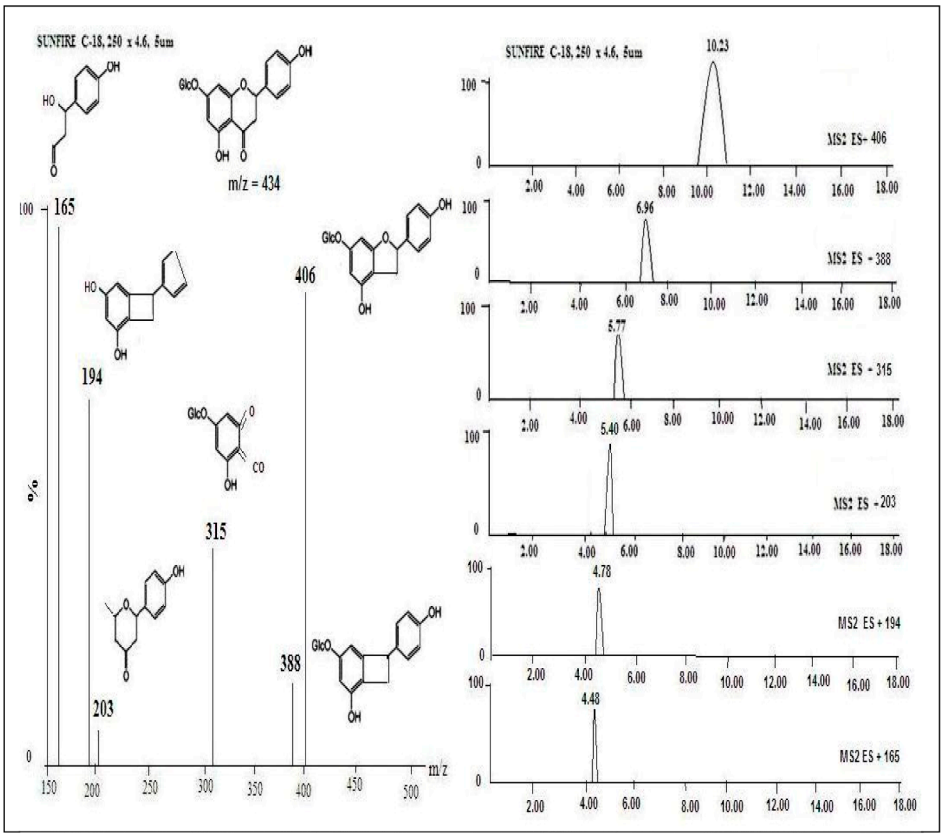


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Fig. 6. Identification of de-rhamnosylated products of naringin, rutin and hesperidin: (A). TLC Chromatogram of naringin, rutin, hesperidin, their derhamnosylated product and their aglycons. Naringin (lane 1), naringenin (lane 2), reaction product of naringin (lane 3), pure rutin (lane 4), reaction product of rutin (lane 5) and pure quercetin (lane 6), reaction product of hesperidin (lane 7), hesperidin (lane 8) and hesperetin (lane 9). (B) HPLC-Mass analysis result of the de-rhamnosylated product of naringin (prunin). (C) HPLC-Mass analysis result of the de-rhamnosylated product of rutin (isoquercetrin). (D) HPLC-Mass analysis result of the de-rhamnosylated product of hesperidin (hesperetin-glucoside).

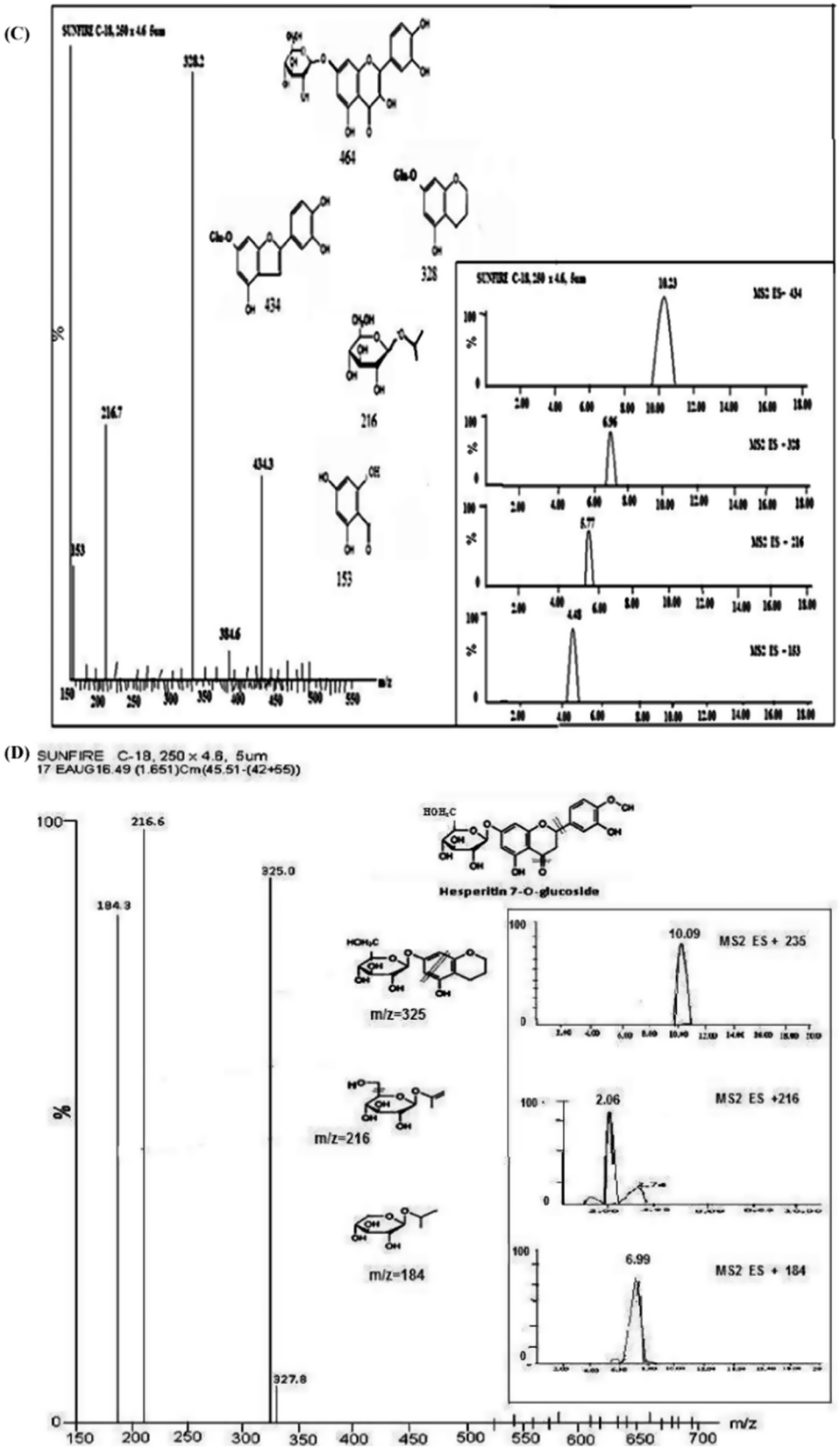

Fig. 6. (*continued*)

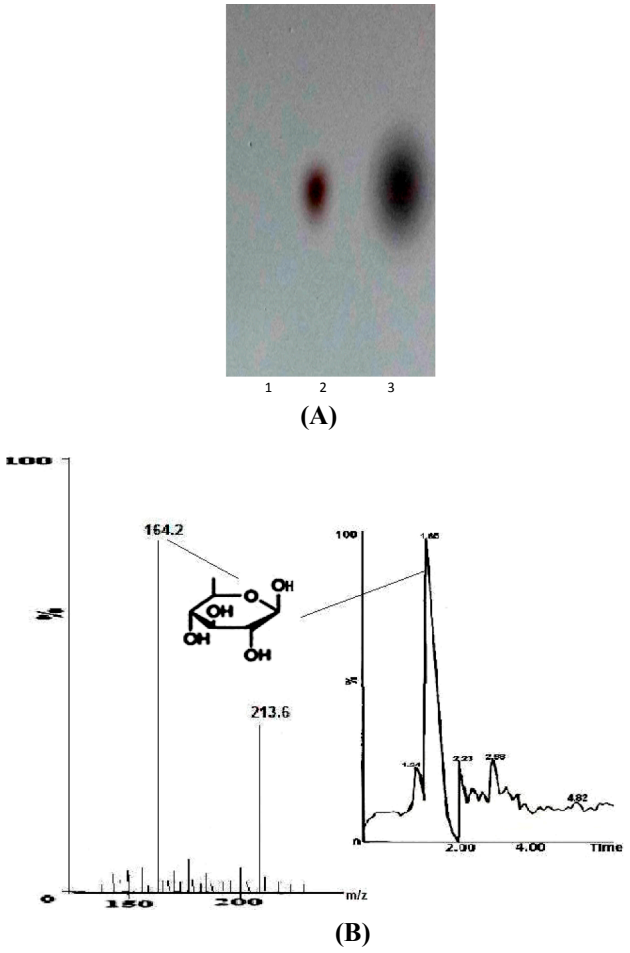

Fig. 7. Results of the release of L-rhamnose from enzyme treated wine. (A) TLC Chromatogram of wine (lane 1), wine treated with pure enzyme (lane 2) and standard sample of *L*-rhamnose. (B) HPLC-Mass analysis result of the drhamnosylated product L-rhamnose.

which is suitable for derhamnosylation of naringin, rutin, and hesperidin.

Disclosure statement

No potential conflict of interest was reported by the authors.

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