Appl Biochem Biotechnol (2009) 159:270–283
DOI 10.1007/s12010-008-8471-1

Purification and Characterization of Pectin Lyase Produced by *Aspergillus terricola* and its Application in Retting of Natural Fibers

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Received: 25 August 2008 / Accepted: 2 December 2008 /
Published online: 17 December 2008

Abstract An indigenously isolated fungal strain identified as *Aspergillus terricola* with assigned fungal strain number MTCC 7588 has been used as source for pectin lyase production. The extracellular pectin lyase was purified to homogeneity from the culture filtrate of *A. terricola* by ion exchange and gel filtration chromatography. The determined molecular weight was 35±01 kDa. The K_m and k_{cat} (turnover) values of the purified enzyme at 37 °C using citrus pectin as the substrate were found to be 1.0 mg/ml and 110.0 s^{-1}, respectively. The pH and temperature optima of the enzyme were 8.0 and 50 °C, respectively. The retting ability of the purified pectin lyase for natural fibers viz. *Cannabis sativa* and *Linum usitatissimum* has been demonstrated for the first time.

Keywords Pectin lyase · Retting · *Aspergillus terricola* · *Cannabis sativa* · *Crotalaria juncea* · *Linum usitatissimum*

Introduction

The groups of pectinolytic enzymes broadly known as pectinases are involved in pectin degradation. Due to great diversity in the structure of pectins, the pectin degrading enzymes can be categorized as those acting on the "smooth regions" composed of homogalacturonan and enzymes acting on the "hairy region" composed of rhamnogalacturonan and side chains. The group of enzymes which are involved in the degradation of "smooth region"

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(homogalacturonan) include deesterifying enzymes, i.e., pectin methyl esterases (PME, E.C 3.1.1.11) and pectin acetyl esterase (PAE, E.C 3.1.1.6) which removes methoxyl and acetyl residues from pectin producing polygalacturonic acid. The other subclass of homogalacturonan degrading group of enzymes are broadly termed depolymerases which break the α-1,4 linkages either by hydrolysis, i.e., polygalacturonases (PG, E.C 3.2.1.15) or via trans-elimination mechanism, namely, pectate lyases (PL, E.C 4.2.2.2) and pectin lyases (PNL, E. C 4.2.2.10). The groups of enzymes which are involved in the degradation of the hairy region of pectins are rhamnogalacturonan hydrolase, rhamnogalacturonan lyase, rhamnogalacturonan rhamnohydrolase, rhamnogalacturonan galactohydrolase. These group of enzymes have not been studied extensively [1–4], and hence, much is not known about their structures and functions. There are other accessory enzymes involved in degradation of side chains of pectins which include α-arabinofuranosidase (E.C 3.2.1.55), endoarabinase (E.C 3.2.1.99), β-galactosidase (E.C 3.2.1.23), endogalactanase (E.C 3.2.1.89), and feruloyl and *p*-coumaroyl esterases [5].

Pectinases, in general, are basically involved in degradation of pectin that occurs as structural polysaccharides in the middle lamella and primary cell wall of young plant cells. These enzymes have potential applications in clarification of fruit juices, retting of natural fibers (ramie, hemp, flax, bast), in treatment of pectic waste water, coffee and tea leaf fermentation, oil extraction, virus purification, etc. [6–8]. Among all pectinases, pectin lyases are the only enzymes capable of depolymerizing highly esterified pectin into small molecules without prior action of other enzymes [9]. Pectin lyase cleaves pectin by β-elimination mechanism that results in the formation of 4,5 unsaturated oligogalacturonates without affecting the ester content of the polymer chain which is responsible for specific aroma of fruits [10]. Moreover, pectin lyase does not produce methanol which is toxic, and hence, it is better suited for fruit juice industry. Pectin lyase has been extensively reviewed recently [11]

Retting is a process of fiber separation from nonfiber parts of plants. The plant fibers are held together with the help of pectin, and pectin plays an important role as a binder between adjacent cells [12]. The current commercial process, i.e., dew retting have many disadvantages. It is restricted to geographical regions with appropriate climates, and the resulting fibers are often inconsistent in quality with significant amount of dirt and contaminants [13, 14]. Therefore, enzymatic retting is a better option as it does not damage the fibers, it is energy conserving, and also ecofriendly. There are many reports of retting using polygalacturonases [14–19] and pectate lyase [20]. The enzymatic retting of *Cannabis sativa* (Indian hemp) using pectin lyase (PNL, E.C. 4.2.2.10), has been recently reported for the first time [21]. In this communication, the authors report purification and characterization of a pectin lyase produced from indigenously isolated fungal strain *Aspergillus terricola* MTCC 7588 and studied the retting ability of the purified enzyme for three natural fibers, i.e., *C. sativa* (Indian hemp), *Crotalaria juncea* (Sunn hemp), and *Linum usitatissimum* (flax).

Materials and Methods

Chemicals

Citrus pectin (70% esterification), DEAE cellulose, and Sephadex G-100 were purchased from Sigma Chemical Company, St. Louis, USA. The rest of the chemicals were procured either from Merck, Navi Mumbai, or S.D. Fine, Mumbai, India and were used without further purifications.

Organism and Growth Conditions

Twenty fungal strains were isolated from soil samples containing decaying vegetation from the campus of D.D.U. Gorakhpur University, Gorakhpur, India using serial dilution technique [22]. The isolated and purified fungal strains were screened for the secretion of pectinases by plate assay method [23]. The fungal strains showing prominent halo zones were further screened for PNL production in liquid culture medium as described in the "Enzyme Purification" section. The most promising PNL producing fungal strain was identified as *A. terricola* and was assigned fungal strain number MTCC 7588 at the Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh, India. The culture was maintained by subculturing on potato dextrose agar slants at 30 °C fortnightly.

Enzyme Assay

Enzyme activity of PNL was assayed by the method reported in the literature [24]. Assay was performed by monitoring the increase in optical density at 235 nm due to the formation of unsaturated uronide product using UV/Vis Spectrophotometer Hitachi (Japan) model U-2000, which was fitted with electronic temperature control unit and had a least count of 0.001 absorbance unit. Enzyme solution (0.2 ml) was added to a reaction mixture containing 0.8 ml citrus pectin (1% *w*/*v*) and 2.0 ml of the 100 mM sodium phosphate buffer (pH 8.0) maintained at 37 °C. Optical density was measured at zero time and after 20 min, and the steady-state velocity was calculated by absorbance change per minute. Enzyme activity was defined in terms of micromole of unsaturated product released per minute, based on its molar extinction coefficient value of 5,500 M^{-1} cm^{-1}. Protein was determined by the Lowry method [25] taking bovine serum albumin (BSA) as the standard.

Enzyme Purification

Extracellular PNL was produced under submerged culture conditions in liquid culture growth medium consisting of pectin 10 g, L-asparagine 2 g, $MgSO_4$ $7H_2O$ 0.5 g, KH_2PO_4 3 g in 1 L double distilled water and initial pH was adjusted to 4.5. One milliliter of spore suspension (spore density 5×10^6spores/ml) from agar slant was inoculated aseptically into ten 100 ml culture flasks each containing 25 ml of sterilized liquid medium. The flasks were incubated at 25 °C in BOD incubator, and cultures were allowed to grow under stationary conditions. The maximum activity of pectin lyase appeared on the eighth day of inoculation of the fungal strain. On the same day, all the cultures were pooled and filtered using Whatmann filter paper no. 41 and cell-free filtrate was used as crude enzyme source. The culture filtrate, 200 ml was concentrated to 10 ml with Amicon Cell Model 8200 using PM 10-ultrafiltration membrane having 10-kDa molecular weight cutoff value.

The concentrated enzyme preparation was dialyzed against 10.0 L of 50 mM sodium phosphate buffer with three changes of buffer at the intervals of 8 h (pH 6.0). The dialysate was centrifuged for 10 min at 10,000×*g* to remove the particles, and the supernatant was loaded on DEAE cellulose column (6.5×2.0 cm) equilibrated with 50 mM sodium phosphate buffer pH 6.0. The adsorbed protein was washed with 100 ml of the same buffer. The protein was eluted using linear gradient of 1 M in 100 ml (50 ml buffer + 50 ml buffer containing 1 M NaCl) using Pharmacia, Gradient mixer GM-1, at the flow rate of 10 ml/h. Fractions of 5.0 ml were collected and analyzed for the activity of pectin lyase and protein.

The active fractions (13 to 17) obtained after the elution from DEAE cellulose chromatography were pooled, concentrated by Amicon Ultrafiltration Cell using PM 10 membrane to 2 ml. The concentrated enzyme solution was dialyzed against 20 mM sodium phosphate buffer pH 7.0 and the dialysate was loaded on Sephadex G-100 column (78× 1.6 cm) equilibrated with 20 mM sodium phosphate buffer pH 7.0. The flow rate was 7.0 ml/h; fractions of 5.0 ml were collected and analyzed for the activity of pectin lyase and protein. The active fractions were pooled, concentrated, and stored in deep freezer at −20 °C. The enzyme purity and molecular weight were estimated by the 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis technique (SDS-PAGE) [26, 27], using phosphorylase B (97.4 kDa), BSA (66.0 kDa), ovalbumin (43.0 kDa), carbonic anhydrase (29.0 kDa), soya bean trypsin inhibitor (20.1 kDa), and lysozyme (14.3 kDa) as molecular weight markers. Silver staining was done for locating the protein bands.

Characterization of Purified PNL

The K_m and k_{cat} values of the purified enzyme were determined by measuring steady-state velocities of the enzyme-catalyzed reaction at different concentrations of citrus pectin and analyzed the data using nonlinear regression method. The pH optimum was determined by measuring the steady-state velocity in the buffered reaction solutions in the pH range 1.0–12.0 and plotting the steady-state velocity against the pH of the reaction solution. The different buffers used were glycine–HCl (1.0–2.0), citrate phosphate (3.0–5.0), sodium phosphate (6.0–8.0), sodium carbonate (9.0–10.0), and sodium phosphate–NaOH (11.0–12.0) all at 100 mM. The temperature optimum was determined by measuring steady-state velocity of the enzyme-catalyzed reaction at different temperatures and plotting steady-state velocity against temperature. The pH stability of the purified pectin lyase was studied by exposing the enzyme at a particular pH for 24 h at 20 °C and monitoring the enzyme activity and plotting the percentage residual activity against the pH at which the enzyme has been exposed.

Thermal stability of the enzyme was tested by incubating an enzyme aliquot at a particular temperature for 1 h and assaying its residual activity and plotting the percent residual activity against temperature. The values of half lives ($t_{1/2}$) were calculated for higher temperatures (i.e., 60 °C, 70 °C, 80 °C, and 85 °C), and the activation energy for the purified PNL was also determined by using reported method [28].

In order to know in which way metal ions and protein inhibitors affect the activity of the purified pectin lyase, the effect of metal ions like Ca^{++}, Mg^{++}, Mn^{++}, Co^{++}, Zn^{++}, Hg^{++}, Cu^{++}, K^{+}, Na^{+}, Ag^{+}, and protein inhibitors like sodium arsenate, sodium azide, potassium permanganate, potassium ferrocyanide, and ethylenediaminetetraacetic acid (EDTA) were studied. The steady-state velocity in the reaction solutions containing 1 mM of the metal ions or protein inhibitors was determined and compared with the value in the absence of these ions or inhibitors.

Effect of Ammonium Sulfate on Thermostability of the Purified Enzyme

In order to enhance the thermal stability of the purified enzyme, the effect of ammonium sulfate on the thermal stability of the enzyme was studied by incubating the enzyme along with different concentrations of ammonium sulfate in the range of 0.1 to 4.0 M for 1 h in the temperature range 60–100°C and determining the residual activity by standard assay method. All experiments were performed in triplicates and the variation coefficient was between 3% and 5%.

Table 1 Purification of pectin lyase secreted by *Aspergillus terricola* MTCC 7588.

Fraction	Total activity	Total protein (mg)	Specific activity (U/mg)	Yield (%)	Purification fold
Crude	21.4	184.0	0.116	100	–
DEAE-cellulose	15.36	6.0	2.56	71.78	22.07
Sephadex-G_{100}	9.8	2.4	4.08	45.79	35.17

Retting Ability of the Purified PNL

The retting of natural fibers of mature sticks of *C. sativa* (Indian hemp), *C. juncea* (Sunn hemp), and *L. usitatissimum* (Flax) were studied by using a reported method [29] with minor modifications. Four approximately 10-cm long sticks (stem material) of each natural fiber were entirely submerged in test tubes containing 10 ml of 100 mM sodium phosphate buffer (pH 8.0). One test tube was made control designated as C and contained 0.24 IU of deactivated enzyme for each of the three fibers. The deactivated enzyme was prepared by heating it in the water bath at 100 °C for 15 min. The other three test tubes were designated as E_1, E_2, and E_3 containing 0.08, 0.16, 0.24 IU of the purified enzyme, respectively, for each of the three fibers. These test tubes were incubated in a water bath at 37 °C for 24 h under static conditions. After 24 h, the sticks were shaken vigorously each with 10 ml hot water for 1 min, hot water was poured off, and the resulting sticks were photographed. A modified version of the Fried test [30] was used to monitor the fiber separation.

Results

Enzyme Purification

The different steps of purification of pectin lyase produced by *A. terricola* MTCC 7588 are shown in Table 1. The elution profile of the enzyme from DEAE-cellulose column is shown in Fig. 1 and from the gel filtration sephadex G-100 column is shown in Fig. 2. In the final purification step, most of the activity was present in a single protein peak, which was well separated from other protein peaks indicating that the enzyme preparation was relatively pure. The specific activity went up to 4.08 IU/mg, and the recovery was 45.79%. The homogeneity of the enzyme preparation was confirmed by performing 10% SDS-PAGE

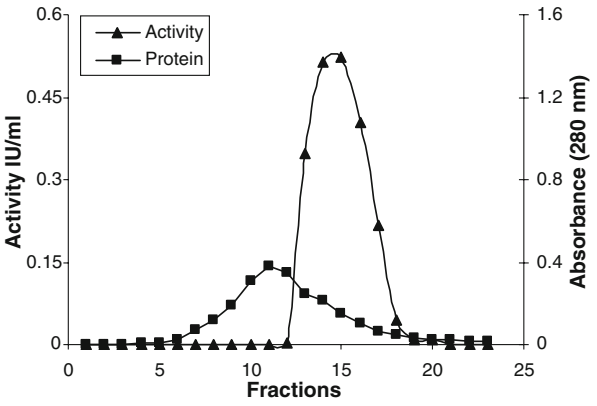

Fig. 1 Anion exchange chromatography on DEAE-cellulose column of Pectin lyase produced by *A. terricola* MTCC 7588 [Proteins (*filled squares*) and PNL activity (*filled triangles*)]

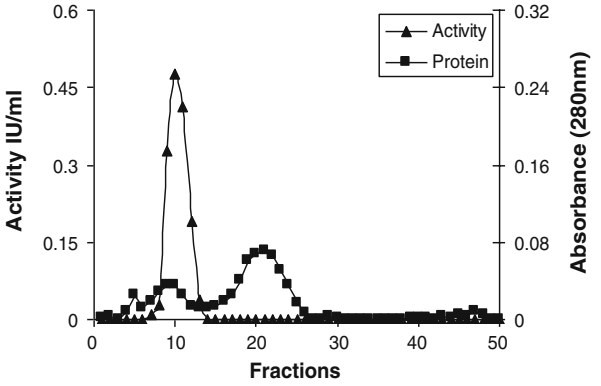

Fig. 2 Gel filtration chromatography of pectin lyase produced by *A. terricola* MTCC 7588 [Proteins (*filled squares*) and PNL activity (*filled triangles*)]

where a single prominent band was obtained (Fig. 3). The molecular weight of the purified enzyme, as determined by plotting the log of molecular weights of the markers versus their relative mobilities, was 35±01 kDa.

Characterization of Pectin Lyase

For citrus pectin as the substrate, the steady-state velocity of the purified pectin lyase-catalyzed reaction versus the different concentrations of the citrus pectin is shown in Fig. 4. The calculated values of K_m and k_{cat}, ignoring inhibition at higher concentration of citrus pectin and using nonlinear regression analysis, are 1.0 mg/ml and 110.0 s^{-1}, respectively. The optimum pH was found to be 8.0. The enzyme retains approximately 100% of its maximum activity for 24 h in a buffer of pH range 4.0–9.0 (Fig. 5). The optimum temperature of the enzyme was found to be 50 °C (Fig. 6a). The percentage residual activity versus the temperature at which the enzyme has been kept for 1 h showed that the enzyme retains its full activity up to 50 °C, and thereafter, activity decreased with increase in temperature (Fig. 6a). However 75% of the activity is lost after incubating the purified PNL at 60 °C for 1 h. The values of half lives for 60 °C, 70 °C, 80 °C, and 85 °C are given in Table 3, and the activation energy of the purified enzyme was found to be 61.1 kJ mol^{-1}.

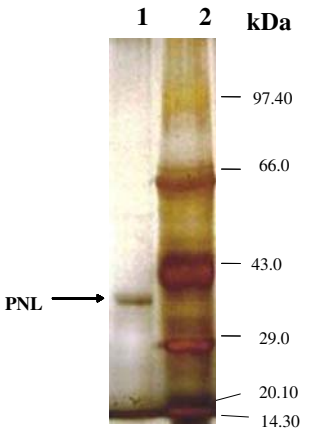

Fig. 3 SDS-PAGE of purified pectin lyase. *lane 1* purified pectin lyase, *lane 2* markers

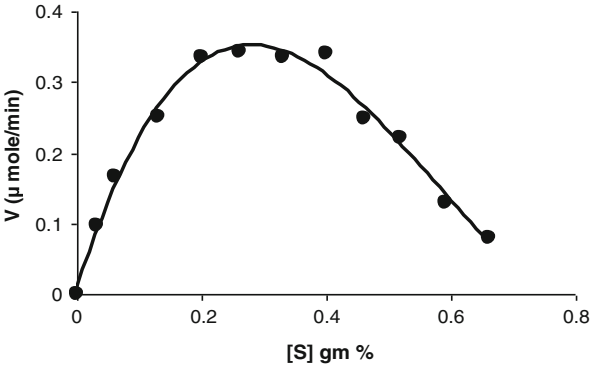

Fig. 4 Effect of substrate (citrus pectin) concentration on the steady-state velocity of pectin lyase-catalyzed reaction

The results of the effects of metal ions Ag^{+}, Ca^{++}, Co^{++}, Cu^{++}, Hg^{++}, K^{+}, Mg^{++}, Zn^{++}, Na^{+}, Mn^{++}, and protein inhibitors such as EDTA, sodium arsenate, sodium azide, potassium permanganate, and potassium ferrocyanide on the activity of pectin lyase using 1.0 mM concentrations are summarized in Table 2. It was observed that Co^{++}, Na^{+}, and sodium arsenate slightly promotes the activity of PNL, while Ag^{+}, Cu^{++}, Hg^{++}, potassium ferrocyanide, and potassium permanganate inhibited the activity of the purified pectin lyase completely.

Effect of Ammonium Sulfate on Thermostability of the Purified Enzyme

The result of studies of the effects of ammonium sulfate on the thermal stability of the purified PNL is shown in Fig. 7 (Table 3). The purified enzyme retains its full activity after exposure for 1 h at 60 °C in 1.0 M ammonium sulfate. However, in the absence of ammonium sulfate, the enzyme loses its 77% activity at 60 °C. At higher temperatures (70–100 °C), ammonium sulfate is not able to stabilize the activity of the pectin lyase purified from *A. terricola*.

Retting Ability of the Purified PNL

The results of studies of retting experiments on stem (bast) fibers of Indian hemp (*C. sativa*), Sunn hemp (*C. juncea*), and Flax (*L. usitatissimum*) representing Cannabaceae,

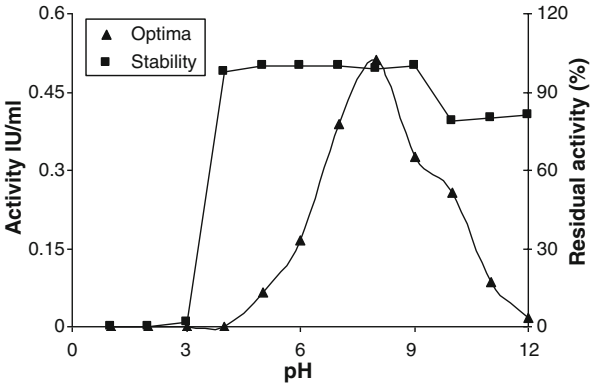

Fig. 5 pH optimum and pH stability of the purified pectin lyase. Optimal pH (*filled triangles*), pH stability (*filled squares*)

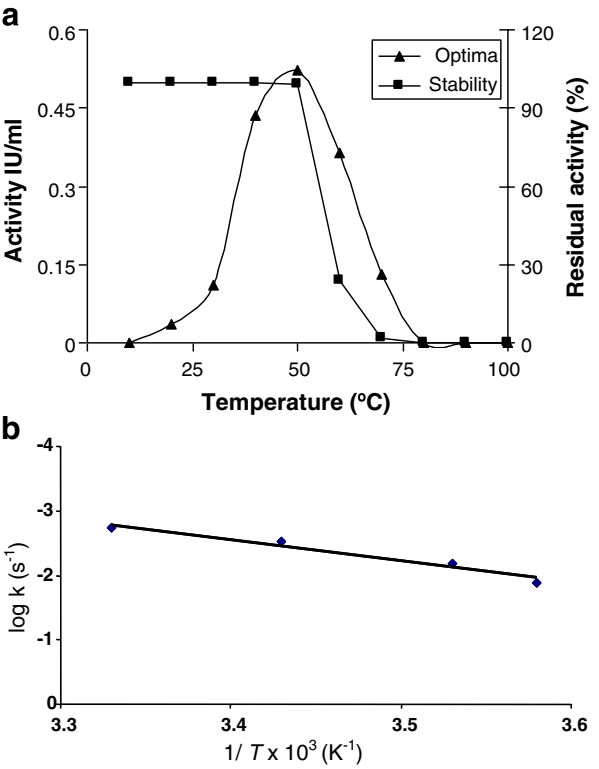

Fig. 6 **a** Optimum temperature and thermostability of purified pectin lyase. Optimal temperature (*filled triangles*), thermostability (*filled squares*). **b** Arrhenius plot for heat inactivation of purified pectin lyase

Table 2 Effect of metal ions and protein inhibitors on pectin lyase activity.

S. No.	Relative activity (%)
Metal ions (1.0 mM)	
Control	100.0
Ag^{+}	0.0
Ca^{++}	105.0
Co^{++}	114.0
Cu^{++}	10.0
Hg^{++}	24.0
K^{+}	91.0
Mg^{++}	97.0
Zn^{++}	69.0
Na^{+}	108.3
Mn^{+}	93.0
Protein inhibitors (1.0 mM)	
EDTA	85.0
Sodium arsenate	110.0
Sodium azide	79.0
Potassium permanganate	0.0
Potassium ferrocyanide	0.0

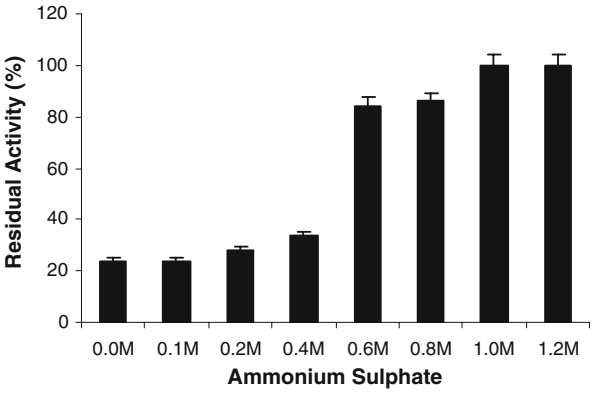

Fig. 7 Effects of ammonium sulfate (0.1–1.2 M) on the thermal stability of the purified pectin lyase after exposure of the enzyme at 60 °C for 1 h

Fabaceae, and Linaceae family, respectively, by the purified enzyme in 24 h is shown in Fig. 8a, b, c. The retting ability has been compared subjecting the fibers to the same aliquots of enzyme, namely, 0.08 IU (E_1), 0.16 IU (E_2), and 0.24 IU (E_3). In the case of *C. sativa* and *L. usitatissimum* fibers, complete retting was observed with 0.24 IU of purified enzyme in 24 h, whereas in the case of *C. juncea*, only partial retting was observed subjected to similar condition which is also clearly evident from the Fried score's determined for all the three fibers as mentioned in Table 4.

Discussion

The results of the purification of the pectin lyase from the culture filtrate of *A. terricola* MTCC 7588 are summarized in Table 1, and the elution profiles from the anion exchange DEAE Cellulose column and Gel filtration sephadex G-100 column are shown in Figs. 1 and 2, respectively. A 35-fold purification with 45.8% yield has been achieved. The purification procedure is simpler than the procedures reported for the pectin lyases of *Aspergillus flavus*, *Penicillium italicum*, *Penicillium expansum*, *Pythium splendens*, *Lasidioplodia theobromae* [21, 31–34] but similar to the procedures reported for the purification of the pectin lyase from *Aspergillus ficcum* [35]. A single protein band in lane 1 (Fig. 3) clearly indicates homogeneity of enzyme preparation. The calculated molecular weight (35±01 kDa) of *A. terricola* MTCC 7588 pectin lyase is similar to other fungal pectin lyases: *A. flavus* (38.0 kDa), *A. ficcum* (31.6 kDa), *Aspergillus niger* (38.0 kDa), *Rizopus oryzae* (31.0 kDa), and *Penicillium canescens* (38.0 kDa). [21, 35–38].

The calculated K_m and k_{cat} (turnover) were 1.0 mg/ml and 110.0 s^{-1}, respectively, which are comparable to the values obtained by the authors for an acidic PNL purified from *A.*

Table 3 Thermal inactivation half-lives for purified pectin lyase.

Temperature (°C)	Half-life (min)
60	7.03±0.21
70	3.9±0.18
80	1.8±0.06
85	0.89±0.04

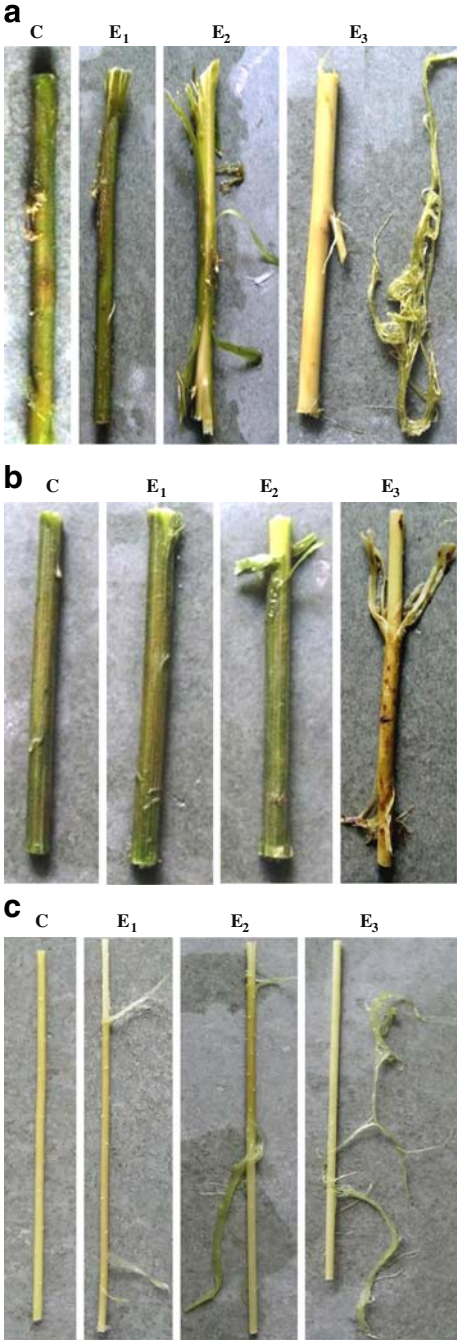

Fig. 8 **a** Effect of purified pectin lyase produced from *A. terricola* on retting of *C. sativa* (Indian hemp) E_1, E_2, and E_3 are enzyme-treated sticks subjected to 0.08, 0.16, and 0.24 IU of enzymes, respectively. *C* is control stick treated by inactivated enzyme. **b** Effect of purified pectin lyase produced from *A. terricola* on retting of *C. juncea* (Sunn hemp). E_1, E_2, and E_3 are enzyme-treated sticks subjected to 0.08, 0.16, and 0.24 IU of enzymes, respectively. *C* is control stick treated by inactivated enzyme. **c** Effect of purified pectin lyase produced from *A. terricola* on retting of *L. Usitatissimum* (Flax). E_1, E_2, and E_3 are enzyme-treated sticks subjected to 0.08, 0.16, and 0.24 IU of enzymes, respectively. *C* is control stick treated by inactivated enzyme

ficuum [35] and also to an alkaline PNL purified from *A. flavus* [21]. It is worth mentioning that the activity of the purified pectin lyase was inhibited by the citrus pectin above 4.0 mg/ml (Fig. 4), and the data points corresponding to inhibition were ignored in the calculation of K_m and k_{cat}.

Table 4 Retting ability of purified pectin lyase.

Enzyme unit used (IU)	Retting ability of purified PNL in case of		
	Cannabis sativa	*Crotalaria juncea*	*Linum usitatissimum*
0.0	0.0	0.0	0.0
0.08	0.5	0.25	1.0
0.16	3.5	1.25	4.0
0.24	6.0	3.5	5.5

The pH optimum of the enzyme was found to be 8.0 (Fig. 5). However, the enzyme showed more than 50% of its activity in the pH range 7–10. A decreased activity on either side of optimum pH could be ascribed to decreased saturation of the enzyme with the substrate due to a decreased affinity and/or decreased stability of the enzyme [39]. Thus, the purified pectin lyase has an alkaline pH optimum and should be suitable for retting of natural fibers. The pH optima of the previously reported pectin lyases have been found to be acidic for *Penicillium paxilli* (5.0) [40], *Aspergillus sojae* (5.5) [41], *P. canescens* (5.5) [38], *Aspergillus japonicas* (6.0) [42], neutral for *Rhizoctonia solani* [43], and *P. expansum* [32] and basic for *Penicillium oxalicum* (8.0) [44], *Fusarium oxysporum* (9.5) [45], *A. oryzae* (8.5) [46].

The enzyme was found to be stable for 24 h in the pH range 4.0–9.0.This value is similar to our earlier reported pectin lyase purified from *A. flavus* [21]. This property of enzyme could be exploited for biotechnological applications. The optimum temperature of the enzyme was found to be 50 °C above wherein it looses its activity gradually (Fig. 6a). The half-life values of the purified PNL in the temperature range 60–80 °C was found to be higher (7.03±0.21 min to 1.8±0.06 min) than reported for commercial pectin lyases, i.e., Rapidase C80 (2.24±0.07 min to 0.11±0.01 min), Pectinase CCM (1.48±0.06 min to 0.39±0.02 min), Pectinex 3XL (5.04±0.06 min to 1.38±0.07 min) and comparable to Grindamyl 3PA (7.68± 0.21 min to 2.21±0.12 min) [28]. The activation energy of the purified pectin lyase was 61.1 kJ mol^{-1} (Fig. 6b), which is higher than the values reported for the aforementioned commercial preparations of pectin lyase.

The activity was 100% retained for 1 h exposure in the presence of 1 M ammonium sulfate (Fig. 7). It is worth mentioning that in the absence of ammonium sulfate the enzyme lost 77% activity by exposing the enzyme for 1 h at 60 °C. However, above 60 °C, even the addition of 4 M ammonium sulfate could not stabilize the enzyme. The thermal stability of *A. terricola* pectin lyase has been found to be less than the thermal stability of the pectin lyase purified from *A. flavus* [21]. In the case of *A. flavus*, it is completely stable at 70 °C after addition of 1.8 M ammonium sulfate, while in present case, the purified pectin lyase is not stable at 70 °C even after addition of 4.0 M ammonium sulfate.

The results of effects of protein inhibitors and metal ions are summarized in Table 2. It has been observed that at 1 mM concentration, K^+, Mg^{++}, Na^+, and sodium arsenate slightly enhance the activity, but Ag^+, Cu^{++}, potassium ferrocynide, and potassium permanganate totally inhibited the activity. A species of *Aspergillus* has been reported to produce a calcium-dependent pectin lyase [47]. A similar observation for Ca^{++} (0.2 mM) dependence has been reported for PNL produced by *P. splendens* also [33]. Sodium arsenate was found to enhance the activity of purified PNL enzyme which is quite contrasting compared to the PNL produced by *R. oryzae*. The total inhibition of PNL produced by *R. oryzae* by sodium arsenite, Hg^{2+}, and sodium arsenate indicates the possibility of –SH groups at the active site of the enzyme [37]. EDTA at 1 mM inhibited the activity of pectin lyase, in this case, which

is similar to the behavior of pectin lyases from *R. oryzae* [37], *A. flavus* [21], but is different from the other reports where no inhibition of pectin lyase activity has been found by EDTA [32, 33, 35]. This clearly indicates the possibility of efficient retting in the absence of EDTA and, hence, should be omitted from the retting solution in the case of this enzyme.

With the exception of synthetic polymers, most economically important products, such as paper, cordage (cords and rope), and textiles are derived from plant fibers. Fibers present in the stems of many herbaceous dicots, such as flax, Indian hemp, Sunn hemp are potent source for textile industries worldwide. Due to several constraints associated with both water and dew-retting, efforts have been made to employ enzyme retting [14, 48, 49] of natural fibers. Prior information about the structural and chemical characteristics of natural fibers is important for employing enzyme retting for producing fibers with specific properties as desired for its industrial applications. Since pectin serves as a glue to hold fibers together in bundles and the bundles to nonfiber tissue [14], pectinases are the potential enzymes used in retting [50]. Though polygalacturonases and pectate lyases have been studied for enzymatic retting, reports on pectin lyase for enzymatic retting is rare [21]. In the present study, retting ability was compared for three natural fibers as shown in Fig. 8a, b, c and Table 4.

Of the purified pectin lyase, 0.24 IU gave complete retting of approximately 10-cm long stick of *C. sativa* and *L. usitatissimum* (Fig. 8a, c) in 24 h at 37 °C, while in the case *C. juncea* fibers, only partial retting was observed (Fig. 8b). It is worth mentioning that *C. juncea* when subjected to pectin layse purified from *A. flavus* [21] showed complete retting. Use of EDTA has been reported as one of the essential component in enzymatic retting with polygalacturonases [50–52]. The pectin lyase of *A. terricola* showed inhibition by EDTA which provides a possible reason for efficient retting even in the absence of EDTA. This is similar to our earlier report [21]. Thus, in this communication, authors report purification, characterization, and retting ability of purified pectin lyase produced by *A. terricola* MTCC7588 for three natural fibers.

Acknowledgements Sangeeta Yadav is thankful to the Department of Science and Technology, Government of India, New Delhi for the financial support in the form of a Women Scientist Fellowship SR/WOS-A/LS-34/2004. The laboratory facilities provided by the Heads of the Chemistry Department and Biotechnology Department, D.D.U. Gorakhpur University, Gorakhpur are also thankfully acknowledged.

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