

# Purification, characterization and retting of *Crotolaria juncea* fibres by an alkaline pectin lyase from *Fusarium oxysporum* MTCC 1755

S. Yadav<sup>1</sup> · S. K. Maurya<sup>1</sup> · G. Anand<sup>1</sup> · R. Dwivedi<sup>1</sup> · D. Yadav<sup>1</sup>

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**Abstract** Using solid-state fermentation, production of an industrially important pectin lyase from a fungal strain *Fusarium oxysporum* MTCC 1755 was attempted, which was further subjected to purification and characterization. The enzyme was purified by three steps, namely ammonium sulfate fractionation, cation-exchange chromatography on CM cellulose followed by gel filtration chromatography using Sephadex G-100 column. A 16-fold purification with 31.2% yield and 3.2 U/mg specific activity was achieved. The optimum pH of the purified enzyme was 9.0 and stability ranged from pH 5.0–7.0 for 24 h. Optimum temperature of purified enzyme was found to be 40 °C while temperature stability ranged from 10 to 50 °C for 30 min. The  $K_m$  and  $k_{cat}$  of the enzyme was 1.75 mg/ml and 83.3 s<sup>-1</sup>, respectively. The purified enzyme was found to be highly stimulated by Ca<sup>2+</sup> ions while sugars like mannitol and sorbitol, and salts like NaCl and CaCl<sub>2</sub> enhanced the thermostability. The purified pectin lyase was found suitable for retting of *Crotolaria juncea* fiber.

**Keywords** Pectin lyase · *Fusarium oxysporum* MTCC 1755 · Retting · Purification

## Introduction

Pectic substances are high molecular weight, negatively charged, acidic, complex glycosidic macromolecules (polysaccharides) that are present in the plant kingdom. They are present as the major components of middle lamella between the cells in the form of calcium pectate and magnesium pectate (Bonnin et al. 2014). Pectic polysaccharides exist in the cell wall as either “smooth region” of linear copolymer  $\alpha$  (1–4)-linked GalUA (galacturonic acid) or “hairy region” that have attached  $\alpha$  (1–2)-linked rhamnosyl residues that may be substituted with Ara (arabinan)- and Gal (galactan)-rich side chains (Caffall and Mohnen 2009). A group of enzymes popularly known as pectinases are associated with degradation of pectin and includes exo- and endo-polygalacturonases (PGs, E.C 3.2.1.15), pectin methyl esterases (PME.E.C 3.2.1.11), pectate lyases (PL.E.C 4.2.2.9) and pectin lyases (PNL 4.2.2.10). These enzymes are widely distributed in higher plants and microorganisms (Whitaker 1990) and are of prime importance for plants as they help in cell wall extension and softening of some plant tissues during maturation and storage (Aguilar and Huirton 1990; Sakai 1992). They also aid in maintaining ecological balance by causing decomposition and recycling of waste plant materials. Pectin lyase is unique among all pectinases based on the  $\beta$ -elimination reaction mechanism and since it does not produce methanol, it is preferred in fruit juice industries (Yadav et al. 2009).

Several potential industrial applications of pectinases such as clarification of fruit juices, retting of natural fibers, treatment of pectic waste water, coffee and tea leaf fermentation, oil extraction, virus purifications and developing functional foods have been reported (Kohli and Gupta 2015; Sharma et al. 2013; Kashyap et al. 2001).

✉ S. Yadav  
sangeeta\_rahul@rediffmail.com

<sup>1</sup> Department of Biotechnology, DDU Gorakhpur University, Gorakhpur 273009, India

Solid-state fermentation (SSF) and submerged fermentation (SmF) are the two popular conventional methods for the production of enzymes from microbial sources. SSF has advantage over SmF as it aims to achieve the highest substrate concentration for fermentation by bringing a cultivated fungus or bacterium into close contact with the insoluble substrate and is also economically more feasible. It involves use of abundant and cheap substrates, which are mostly the waste products, such as wheat bran, sugarcane bagasse and orange peel (Meenakshisundaram 2012; Tivkaa et al. 2013; Kashyap et al. 2003; Martin et al. 2004).

In general, enzyme production by microbes depends on several factors such as composition of growth medium, cultivation conditions like pH, temperature, aeration, agitation, and incubation time. Filamentous fungi are the most preferred microorganism in the enzyme industry due to the fact that as much as 90% of the enzymes produced by these fungi are extracellular in nature (Jun et al. 2011). Several fungal strains have been reported for the production of pectin lyases as reviewed (Yadav et al. 2009); however, strains representing *Fusarium* genera are few (Yadav et al. 2014). The acidic and alkaline pectin lyases are generally used for fruit juice clarification and retting of fibers, respectively. In the present manuscript, purification and enzymatic characterization of an alkaline pectin lyase from *Fusarium oxysporum* MTCC 1755 with its possible application in retting have been discussed.

## Materials and methods

### Chemicals

All the chemicals used were either procured from Sigma-Aldrich (USA) or HIMEDIA (Mumbai, India) and were of high analytical grade. Pectin from citrus fruit (Sigma) was used as the substrate. The fungus *Fusarium oxysporum* MTCC 1755 was procured from MTCC Centre and Gene Bank, Institute of Microbial Technology, Chandigarh, India.

### Maintenance of *Fusarium oxysporum* MTCC 1755 and PNL production

The fungal strain was cultured on Potato Sucrose Agar (PSA) slants and incubated at 25 °C for 7 days. The heavily sporulated slants were stored at 4 °C and subculturing was done after every 3 months. The enzyme was produced by solid-state fermentation and the production medium consisted of 5 g of wheat bran and 7 ml of salt solution. The salt solution consisted of 4 gL<sup>-1</sup> each of K<sub>2</sub>HPO<sub>4</sub>, KH<sub>2</sub>PO<sub>4</sub>, and (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>. The spores were harvested by adding 1 ml of sterile distilled water. About

5 × 10<sup>5</sup> spores were required for maximum production of the enzyme. A total of 10 × 250-ml Erlenmeyer flasks containing solid medium were inoculated and were kept at 25 °C in a biological oxygen demand incubator (BOD). Maximum production occurred on the 7th day of incubation.

### Enzyme extraction and assay

For extraction of crude enzyme, 15 ml of chilled distilled water was added to each flask and the resulting mixture was homogenized by a glass rod. The homogenate was extracted through four layers of cheese cloth and the filtrate was centrifuged at 10,000 rpm for 20 min. The clear supernatant obtained was used as crude enzyme for purification. Enzyme activity of PNL was assayed by the method reported in the literature (Albersheim 1966). Enzyme solution (0.2 ml) was added to a reaction mixture containing 0.8 ml of citrus pectin (1%) and 2.0 ml of the 100 mM glycine–NaOH buffer (pH 9.0). It was incubated at 37 °C in a water bath and the optical density was measured. Enzyme activity was measured at zero time and after 20 min at 235 nm and the steady-state velocity was calculated by absorbance change per minute. Enzyme activity was defined in terms of μmole of unsaturated product released per min, based on molar extinction coefficient value of 5500 M<sup>-1</sup> cm<sup>-1</sup>. Protein was determined by the standard Lowry–Folin method (Lowry et al. 1951) using bovine serum albumin as standard. All the experiments were performed in triplicates and were found to have standard deviation of less than 5%.

### Enzyme purification

Finely powdered ammonium sulfate was added to 100 ml of crude enzyme preparation with gradual stirring up to 30% saturation at 4 °C and the resulting solution was kept in the fridge overnight. Next morning it was centrifuged at 10,000 rpm for 15 min. The pellet was discarded and the supernatant was saturated up to 90% with ammonium sulfate and allowed to stand overnight. It was again centrifuged at 10,000 rpm for 20 min. The pellet was dissolved in 10 mM citrate buffer (pH 5.0). This fraction was dialysed against 1000 times excess of 10 mM citrate buffer (pH 5.0) with sequential three changes to remove the salt completely.

The dialysate was centrifuged at 5000 rpm for 5 min to get a clear transparent concentrated enzyme. This was allowed to bind to the CM cellulose resin (3.0 g) in a beaker (100 ml) by batch procedure. The resin was pre-equilibrated with 20 mM citrate phosphate buffer (pH 5.0), as this was the pH at which the enzyme was found to bind maximally. The resin was washed with 15 ml of buffer to

remove other undesired protein. The resin was allowed to settle down at 4 °C and again washed with 15 ml of 20 mM citrate buffer (pH 5.0) with 1.5 M NaCl to elute out the bound protein. This eluted product was tested for PNL activity, protein concentration and also for homogeneity.

The eluent obtained was concentrated by Amicon ultrafiltration unit and was used for next step of purification. The concentrated enzyme was dialysed against 10 mM citrate phosphate buffer (pH 6.8) and was loaded on Sephadex G-100 column (22 cm × 1.1 cm) equilibrated with 50 mM citrate phosphate buffer (pH 6.8). The flow rate was maintained at 10 ml hr<sup>-1</sup> and fractions of 2 ml each were collected, assayed, pooled and stored in deep fridge at -20 °C.

### Biochemical characterization of purified PNL

#### *Determination of homogeneity and molecular mass of purified PNL*

The enzyme purity and relative molecular weight were estimated by the SDS-PAGE method of Laemmli (Laemmli 1970). A standard protein marker comprising protein bands in the range of 10–250 kDa, with a distinguished color reference bands, namely orange for 71 and 29 kDa and green for 10 kDa band was used. Silver staining was done for locating the protein bands.

#### *Optimum pH and pH stability of purified PNL*

Optimum pH was determined by measuring the steady-state velocity in the buffered reaction solution in the pH range 1.0–12.0 and plotting steady-state velocity against the pH of the reaction solution. The different buffers used were: HCl–KCl (1.0–2.0), citrate phosphate buffer (3.0–5.0), sodium phosphate (6.0–8.0), glycine–NaOH (9.0–10.0) and sodium phosphate–NaOH (11.0–12.0), all at 100 mM concentration. The pH stability of the purified pectin lyase was studied by exposing the enzyme at a particular pH for 24 h at 4 °C and consequently plotting the percent residual activity against the pH at which the enzyme has been exposed.

#### *Optimum temperature and thermostability of the purified PNL*

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 thermal stability of the enzyme was tested by two separate experiments: in one experiment enzyme aliquots were allowed to remain at different temperature for half an hour and their activities were assayed

using the standard assay method. The percent residual activity after half an hour was plotted against temperature. In the second experiment, an enzyme sample was kept at different temperatures (viz., 40, 50 and 60 °C), aliquots were withdrawn after 15, 30, 60 and 120 min and were assayed using standard assay method.

#### *Effects of substrate concentration*

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 drawing Michaelis–Menten curve and double reciprocal Lineweaver–Burk plot.

#### *Effect of divalent cations and protein inhibitors*

The enzyme activity was determined in the presence of various metal ions, namely Ag<sup>+</sup>, Ca<sup>2+</sup>, Co<sup>2+</sup>, Cu<sup>2+</sup>, Hg<sup>2+</sup>, K<sup>+</sup>, Mg<sup>2+</sup>, Zn<sup>2+</sup>, Na<sup>+</sup>, Mn<sup>2+</sup>, and protein inhibitors such as EDTA, sodium azide, potassium permanganate and potassium ferrocyanide at 1 mM concentration.

#### *Effect of salts and sugars on thermostability of the purified PNL*

Salts and sugars like magnesium chloride, calcium chloride (5–10 mM); sodium chloride, glycine (0.5–1.0 M); glycerol, mannitol and sorbitol (10–20%) were incorporated in the reaction mixture and incubated at 50 °C for one hour to study the thermal stability of the purified enzyme.

#### *Retting of natural fiber*

The retting of *Crotalaria juncea* (Sunnhemp) fiber was studied (Zhang et al. 2005). The fiber sticks of 5 cm were incubated in 10 ml of 100 mM glycine–NaOH buffer (pH 9.0) in two sets, one containing 0.01 M EDTA and other lacking EDTA. An aliquot (100 µl) of enzyme was added in both the sets and the reaction mixtures were incubated for 24 h at 37 °C in water bath. Control tubes with inactivated enzyme were simultaneously maintained. After 24 h, the sticks were shaken vigorously with 10 ml of hot water for one minute and the hot water was poured off and the resulting sticks were photographed.

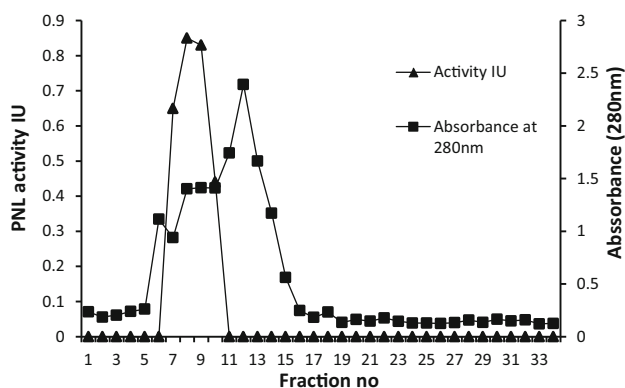
## Results

### Enzyme purification

The different steps of purification of pectin lyase produced by *Fusarium oxysporum* MTCC 1755 is shown in Table 1.

**Table 1** Purification chart of PNL secreted by *Fusarium oxysporum* MTCC 1755

Fraction	Total activity (IU)	Total protein (mg)	Specific activity	Fold purification	% yield
Crude	54	26	0.2	–	–
Ammonium sulfate (30–90%)	27.5	21.25	1.29	6.45	50
CM cellulose	11.2	6.2	1.8	9.0	20.7
Sephadex G-100	16.8	5.2	3.2	16	31.2

**Fig. 1** Gel filtration purification profile of PNL from *Fusarium oxysporum* MTCC 1755

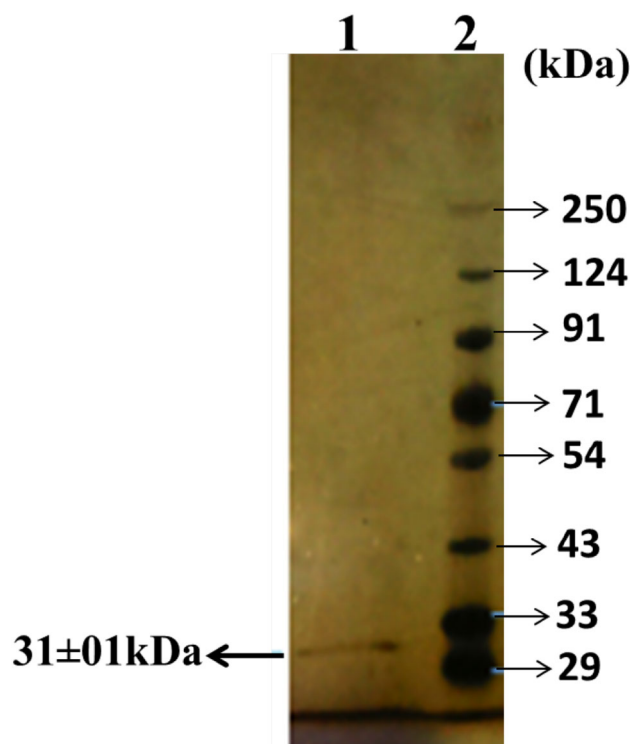
The crude enzyme had specific activity of 0.2 U/mg. 50% of the enzyme gets precipitated between 30 and 90% of ammonium sulfate saturation and the specific activity increased to 1.2 U/mg. The specific activity increased to 1.8 U/mg and the recovery was 20.7% after ion exchange in batch technique. In the final purification step by gel filtration chromatography by Sephadex G-100 column, 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 pure as shown in Fig. 1.

The specific activity was enhanced to 3.2 U/mg and 16-fold purification fold was achieved. The homogeneity of the purified enzyme was confirmed by performing 10% SDS-PAGE, where a single prominent band was obtained (Fig. 2). The molecular weight of the purified enzyme was determined by plotting the log of molecular weights of the markers versus their relative mobility (Werber and Osborn 1969) which was determined to be approximately  $31 \pm 01$  kDa.

### Biochemical properties of purified pectin lyase of *Fusarium oxysporum* MTCC 1755

The variation in activity of purified pectin lyase with pH of the reaction medium is shown in Fig. 3.

The pH optimum was found to be 9.0 and the enzyme shows 98% of its activity in pH range 9.0–10.0. The results

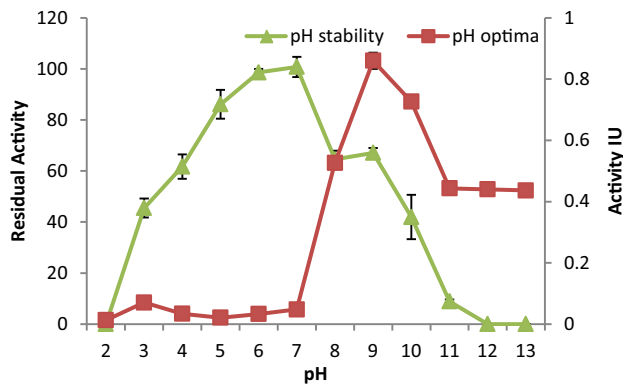
**Fig. 2** SDS-PAGE of PNL purified from *F. oxysporum* 1755. Lane 1 purified PNL; Lane 2 protein marker

of the studies on pH stability of purified pectin lyase are also shown in Fig. 3. The graph shows that the enzyme retains most of its activity if left for 24 h in a buffer of pH range 5.0–7.0.

The temperature optimum of the enzyme is 40 °C (Fig. 4a). The percentage residual activity versus temperature at which enzyme has been kept for half an hour shows that the enzyme retains its full activity up to 50 °C and thereafter activity is decreased, with increase in temperature (Fig. 4b). However, 32% of residual activity is lost after incubating the purified PNL at 60 °C for half an hour.

The  $K_m$  and  $k_{cat}$  calculated from Lineweaver–Burk plots (Fig. 5) were 1.75 mg/mL and  $83.3 \text{ s}^{-1}$ , respectively.

The results of the effects of metal ions at 1 mM concentration of  $\text{Ag}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Hg}^{2+}$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Na}^+$ ,  $\text{Mn}^{2+}$  and protein inhibitors such as EDTA, sodium azide, potassium permanganate and potassium

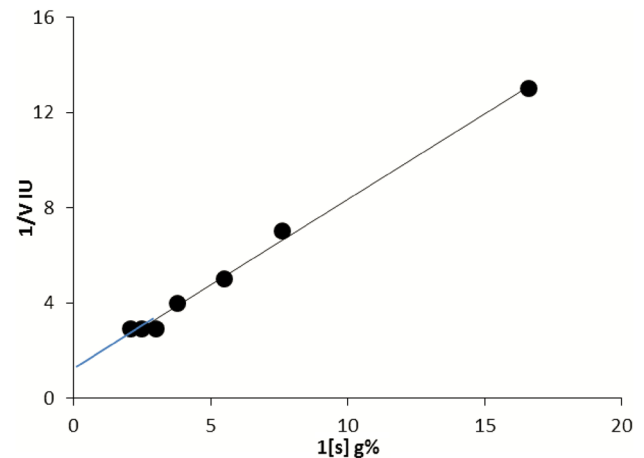
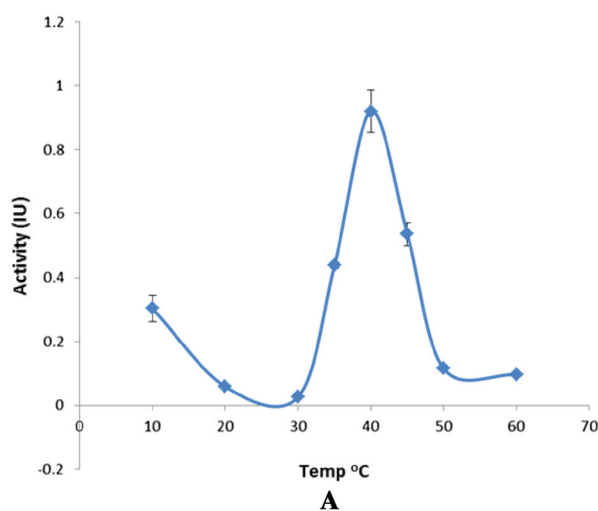


**Fig. 3** Optimum pH black filled square and pH stability black filled triangle of purified PNL from *F. oxysporum* MTCC 1755. The vertical bars indicate standard deviation (SD) of the mean calculated for three readings

ferrocyanide on the activity of pectin lyase are summarized in Table 2. It was observed that  $\text{Ca}^{2+}$  significantly increased the PNL activity while  $\text{K}^+$ ,  $\text{Na}^+$ ,  $\text{Zn}^{2+}$ ,  $\text{Ag}^+$ ,  $\text{Hg}^{2+}$ ,  $\text{Co}^{2+}$  and  $(\text{NH}_4)_2\text{SO}_4$  were also found to be stimulating the PNL activity whereas, potassium ferrocyanide and potassium permanganate inhibited the activity of the purified pectin lyase completely.

#### Effect of salts and sugars on thermostability of the purified PNL

As shown in Fig. 4b, the purified enzyme was thermally stable for half an hour up to 50 °C and activity decreased sharply thereafter. Effects of a few salts and sugars were tested when exposed for an hour at 50 °C. It was observed

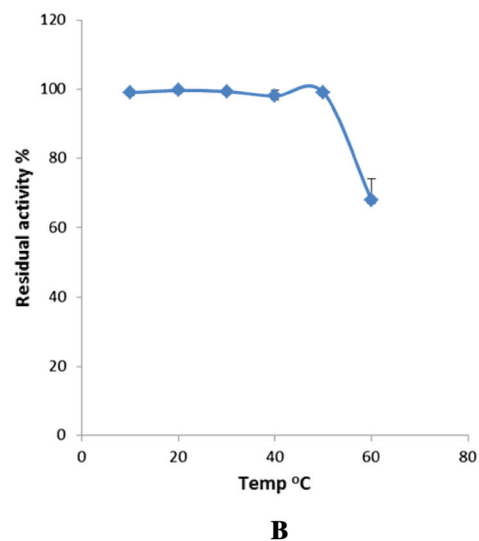


**Fig. 5** Double reciprocal Lineweaver–Burk plot of purified PNL of *F. oxysporum* MTCC 1755

that  $\text{CaCl}_2$  increased the PNL activity at 10 mM concentration while sugars like mannitol and sorbitol maintain the stability.  $\text{MgCl}_2$  and glycine were found to decrease the activity (Table 3).

#### Retting of natural fiber *Crotolaria juncea* in the presence of purified pectin lyase

The results of studies of retting experiments on mature *Crotolaria juncea* sticks by the purified enzyme in 24 h are shown in Fig. 6. It is quite interesting to note that the retting of *Crotolaria juncea* sticks by the purified enzyme is accomplished in the absence of EDTA, which is generally required for retting.



**Fig. 4** The optimum temperature (a) and thermostability (b) of purified PNL of *F. oxysporum* MTCC 1755. The vertical bars indicate standard deviation (SD) of the mean calculated for three readings



**Table 2** Effect of metal ions and protein inhibitors on activity of purified PNL

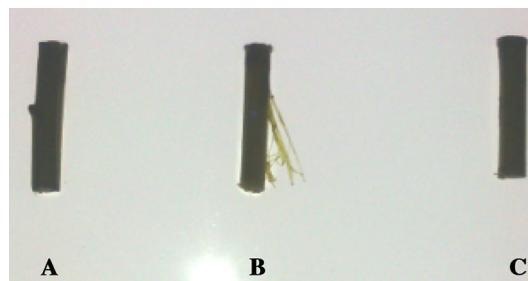
Metal ions/protein inhibitor	Relative activity (%)
<b>Metal ions (1.0 mM)</b>	
Control	100
CaCl <sub>2</sub>	2886
KCl	200
CuSO <sub>4</sub>	100
NaCl	181
ZnSO <sub>4</sub>	131
AgNO <sub>3</sub>	416
CoCl <sub>2</sub>	336
HgCl <sub>2</sub>	360
Citric acid	78
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	203
<b>Protein inhibitors (1.0 mM)</b>	
EDTA	10.2
Sodium arsenate	91.7
Sodium azide	125
Potassium permanganate	0.0
Potassium ferrocyanide	0.0

**Table 3** Effects of different concentration of salts and sugars on thermostability of purified PNL of *Fusarium oxysporum* 1755

Sl no.	Salt/sugar	Concentration	Residual activity (%)
1	Control	–	100
2	MgCl <sub>2</sub>	5 mM	72.2
3	MgCl <sub>2</sub>	10 mM	25.9
4	CaCl <sub>2</sub>	5 mM	44.0
5	CaCl <sub>2</sub>	10 mM	111.1
6	NaCl	0.5 M	100.0
7	NaCl	1.0 M	50.0
8	Glycine	0.5 M	61.1
9	Glycine	1.0 M	50.0
10	Glycerol	10%	24.3
11	Glycerol	20%	29.1
12	Mannitol	10%	103.5
13	Mannitol	20%	100.0
14	Sorbitol	10%	100.0
15	Sorbitol	20%	54.0
16	Ammonium	10%	99.0
17	Sulfate ammonium sulfate	20%	95.0

## Discussion

The purification procedure summarized in Table 1 shows that pectin lyase purified from culture filtrate of *Fusarium oxysporum* MTCC 1755 achieved 16-fold purification with 31.2% yield and 3.2 U/mg specific activity. The

**Fig. 6** Retting of *Crotalaria juncea* fiber by purified PNL of *F. oxysporum* MTCC 1755. **a** Control, **b** treated with enzyme without EDTA and **c** treated with enzyme with 0.01 M EDTA

homogeneity of the enzyme confirmed by SDS-PAGE revealed a single prominent band (Fig. 2). The purification procedure involved in the present enzyme has three steps, i.e., ammonium sulfate fractionation, cation-exchange chromatography on CM cellulose and gel filtration chromatography on Sephadex G-100. Similar purification procedure has been reported in the literature for the purification of the pectin lyases from *Pleurotus ostreatus* and *Aspergillus giganteus* (Rashad et al. 2009; Pedrolli and Carmona 2014). The molecular weight determined for the purified pectin lyase was  $31 \pm 01$  kDa, which is similar to pectin lyase of *Rhizopus oryzae* (Hamdy 2005) as determined by gel filtration chromatography. An alkaline pectin lyase from *A. terricola* is reported to have molecular weight of 35 kDa (Yadav et al. 2009).

The values of  $K_m$  and  $k_{cat}$  determined for pectin lyase from *F. oxysporum* MTCC 1755 were 1.75 mg/mL and  $83.3 \text{ s}^{-1}$ , respectively, using citrus pectin as the substrate at 40 °C. However, affinity of the enzyme for the substrate cannot be compared accurately as the substrate is a polymer with variable molecular weights (20,000–4,00,000). Therefore, comparison of  $K_m$  values does not give accurate estimate of affinity of the enzyme for the substrate. The variable  $K_m$  values of 3.2, 1.7, 1.2 and 0.25 mg/ml has been reported for pectin lyase of *Penicillium italicum*, *Oidiodendron echinulatum* MTCC 1356, *Aspergillus flavus* MTCC 10938 and *Fusarium decemcellulare* MTCC 2079 (Yadav et al. 2012, 2013, 2014; Alana et al. 1991). The  $k_{cat}$  value of pectin lyase purified from *Fusarium oxysporum* MTCC 1755 was  $83.3 \text{ s}^{-1}$ , which is in the range of 25–133  $\text{s}^{-1}$  reported for pectin lyase of *Penicillium canescens* using pectins with variable degrees of esterification (Sinitsyna et al. 2007).

The pH optimum of *F. oxysporum* pectin lyase was found to be 9.0 which is quite similar to what has been reported for PNL purified from another strain of *F. oxysporum* and *F. decemcellulare* MTCC 2079 (Yadav et al. 2014; GuevaraMA and Estevez 1996). Optimum pH of 8.0–8.5 have been reported from a number of microbial sources, viz., *A. flavus*, *A. terricola*, *Bacillus clausii*,

*Pythium splendens*, *P. oxalicum* and *Cystoflobasidium capitatum* (Chen and Tseng 1998; Yadav and Shastri 2007; Nakagawa et al. 2005; Yadav et al. 2009, 2013; Li et al. 2012).

Temperature optimum of the purified pectin lyase was determined to be 40 °C, which is in the same range 40–60 °C as reported in the literature for PNLs of *Thermoascus aurantiacus*, *Aspergillus giganteus* and *Fusarium decemcellulare* MTCC 2079 (Yadav et al. 2014; Pedrolli and Carmona 2014; Martins et al. 2002) but different from temperature optima of 35 °C for PNL of *Lasiodiplodia theobromae* (Adejuwon and Olutiola 2007). The present enzyme was found to be quite stable in the pH range 5.0–7.0 when exposed for 24 h; however, it loses its activity above pH 9.0 as shown in Fig. 3. Pectin lyase from *Aspergillus giganteus* and *Aspergillus niger* have been reported to show a pH stability in the range of 3.0–7.0 (Pedrolli and Carmona 2014; Xu et al. 2015). Recent reports on purification strategies along with biochemical attributes of pectin lyase from few microbial strains have been summarized in Table 4.

When the purified enzyme was exposed for half an hour at various temperatures (10–100 °C) and then assayed for pectin lyase activity, it was found to retain 98% of the activity up to 50 °C but the activity started to decrease thereafter. Similar value of temperature stability has been reported in the literature for PNL from *Oidiodendron echinulatum* MTCC 1356 (Yadav et al. 2008) though this value was higher than PNL of *Aspergillus giganteus* (Pedrolli and Carmona 2014). The thermostability of the purified enzyme was enhanced when exposed to 10 mM concentration of CaCl<sub>2</sub> while 1 M NaCl decreases the thermal stability. It was observed that 10% ammonium sulfate retains the activity. Sugars like mannitol and 5 mM sorbitol showed no effect on thermostability while glycerol at 10 and 20 mM decreased the stability at 50 °C.

Among metal ions and protein inhibitors, Ca<sup>2+</sup>, K<sup>+</sup>, Na<sup>+</sup>, Zn<sup>2+</sup>, Ag<sup>+</sup>, Hg<sup>+</sup>, Co<sup>2+</sup> ions were found to stimulate the enzyme activity to a significant level whereas citric acid, EDTA, potassium permanganate and potassium ferrocyanide inhibited the total activity of the pectin lyase at 1 mM. Cu<sup>2+</sup> does not affect the PNL activity. It was interesting to note that particularly Ca<sup>2+</sup> increased the activity up to 35-fold as compared to activity in native form, which is a distinguishing feature of pectate lyases; however, a calcium-dependent pectin lyase has also been reported in the literature (Pedrolli and Carmona 2014). The effects of metal ions and protein inhibitors on the activity of the purified PNL are reported to be quite variable from different microbial sources. The effect of K<sup>+</sup>, Ca<sup>2+</sup>, Na<sup>+</sup> and Mg<sup>2+</sup> on the activity of pectin lyase from *L. theobromae* (Adejuwon and Olutiola 2007) has been studied in the concentration range of 0–30 mM. The activity of pectin lyase was found to increase when subjected to 15 mM of

**Table 4** Purification strategies and biochemical attributes of purified pectin lyases from different microbial sources

Source organism	Purification strategy	pH optima	pH stability	Temp optima (°C)	Temp stability (°C)	K <sub>m</sub> (mg/mL)	MW (kD)	Ref.
<i>Aspergillus niger</i>	FPLC, gel chromatography	5.0	3.0–7.0	43	25–50	0.66	47	(Xu et al. 2015)
<i>Fusarium decemcellulare</i>	Ion exchange, gel filtration	9.0	9.0–12.0	50	10–40	0.125	45	(Yadav et al. 2014)
<i>Penicillium purpurogenum</i>	Phenyl agarose chromatography	6.0	–	50	Upto 50	–	45	(Pérez- Fuentes et al. 2014)
<i>Aspergillus flavus</i>	Ion exchange, gel filtration	8.0	3.0–11.0	55	10–40	1.70	50	(Yadav et al. 2013)
<i>Bacillus clausii</i>	Ion exchange, gel filtration	10.0	8.0–10.0	60	Upto 40	0.87	35	(Li et al. 2012)
<i>Oidiodendron echinulatum</i>	Ion exchange, gel filtration	7.0	Upto 5.0	50	10–50	1.20	–	(Yadav et al. 2012)
<i>Aspergillus giganteus</i>	Ion exchange, gel filtration	8.5–9.0	6.0–10	50	–	–	–	(Pedrolli and Carmona 2014)
<i>Aspergillus terricola</i>	Ion exchange, gel filtration	8.0	4.0–9.0	50	10–50	1.0	35	Yadhav et al. (2009)
<i>Penicillium citrinum</i>	Gel filtration, ion exchange	9.0	9.0–12.0	50	10–40	1.0	31	Yadhav et al. (2009)
<i>Penicillium canescens</i>	Ion exchange, hydrophobic chromatography	5.0–5.5	5.0–6.0	60	Upto 50	–	37.5	(Simitsyna et al. 2007)
<i>Penicillium oxalicum</i>	Ion exchange, gel filtration	8.0	–	50	–	1.1	50	(Yadav and Shastri 2007)
<i>Cystoflobasidium Capitatum</i>	Ion exchange, hydrophobic interaction chromatography, gel filtration	8.0	3.0–9.0	40	–	36.6	42	(Nakagawa et al. 2005)

Ca<sup>2+</sup>, 20 mM of K<sup>+</sup> and 25 mM of Na<sup>+</sup> and Mg<sup>2+</sup> while the activity declined at 30 mM concentration for all the ions. The activity of the enzyme was inhibited by 10 mM EDTA and 6 mM Hg<sup>2+</sup> (Adejuwon and Olutiola 2007).

Microbial pectinases are important group of enzymes with potential applications in various industries such as wine industry, paper industry, textile industry and food industry (Garg et al. 2016). Alkaline pectin lyases are promising enzyme for retting of fibers as reviewed recently (Kohli and Gupta 2015; Yadav et al. 2016). Based on alkaline nature of purified pectin lyase of *F. oxysporum* MTCC 1755, attempt was made to investigate its application in retting of available natural fiber, i.e., *Crotolaria juncea*. The results of retting experiments using purified pectin lyase in the absence of EDTA for *Crotolaria juncea* sticks shown in Fig. 6 reveal efficient retting, which could be commercially exploited after scale-up for textile industries. These results are in agreement with our previous observation that EDTA inhibits the activity of pectin lyase (Table 2) produced by *F.oxysporum*. Retting of *Crotolaria juncea* fiber in the absence of EDTA has earlier been reported for fungal pectin lyases, namely *Aspergillus flavus* MTCC 7589, *Aspergillus terricola* MTCC 7588, *Penicillium citrinum* MTCC 8897, *Aspergillus flavus* MTCC 10938, and *Fusarium decemcellulare* MTCC 2079 (Yadav et al. 2008, 2009a, b, 2013, 2014). In contrast, efficient retting in the presence of EDTA has also been reported in the literature (Yadav et al. 2012; DE Henriksson et al. 1997). Since the efficiency of enzymatic retting is influenced by several factors like nature of fibers, enzyme composition, treatment duration, concentration of enzymes, use of additives like EDTA, etc. (Garg et al. 2016), there is a need for further investigation of the purified PNL of *F. oxysporum* MTCC 1755 using different fibers.

Thus, this communication reports about the purification and enzymatic characterization of an alkaline pectin lyase from a new fungal strain *Fusarium oxysporum* MTCC 1755. Further, its potential application in retting of *Crotolaria juncea* sticks in the absence of EDTA has been elucidated. The purified pectin lyase also reveals enhanced thermostability in the presence of ammonium sulfate and Ca<sup>2+</sup> seems to be drastically influencing the enzyme activity, which is a matter of further investigation.

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**Compliance with ethical standards**

**Conflict of interest** Authors have no conflict of interest.

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