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A novel thermostable, alkaline pectate lyase from *Bacillus tequilensis* SV11 with potential in textile industry

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ABSTRACT

An extracellular pectate lyase was purified and characterized from a UV mutant of *Bacillus tequilensis* SV11. Purification resulted in a 16.2-fold improvement in the enzyme specific activity, with approximately 40.2% yield. SDS-PAGE showed that the enzyme had two subunits with molecular masses of 135 ± 2 and 43 ± 2 kDa. Further, MALDI-TOF MS experiments revealed that the mass spectrum of the second peptide significantly (91% score) matched with the unsaturated rhamnogalacturonyl hydrolase YteR OS-*Bacillus subtilis* (strain 168) by 27% sequence coverage, nominal mass 43,231 Da, and PI 5.91. The enzyme was optimally active at 60 °C, pH 9. K_m and V_{max} of the purified pectate lyase was found to be 1.220 mg/mL and 1773 U/mL, respectively. The enzyme was studied for its applicability in bioscouring and found to be efficient in the removal of 97.91% pectin of cotton fabric when compared with alkali-treated fabric.

1. Introduction

Plant cell walls generally contain three types of polysaccharides: cellulose, pectin, and hemicellulose. Among these, pectins or pectic substances are the most complex polysaccharides and often have a high molecular weight. Additionally, these negatively charged, acidic polysaccharides are present as the major components of the middle lamella of plants in the form of calcium pectate and magnesium pectate (Jayani, Saxena, & Gupta, 2005; Murad & Azzaz, 2011). The primary structure of pectin consists of both linear (homogalacturonan, HG) and ramified (rhamnogalacturonan, RG) regions. HG is a linear homopolymer of α-1,4-linked D-galacturonic acid (polygalacturonic acid) and is thought to contain about 100–200 galacturonic acid residues with 70–80% methylesterification. Within the ramified (hairy) regions, two types of polysaccharides can be found, RG-I and RG-II. The main chain of RG-I has a repeating disaccharide unit containing galacturonic acid (GalA) and rhamnose (Rha) residues, while RG-II is a branched pectic domain containing an HG backbone with 11 different types of sugars as side chains (Itoh, Ochiai, Mikami, Hashimoto, & Murata, 2006).

The enzymes that hydrolyze pectic substances are broadly classified as pectinases or pectinolytic enzymes and account for 25% of global food enzyme sales (Jayani et al., 2005; Murad & Azzaz, 2011). These enzymes can be classified into two main groups: methyl esterases, which catalyze the removal of the methoxyl group from pectin to form pectate, and depolymerases, which split the backbone of both pectin and pectate by hydrolytic cleavage or transeliminative cleavage (Favela-Torres, Aguilar, Contreras-Esquivel, & Viniegra-Gonzalez, 2005). Pectate lyase, a critical enzyme involved in the depolymerization of pectic substances, is widely found in bacteria (Arijit, Sourav, Naimisha, & Rajan, 2013), yeast (Gummadi & Kumar, 2010), fungi (Wang, Fu, & Zhang, 2011), and actinomycetes (Bruhlmann, 1995) and has been recently

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identified in plants (Payasi, Misra, & Sanwal, 2006). The activity of pectate lyase depends on calcium ions and functions to catalyze the cleavage of α-1,4 glycosidic linkages in pectic acid by transelimination, resulting in the synthesis of unsaturated oligogalacturonates with a double bond at C_4 and C_5 (Yuan et al., 2011).

Based on the optimal pH for enzymatic activity, pectinases can be classified into acidic and alkaline enzymes. Acidic pectinases are generally used in the fruit juice industry, wine making, and animal feed (Kashyap, Vohra, Chopra, & Tewari, 2001). In contrast, alkaline pectinases have received more attention due to their extensive applications in the textile industry; indeed, alkaline pectinases have been used for cotton fiber bioscouring (Yuan et al., 2011); retting and degumming of plant fibers, such as ramie, sunn hemp, buel, flax, and jute (Saleem, Rennebaum, Pudel, & Grimm, 2008); paper and pulp production; pectic waste water treatment; coffee/tea fermentation (Thakur & Gupta, 2012); plant virus purification; and vegetable oil extraction (Damasio et al., 2011). In the plant fiber and textile processing industries, alkaline pectinolytic enzymes in particular can be used in bioscouring, an important preparatory step that uses living organisms for complete or partial removal of the noncellulosic components and natural or unnatural impurities of native cotton. Compared to traditional scouring, which is achieved through a series of chemical treatments, bioscouring is a more environmentally friendly method, produces higher-quality fibers (softer to the touch and better strength) and less waste water, saves energy, and is compatible with other procedures, equipment, and materials (Klug-Santner et al., 2006; Tzanov, Calafell, Guebitz, & Cavaco-Paulo, 2001).

While microbial systems are generally useful for the production of such enzymes, current systems are not sufficient to keep up with the increasing demand for pectinolytic enzymes. Therefore, we sought to isolate a microorganism with high potential to produce pectate lyase from low-cost substrates in order to improve the efficiency of pectate lyase production for textile and fiber applications.

2. Material and methods

2.1. Micro-organism used

A UV mutant of *Bacillus* strain SV11 that had been isolated from the Godavari river of Walgonda Village, Karimnagar, India, was used for the present study and was identified by morphological and biochemical characterization (Rani, Hasini, & Rao, 2012). The strain was further characterized to species level by 16S rRNA sequencing. The total genomic DNA was extracted from the strain SV11 for PCR amplification of 16S rDNA (Sambrook, Fritch, & Maniatis, 1982). The primers used were universal primers such as forward primer, 518F (CCAGCAGCCGCGGTAATACG), and reverse primer, 800R (TACCAGGGTATCTAATCC).

The selected strain was identified by matching the 16S rRNA sequence using EZ taxon server 2.1 database and by constructing a phylogenic tree (boot strap analysis) using Mega 4.0.2 software.

2.2. UV mutagenesis

UV mutagenesis was carried out for strain development for the improved enzyme production. An overnight grown culture of 5 mL was washed twice with sterile distilled water, re-suspended in 0.1 M phosphate buffer (pH 7), and resulted in 10^8 cells/mL. The earlier mentioned 2 mL of suspension was placed in a sterile Petri plate and exposed to UV rays at a distance of 20 cm. The samples were collected at different time intervals (5, 10, 15, 30, and 60 min), serially diluted, and plated on nutrient agar by spread-plate method; then, they were incubated at 37 °C for 24 h (Winston & Ausubel, 1990). Isolated colonies were randomly picked and subjected to submerged fermentation (SmF) for the production of pectate lyase.

2.3. Production of pectate lyase using strain SV11-UV37

Inoculum was prepared by adding a 24-h-grown culture from the nutrient agar slant into nutrient broth and incubating at 37 °C for 18 h. After incubation, 1.5% of inoculum was added to fermentation medium (lemon peel powder 1.12%, tryptone 1%, and $MnSO_4$ 0.05%; pH 6) and incubated at 35 °C in a rotary shaker at 200 rpm. After 33 h of shaking, a 1-L fermented broth was used as the enzyme source for purification.

2.4. Purification of pectate lyase from SV11-UV37

One liter of fermented broth obtained by SmF was used for clarification, concentration, and purification of the enzyme by using membrane-based Tangential Flow Filtration unit. Before ultrafiltration, the fermented broth was clarified by 0.2-μ membrane filters to separate intact cells and debris. Then, the crude culture filtrate was concentrated by using 10 kDa NMWL (Amicon) cut off membranes. The concentrated enzyme was applied to Q-sepharose pre-packed column (HiTrap Q FF-5 × 1 mL, GE Healthcare Life Sciences) in AKTA purifier, pre-equilibrated with 20 mM Tris–HCl buffer (pH 7.5) at a flow rate of 0.5 mL/min. Elution was carried out with a linear gradient of 0 to 100% 0.5 M NaCl at a flow rate of 2 mL/min, and 1-mL fractions were collected and analyzed for enzyme and protein activity. The purified enzyme was stored at −20 °C until use. Total protein content was determined according to Lowry's (Lowry, Rosenbrough, Farr, & Randall, 1951) method using bovine serum albumin as the standard. Sodium dodecyl sulfate polyacrylamide gel (12%) electrophoresis (SDS-PAGE) was performed using the Bio-Rad apparatus to check the homogeneity of the enzyme and for the determination of molecular mass (Laemmli, 1970). Protein bands were visualized by staining with Coomassie brilliant blue R-250. Broad-range molecular mass markers (Bio-Rad) were used for the estimation of the molecular mass of the pectate lyase. Zymogram analysis was performed by 12% SDS PAGE containing 0.1% polygalacturonic acid (PGA), and the zone of clearance was visualized by staining with 0.05% ruthenium red solution.

2.5. Activity assay of pectate lyase

Pectate lyase activity was determined spectrophotometrically by measuring the increase in absorbance at 235 nm (Sittidilokratna et al., 2007; Songpim, Vaithanomsat, & Chuntranuluck, 2010). The reaction mixture (1 mL) containing 25 mM Tris–HCl buffer and 1 mM $CaCl_2$ of pH 9, 0.4% (w/v) PGA, and 0.04 mL of crude enzyme was incubated at 60 °C for 10 min. The reaction was terminated by adding 4 mL of 0.01 M HCl to the mixture. Crude enzyme was inactivated by boiling it for 10 min so that it could be used as a control in the reaction.

One unit of pectate lyase corresponds to the amount of enzyme that lyses 0.4% PGA solution and releases products with an absorbance increase of 0.2 at 235 nm within 10 min under the standard assay conditions (Sittidilokratna et al., 2007; Songpim et al., 2010).

2.6. MS and MS–MS analysis using MALDI-TOF

Matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) is a relatively novel technique for the analysis of biomolecules, in which a co-precipitate of a UV-light absorbing matrix and a biomolecule is irradiated by a nanosecond laser pulse. The MALDI is an efficient process that is used for

generating gas-phase ions of peptides and proteins for mass spectrometric detection. Before to MALDI analysis, protein bands of interest were excised from the gel with a clean blade and made into smaller pieces of approximately 1 mm for In-gel trypsin digestion, which was carried out by the method adopted from Shevchenko, Wilm, Vorm, and Mann (1996). Then, MALDI-TOF (Bruker) MS analysis was performed by using α-cyano-4-hydroxy cinnamic acid as a matrix, where a 1:1 ratio of matrix and the pepmix/sample mix was prepared and spotted (1–2 μL) onto the ground steel plate. Further, the samples were dried at room temperature, fired and a mass list was generated using the Mascot search engine. Significant scores obtained were checked for matching of peptide sequence using the NCBI blast tool, and further significant scores were analyzed for MS–MS by firing the same spot.

2.7. Effect of pH, temperature on pectate lyase activity and stability

Influence of pH on pectate lyase activity was measured by performing the assay at pH values ranging from 3.0 to 11.0 using different buffers (citrate–phosphate −3 to 8, Tris–HCl −8.5 and 9, and carbonate–bicarbonate −9.5 to 11) at a fixed assay temperature (60 °C). The pH stability of the enzyme was determined by incubating the enzyme in different buffers and measuring the residual activity after 24 h.

The optimum temperature for enzyme activity was determined by conducting the assay in Tris–HCl buffer (pH 9) at temperatures ranging from 30 °C to 80 °C. Temperature stability was studied by incubating the enzyme at different temperatures (30–80 °C) and measuring the residual activity at different time intervals for 24 h.

2.8. Substrate specificity

The substrate specificity of the enzyme was studied by using PGA and pectin at a fixed concentration and conducting the enzyme assay under standard conditions.

2.9. Influence of metal ions and reagents on pectate lyase activity

Influence of metal ions and some reagents on enzyme activity was determined by incubating the enzyme in 1 mM of $CuSO_4$, $ZnCl_2$, $MnSO_4$, $CaCl_2$, $MgSO_4$, $FeCl_3$, $CoCl_2$, SDS, β-mercapto ethanol, ascorbic acid, EDTA, and L-cystine and by measuring the relative activity.

2.10. Determination of K_m and V_{max}

The kinetic parameters, K_m and V_{max} of the purified pectate lyase were determined using Graph Pad Prism 4 software by fitting the activity data at different substrate concentrations (0.5 to 10 mg/mL) to a linear regression on a Line-weaver burk plot. Here, V_{max} represents the maximum rate achieved by the system, at maximum (saturating) substrate concentrations, and K_m is the substrate concentration at which the reaction rate is half of the V_{max}.

2.11. Application of pectate lyase in bioscouring of cotton fabric

The plain woven 100% cotton fabric was purchased from Khadi bhandar, Metpally, India. Before bioscouring, fabrics were pretreated with *n*-hexane, triton-X-100, and distilled water (2 min at 100 °C) to study their effect on wax removal. Fabrics (6 × 9 cm, one fabric per beaker) were treated with pectate lyase (100 U/g fabric) in a 500-mL beaker containing 250 mL of 25 mM Tris–HCl buffer (pH 9). The incubation was carried out in a temperature-controlled water bath at 50 °C for 30 min by intermittent shaking. Then the fabric was rinsed with water for 15 min at 90 °C to stop the reaction. Two subsequent washing steps were performed at room temperature in a water bath at 100 rpm for 5 min. The treated and rinsed fabrics were dried for 24 h before the fabric was used for further measurements (Klug-Santner et al., 2006). Alkaline scouring was carried out by boiling at 90 °C for 45 min in sodium hydroxide solution (0.2N) with 0.1 g/L of surfactant (Tzanov et al., 2001).

2.11.1. Ruthenium red dyeing and measurement of K/S values

Residual pectin on the fabric was analyzed by staining with ruthenium red (Klug-Santner et al., 2006). The fabric was dyed with ruthenium red solution for 15 min at room temperature and rinsed with 1 L of demineralized water. Then, each swatch was washed with demineralized water for 10 min at 60 °C. After drying for overnight, the reflectance (*R*%) at 540 nm was measured, and K/S [$K/S = (1-R)^2/2R$] values were calculated. The extent of pectin removal was calculated by considering the untreated fabric as 100% pectin control (0% pectin removal) and alkali-treated fabric as 0% pectin control (100% pectin removal).

3. Results and discussion

3.1. Identification of strain SV11

The identity of the strain SV11 was confirmed by molecular characterization with 16S rRNA gene sequencing analysis. The 16S rRNA gene sequence of the strain was 1388 bp long and showed 100% sequence similarity with *B. tequilensis* 10b(T) using EZ taxon server 2.1. Phylogenic tree of strain SV11 also showed that the strain was clustered with the type strain of *B. tequilensis*. The 16S rRNA sequence was deposited in the GenBank under the accession number JX473585, and the culture was also deposited in the MTCC with the deposition number MTCC-11716.

The mutation and screening of industrially useful microorganisms are important for the successful development of various strains that are required for use in the fermentation industry (Kumar, Chandra, Sujana, Reddy, & Choi, 2009). It has been reported that the economics of the pectinase production may be enhanced by the selection of more productive mutants or genetically modified organisms (Blanco, Sieiro, Reboredo, & Villa, 1998). In the present study, the productivity of the wild strain *B. tequilensis* SV11 was improved by the application of UV irradiation. Among all the mutants, UV37 was selected for this study as it showed a 1.4-fold improvement in pectate lyase production compared with the wild strain.

3.2. Properties of purified pectate lyase from Bacillus tequilensis SV11-UV37

Pectate lyase could be successfully purified from the culture broth of *B. tequilensis* SV11-UV37 by ultrafiltration and Q-sepharose ion exchange chromatography. After clarification of fermented broth, a 970-mL cell-free filtrate was obtained and concentrated 50 fold by ultrafiltration with the removal of water and low-molecular-weight solutes. The retentate obtained from ultrafiltration was subjected to anion exchange (Q-sepharose) chromatography, where a major peak of pectate lyase activity was detected when buffer volume reaches 42.54 mL (Fig. 1). Purification of pectate lyase from SV11-UV37 resulted in a 16.2-fold increase in specific activity (168.3 U/mg protein) and approximately 40.2% yield (Table 1). Usually, the specific activity of the purified enzyme from microbial sources ranges between 45 and 600 U/mg of protein (Payasi, Sanwal, & Sanwal, 2009).

The purified pectate lyase showed two bands on SDS-PAGE with molecular masses of 135 ± 2 and 43 ± 2 kDa (Fig. 2), while activity staining (zymogram) showed that the zone of clearance was corresponding to the molecular mass ranges from 211 to 100 kDa (Fig. 2).

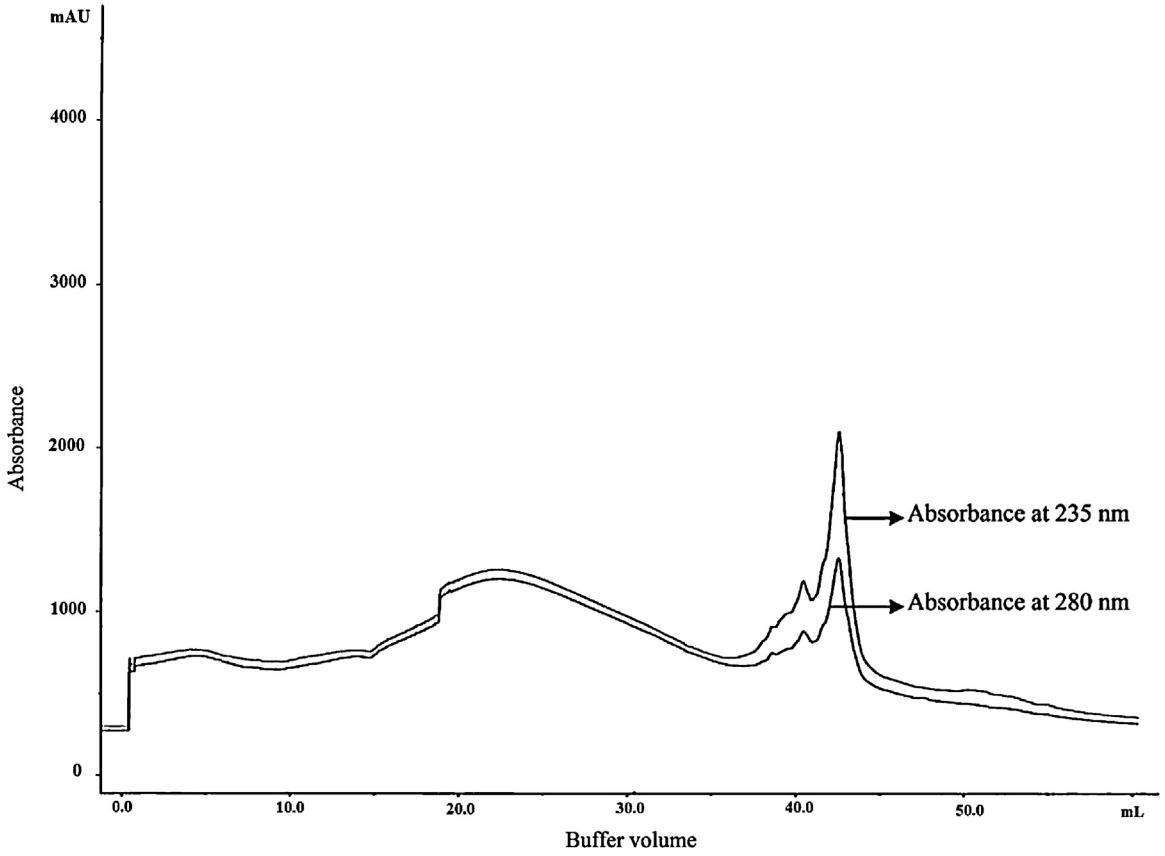

Fig. 1. Elution profile of the pectate lyase during Q-sepharose chromatography.

Table 1
Summary of purification of pectate lyase produced by *Bacillus tequilensis* SV11-UV37.

Purification step	Total activity (U)	Total protein (mg)	Specific activity (U/mg)	Yield (%)	Purification fold
Crude	118,000	11,395	10.355	100	1
Ultrafiltration	87,075	1383.5	62.938	73.79	6.078
Q-sepharose	47,421	281.743	168.312	40.187	16.254

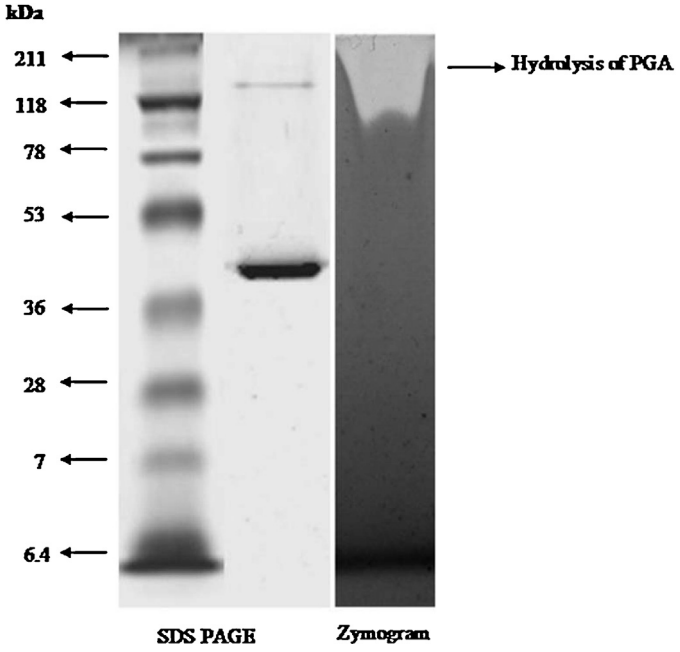

Fig. 2. SDS-PAGE and zymogram of pectate lyase.

The nominal mass of the two protein bands was further measured by MS analysis, in which mass spectrum of the second band was matched significantly; while the other was not matched to any of the peptides/proteins reported in the database. The mass spectrum of the second protein band (Fig. 3) showed 27% sequence coverage with unsaturated rhamnogalacturonyl hydrolase YteR OS-*B. subtilis* strain 168 with a 91% score, which had a nominal mass of 43,231 Da and PI 5.91. The sequence is provided next, and matched peptide sequences are shown in bold.

1 MGSMDQSIAV KSPLTYAEAL ANTIMNTYTV EELPPANRWH
41 YHQGVFLCGV LR**LWEATGEK RYFEYAK**AYA DLLIDDNGNL
81 LFRRDELDAI QAGLILFPLY EQTKDERYVK AAKRLRSLYG
121 TLNR**TSEGGF WHK**DGYPYQM WLDGLYMGGP FALKYANLKQ
161 ETELFDQVVL QESLMRKHTK DAK**TGLFYHA WDEAKK**MPWA
201 NEETGCSPEF WAR**SIGWYVM SLADMIEELP KK**HPNRHVWK
241 NTLQDMIKSI CR**YQDKETGL WYQIVDKGDR SDNWLESSGS**
281 **CLYMYAIAK**G INKGYLDRAY ETTLLK**AYQG LIQHK**TETSE
321 DGAFLVKDIC VGTSAGFYDY YVSRERSTND LHGAGAFILA
361 MTELEPLFRS AGK

Further, MS–MS analysis of the same spot confirmed that the two peptides with nominal mass 1048.503 Da and 1437.708 Da matched with the sequences R.TSEGGFWHK.D and K.TGLFYHAWDEAK.K of unsaturated rhamnogalacturonyl hydrolase YteR OS-*B. subtilis*, with significant scores 24% and 50%, respectively. The mass spectrums of these two peptides are shown

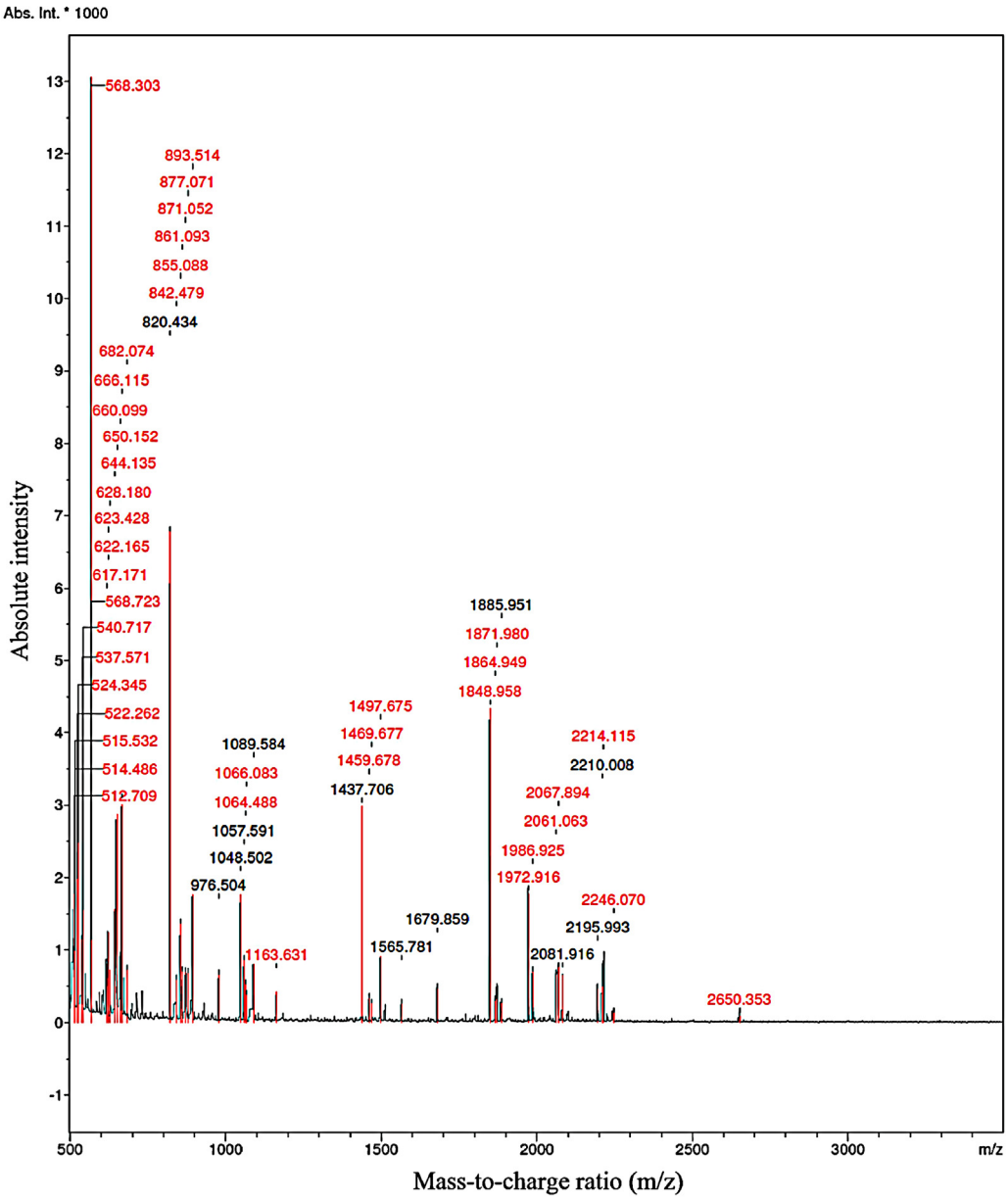

Fig. 3. Mass spectrum (MS) of the 43 kDa peptide.

in Fig. 4a and b. Unsaturated rhamnogalacturonyl hydrolase belongs to glycoside hydrolase family 105, which acts specifically on unsaturated rhamnogalacturonan (RG) that is produced from plant cell wall RG-I treated with RG lyases, releasing unsaturated galacturonic acid (DGalA) from the substrate (Itoh et al., 2006). Hence, in the present study, the action of a peptide (43 kDa) of pectate lyase from *B. tequilensis* SV11 was identified as hydrolysis of unsaturated rhamnogalacturonan, which is a structural part of the ramified (hairy) region of the pectin.

The molecular mass of pectate lyase from various bacterial, fungal, and plant sources generally varies from 20 to 70 kDa. However, Kozianowsky, Canganella, Rainey, Hippe, and Antranikian (1997) reported higher molecular mass by SDS gel electrophoresis, such as pectate lyase-a appeared as a single polypeptide with 135 kDa; whereas pectate lyase-b consisted of two subunits with 93 kDa and 158 kDa. The molecular mass of pectate lyase from the hyper-thermophilic bacterium *Thermotoga maritima* is reported as 152 kDa as analyzed by gel filtration (Kluskens, van-Alebeek, Voragen, de Vos, & van der Oost, 2003). In this study, we report that the pectate lyase from *B. tequilensis* SV11 was found to be novel, as it has a molecular mass of 178 ± 2 kDa, which has not been matched with any of the reported literature till now.

3.3. Enzyme characterization and kinetics

pH and temperature are the two important physical factors which influences the enzyme activity. It has been reported that alkaline pectate lyases are predominantly produced from bacteria, such as the genus *Bacillus*, and most of them had an optimum pH between 8.0 and 10.5 (Gang et al., 2010; Yuan et al., 2011). Generally, the optimum temperature of pectate lyases varies between 40 °C and 70 °C (Payasi et al., 2009). In the present study, the purified pectate lyase exhibited maximum activity at pH 9.0 (Fig. 5a), which is in accordance to the results reported earlier (Yuan et al., 2011). The optimal temperature for the pectate lyase activity was found to be 60 °C (Fig. 5b), which was similar to that reported for high alkaline pectate lyase from alkaliphilic *Bacillus* sp. strain KSM-P7 (Kobayashi et al., 1999). The enzyme was stable for 24 h in a

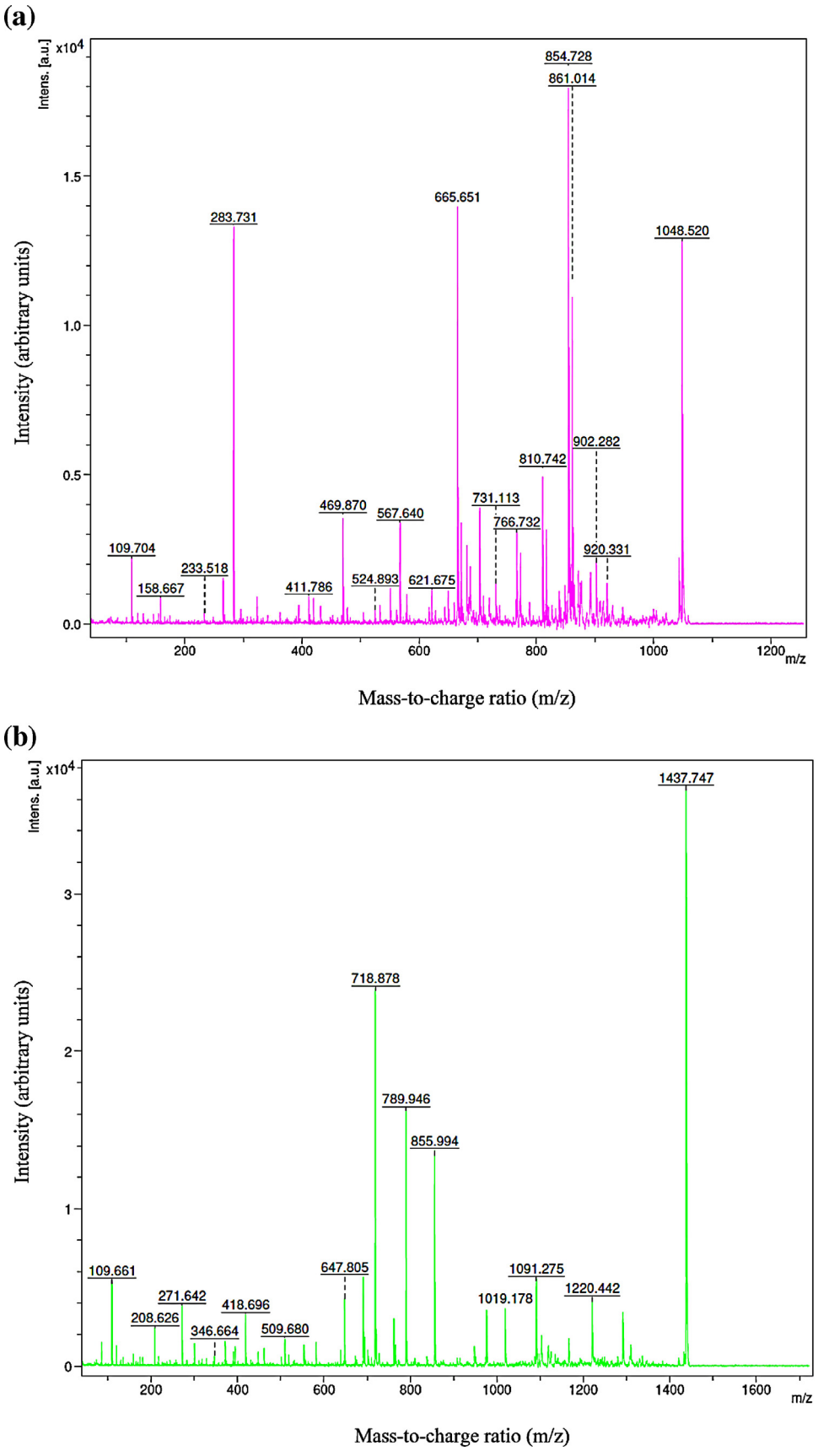

Fig. 4. Mass spectrum (MS–MS) of the (a) 1048.503 Da, (b) 1437.708 Da peptide.

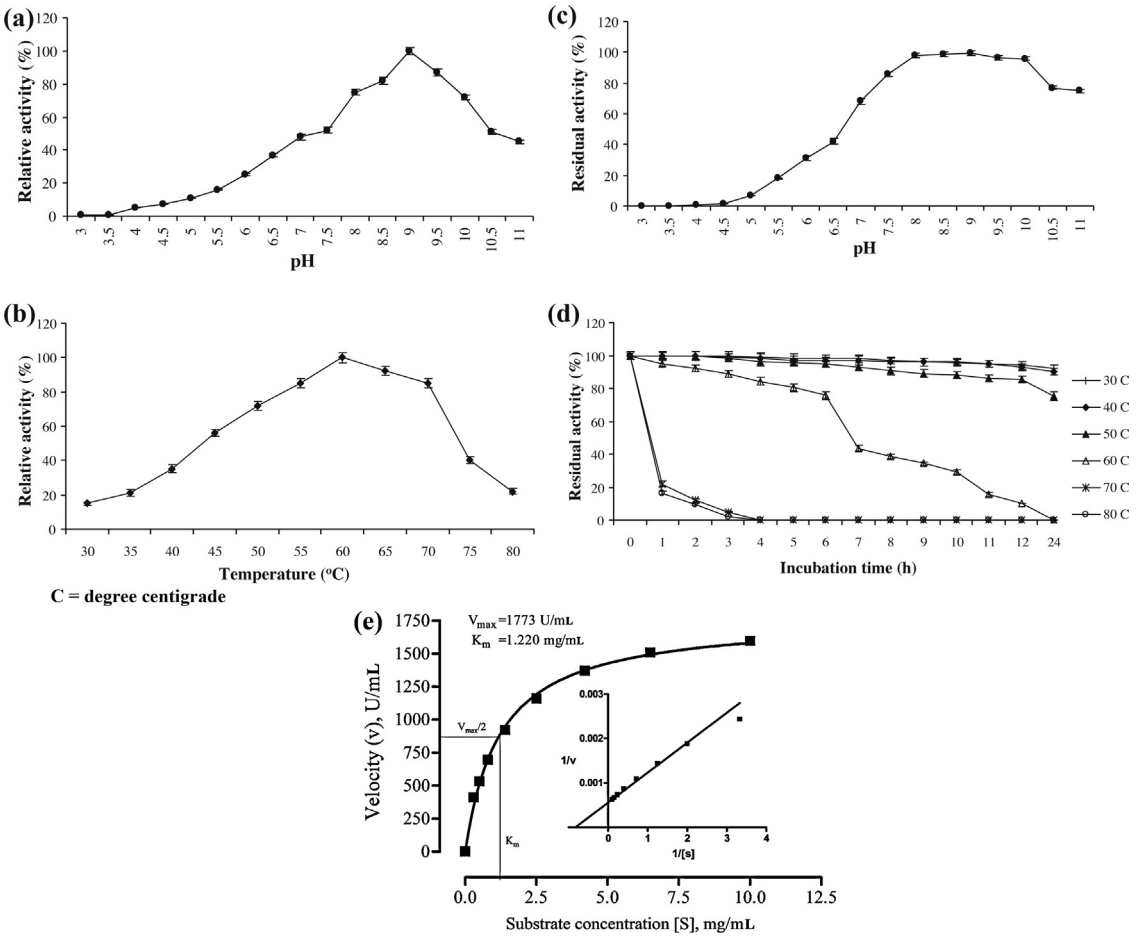

Fig. 5. Effect of (a) pH, (b) temperature on purified pectate lyase activity, (c) pH stability, (d) temperature stability, (e) substrate-velocity curve and Lineweaver–Burk plot of purified pectate lyase. Values are averages of results from triplicate trials; error bars indicate the SD values.

range of pH from 7.0 to 11.00, with a residual activity of 75% at pH 11 (Fig. 5c). The enzyme exhibited thermostability at 50 °C for 24 h and 60 °C for 6 h with 75% residual activity (Fig. 5d). In contrast to the broad temperature optimum curve, the pectate lyase was found to have sharp pH optimum at 9.0 with rapid loss of activity on either side of the optima. The results are close to the pectate lyases that have been reported earlier by other research groups.

In case of substrate specificity of the pectate lyase, PGA was found to be the preferred substrate over the pectin, which had a 67.64% relative activity compared with PGA. Pectate lyase can use both PGA and esterified pectins as substrates, but the affinity is more for intermediate esterified pectins (Klug-Santner et al., 2006). It has been reported that the PGA was a better substrate than methyl esterified pectins for the pectate lyase of banana and Bsp165PelA (Gang et al., 2010; Payasi et al., 2006).

Among the different metal ions tested, the enzyme activity was stimulated by Mg^{2+}, Mn^{2+}, and Ca^{2+} with the relative activities of 166.6, 150, and 115.5%, respectively, whereas the Co^{2+}, Zn^{2+}, Fe^{3+}, and Cu^{2+} ions strongly inhibited it. These results are in accordance to the earlier reports (Payasi et al., 2006; Basu et al., 2008). The enzyme is Ca dependent for its activity as it is evident from the reduction of enzyme activity (10%) by EDTA. Most microbial pectate lyases have a requirement of Ca^{2+} for its activity (Yuan et al., 2011). Generally, Ca^{2+} is necessary for enzyme binding or for salt bridge formation between polygalacturonic chains. Consequently, a complexing agent, such as EDTA had the strongest inhibition effect on the pectate lyase as it chelates the Ca^{2+} ions (Klug-Santner et al., 2006; Zhang et al., 2013). Slight activation of enzyme activity by L-cystine and inhibition by β-mercaptoethanol suggests no role of sulfhydril groups at the active site of the enzyme. Inhibition of pectate lyase activity by SDS and ascorbic acid (mild reducing agent) indicates that the denaturation of the enzyme and in turn necessity of disulfide bond maintenance.

The K_m and V_{max} values of the pectate lyase from *B. tequilensis* SV11-UV37 were found to be 1.220 mg/mL and 1773 U/mL (Fig. 5e), respectively, using PGA as a substrate with good catalytic efficiency (1453 U/mg/mL PGA). It has been reported that the K_m values for *Bacillus* pectate lyases vary between 0.15 and 1.87 mg/mL (Payasi et al., 2009). Similarly, Basu et al. (2008) have shown K_m and V_{max} values of pectae lyase from *Bacillus pumilus* DKS1 as 0.44 mg/mL and 909 U/mL, respectively.

3.4. Application studies

Bioscouring with alkaline pectate lyase from *B. tequilensis* SV11-UV37 was tested on cotton fabric and the amount of pectin removal was analyzed by ruthenium red dyeing, *K*/*S* value determination. As evident from Fig. 6a, the amount of ruthenium red dyeing was less in alkali and enzyme-treated fabrics when compared with the control (untreated). The reflectance (*R*%) spectrum of the fabric is shown in Fig. 6b. Table 2 shows that the higher the *K*/*S* value, the higher is the fabric dyeing and the higher is the pectin present in it. Fabric treated with triton-X-100 showed maximum pectin removal (97.91%), than fabric treated with n-hexane (96.25%) and distilled water (71.93%). Percentage weight loss in enzyme-treated fabric was 62.5% and the percentage of galacturonic acid released was 78.2% when compared with the alkali-treated fabric (100%).

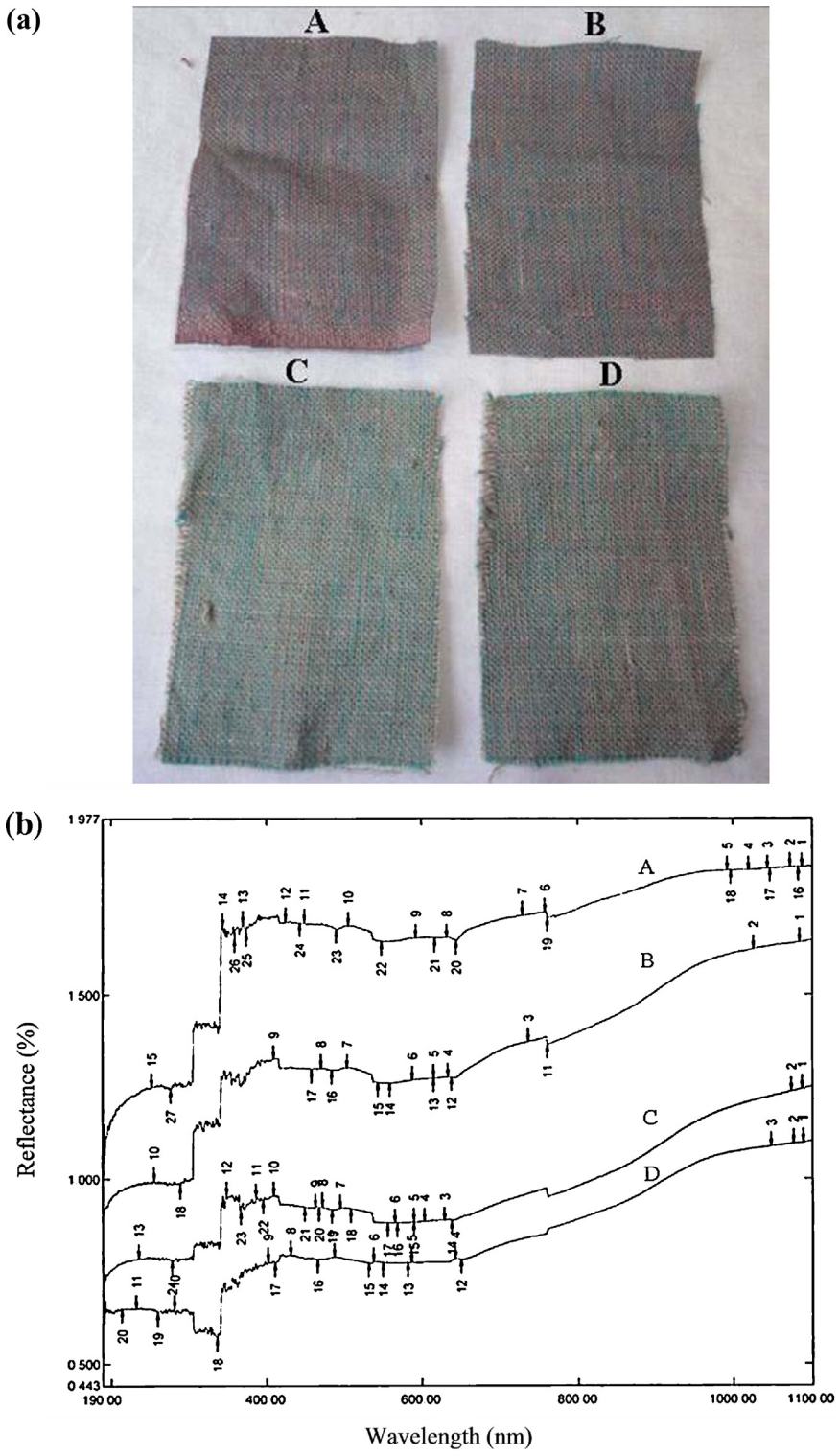

Fig. 6. Bioscouring (a) ruthenium red dyed swatch, (b) reflectance spectrum of a cotton fabric.

The purpose of assuming alkaline scoured fabric as 100% pectin removal is to consider it as a benchmark that needs to be achieved during enzymatic scouring. The indirect ruthenium red dyeing method that is used to measure the pectin removal is well adopted among the researchers in this field and is considered one of the important parameters for studying the scouring effect on cotton fabric (Klug-Santner et al., 2006). Klug-Santner et al. (2006) reported 80% pectin removal by means of ruthenium red dyeing with the application of pectate lyase from *B. pumilus* BK2. Tzanov et al. (2001) employed two types of pectinases such as Bioprep 3000 L (alkaline pectate lyase 20 APSU/g) and Pect 062 L (acidic pectinase 70 U/g). Both commercial pectinases are found to be equally efficient in terms of improved absorbency as the conventional alkaline scouring. The enzymatic treatment has no effect on

Table 2
Percentage of pectin removal after bioscouring with pectate lyase.

Treatment	*R* value	*K*/*S*	% Of pectin removed	% Of weight loss	GA released (μmol/mL)	% Of GA released
Control	1.645	0.126451	–	0	0	0
0.2N NaOH	0.88	0.008182	100	8	8.42	100
0.1% Triton-X-100 + enzyme	1.157	0.010652	97.91	5	6.59	78.26
DW + Enzyme	1.172	0.012621	96.25			
0.1% *n*-Hexane + enzyme	1.332	0.041375	71.93			

the tensile strength; moreover, it slightly enhances the whiteness (Traore & Buschle-Diller, 2000). On the basis of their studies, they concluded that a simple procedure with pectinases in the presence of a non-ionic surfactant is sufficient to attain good absorbency (Galante & Formantici, 2003).

4. Conclusion

The present study led to the isolation of a new potent pectate lyase producer *Bacillus tequilensis* SV11, which is the first report on pectate lyase production from this organism. The enzyme was found to be novel with a high molecular weight in contrast to the results reported earlier and showed 27% sequence similarity with rhamnogalacturonyl hydrolase. From the results, it is clearly evident that the enzyme has high potential to replace and is found to be the best alternative to the conventional scouring process, which can be beneficial for the textile and fabric industry.

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