*Bulgarian Journal of Agricultural Science, 20 (No 1) 2014, 193-198*
*Agricultural Academy*

# PURIFICATION AND CHARACTERIZATION OF AN EXTRACELLULAR ALKALINE PECTATE LYASE FROM *BACILLUS SUBTILIS* BPLSY1

B. AL BALAA[1], R. ESMAIL[2*] and S. YAZAJI[2]
[1]*Department of Molecular Biology & Biotechnology, Atomic Energy Commission of Syria (AECS), Damascus, Syria*
[2] *Damascus University, Department of Food Sciences, Faculty of Agriculture, Damascus, Syria*

## Abstract

AL BALAA, B., R. ESMAIL and S. YAZAJI, 2014. Purification and characterization of an extracellular alkaline pectate lyase from *Bacillus subtilis* BPLSY1. *Bulg. J. Agric. Sci.,* 20: 193-198

Pectinases are among the most important enzymes of food and textile industries. Alkaline pectinases are widely used in the vegetable oil extraction, treatment of food industry wastewaters, coffee and tea fermentations, papermaking and textile processing. A newly isolated *Bacillus subtilis*, designated as BPLSY1, was cultivated for production of pectinases. An extracellular pectate lyase enzyme (EC 4.2.2.2) from the culture supernatant was purified to homogeneity in three steps using acetone precipitation, ion exchange on HiTrap SP and size exclusion chromatography on Superdex 75. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of the purified enzyme showed a single protein band with a molecular weight of 42 kDa. The specific activity of purified enzyme was 58.85 U/mg after 9.86 fold purification. The enzyme characteristics were explored by varying the pH and temperatures. The pectate lyase exhibited maximal catalytic activity at a temperature of 60°C and an alkaline pH 9.5. The presence of 0.5 mM of $CaCl_2$ significantly enhanced pectate lyase activity of the purified enzyme. These findings indicate the enzyme to be alkaline pectate lyase and can be used further for various applications.

*Key words*: *Bacillus subtilis*, activity, characterization, pectate lyase, purification

*Abbreviations*: Pectate lyase (PEL); fast-protein liquid chromatography (FPLC); sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE); bovine serum albumin (BSA)

## Introduction

Pectinases can degrade pectic substances which is a major component of the primary cell wall and middle lamella of plants (Walton, 1994). Galacturonic acid which is the main structure unit of pectic substances (Kashyap et al., 2001) are partially esterified with methyl in pectin, while the pectic substances with unesterified units called pectate or polygalactuoronic acid (Gummandi and Kumar, 2006). According to the action mechanism, pectinase can be divided into two main groups: Esterases which remove the methoxyl groups from pectin, and depolymerases (hydrolases and lyases), which split the α-(1,4) glycosidic bonds in both of pectin and pectate (Hoondal et al., 2002), lyases split the α-(1,4) glycosidic bonds of pectic substances via a transe-elimination mechanism releases unsaturated products (Payasi et al., 2009). Pectate lyase enzymes catalyze the cleavage of the α-1,4-glycosidic linkages in the unesterified polygalacturonic acid and produce an unsaturated bond between C4 and C5 of each d-galacturonic acid unit (Soriano et al., 2006; Sukhumsirichart et al., 2009). Pectinases are widely distributed among microorganisms, such as yeast (Blancoa et al., 1999), bacteria (Takao et al., 2000), fungi (Patil and Dayanand, 2006). Pectinases have been used in various processes and industries, which in the degradation of pectic substances is essential (Favela-Torres et al., 2005; Sharma and Satyanarayana, 2006). Pectate lyase have a great commercial significance in industrial applications, extraction and clarification of fruit juices (Henrissat, 1991; Henrissat and Davies, 1997), maceration of vegetables, scoring of cotton fabric, and retting of

E-mail: esbsmail@yahoo.com

flax (Pilnik, 1990) and in other applications where alkaline pH processes are common such as paper making, and the textile industry to release fibers as an alternative to chemical conventional retting which cause many problems especially environmental pollution and high cost (Alkorta et al., 1998). This paper describes the purification and characterization of an extracellular pectate lyase from *Bacillus subtilis*.

## Materials and Methods

***Chemicals and reagents***

D-galacturonic acid, and Polygalacturonic acid were obtained from Sigma Co., All other chemicals were of analytical grade.

***Microorganism and Pectate lyase (PEL) production***

The bacterial strain, *Bacillus subtilis* (BPLSY1), used in this study was isolated from a soil sample in our laboratory for pectinases production and identified by 16S rRNA analysis as *Bacillus subtilis.* Submerged fermentation was performed to produce pectate lyase in 1L Erlenmeyer flask containing 250 ml of sterile production medium containing 0.5% Polygalacturonic acid as a substrate, 0.14% $(NH_4)_2SO_4$, 0.6% $K_2HPO_4$, 0.2% $KH_2PO_4$, 0.02% $MgSO_4.7H_2O$, 0.1% yeast extract, at pH 6.8. Production medium was inoculated with $10^6$ CFU of overnight-grown *B. subtilis* (BPLSY1). Culture was incubated in a rotary shaker and maintained for 72 h at 37°C with agitation, 150 rpm. Then culture was centrifuged at 10500 rpm, 4°C, for 10 min. The cell-free supernatant was used as a crude enzyme extract preparation.

***Enzyme assay***

Pectate lyase activity was assayed spectrophotometrically by measuring the increased absorbance at 232 nm of Δ 4,5-unsaturated reaction products due to *β*-elimination reaction. The molar extinction coefficient for the unsaturated product at 232 nm is 4600 $M^{-1}$ $cm^{-1}$ (Collmer et al., 1988). Reaction mixture containing 0.5 ml of enzyme solution and 2 ml of 0.24% (w/v) polygalacturonic acid as a substrate dissolved in 50 mM tris-HCl buffer, pH 8.0, and 0.5 mM $CaCl_2$. The reaction mixture was incubated at 50°C for 10 min. One unit of enzyme activity (U) was defined as the production of 1 µmol of unsaturated uronide per minute.

***Enzyme purification***

Enzymatic extract obtained previously was used for purification of pectate lyase, which performed through three steps as described below.

***Cold acetone precipitation***. Cell free supernatant obtained from 250 ml of cultivation broth was precipitated using chilled acetone at final concentration 80% with gentle agitation and then was kept at -20°C for 1 hour**.** The precipitate was collected by centrifugation at 14000 rpm for 20 min, at 4°C. Then the pellet obtained was redissolved in 50 ml of 50 mM sodium acetate buffer, pH 5.2. The concentrated enzyme solution was filtrated through 0.45 µm filter, and then was desalted by dialysis membrane with a cutoff of 2000 D against same buffer at 4°C for 24 h with continuous stirring. The buffer was exchanged 4 times at interval times, and kept at 4°C for further purification**.** Concentrated and dialyzed enzymatic broth was subjected to successive purification steps using fast-protein liquid chromatography (FPLC) through Hi-Trap SP and Superdex 75 10/300 GL columns. All steps of purification were carried out by (FPLC) at room temperature.

***Ion-exchange chromatography.*** 50 milliliter of the concentrated and dialyzed crude enzyme was loaded on a HiTrap SP (GE Healthcare, Uppsala, Sweden) column equilibrated with 50 mM sodium acetate buffer, pH 5.2. The protein adsorbed on the column was washed with the same buffer, and then eluted with a linear gradient of 0~500 mM NaCl in the same buffer at a flow rate of 1 ml/min. The eluted fraction containing PEL activity was pooled, concentrated and dialyzed against 50 mM sodium acetate buffer, pH 5.2 using Centricon-10 (Millipore, Bedford, Missouri, USA).

***Gel filtration chromatography.*** The desalted concentrate fraction containing PEL activity obtained from ion exchange column was put on Superdex 75 10/300 GL (GE Healthcare, Uppsala, Sweden) column (a high-resolution chromatography column) equilibrated with 50 mM sodium acetate buffer, pH 5.2. The enzyme was eluted with the same buffer at a flow rate of 0.5 ml/min. Fractions were analyzed for PEL activity, and the fraction containing PEL activity was used for the characterization as the final preparation of purified enzyme.

***Electrophoresis of proteins***

The Homogeneity and molecular mass of the enzyme were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on a 12.5% polyacrylamide gel (Laemmli, 1970), since silver staining was employed to identify protein bands. The molecular mass of the denatured pectate lyase was estimated from its position relative to those of standard protein marker (BioLabs P7708L) with known molecular mass ranging from 7–175 kDa.

***Protein assay***

Protein concentration was determined by Bradford method using bovine serum albumin (BSA) as a standard (Bradford, 1976). During purification, the protein concentration was estimated by assuming that a 1 mg/ml solution of protein will have an absorbance of 1 at 280 nm.

***Characterization of purified PEL***

Purified enzyme was characterized by studying the effects of each of $CaCl_2$ concentration, pH, and temperature on PEL activity. The pectate lyase activity assayed as described above. All experiments were performed in triplicate.

***Effect of $CaCl_2$.*** The effect of different concentrations of $CaCl_2$ (0, 0.5, 1.0, 1.5, 2) mM on activity of purified PEL was studied at pH 8, and 50°C.

***Determination of optimum pH.*** The influence of pH on the activity of purified PEL enzyme was probed at different pH, either in 50 mM Tris-HCl (pH 7-9), in 50 mM glycin-NaOH buffer (pH 9-10) or in 50 mM Carbonate-Bicarbonate buffer (pH 10-11). The reaction mixtures were incubated for 10 min at 50°C in presence of 0.5 mM $CaCl_2$, and then the change of absorbance at 232 nm was measured.

***Determination of optimum temperature.*** The temperature dependence of the purified PEL activity was tested at different temperatures (40°C, 45°C, 50°C, 55°C, 60°C and 65°C) at 0.5 mM $CaCl_2$ and optimum pH 9.5 for 10 min, and then the change of absorbance at 232 nm was measured.

## Results and Discussion

***Enzyme Purification***

The pectate lyase secreted by *Bacillus subtilis* strain (BPSY1) purified to homogeneity by cold acetone precipitation, ion exchange chromatography on HiTrap SP, and high-resolution size exclusion chromatography on Superdex 75. A summary of purification results was recorded in Table 1. The first chromatography yielded two main peaks, $P_1$ and $P_2$ (Figure 1). Enzyme assay for all peaks showed that $P_2$ had a specific activity of 28.8 $U.mg^{-1}$ of protein. The desalted $P_2$ was polished on a column of Superdex 75 (Figure 2). The resultant PEL enzyme was purified 9.86-fold with a yield of 2.59% to a specific activity of 58.85 $U.mg^{-1}$ of protein. Pectate lyase from *Bacillus pumilus* BK2 was purified seventeen fold

**Table 1**
**Purification of pectate lyase from *Bacillus subtilis*.**

| Steps | Total protein, mg | Total activity, U | Specific activity, $U.mg^{-1}$ | Yield, % | Purification, fold |
|---|---|---|---|---|---|
| Crude Extract | 80.00 | 477.50 | 5.97 | 100.00 | 1.00 |
| Cold acetone | 46.50 | 345.00 | 7.42 | 72.25 | 1.24 |
| Hi-trap SP | 1.20 | 34.56 | 28.80 | 7.24 | 4.82 |
| Superdex 75 | 0.21 | 12.36 | 58.85 | 2.59 | 9.86 |

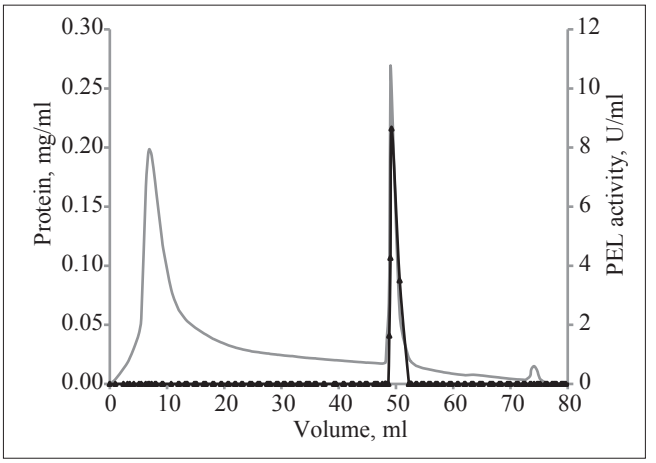


**Fig. 1. Elution profile of *Bacillus subtilis* BPSY1 pectate lyase on HiTrap SP**

The column was equilibrated with 50 mM sodium acetate buffer, pH 5.2. The enzyme solution was eluted with a linear salt gradient (0 to 500 mM NaCl) in the same buffer. Protein was estimated by absorbance at 280 nm, and enzyme activity was determined as described in the text. ——, Protein (mg/ml); —►—, PEL activity (U/ml).

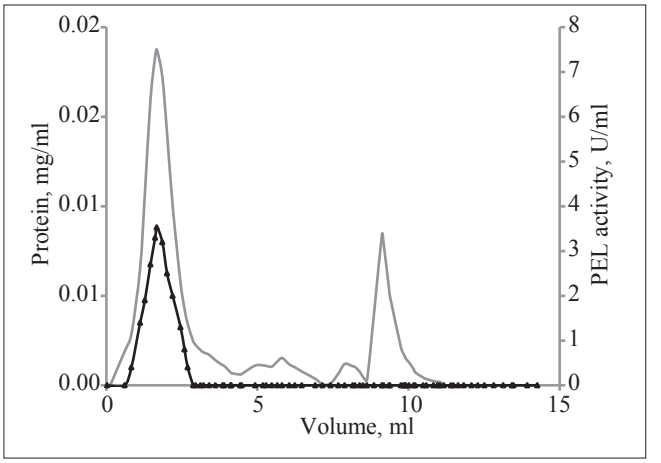


**Fig. 2. Size exclusion profile of *Bacillus subtilis* BPSY1 pectate lyase on Superdex75**

The column was equilibrated with 50 mM sodium acetate buffer, pH 5.2. The enzyme solution of HiTrap Sp column ($P_2$) was eluted in the same buffer. Protein was estimated by absorbance at 280 nm, and enzyme activity was determined as described in the text.
——, protein (mg/ml); —►— PEL activity (U/ml).

by using ammonium sulfate precipitation, hydrophobic interaction and size exclusion chromatography (Klug-Santner et al., 2006). Whereas 25 fold increase in specific activity for the pectate lyase by *Bacillus sp.* N16-5 was obtained (Li et al., 2010). At the end of the steps, the purity of enzyme was confirmed by using SDS-PAGE, which visualized by silver staining, only one band at an apparent molecular weight of 42 kDa was detected, indicating complete purification of the enzyme (Figure 3). Many papers reported that the purified PEL enzymes synthesized by *B. subtilis* have a molecular weight of either 23 kDa (Soriano et al., 2006) or 42 kDa (Nasser et al., 1990; Zhuge et al., 2007; Li et al., 2010).

### *Characterization of purified Pectate Lyase (PEL)*

#### *Effect of $CaCl_2$ concentration*

The effect of $Ca^{2+}$ ions on PEL activity from BPSY1 was determined at different concentrations of $CaCl_2$. Results showed that purified enzyme has an absolute requirement of $Ca^{2+}$ ions for polygalacturonic acid degradation. The main feature, which distinguishes pectate lyase from pectin lyase, is the absolute requirement of $Ca^{2+}$ ions for the activity of pectate lyase (Kita et al., 1996). This ion is needed for calcium binding site of the enzyme (Sakai et al., 1993). The maximum activity was found at 0.5 mM $CaCl_2$, and then the activity was decreased at higher $CaCl_2$ concentrations as illustrated by Figure 4. No activity was detected in absence of this ion, PEL exhibited maximum activity at 0.5 - 0.75 mM of $CaCl_2$ (Margarita et al., 2006). A 0.6 mM of $CaCl_2$ was the optimum concentration for activity of PEL from *Bacillus sp.* KSM-P15 (Sawada et al., 2000), while 2 mM of $CaCl_2$ has been reported as optimum concentration for same enzyme from *B. subtilis* 168 (Soriano et al., 2006).

#### *Effect of pH on the enzyme activity*

The effect of pH on the activity of purified extracellular PEL from *B. subtilis* BPSY1 was determined at different pH values ranging between 7 and 11. The purified PEL exhibited maximum activity at pH 9.5. When pH is altered below or above the optimum, the activity of pectate lyase is decreased (Figure 5.). The range of pH (8–10.5) reported as optimum for microbial pectate lyases (Payasi et al., 2009). Whereas the pH 8.4 was the optimum for the same enzyme from *B. subtilis* (Nasser et al., 1990), at pH 10 (Soriano et al., 2006) and pH 8 for PEL from *Bacillus sp.* TS 47 (Takao et al., 2000). The decreasing of enzyme activity at pH values away of the optimum pH could be due to the effect of pH on the stability of the enzyme (Dixon and webb, 1979).

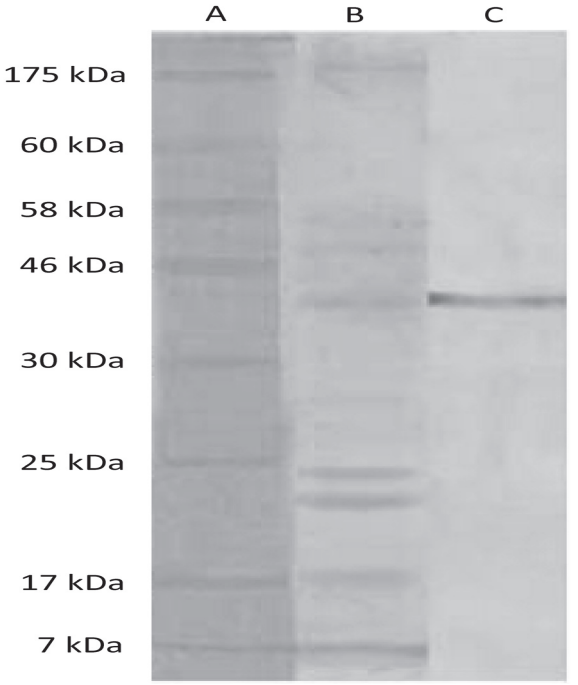


**Fig. 3. Silver stained SDS-PAGE (12.5%) of the crude and puirified enzyme**

Lane A, molecular weight markers from Biolabs; Lane B, crude enzyme; Lane C, purified enzyme.

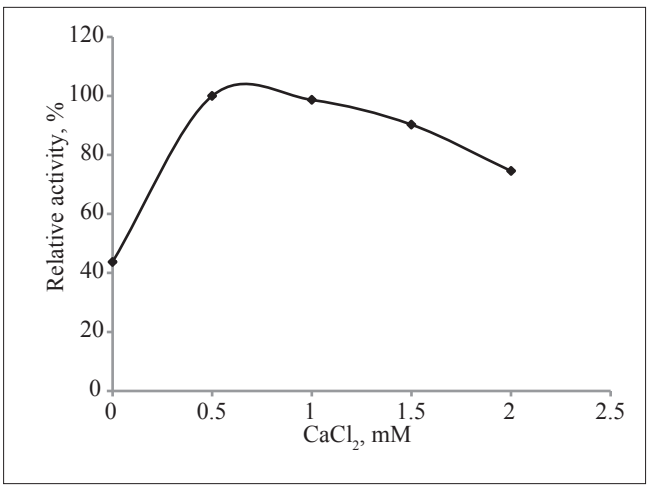


**Fig. 4. Effect of $CaCl_2$ concentration on activity of purified extracellular PEL from BPSY1 strain**

PEL activity was assayed at 50°C, pH 8.0 up to 10 min at the indicated $CaCl_2$ concentrations. The graphics are obtained by fitting the experimental data (mean values of three different experiments) to the following equation: Relative activity = (Am/Amax) x100, Am represents the activity of the PEL measured at different concentration of $CaCl_2$, and Amax, the maximum activity observed for the PEL at its optimum $CaCl_2$ concentration (0.5 mM).

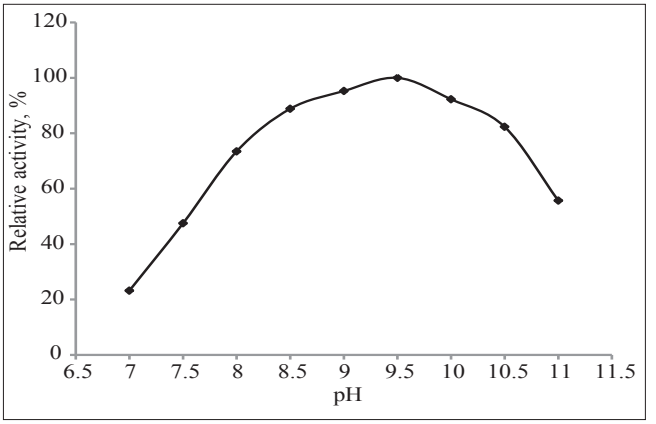


**Fig. 5. Effect of pH on activity of purified extracellular PEL from BPSY1 strain**

PEL activity was assayed at 50°C up to 10 min in presence of 0.5 mM $CaCl_2$ and at the indicated pH either in 50 mM Tris-HCl (pH 7-9), in 50 mM glycin-NaOH buffer (pH 9–10), or in 50 mM Carbonate-Bicarbonate buffer (10-11). The graphics are obtained by fitting the experimental data (mean values of three different experiments) to the following equation:

Relative activity = (Am/Amax) x100, Am represents the activity of the PEL measured at different pH (7-11), and Amax, the maximum activity observed for the PEL at its optimum pH (pH 9.5).

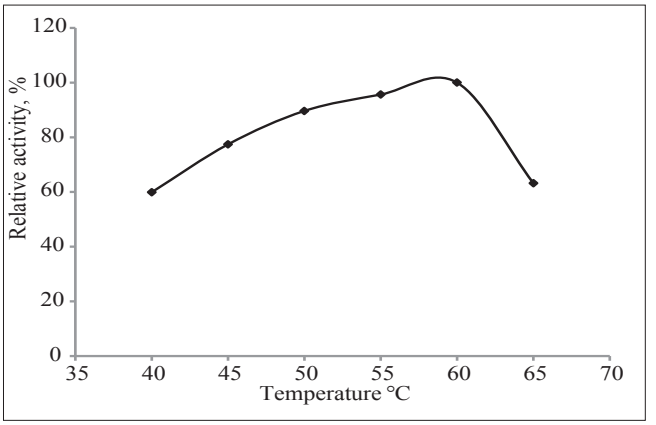


**Fig. 6. Effect of temperature on activity of purified extracellular PEL from BPSY1 strain**

PEL activity was assayed at optimum pH 9.5 up to 10 min in presence of 0.5 mM $CaCl_2$ and at the temperature range (40°C-65°C). The graphics are obtained by fitting the experimental data (mean values of three different experiments) to the following equation:

Relative activity = (Am/Amax) x100, Am represents the activity of the PEL measured at different temperature (40°C-65°C), and Amax, the maximum activity observed for the PEL at its optimum temperature (60°C).

***Effect of temperature***

In order to determine the effect of temperature on the activity of purified pectate lyase, the standard PEL assay procedure at different temperatures ranging from 40°C to 65°C was performed at optimum pH and $CaCl_2$ concentration, 9.5 and 0.5 mM, respectively. The purified PEL enzyme exhibited optimum activity at a temperature of 60°C. With further increase of temperature, the PEL activity was decreased (Figure 6.), This may be a result of thermal denaturation of the enzyme possibly due to disruption of non-covalent linkages, including hydrophobic interactions (Georis et al., 2000; Bhatti et al., 2006). Several studies on pectate lyase from *B. Subtilis* showed optimum temperature at 40°C (Nasser et al., 1990), 70°C (Takao et al., 2000) and 65°C (Soriano et al., 2006).

## Conclusion

In this experimental work, the purification and some properties of alkaline pectate lyase from *Bacillus subtilis* were studied, pectate lyase exhibited optimal activity at alkaline pH 9.5, 60°C and 0.5 mM $CaCl_2$. From this characterization of pectate lyase from *B. subtilis* (BPSY1), it may be a potential candidate for industrial uses. Further studies on the pectate lyase of *B. subtilis* are necessary. Cloning and sequencing of the pectate lyase gene are in progress in our laboratory.

***Acknowledgements***

The authors would like to thank the Director General of the AECS and the Head of the Molecular Biology and Biotechnology Department for their support.

## References

**Alkorta, I., C. Garbisu, M. J. Llama and J. L. Serra**, 1998. Industrial applications of pectic enzymes: A review. *Process Biochemistry*, **33**: 21-28.

**Bhatti, H. N., M. Asgher, A. Abbas, R. Nawaz and M. A. Sheikh**, 2006. Studies on kinetics and thermostability of a novel acid invertase from *Fusarium solani*. *Journal of agricultural and food chemistry*, **54**: 4617-4623.

**Blancoa, P., C. Sieiroa and T. G. Villaa,** 1999. Production of pectic enzymes in yeasts. *FEMS Microbiol. Lett*., **175**: 1-9.

**Bradford, H. M.**, 1976. Arapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein–dye binding. *Anal Biochem*., **72**: 248-254.

**Collmer, A., J. L. Ried and M. S. Mount**, 1988. Assay methods for pectic enzymes. *Methods in enzymology*, **161**: 329-335.

**Dixon, M. and E. C. Webb**, 1979. Enzyme kinetics, In: Enzymes, 3d. Academic Press. New York, San Francisco, A subsidiary of Harcourt Brace Jovanovich, Publishers,Longman Group, p. 47

**Favela-Torres, E., C. Aguilar, J. C. Contreras-Esqivel and G. Viniegra-González**, 2005. Pectinase. In: Enzyme Technology,

ed. by Pandey, A., Webb, C., Soccol, C.R., and Larroche, C., Springer Verlag, Berlin, Germany, pp. 273–296.

**Georis, J., F. L. Esteves, J. L. Brasscur, V. Bougnet, J. L. B. Devreese and F. Giannotta**, 2000. An additional aromatic interaction improves the thermostability and thermophilicity of a mesophilic family xylanase: Structural and molecular study Protein Science. **9**: 466-475.

Gummandi, S. N. and D. S. Kumar, 2006. Optimization of chemical and physical parameters affecting the activity of pectin lyase and pectate lyase from *Debaryomyces nepalensis*: A statistical approach. *J Biochem Eng*., **30**: 130-137.

**Henrissat, B**., 1991. A classification of glycosyl hydrolases based on amino acid sequence similarities. *Biochem J*., **280**: 309-316.

**Henrissat, B. and G. J. Davies,** 1997. Structural and sequence-based classification of glycoside hydrolases. *Curr Opin Struc Biol*., **7**: 637-644.

**Hoondal, G., R. Tiwari, R. Tewari, N. Dahiya and Q. Beg**, 2002. Microbial alkaline pectinases and their industrial applications: a review. *Applied Microbiology and Biotechnology,* **59**: 409-418.

**Kashyap, D. R., P. K. Vohra, S. Chopra and R. Tewari**, 2001. Applications of pectinases in the commercial sector: a review. *Bioresource Technology*, **77**: 215-227.

**Kita, N., C. M. Boyd, M. R. Garrett, F. Jurnak and N. T. Keen**, 1996. Differential effect of site-directed mutations in Pel C of pectate lyase activity, plant tissue maceration, and elicitor activity. *Journal of Biological Chemistry*, **271**: 529-535.

**Klug-Santner, B. G., W. Schnitzhofer, M. Vrľanská, J. Weber, P. B. Agrawal, V. A. Nierstrasz and G. M. Guebitz**, 2006. Purification and characterization of a new bioscouring pectate lyase from *Bacillus pumilus* BK2. *Journal of biotechnology* **121**: 390-401.

**Laemmli, U. K**.. 1970. Cleavage of structural proteins during the assembly of head bacteriophage T4. *Nature,* **22**: 680-685.

**Li, G., L. Rao, Y. Xue, C. Zhou, Y. Zhang and Y. Ma**, 2010. Cloning, expression, and characterization of a highly active alkaline pectate lyase from alkaliphilic *Bacillus sp.* N16-5. *J. Microbiol. Biotechnol*., **20**: 670-677.

**Margarita, S., D. Pilar and I. J. P. Francisco**, 2006. Pectate lyase C from *Bacillus subtilis*: a novel endo-cleaving enzyme with activity on highly methylated pectin. *Microbiology*, **152**: 617-625.

**Nasser, W., F. Chalet and J. Robert-Baudouy**, 1990. Purification and characterization of extracellular pectate lyase from *Bacillus subtilis*. *Biochimie.*, **72**: 689-695.

**Patil, S. R. and A. Dayanand**, 2006. Optimization of process for the production of fungal pectinases from deseeded sunflower head in submerged and solid-state conditions. *Bioresource technology*, **97**: 2340-2344.

**Payasi, A., R. Sanwal and G. Sanwal**, 2009. Microbial pectate lyases: characterization and enzymological properties. *World Journal of Microbiology and Biotechnology*, **25**: 1-14.

**Pilnik, W**., 1990. Pectin: a many splendoured thing. In Gums and stabilizers in the food industry, ed. by: Wedlock, D .J. and Willima, P.A., Oxford: Oxford University press, pp. 209-211.

**Sakai, T., T. Sakamoto, J. Hallaert and E. J. Vandamme**, 1993. Pectin, pectinase and protopectinase: production, properties and applications. *Advances in Applied Microbiology*, **39**: 213-294.

**Sawada, K., A. Ogawa, T. Ozawa, N. Sumitomo, Y. Hatada, T. Kobayashi and S. Ito**, 2000. Nucleotide and amino-acid sequences of a new-type pectate lyase from an alkaliphilic strain of *Bacillus*. *European Journal of Biochemistry*, **267**: 1510-1515.

**Sharma, D. C. and T. Satyanarayana,** 2006. A marked enhancement in the production of a highly alkaline and thermostable pectinase by *Bacillus pumilus* dcsr1 in submerged fermentation by using statistical methods. *Bioresour. Technol*., **97**: 727-733.

**Soriano, M., P. Diaz and F. Pastor,** 2006. Pectate lyase C from *Bacillus subtilis*: a novel endo-cleaving enzyme with activity on highly methylated pectin. *Microbiology*, **152**: 617-625.

**Sukhumsiirchart, W., S. Kawanishi, W. Deesukon, K. Chansiri, H. Kawasaki and T. Sakamoto**, 2009. Purification, characterization, and overexpression of thermophilic pectate lyase of *Bacillus sp.* RN1 isolated from a hot spring in Thailand. *Bioscience, biotechnology, and biochemistry*, **73**: 268-273.

**Takao, M., T. Nakaniwa, K. Yoshikawa, T. Terashita and T. Sakai**, 2000. Purification and Characterization of Thermostable Pectate Lyase with Protopectinase Activity from Thermophilic *Bacillus sp.* TS 47. *Bioscience, Biotechnology, and Biochemistry*, **64**: 2360-2367.

**Walton, J. D**., 1994. Deconstructing the cell wall. *Plant Physiology*, **104**: 1113-1118.

**Zhuge, B., G. Du, W. Shen, J. Zhuge and J. Chen**, 2007. Efficient secretory expression of an alkaline pectate lyase gene from *Bacillus subtilis* in E. coli and the purification and characterization of the protein. *Biotechnology Letters*, **29**: 405-410.

*Received September, 2, 2013; accepted for printing December, 2, 2013.*