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Production, Purification and Characterization of Polygalacturonase from Bacillus licheniformis SHG10

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ABSTRACT

Background*:** Microorganisms are the best source of pectinolytic enzymes as they allow an economical technology with low resource consumption. ***Objectives: The present study was carried out to isolate, purify and characterize polygalacturonase (PGase) form *Bacillus licheniformis* SHG10. ***Methods:*** The concentrated dialysed cell free extract was loaded on prepacked DEAE-Sepharose Fast-Flow ion exchange chromatography. The active PGase fractions were concentrated and loaded again on sephacryl Fast-Flow High Resolution (FF S-100 HR) chromatography. Molecular weight was determined using slab-gel SDS-Polyacrylamid gel electrophoresis and Characterization of the purified enzyme was performed. ***Results:*** The results showed that the enzyme was purified with a purification fold (33.34) and yield (41.73%) with specific activity (8.67 µ moles/min/mg protein). It has a molecular mass of about 68.0 kDa. While, on SDS-PAGE electrophoresis, the purified PGase appeared as single band with molecular mass of about 34.0 kDa, suggesting that the purified PGase is a dimer protein. The purified enzyme exhibited maximal activity at a temperature of 45^{o}C and pH 8.5. The maximum velocity of the enzyme in presence of citrus pectin and pectate as substrates were 10.98 and 14.7 µ mol galacturonate/min/mg protein, respectively. The Michaelis constant (Km) values were 0.085 and 0.039 mM, respectively, indicating that the purified PGase has higher affinity to pectate (non-methylated pectic substance) than citrus pectin as substrate.

Introduction:

Pectinases catalyze the degradation of pectin, a polysaccharide, found in plant cell walls. They include pectolyase, pectozyme and polygalacturonase depending on the substrate specificity and mode of action [(1)]. Plant and microorganisms are the major sources of pectinases. Industrial economic pectinases are produced by eukaryotic microorganisms, mostly fungi such as *Aspergillus japonicus*, *Rhizopus stolonifer*, *Fusarium oxysporum* and *Neurospora crassa* [(2)]. Also, pectinases are produced by prokaryotes *as Agrobacterium tumefaciens*, *Bacteroides thetaiotamicron*, *Ralstonia solanacearum*, and *Bacillus* species [(3)]. Furthermore, the production of these enzymes has also been described in yeast, insects, nematodes and protozoa [(4, 5)].

Microbial pectinases account for 10-25% of the global food and

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industrial enzyme [6]. Among pectinolytic enzymes, polygacturonases (PGases) are the enzymes of particular interest to industry. PGases catalyze the hydrolysis of α-1,4 glycosidic bond linking α-galacturonic acid residues in pectin. The pectic substances of plant cell walls contain mostly polymerized galacturonic acid (polygalacturonic acid), producing shorter pectin molecular structures. Thus, decreasing the viscosity, increasing the yield of juices and determining the crystalline structure of the final product. They have a share of 25% in the global sale of the commercial food enzymes [7]. Bacterial PGases are mainly reported to be extracellular enzymes, often secreted in several isoforms [8] [9].

Our previous study, Embaby et al., (2014) showed the optimum condition for PGase production from *Bacillus licheniformis* SHG10 through a sequential statistical optimization [10]. Therefore, the present study extended to isolate, purify and characterize *Bacillus licheniformis* SHG10 polygalacturonase.

Materials and Methods:

Materials:

Bacillus licheniformis strain SHG10, was isolated and identified from Egyptian soil. Its 16S rDNA nucleotide sequence was submitted in the GenBank at the National Centre of Biotechnology Information (NCBI), under the accession number [GenBank: JN853580]. Labscale Tangential Flow Diafiltrator ultrafiltration Centricon 10, membrane cut-off of 10 kDa, (Labscale TFF system). Prepacked (1.6×10 cm) DEAE- Sepharose column and pre-packed HiPrep (1.6 x 60 cm) sephacryl FF S-100 HR, were purchased from Amersham Biosciences AB, Uppsala, Sweden. The DEAE-Sepharose and the Sephacryl chromatographic columns were conducted to Sigma-FF system (AKTA Purifier, GE Healthcare). Pectin, standard galacturonate sodium salt, Bovine serum albumin (BSA), Folin-Ciocalteauphenol, acrylamide, were purchased from Sigma-Aldrich.

Protein Assay:

Protein content was determined colorimetrically according to Lowry *et al.* (1951) [11].

Polygalacturonase Assay:

Polygalacturonase was assayed in a reaction mixture containing, citrus pectin (0.1mM) as substrate and 0.1 ml enzyme in 50 mM Tris-HCl buffer (pH 8.5). The reaction mixture was incubated at 37°C for 20 min. The activity was estimated colorimetrically via determining the released reducing sugar galacturonate [12], using 3, 5-dinitrosalycilic acid (DNS) determination [13]. Standard curve of galacturonate (product) was established with spectrophotometric measurements at 540 nm. Enzyme activity was expressed in terms of specific activity (μmole galacturonate/ min./ mg protein).

Purification of Bacillus licheniformis SHG10 Polygalacturonase:

The purification of *B. licheniformis* was performed according to Greiner *et al.,* (1993) through three main steps [14], including:

Step (1): Production, Concentration and Dialysis

Our previous study; Embaby et al. (2014) optimized the production of PGase from *Bacillus licthenifomis* SHG10 [10]. The crude extract (cell free extract) was prepared by centrifugation of the culture broth at 10,000 rpm and 4°C for 15 minutes. The supenatent (cell free extract, 250 ml) was concentrated using ultrafiltration to fifth of its volume. Protein content and enzyme activity were determined. Then, the

crude extract was subjected to the second purification step.

Step (2): DEAE- Sepharose Fast Flow Anion Exchange Chromatography

The crude cell free extract resulted from step (1) was applied on pre-packed column with DEAE-Sepharose (1.6 x 10 cm), pre-equilibrated with 20 mM Tris-buffer (pH 7.5). The protein samples were eluted with a salt gradient from 0 to 1.0 M NaCl at flow rate 1 ml/min at 4°C. Protein absorbance was measured at 280 nm and the enzyme activity was determined. The fractions of the peak active PGase activity were pooled, concentrated by ultrafiltration to 20 ml and subjected to the third step.

Step (3): Sephacryl Fast Flow High Resolution (FF S-100 HR) Chromatography

The partial purified enzyme from step (2) was applied on pre-packed HiPrep (1.6 x 60 cm) sephacryl FF S-100 HR column, pre-equilibrated with 20 mM Tris buffer (pH 7.5). The protein samples were eluted using 20mM Tris buffer containig 0.2 M NaCl with a flow rate 1 mL/min at 4°C. Protein absorbance was measured at 280 nm and the enzyme activity was determined. The fractions of the peak active purified PGase were pooled and concentrated by ultrafiltration to 4ml and used for studying the enzyme characterisitics.

Sodium Dodecyl Sulfate–Polyacrylamide Slab Gel Electrophoresis (SDS-PAGE)

The purified PGase as well as the standard proteins were subjected to slab gel SDS-PAGE **(15)** for molecular weight determination according to Maniatis *et al.,* (1989) **(16)**. The electrophoresis was applied at 5 mA for 10 min then the current was increased to 15 mA till the end of the electromigration. The gel was stained with bromophenol blue and destained with acetic acid.

Characteristics of the Purified Bacillus licheniformis SHG10 PGase

Optimum pH and temperature

Citrus pectin (5 mg/mL) as substrate was incubated with 0.1 mL of the purified enzyme (30 µg protein). Different pH (3.5-11) were used for the optimum pH determination. The incubation was carried out at 45°C for 20 min. For the determination of the optimum temperature, incubation was carried out at different temperature degrees (25 – 80°C) for 20 min at pH 8.5.

pH, thermal and storage stabilities

For detection of pH stability, purified PGase fractions (30 µg each) in 0.3 mL of 50 mM Tris-HCl, were incubated at different pH-values (6.5-10.5) and different time intervals (0, 30, 60, 120 min) at 45°C. For temperature stability, PGase fractions (30 µg each) were preincubated at different temperature degrees (30-80°C) at pH 8.5 for the same different time intervals (0, 30, 60, and 120 min) **(17)**. For examining storage stability, PGase fractions were kept at -20°C for 1, 2 weeks and 2 months and the enzyme activity was determined at the optimum conditions (pH 8.5 and 45°C).

Effect of a chelating agent metal cations and detergent:

Effect of different concentrations (0.01-1mM) of ethylenediaminetetraacetic acid (EDTA) and mercuric chloride, as well as magnesium and calcium chloride were studied. The enzyme was incubated with each compound for 10 min before the enzyme assay. Also, different concentrations (0-5 mM) of Tween-20 as a non ionic surfactant were examined.

Substrates Specificity

The PGase specific activities (μmole galacturonate/ min./mg ptotein) were determined with three different substrates, (citrus pectin, pectate and chitosan) at a concentration of 0.1mM each in 50 mM Tris-HCl (pH 8.5) with 0.1 mL of the purified enzyme (30 μg protein) at optimum conditions. A control for each substrate was done.

Determination of the Kinetic Parameters of the Purified Polygalacturonase

Kinetic parameters were performed at optimum conditions of pH 8.5 and temperature 45°C in prescence of two substrates (citrus pectin and pectate) with concentration range from 0.5 to 10 mg/mL in 50 mM Tris-HCl (pH 8.5) and 0.1 mL of the purified enzyme (30 μg protein content).

Results

Production and concentration of Bacillus licheniformis SHG10 Polygalacturonase

The volume of *Bacillus licheinformis* SHG10 cell free extract (crude enzyme) was 250 mL, with a total protein content 287.5 mg and total PGase activity 75 U. The yield was 100% and the purification factor was one. This crude enzyme volume was concentrated to 50 mL by ultrafiltration giving a total protein content of 271.5 mg, while the total PGase activity was reduced to 72 U (about 96.53% of the total PGase activity of the previous step) with a purification fold 1.02 (Table 1).

Purification of Bacillus licheniformis SHG10 Polygalacturonase:

Step (1): DEAE-Sepharose Fast Flow ion exchange chromatography

The elution profile of DEAE-Sepharose FF (Figure (1)), indicates several peaks of protein with a single peak of PGase activity, giving a maximum activity at gradient between 0.5-0.625 M NaCl. PGase peak fractions were collected giving total volume of 16 mL that was concentrated to 2 mL by ultrafiltration. This step gave a yield 0f 57.7% with purification fold reached to 27.4 (Table 1).

Step (2): Sephacryl FF S-100 HR Chromatography

The elution profile of Sephacryl S-100 chromatography eluent (Figure (2)), indicates a sharp single peak of protein and PGase activity estimating a molecular mass of 68 kDa for the purified PGase by comparing with the standard curve of proteins separated by the same column at the same elution conditions.

PGase fractions (17 to 20) were collected giving total volume of 10 ml with a protein concentration of 0.361 mg protein/mL and specific activity of 8.67 μmole galacturonate/ min./ mg protein and a yield of 41.73%. The purification fold was equal to 33.34 (Table 1).

SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE plate (Figure (3)) shows a single band for the purified *Bacillus licheniformis* SHG10 PGase with a molecular mass of about 34.0 kDa, in comparison to standard proteins. Comparing the molecular mass of PGase calculated by gel filtration with that by SDS-PAGE, it was concluded that the purified PGase is a dimer protein.

Characteristics of the Purified Bacillus licheniformis SHG10 Polygalacturonase

Optimum pH and temperature

Bacillus licheniformis SHG10 PGase showed maximum activity at optimal pH of 8.5 (Tris-HCl buffer), so it is an alkaline polygalacturonase. The activity declined gradually between pH

9-11 (Figure (4a)). However, Figure (4b) discloses that the enzyme activity increased gradually from 25°C, reaching maximum value at 45°C. Thereafter, it decreased.

pH, thermal and storage Stabilities

The results recorded in Figure (5) showed that at pH 8.5 (using Tris-HCl buffer), the enzyme activity exhibited highest stability at 120 min exposure. At pH 6.5 and 7.5 the enzyme retained about 84.4 and 87.5% activity, respectively, after 120 min exposure. It is also obvious that the enzyme lost about 15 and 21%, respectively at pH 9.5 and 10.5, of its full activity after 120 min.

Figure (5b) shows that the purified PGase was stable after incubation for 120 min at 45°C. While, it retained only about 64.3, 61 and 53.4% of its activity when incubated at 60°C for 30, 60 and 120 min, respectively.

The specific activity of purified PGase (8.96 µmole galacturonate/min/mg protein) after storage for 2 months in 50 mM Tris-HCl at -20°C and pH 8.5 retained about 96.8% of its original zero time activity (9.261µmole galacturonate/min/mg protein as 100% activity).

Effect of a chelating agent, metal cations and detergent:

Bacillus licheniformis SHG10 PGase activities were decreased by about 36.7, 41 and 69.8 %, respectively, in the presence of 0.01, 0.5 and 0.1 mM of EDTA (Figure (6)) comparing with that of control.

The addition of 0.01, 0.05 and 0.1 mM of $HgCl_2$ (Figure (6A)) inhibited the PGase activity by 28.5, 50.8 and 87.06 %, respectively compared to the control. Complete inhibition of PGase activity was noticed at higher concentrations of EDTA and $HgCl_2$.

PGase activity was increased with increasing Ca^{+2} concentration up to 183.2%, compared to the control (taken as 100%) at 10 mM $CaCl_2$. At 20 mM concentration, the stimulatory effect of this metal cation was decreased to be non-significant with respect to control (Figure (6B)). However, an inhibitory effect of high concentrations of $MgSO_4$ was detected.

The PGase activity was increased with increasing the concentration of Tween-20 till a maximum value (219.95% increase) recorded at 0.1 mM (Figure (6C)). Then, the activity was decreased but still higher than the control by about 28.7 %.

Substrate specificity

Both citrus pectin (67% esterification) and Sodium pectate were hydrolyzed by the purified PGase with different rates 100 and 136.5%, respectively. But chitosan was not hydrolyzed.

Kinetic Parameters of the Purified Bacillus licheniformis SHG10 PGase

Lineweaver-Burk reciprocal plot of the purified PGase with two different substrstes, citrus pectin and pectate is shown in Figure (7). Through Km values, it may be postulated that the purified *B. licheniformis* SHG10 PGase has higher affinity to pectate (Km= 0.039 mM and Vmax= 14.7 µmole galacturonate/ min. mg protein) than citrus pectin (Km=0.085 mM and Vmax= 10.98 µmole galacturonate/ min. mg protein), indicating that pectate is more specific substrate. Comparing the present findings with previous reports, it may be concluded that this purified enzyme is a polygalacturonase (PGase) rather than a polymethlylgalacturonase (PMGase). Where, the later has higher affinity to citrus pectin (methylated pectic substrate).

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DISCUSSION

The appearance of the PGase enzyme as one peak in the elution profile of DEAE-Sepharose FF (Figure (1)) indicates that this enzyme is present in one form i.e. there is no isoenzyme. Comparing the molecular mass of PGase calculated by gel filtration with that by SDS-PAGE, it was concluded that the purified PGase suggested to be dimer protein.

Mohsen *et al.*, (2009) purified a dimer protein of *Aspergillus niger* U-86 PGase which appeared as two bands on SDS-PAGE with the molecular mass of about 36 and 38 kDa, after ammonium sulfate fractionation followed by gel filtration on sephadex G-75 [(18)].

The molecular mass of *Mucorcir cinelloides* PGases was reported to be about 63 and 66 kDa [(19)]. Additionally, the molecular mass of most purified PGases was in range of 30-35 kDa as PGases purified from *Fusarium monilifor* [(20)], *Saccharomyces cerevisiae* [(21)] and *Risomucor pusilis* [(22)].

The decline of *Bacillus licheniformis* SHG10 PGase activity between pH 9-11 (Figure (4a)) is in agreement with previous results for enzymes of bacterial origins, showing optimal pH values in alkaline conditions. Tariq and Latif, (2012) recorded higher hydrolytic activity of *Bacillus* strains Z-AT23, Z-AT33 and Z-AT35 on pectin at pH 7.0 to 8.0 [(23)].

Concerning optimum temperature detection, Figure (4b) reveals that the decrease in PGase at temperatures above 50^{o}C may be due to partial denaturation. This is consensual with that previously recorded for optimal temperatures of PGases from different sources, *Mucor flavus*, *Sclerotinia sclerotiorum* and *Trichoderma reesei* [(24-26)] all were around 40 and 50^{o}C.

The present study proved that the enzyme activity exhibit highest stability at pH 8.5. Similarly, Kashyap *et al.*,(2000) reported that pectinase isolated from *Bacillus* sp. DT7 had maximal stability under alkaline condition of pH 7.5-8.5; enzyme retained 50% of its activity at neutral pH with complete loss of activity at pH values less than 6 [(27)]. However, fungal PGases showed stability at acidic conditions. It was reported that *Mucor flavus* PGase was completely stable between pH 2.5 and 6.0 for 20 h at 20^{o}C but at pH 7.0, the stability was decreased to about 60% [(24)].

The thermal inactivation of PGase at extremely high temperatures may be related to deamination, hydrolysis of the peptide bonds, interchange, and destruction of disulphide bonds and oxidation of the amino acids side chains of the enzyme protein molecules [(28)].

PGaseII of *Trichoderma harzianum*; was found to have temperature optimum at 40^{o}C and was approximately stable up to 30^{o}C for 60 min [(29)].

The high stability of purified PGase after storage for 2 months in 50 mM Tris-Hcl at -20^{o}C and pH 8.5 is in good concurrence with that of Mohamed et. al. (2006) who reported high storage stability for liquid form (3 months) of *Trichoderma harzianum* PGase II and III and powder form (1 year), however PGase I was unstable and could not be stored.

The inhibitory effect of chelating agent EDTA on *Bacillus licheniformis* SHG10 PGase activities demonstrated the ion requirements of this PGase activity. This is in agreement with the reports of Juwon *et al.*, (2012) who showed decreasing in the activity of *Aspergillus niger* CSTRF PGase with

EDTA, $PbCl_2$ and $HgCl_2$ addition [30]. Also, Sun (2014) reported that inhibition effect on the purified kiwifruit PGase activity was increased with increasing concentration of EDTA [31], this is also the case in the current study.

The inhibition of PGase by $HgCl_2$ may be related to the reaction of Hg^{2+} with protein sulphydryl group converting it to mercaptides that confirms the essential role of the -SH groups to maintain the catalytic activity of PGase activity. These results are in consonance with the reports of Thakur *et al.* (2010) who reported a strong inhibitory effect of $HgCl_2$ upon *Mucorcircinelloides* ITCC 6025 PGase [19].

The increase of PGase activity with increasing Ca^{+2} concentration may be related to the vital role of Ca^{+2} in maintaining the active confirmation of the purified PGase where Brown and Kelly (1993), showed that the formation of a chelate complex between the substrate and the metal ions could form a more stable metal-enzyme-substrate complex and stabilizing the catalytically active protein conformation [32]. On the other hand, the inhibitory effect of $MgSO_4$ at higher concentrations indicates that the previously noticed stimulatory effect of Mg^{+2} on SHG10 PGase production was due to its effect on bacterial growth in addition to its role in enzyme production [10].

Mucor circinelloides PGase showed 5–15% decrease in enzyme activity in presence of Mn^{+2}, Co^{+2}, and Mg^{+2} [19]. In contrast, studies on *B.gibsonii*S-2 PGases showed that Mg^{2+} (0.1mmol/L $MgSO_4$) stimulated the PGase activity, while Ca^{2+} (0.1 mmol/L $CaCl_2$) stimulated the PGase activity of S-I and S-II, but inhibited the activity of S-III [33]. Aminzadeh *et al.,* (2006) reported that all studied metals (Al^{3+}, Ba^{2+}, Ca^{2+}, Fe^{2+}, $Fe^{3+,}$ $Ni^{2+}$and Mg^{2+}) inhibited PGase of *Tetracoccosporium*sp [34]. This discrepancy in the divalent metal ion preference suggested that these enzymes might have differential flexibility in the active site.

The enhancement of PGase activity under the effect of increasing concentration of Tween-20 can be explained by some previous studies which proved that surfactants may promote the availability of reaction sites and increase the hydrolysis rate [35, 36].

The hydrolysis of citrus pectin and sodium pectate by means of PGase clarified the high specificity of the purified PGase towards pectic substrates with high specificity for their configuration and kind of bonding which is hydrolyzed. It has been reported that PGases from different microbial sources have specificity towards pectins with different degree of esterification and methylation [37-39]. The results showed that the purified enzyme has high specificity to hydrolyze the glycosidic linkage between galacturonate moieties in pectic substances. It could not break the linkage between N-acetyl gluconate moieties in chitosan.

The kinetic parameter Km indicates that the purified *B. licheniformis* SHG10 PGase has higher affinity to pectate than citrus pectin revealing that pectate is more specific substrate. The kinetic constants of the purified *B.licheniformis* SHG10 PGase are quite different from that of PGase from *Aspergillus niger* CSTRF which showed approximately K_m value of 2.7 mg/mL [30]. The apparent K_m value of PGase produced by *Aspergillus niger* U-86 was calculated to be 1.42 mg. ml^{-} [18]. The apparent Km and V_{max} values of PGase from *Rhizomucor pusillus* were 0.22 mg/mL and 4.34 U/mL, respectively [22].

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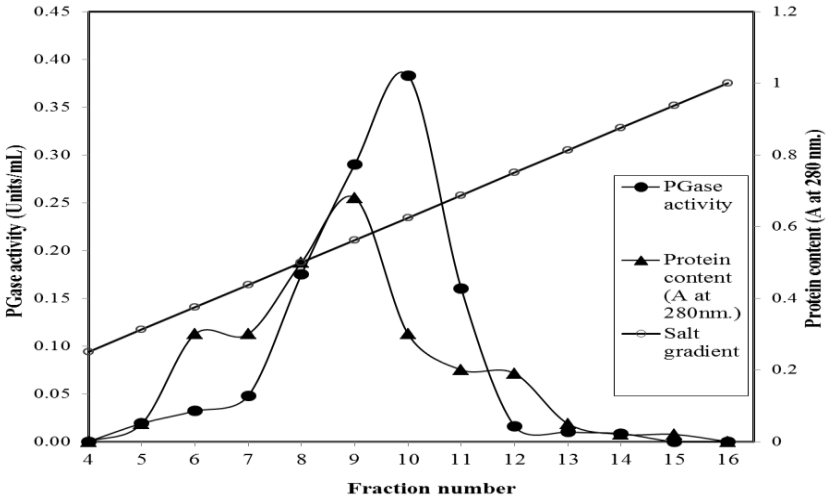

Figure (1): Elution profile of B. licheniformis SHG10 PGase using DEAE-Sepharose FF. The sample was loaded on pre-packed column (1.6 x 10 cm), elution was performed with 20 mM Tris buffer, pH 7.5 with salt gradient from 0.0 to 1.0 M NaCl. Flow rate: 1.0 ml/min. Fractions's volume: 4.0 ml.

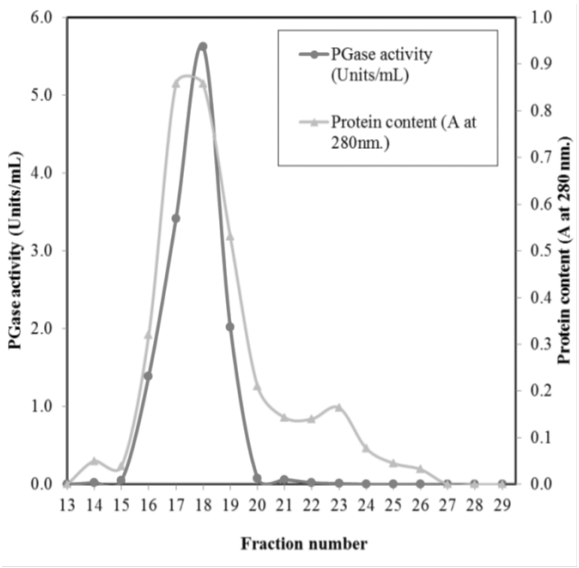

Figure (2): Elution profile of B. licheniformis SHG10 PGase using Sephacryl S-100 HR. The sample was loaded on prepacked column (1.6 x 60),elution was performed with 20 mM Tris buffer containing 0.2M NaCl, pH 7.5. Flow rate: 1.ml/min. Fractions'svolume: 2.5 ml.

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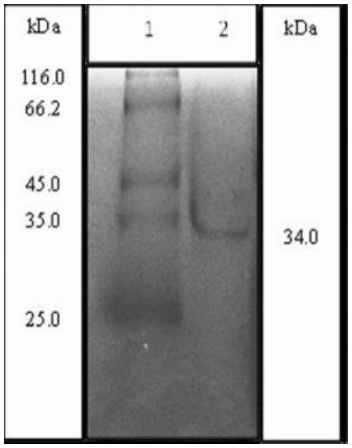
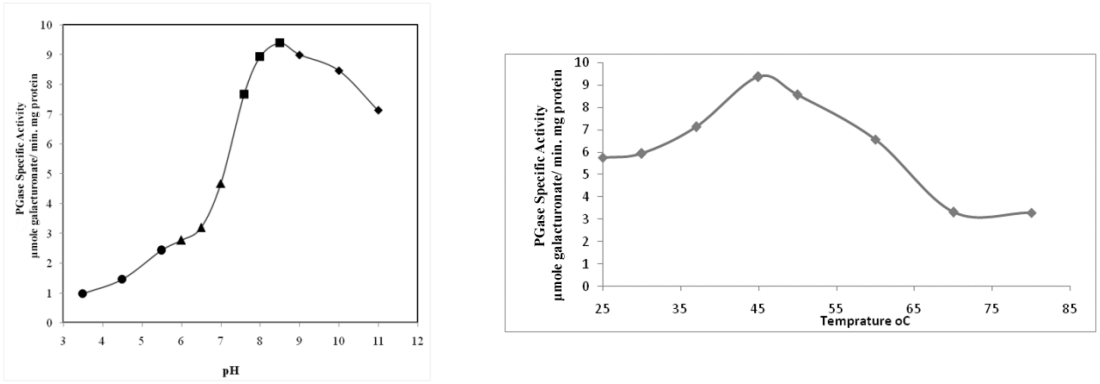


Figure (3): SDS-PAGE of the purified B. licheniformis SHG10 PGase. Lane 1: Showed the protein markers: ß-Galactosidase, Bovine serum Albumin, Ovalbumin, Lactate dehydrogenase, ß-Lactoglobulin of molecular mass 116, 66, 45, 35 and 25 kDa, respectively. Lane 2: Showed a single band of purified SHG10 PGase, revealing a molecular mass of about 34.0 kDa.



A **B**

Figure (4): A: Optimum pH of the purified B. licheniformis SHG10 PGase. B: Optimum temperature of the purified B. licheniformis SHG10 PGase. Different incubation temperatures (25°C – 80°C) were used, at pH 8.5, and 5mg/ml substrate concentration

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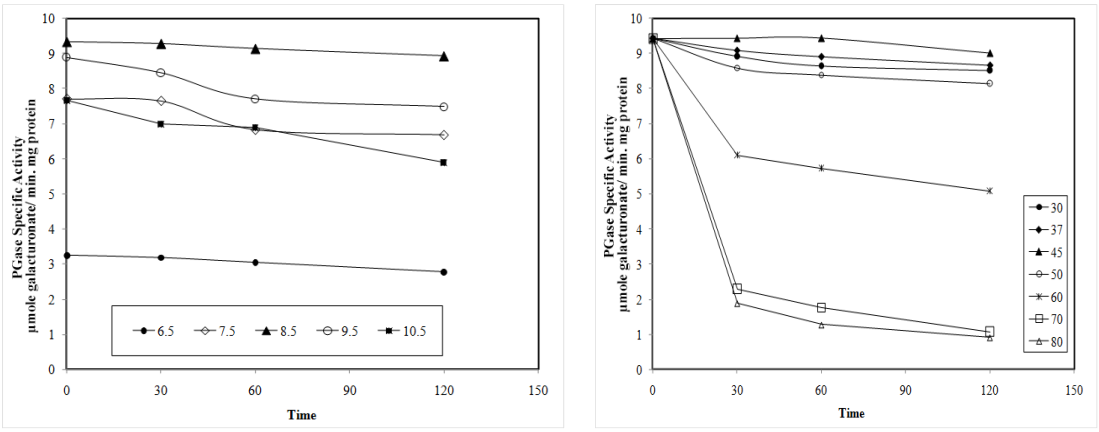

A **B**

Figure (5):A: pH stability of the purified B. licheniformis SHG10 PGase. The purified PGase was preincubated with 50 mMTris-HCl buffer in different pH range (6.5-10.5)for different time intervals (0,30,60,120 min). B: Thermal stability ofthe purified B. licheniformis SHG10 PGase. The purified PGase was preincubated at different temperature degrees (30-80oC) for different time intervals (0, 30, 60, 120 min).

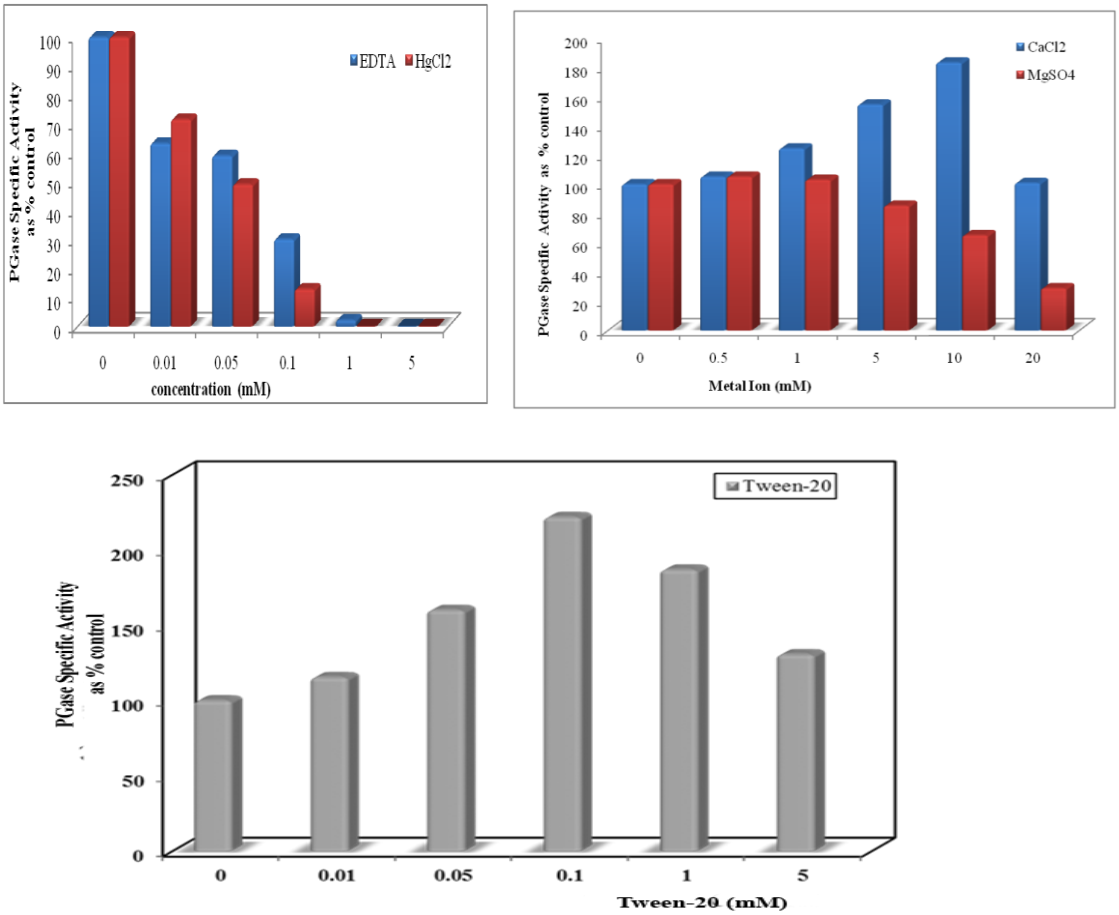

Figure (6): A: Effect of EDTA &HgCl2 B. licheniformis SHG10 PGase activity. The purified PGase was incubated with different concentrations (0- 5 mM) of EDTA & HgCl2 for 15 min at 4oC prior substrate addition. B: Effect ofCa+2 and Mg+2 *B. licheniformis* SHG10 PGase activity. The purified PGase was incubated with different concentrations (0- 5 mM) of Ca+2&Mg+2 for 15 min at 4oC prior substrate addition. C: Effect of Tween-20 on *B. licheniformis* SHG10 PGase activity. Purified PGase was incubated with different concentrations (0- 5 mM) of Tween-20 /ml for 15 min at 40C prior substrate addition.

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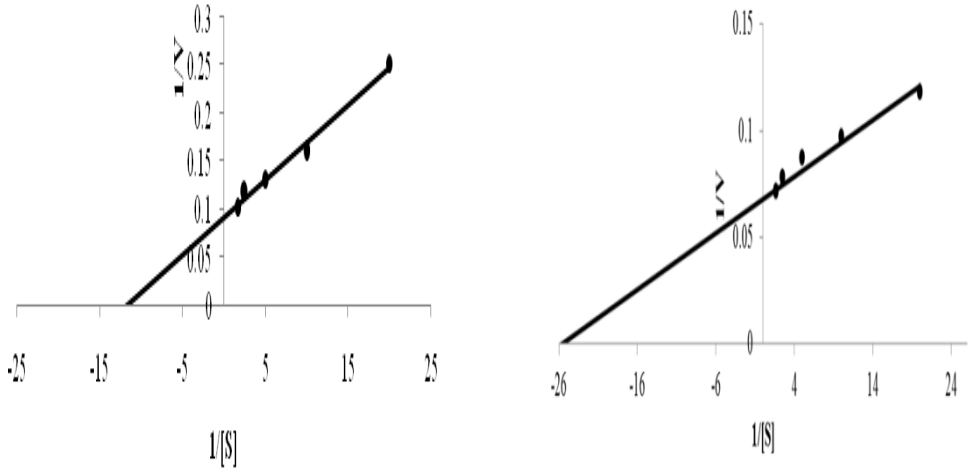

(A)	(B)

Figure (7): Line weaver-Burk Reciprocal Plot: 1/V versus 1/[S]. Purified B.licheniformis SHG10 PGase incubated with different concentrations of substrates. A: citrus pectin 67% esterification and B: pectate pH 8.5 at 45oC for 20 min.

Table (1): Purification Profile of *Bacillus licheniformis*- SHG10 PGase.

Purification step	Total volume (mL)	Protein		Activity			Purification (fold)	Yield %
		mg/mL	Total	U/mL	Total	S.A*		
Step(1) Crude enzyme	250	1.15	287.5	0.300	75	0.261	1	100
Step(2) Concentration	50	5.43	271.5	1.438	72	0.265	1.02	96
Step(3) DEAE-Sepharose FF	16	0.38	6.08	2.71	43.2	7.15	27.4	57.6
Step(4) Sephacryl S-100 HR	10	0.361	3.61	3.13	31.3	8.67	33.34	41.73

S.A*= specific activity of PGase, expressed as μmole galacturonate/min.mg protein.