Section (A)

(A) Qualitative screening for selection the most potent bacterial isolates pectinase producer (First survey by clearing zone technique):

Fifty one bacterial isolates were obtained from *Solanum tuberosum* (ST) peels. These isolates were purified, and subjected to a screening in order to examine their pectinolytic productivities on the basis of mean diameters of clearing zones (mm). As it is observed from the results given in Table (1), the fifty one bacterial isolates had a pectinolytic activity while twenty isolates of them were considered to give good production.

(B) Qualitative screening for selection the most potent bacterial isolates pectinase producer (Second survey by clearing zone technique):

This experiment was carried out to determine the most potent enzyme producers isolates on the basis of mean diameters of clearing zones (mm) by testing the potency of the best bacterial isolates producers selected from the twenty isolates to attack some agriculture, and industrial wastes under solid state fermentation (SSF)

Data recorded in Table (2) showed that the three bacterial isolates numbers 4071, 107 and 10104 out of the twenty isolates gave a higher pectinase productivity by attacking *Solanum tuberosum* (ST) peels compared to other wastes and other isolates, where it reached up to 3.2, 3.4 and 3.4mm respectively.

Table (2): Qualitative data of the pectinolytic productivities of the selected twenty bacterial isolates grown on four agro-industrial wastes. Assay plates were incubated at 37 °C for 24 hours (Second Survey)..

No.	Code no. of bacterial isolates	Pectinolytic productivities Mean diameter of clearing zones (mm).			
		***Solanum tuberosum* (ST) Peels**	***Solanum melongena* (SM) peels**	***Echornia crasipes* (EC)**	**Citrus peels mixture (CPM)**
1	3050	2	2.	2.4	2.2
2	1032A	2	2	2.2	2.6
3	4071	3.2	3	3	2.8
4	109	2.8	2.4	2	2.2
5	1046	2.6	2.2	.4	2.8
6	1032B	2.8	2.6	2.6	2.2
7	107	3.4	2.8	3	3.2
8	3069	2.4	2	2	2.4
9	106	2.8	2.6	2	3.2
10	4087	2.2	0	2	2.4
11	30107	3	2.4	0	0
12	3056	2.2	2.5	2.5	3
13	3062	2	2	3	3
14	3034	2	2.6	2.2	3
15	10104	3.4	3	3	3.4
16	3084	2	2	2.6	2
17	3019	2	2	2.8	2.4
18	3011	0	0	3	2.2
19	3048	2.6	0	3	2.1
20	30102	2	0	3	2.2

(C) Quantitative screening for selection of the most potent pectinase producer bacterial isolates (Second survey by Nelson's technique, 1944):

This experiment was carried out to select the most potent enzyme producers bacterial isolates on the basis of polygalacturonase producitivty (U/ml) by Nelson's technique by testing the potency of the best bacterial isolates producers selected from the twenty isolates to attack some agriculture, and industrial wastes under SSF.

The data presented in Table (3) showed that bacterial isolates numbers 4071, 107 and 10104 also gave a higher polygalacturonase productivity by attacking *Solanum tuberosum* (ST) peels compared to other wastes and other isolates, where it reached up to 515, 515 and 367.5 (U/ml) respectively.

Data recorded in table (4) confirm that, bacterial isolates number 4071, 107 and 10104 gave a higher polygalacturonase productivity by attack *Solanum tuberosum* (ST) peels compared to other wastes, where it reached up to 292, 287 and 297 (U/ml) respectively.

From the previous results concerning the qualitative and quantitative screening of the enzymes production by bacterial isolates B-4071, B-107 and B-10104 were considered more potent for their ability to produce the polygalacturonase productivity.

Table (3): Quantitative data of the pectinase(s) productivities of the best twenty bacterial isolates grown on four agro-industrial wastes. Polygalacturonase productivity was detected by Nelson's technique (1944) (Second Survey).

No.	Code no. of bacterial isolates	Polygalacturonase productivity (U/ml)			
		Citrus peels mixture (CPM)	*Solanum tuberosum* (ST) peels	*Solamum melongena* (SM) peels	*Echornia craspies* (EC)
1	30107	170	335	65	0
2	3069	170	395	0	0
3	3062	170	515	35	0
4	4071	135	515	270	0
5	106	70	200	0	0
6	3034	135	362.5	0	0
7	107	130	367.5	200	0
8	3011	70	235	0	0
9	1032A	100	235	0	0
10	3084	0	235	65	0
11	4087	85	352	132	0
12	3019	135.7	80	42	0
13	30102	100	350	117.5	100
14	1032B	95	187.5	0	0
15	3050	92	342.5	120	0
16	10104	200	515	305	57.5
17	109	7.5	0	0	0
18	3056	100	305	270	127.5
19	1046	150	167.5	72.5	60
20	3048	120	325	205	25

Table (4): Quantitative data of the polygalacturonase productivity of the best three bacterial isolates grown on three agro-industerial wastes. Polygalacturonase productivity was determined by Nelson's technique (1944).

No.	**Code no. of bacterial isolates**	**Polygalacturonase productivities (U/ml)**		
		Citrus peels mixture (CPM)	***Solanum tuberosum* (ST) peels**	***Solamum melongena* (SM) peels**
1	107	105 ± 0.05	287.5 ± 0.02	75 ± 0.01
2	4071	95 ± 0.09	292.5 ± 0	40 ± 0.04
3	10104	115 ± 0.04	297.5 ± 0	177.5 ± 0

Table (5): Morphological, and biochemical properties of the three most potent bacterial isolates.

Character	**Isolate code number**		
	4071	**10104**	**107**
Cell shape	Rods	Rods	Rods
Gram reaction	+	+	+
KOH (3%) reaction	-	-	-
Motility	+	+	+
Spore formation	+	+	+
Oxidase test	-	-	-
Catalase	+	+	+
Carbohydrate fermentation:			
D(-) Galactose	-	-	-
L(-) Mannose	-	-	-
D(-) Fructose	-	-	-
Ribose	-	+A	+A
D(-) Arabinose	-	-	-
Glucose	+A	+A	+A
Trehalose	-	-	+A
Sucrose	+A	-	+A
Maltose	+A	+A	-
Lactose	+A	+A	+A
Raffinose	-	-	-
D(-) Mannitol	+A	+A	-
Aesculin hydrolysis	+A	-	-
Nitrate reduction	+	+	+
Methyl red (MR) test	+	+	+
Voges-Proskauer (VP) test	-	-	-
Enzymes production			
Cellulase	-	-	+
Lipase	+	+	-
Urease	+	+	+
Protease	+	+	+
Amylase	-	+	-
Pectinase	+	+	+
Blood haemolysis	+G	+G	+G
Growth on MacConkey agar	+	+	+
KCN test	+	+	+
Levan formation	+	+	+
Oxidation fermentation(OF) test	+/+	+/+	+/+
Grown on:			
King Ward and Raney's agar			
* Pyocianin	-	-	-
* Florescent	-	-	-
H_2S production	-	-	-
Citrte ultilization	+	+	+

(+): Positive. (-): Negative (±): Doubtful, (A): Acid production (AG): Acid and gas production (PR): Partial reduction (CR): Complete reduction (O/F): Oxidation / Fermentation (SS): (G): Growth.

(D) Identification of the three most potent bacterial isolates:

The three most potent bacterial isolates 4071, 10104 and 107 were subjected to an identification program to the species level. The data obtained were presented in Table (5). The three isolates were found to be rod shaped, *Bacilli*, Gram positive; potassium hydroxide (3%) reaction, negative, motile, spore former, oxidase negative, and catalase positive, ferment of glucose and lactose but do not ferment galactose, mannose, fructose, arabinose, or raffinose, reduced nitrate to nitrite, produce urease, protease and pectinase except isolate 10104 which produce amylase only and isolate 107 which failed to produce lipase. The three isolates were able to grow on blood agar. MacConkey agar, produce levan and utilized citrate while H_2S not produced.

The morphological characteristics and stain reaction led to the fact that the three bacterial isolates are suggestive to being belonging to the genus *Bacillus*, gram positive aerobes to facultative anaerobes, spore formers, catalase, lipase, urease, protease and pectinase positive while, oxidase, cellulase and amylase negative. But the bacterial isolate 10104 produce amylase. Acid produced from glucose, maltose, mannitol, lactose and sucrose. The cells were able to grow at a temperature range 30-60 °C. The cells were able to grow in the presence NaCl at pH 6. Consulting Bergey's Manual of systematic bacteriology, the three isolates are belonging to species *firmus, firmus,* and *laterosporons*. They could be give the tentative name *Bacillus firmus* I-4071, *Bacillus firmus*-I-10104 and *Bacillus laterosporons*-I-107.

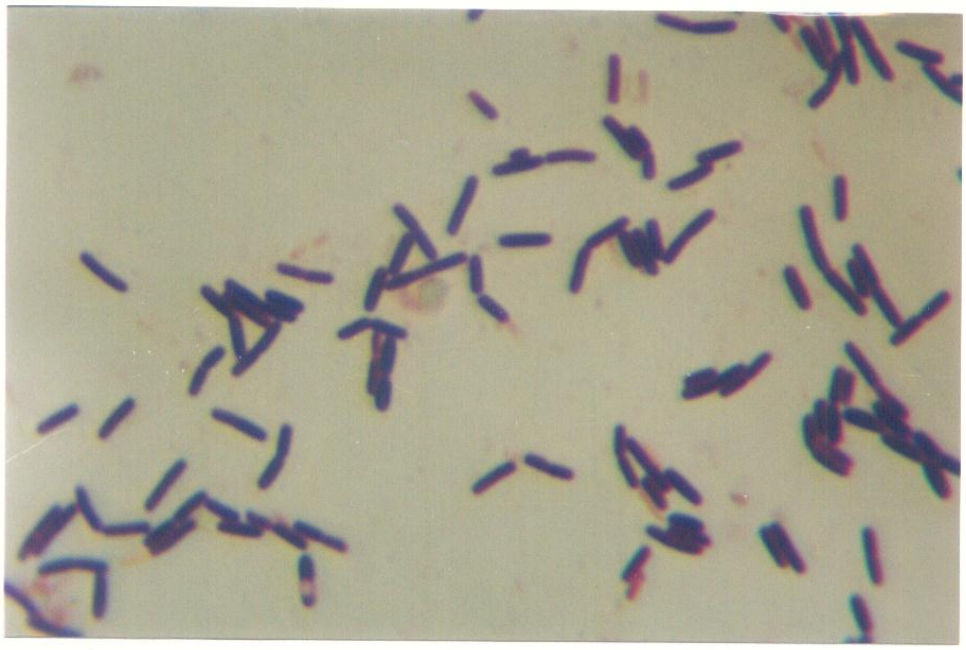

Plate (1): A microphotograph of *Bacillus firmus*-I-4071 (X = 1250).

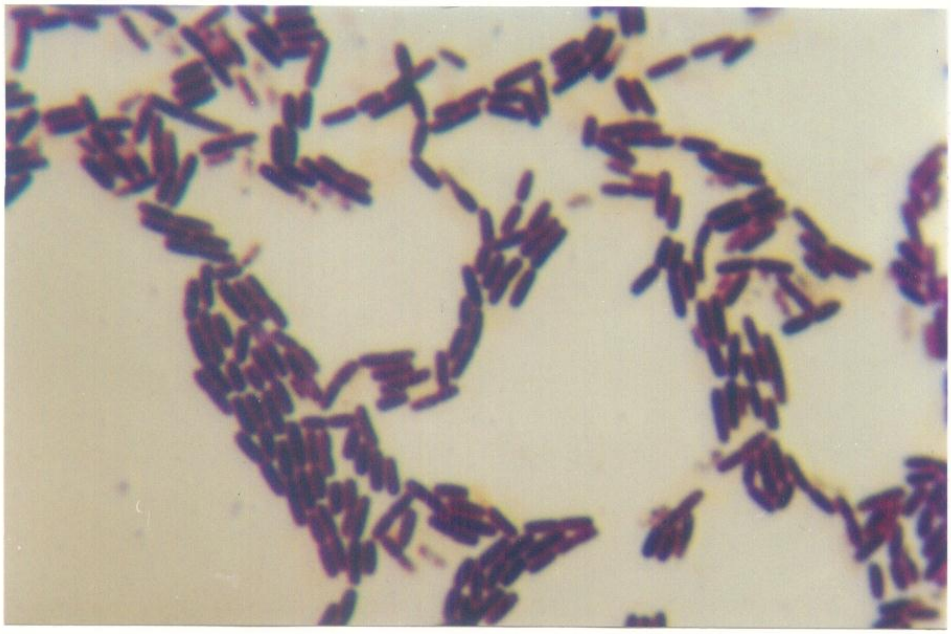

Plate (2): A microphotograph of *Bacillus firmus*-I-10104 (X = 1250).

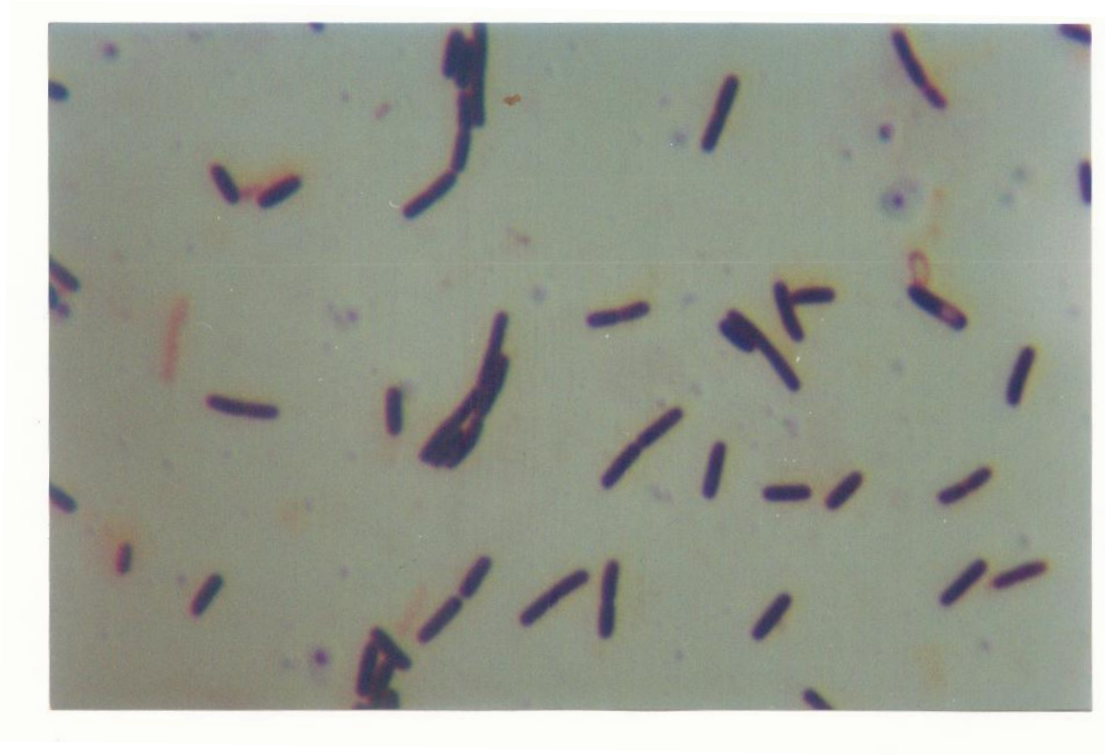

Plate (3): A microphotograph of *Bacillus laterosporons*-I-107 (X = 1250).

Section B

Parameters controlling the polygalacturonase (PG) productivity

1- Different inocula size:

Different volumes of bacterial spore suspension were used as an inocula sizes to inoculate flasks (250 ml) containing the inocula sizes used were 1, 2, 5, 10, 20 and 24 ml. Each one ml of the heavy bacterial cell suspension contained abut 30 x 10^{15} CFU. The optimal inocula sizes need to produce the highest yield of polygalacturonase were 1.0 ml. At this particular inoculum size, the highest yield of polygalacturonase was attained with *Solanum tuberosum* (ST) peels as 350 U/ml. Inocula sizes above the previously optimal recorded value gave value gradually decreasing as compared to that of the optimum one. These data were recorded in Table (6) and represented in Fig. (1).

2- Different substrate concentrations:

It could be concluded from the results given in Table (7) and Fig. (2) that, the maximum polygalacturonase productivity 437.5 U/ml was obtained in presence of 1.25g /25 ml on *Solanum Tuberosum* (ST) peels by *Bacillus firmus*-I-10104 at 37 °C for 96h.

Table (6): Effect of different inocula sizes on the polygalacturonase (PG) productivity using *Solanum tuberosum* (ST) peels under solid state fermentation (SSF) conditions by *Bacillus firmus*-I-10104.

Inoculum size (ml).	Polygalacturonase productivity (U/ml).
1	350 ± 0.02
2	345 ± 0.04
5	345 ± 0.02
10	345 ± 0.02
15	288 ± 0.11
20	222.5 ± 0.13
24	35 ± 0.18

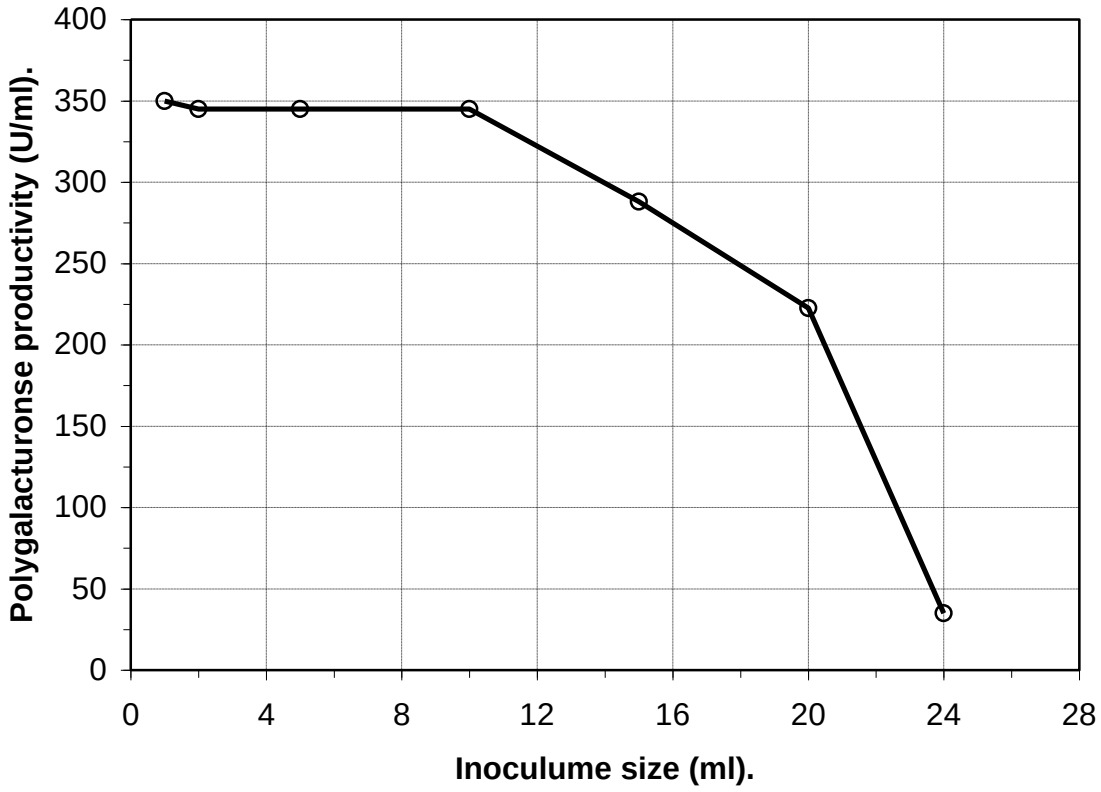

Figure (1): Effect of different inocula sizes on the polygalacturonase (PG) productivity using *Solanum tuberosum* (ST) peels under solid state fermentation (SSF) conditions by *Bacillus firmus*-I-10104.

Table (7): Effect of different substrate concentrations on the polygalacturonase productivity using *Solanum tuberosum* (ST) peels under solid state fermentation conditions by *Bacillus firmus*-I-10104.

Substrate concentration (g/25 ml)	Polygalacturonase productivity (U/ml)
0.1	25 ± 0.01
0.2	52.5 ± 0.04
0.5	262.5 ± 0.26
0.75	385 ± 0.06
1	420 ± 0.03
1.25	437.5 ± 0.03
1.5	415 ± 0.08
2	422.5 ± 0.01
2.5	370 ± 0.02
5	362.5 ± 0.43
10	274 ± 0.43
15	385 ± 0.08

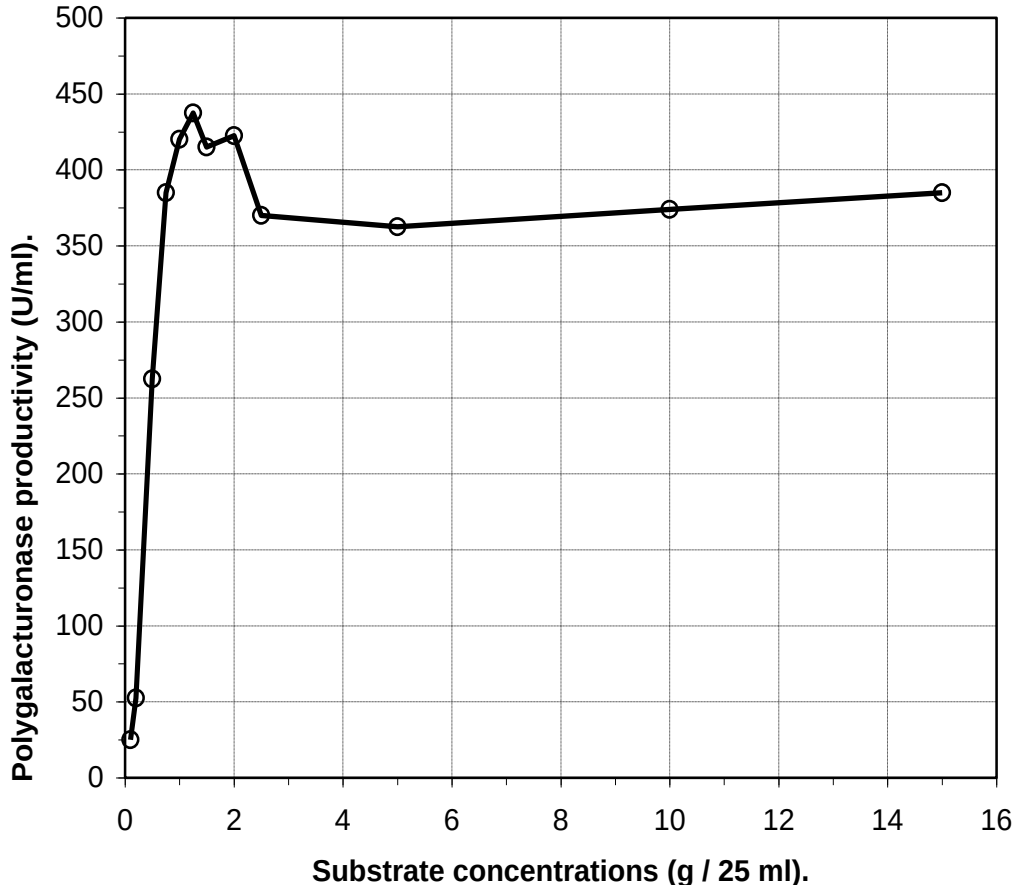

Figure (2): Effect of different substrate concentrations on the polygalacturonase productivity using *Solanum tuberosum* (ST) peels under solid state fermentation conditions by *Bacillus firmus*-I-10104.

3- Different incubation periods:

Effect of different inocubation periods on the polygalacturonase productivity using *Solanum tuberosum* (ST) peels under solid state fermentation conditions by *Bacillus firmus*-I-10104 was tested at time intervals of 6, 12, 24, 48, 72, 96, 120, 144, 168 and 192 hours. The results given in Table (8) and Fig. (3) show that, the level of polygalacturonase increased gradually with increasing the incubation period up to a maximum of 96h. Then gradually decreased after these periods.

4- Different initial pH values:

As it is observed from the results recorded in Table (9) and Fig. (4), the polygalacturonase productivity by *Bacillus firmus*-I-10104 reached its maximum at initial pH 6.0 and 6.2. Since the enzyme yield reached up to 325 U/ml., below and above this optimal pH value, the enzyme productivity gradually decreased.

5- Different incubation temperatures:

It was obvious from the results given in Table (10) and Fig. (5) that, the polygalacturonase productivity reached its optimal value 310 U/ml at an incubation temperature at 37 °C.

Table (8): Effect of different incubation periods on the polygalacturonase productivity using *Solanum tuberosum* (ST) peels under solid state fermentation conditions by *Bacillus firmus*-I-10104.

Incubation period (hours).	Polygalacturonase productivity (U/ml).
6	160 ± 0.03
12	220 ± 0.04
24	315 ± 0.02
48	325 ± 0.01
72	345 ± 0.14
96	375 ± 8.50
120	365 ± 0.02
144	362.5 ± 0.02
168	340 ± 0.01
192	300 ± 8.08

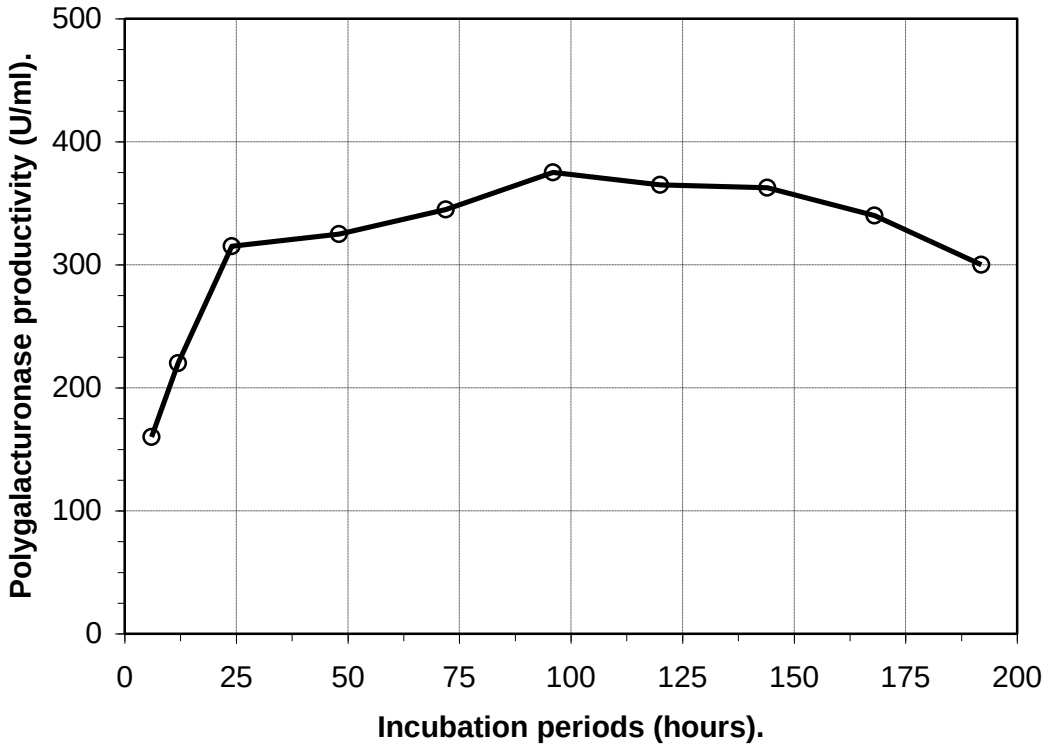

Figure (3): Effect of different incubation periods on the polygalacturonase productivity using *Solanum tuberosum* (ST) peels under solid state fermentation conditions by *Bacillus firmus*-I-10104.

Table (9): Effect of different pH values on the polygalacturonase productivity using *Solanum tuberosum* (ST) peels under solid state fermentation conditions by *Bacillus firmus*-I-10104.

pH value	Polygalacturonase productivity (U/ml).
3	272.5 ± 0.01
5	280 ± 0.01
5.6	322.5 ± 0.01
6	325 ± 0.00
6.2	325 ± 2.51
6.4	322.5 ± 9.71
6.6	297.5 ± 0.09
7.6	297.5 ± 0.02
8.7	250 ± 0.01
9.2	237.5 ± 0.09

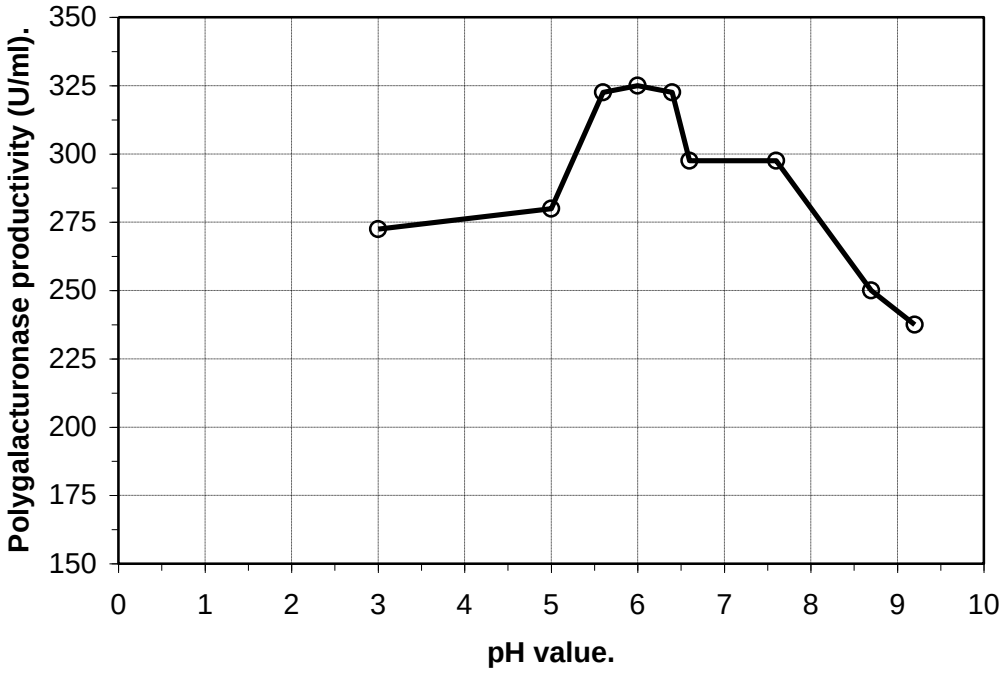

Figure (4): Effect of different pH values on the polygalacturonase productivity using *Solanum tuberosum* (ST) peels under solid state fermentation conditions by *Bacillus firmus*-I-10104.

Table (10): Effect of different temperatures on the polygalacturonase productivity using *Solanum tuberosum* peels (STP) under solid state fermentation conditions by *Bacillus firmus*-I-10104.

Incubation temperature (°C).	Polygalacturonase productivity (U/ml).
0	0
10	42.5 ± 0.04
12	117.5 ± 0.02
28	252.5 ± 0.02
37	310 ± 0.01
40	300 ± 0.03
44	293 ± 0.14
55	275 ± 4.50
65	147.5 ± 3.60
75	117.5 ± 0.10

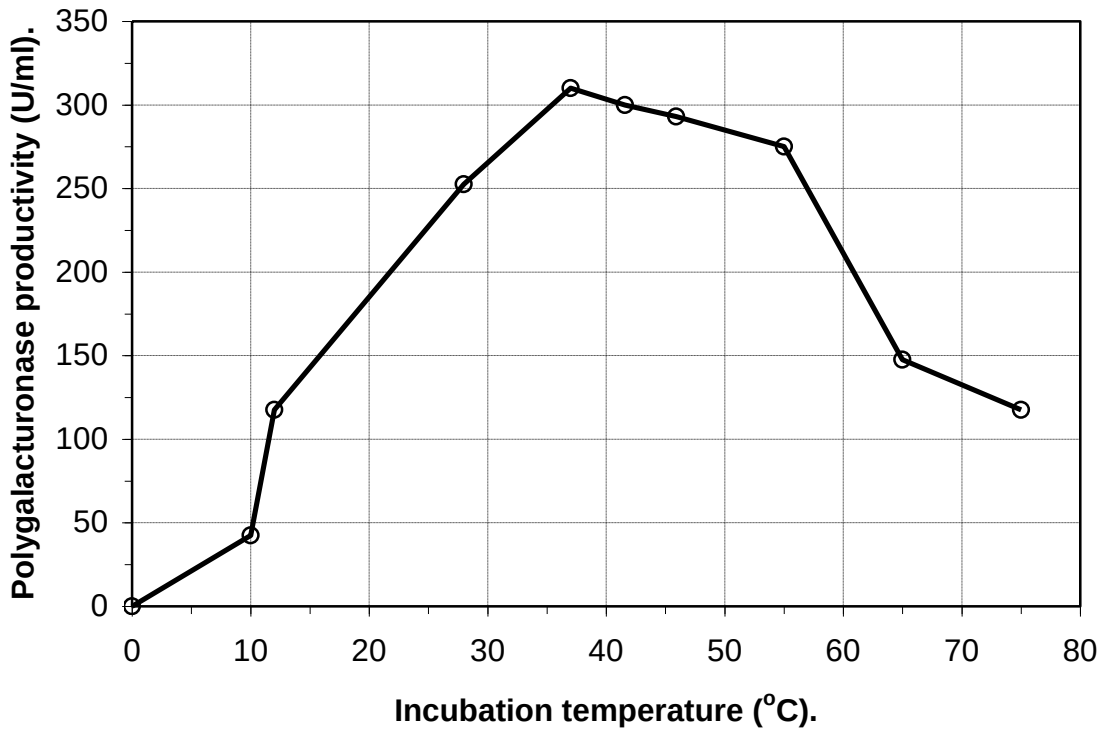

Figure (5): Effect of different temperatures on the polygalacturonase productivity using *Solanum tuberosum* peels (STP) under solid state fermentation conditions by *Bacillus firmus*-I-10104.

6- Different nitrogen sources:

Effect of different organic as well as inorganic nitrogen sources on polygalacturonase productivity by the most potent bacterial strain *Bacillus firmus*-I-10104 were studied. Fourteen different nitrogen sources were applied as equimolecular amount located in Table (11) and represented in Fig. (6). The maximum value of polygalacturonase productivity reached up to 350 U/ml in the presence of peptone.

7- Different carbon sources:

The effect of thirteen different carbon sources listed in Table (12) and illustrated in Fig. (7) were introduced into the applied production medium of polygalacturonase productivity by *Bacillus firmus*-I-10104 under SSF condition were studied. It was clear that all the different carbon sources exhibited various degrees lower than control by the *Bacillus firmus*-I-10104. *Solanum tuberosum* (ST) peels was the best carbon source for polygalactacturonase production where the productivity reached up to 115 U/ml.

8- Different amino acids:

The influence of different eleven amino acids on polygalacturonase productivity by the most potent bacterial strain viz. *Bacillus firmus*-I-10104 was studied. The tested amino acids were introduced as nitrogen sources, at equimolecular amount. The aim of this experiment was to determine the best amino acid that induces the highest enzyme productivity. The results obtained from Table (13) and in Fig. (8), revealed that all the tested amino acids exhibited various degrees of polygalacturonase activity lower than the control.

Table (11): Effect of different nitrogen sources on the polygalacturonase productivity using *Solanum tubersoum* (ST) peels under solid state fermentation conditions by *Bacillus firmus*-I-10104.

Nitrogen source	Polygalacturonase productivity (U/ml)
Control	120 ± 0.09
Gelatin	345 ± 0.04
Peptone	350 ± 0.02
Casine	325 ± 0.11
Diammonium hydrogen phosphate	335 ± 4.24
Ammonium sulphate	250 ± 0.20
Ammonium molybedate	105 ± 0.04
Ammonium chloride	347.5 ± 0.02
Potassium nitrate	342.5 ± 0.03
Ammonium oxalate	310 ± 0.12
Urea	345 ± 0.01
Beef extract	340 ± 0.04
Ammonium citrate	335 ± 0.01
Ammonium tartarate	195 ± 0.07
Ammonium nitrate	332.5 ± 0.08

Fig. 10

Table (12): Effect of different carbon sources on the polygalacturonase productivity using *Solanum tuberosum* (ST) peels under solid state fermentation conditions by *Bacillus firmus*-I-10104.

Carbon source.	Polygalacturonase productivity (U/ml).
Control *Solanum tuberosum* (ST) peel	115 ± 0.15
Monosaccharides	
D(-) Arabinose	0
D (-) Glucose	5 ± 0.01
D (-) Galactose	0
L (-) Mannose	0
D (-) Fructose	0
Trehalose	0
Disaccharides	
Lactose	12.5 ± 0.03
Maltose	0
Sucrose	0
Polysaccharides	
Xylose	12.5 ± 0.0
Starch	0
Cellulose	7.5 ± 5.50
Pectin	0

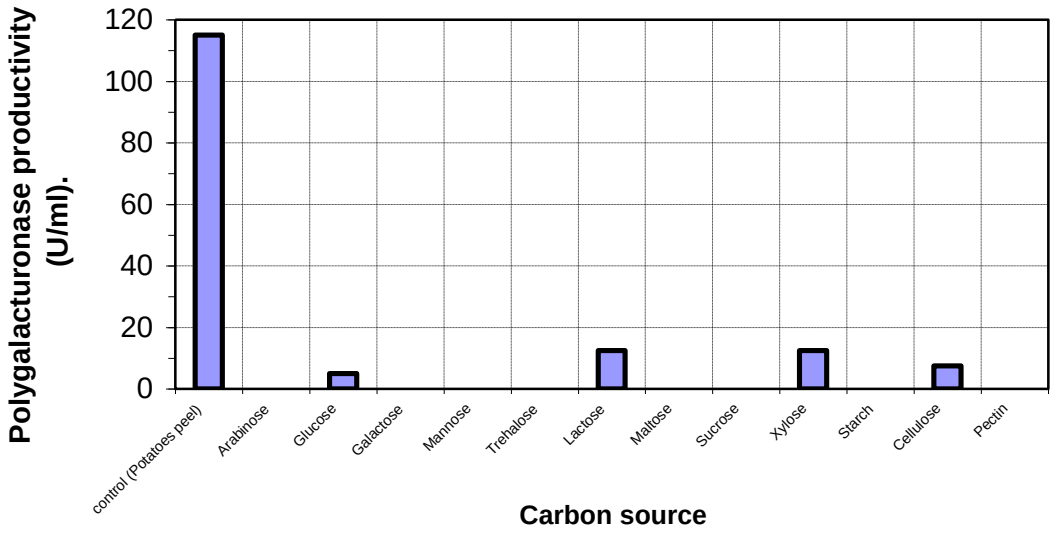

Figure (7): Effect of different carbon sources on the polygalacturonase productivity using *Solanum tuberosum* (ST) peels under solid state fermentation conditions by *Bacillus firmus*-I-10104.

Table (13): Effect of different amino acids on the polygalacturonase productivity using *Solanum tuberosum* (ST) peels under solid state fermentation conditions by *Bacillus firmus*-I-10104.

Amino acid.	Polygalacturonase productivity (U/ml).
Control (without amino acids)	72.5 ± 0
Alanine	30 ± 0
Isoleucine	15 ± 0.01
Methionine	20 ± 0.01
Cystine	10 ± 0
Aspartic acid	25 ± 0
Lysine	20 ± 0
Arginine	20 ± 0.01
Histidine	20 ± 0
Phenylalanine	0
Tryptophan	20 ± 0.01
Glycine	20 ± 0.01

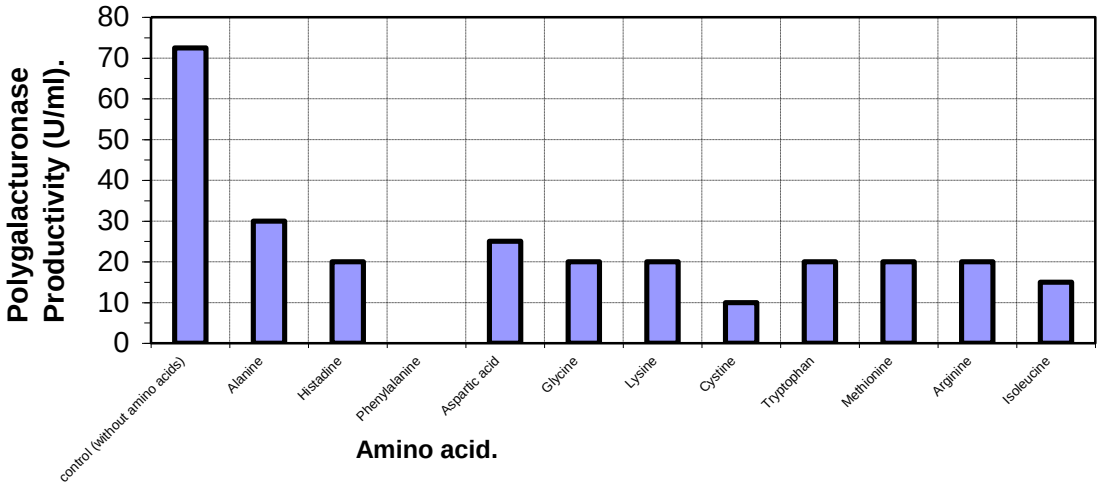

Figure (8): Effect of different amino acids on the polygalacturonase productivity using *Solanum tuberosum* (ST) peels under solid state fermentation conditions by *Bacillus firmus*-I-10104.

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9- Different vitamins:

The results obtained from the Table (14) revealed that, all of the tested vitamins exert suppressive effects on polygalacturonase productivity by *Bacillus firmus*-I-10104 at concentrations 100, 250, 500 and 1000 ppm.

10- Different flask volumes:

As it is shown in Table (15) and Fig. (10) the 500 ml flask volume was more favourable for polygalacturonase productivity, where it reached up to 297.5 U/ml by *Bacillus firmus*-I-10104.

Table (14) Effect of different vitamin concentrations on the polygalacturonase productivity using *Solanum tubersoum* (ST) peels under solid state fermentation conditions by *Bacillus firmus*-I-10104.

Vitamin	Concentration (ppm)	Polygalacturonase productivity (U/ml)
Control	Without vitamins	260 ± 0.11
Vitamin B6	100	0.0
	250	47.5 ± 0.08
	500	102.5 ± 0.03
	1000	172.5 ± 0.01
Folic acid	100	77.5 ± 0.04
	250	47.5 ± 0.11
	500	75 ± 0.06
	1000	120 ± 0.04
Riboflavin	100	95 ± 0.07
	250	127.5 ± 0.04
	500	60 ± 0.06
	1000	100 ± 0.04
Ascorbic acid	100	32.5 ± 0.07
	250	57.5 ± 0.15
	500	35 ± 0.18
	1000	75 ± 0.07

Fig. (9)

Table (15): Effect of different flask volumes on the polygalacturonase productivity using *Solanum tuberosum* (ST) peels under solid state fermentation conditions by *Bacillus fimus*-I-10104.

Flask volume (ml).	Polygalacturonase productivity (U/ml).
Control (250 ml)	260 ± 0.118
100	230 ± 0.108
250	260 ± 0.118
500	297.5 ± 0.061
1000	287.5 ± 0.077

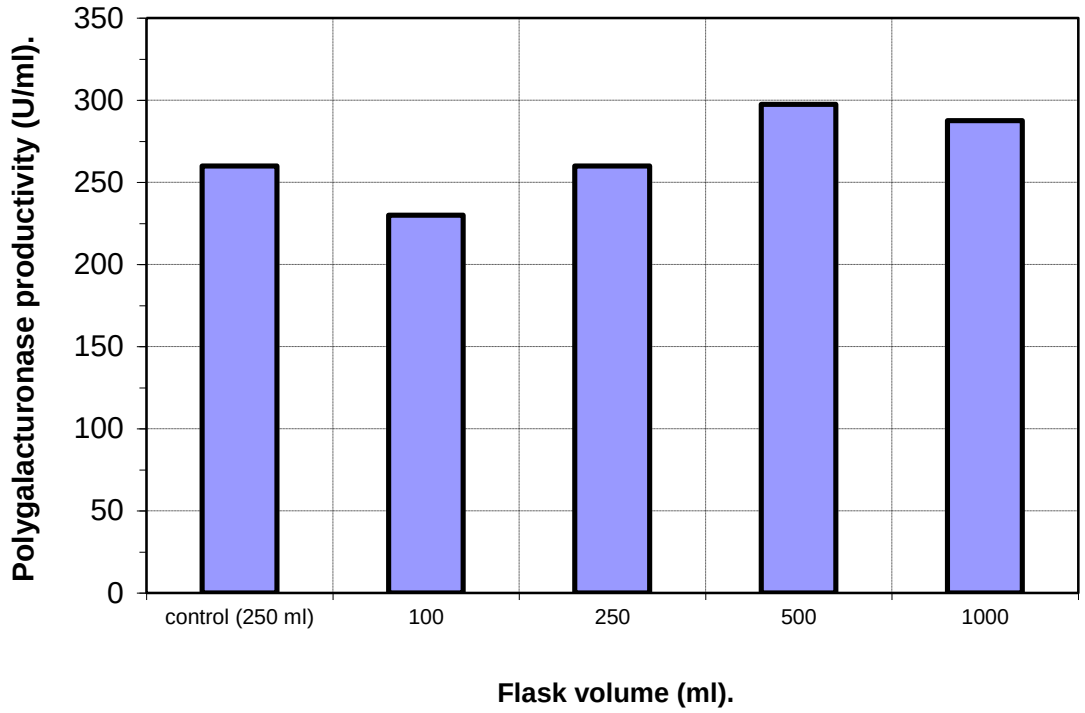


Figure (10): Effect of different flask volumes on the polygalacturonase productivity using *Solanum tuberosum* (ST) peels under solid state fermentation conditions by *Bacillus fimus*-I-10104.

Table (16): The optimal nutritional and environmental parameters controlling polygalacturnase productivity by *Bacillus firmus-I*-10104 under solid state fermentation conditions.

No	**Parameter**	***Bacillus firmus*-I-10104**
1	Inoculum size (ml)	1 ml
2	Substrate concentration (gm/25 ml)	1.25
3	Incubation period (hours)	96
4	pH value	6.2
5	Temperature (°C)	37
6	Nitrogen source	Peptone
7	Carbon source	Control
8	Amino acids	Control
9	Vitamins (100, 250, 500 and 1000 ppm)	Control
10	Flask volume (ml)	500

Section (B)

Purification of polygalacturonase produced by *B. firmus*-I-10104, allowed to grow on *S. tuberosum* (ST) peels under solid state fermentation conditions.

In this study, polygalacturonase, produced by *B. firmus*-I-10104, previously grown on *S. tuberosum* (ST) peels as a preferable substrate supplemnted with mineral salts under the optimum nutritional and environmental conditions recorded in table (16) were purified to homogeneity as previously mentioned, by performing ammonium sulphate fractionation, dialysis against water & sucrose, and applying column chromatography technique on sephadex G200. The obtained purified enzyme were further investigated for some factors affecting its activity. Therefore, data in this section will be discussed in the form of two subsections.

Subsection (I): Purification of polygalacturonase

Subsection (II): Characterization of the purified enzyme.

Subsection (I): Purification of polygalacturonase

The following steps were performed during the course of the purification of the enzyme under study, where polygalacturonase produced by *B. firmus*-I-10104, were allowed to grow on *S. tuberosum* (ST) peels under solid state fermentation conditions at 37 °C under all the optimal conditions.

Data in this subsection will be discussed according to the following steps:

Step (1): Enzyme production and preparation of cell free filtrate (CFF).

Step (2): Fractional precipitation with ammonium sulphate.

Step (3): Concentration by dialysis against distilled water followed by dialysis against sucrose.

Step (4): Preparation of sephadex G-200 gel filtration using column chromatography technique and applying the enzyme sample for purification.

Step (5): Amino acids analysis of the purified enzyme.

Step (6): Gel electrophorasis analysis of the purified enzyme.

Step (1): Enzyme production and preparation of CFF:

In this step the most potent bacterial strain was allowed to grow on the production medium under all the previously mentioned optimal static solid state fermentation conditions as shown in table (16) for production of polygalacturonase.

At the end of incubation period, 600 ml of polyalacturonase production of the obtained extract was done at 6000 rpm for 20 min at 4 °C. The precipitate was collected and tested for determination of both enzyme activity and protein content and corresponding specific activity was calculated.

Step (2): Fractional precipitation by ammonium sulphate:

As it is shown in Table (17) and in Fig. (11), the most active enzyme protein preparation was obtained at ammonium sulphate level at 40-60 % for pectinase enzyme where the activity was reached up to 12180 U/ml and protein content 1.648.8 U/ml corresponding to a specific activity of 1.231 U/mg^{-1} protein.

Only 30 ml were obtained at the end of the process of dialysation against tap water.

Step (3): Concentration by dialysation against sucrose:

The most active ammonium sulphate fractions previously obtained at the best saturation (30 ml) in case of pectinase, were dialyzed against distilled water followed by dialysis against sucrose crystals until a volume of 5 ml was obtained and specific activity was determined as 8.25 U/mg^{-1} protein (Table 19).

Step (4): Purification on sephadex G-200 gel column:

Data recorded in Table (18) and in Fig. (12) showed that, there were two active peaks. It was found that the first beak (fractions 13-18) has the highest specificity and the fraction number 13 was reached to the maximum specific activity up to 5209 Ug/ml^{-1} protein. A summary of the purification steps of pectinase (s) produced by *B. firmus*-I-10104 were presented in table (19).

Step (5): Amino acids analysis of the purified enzyme:

As it is clear from the results in Fig. (13) the 7 amino acids were detected in addition to ammonium sulphate. Proline was the highest value i.e. 8.52 U/ml.

Step (6): Gelectrophorasis analysis of the puified enzyme:

Data presented in plate (4) showed that, two bands were detected. The highest band represented value i.e. (21.500 Dalton) molecular wt.

Table (18) : Fractionation pattern of polygalacturonase produced by *Bacillus firmus*-I-10104 on sephadex G-200 column chromatography technique.

Fraction number	Polygalacturonase activity (U/ml).	Protein Content (U/ml).	Specific activity (U/protein/ml).
1-3	0.0	0.0	0.0
4	110	0.0375	2933.33
5	125	0.0390	3205.12
6	130	0.0455	2857.14
7	140	0.048	2916.66
8	150	0.05	3000.0
9	165	0.065	2538.46
10	280	0.069	4057.97
11	290	0.0785	3694.26
12	305	0.082	3719.51
13	435	0.0835	5209.58
14	405	0.09	4500.0
15	385	0.0905	4254.14
16	380	0.088	4318.18
17	375	0.07	5357.14
18	395	0.0485	8144.32
19	260	0.0325	8000
20	185	0.0205	9024.39
21	165	0.016	10312.5
22	155	0.0145	10689.65
23	115	0.0135	8518.51
24	100	0.0115	8695.65
25	95	0.01	9500.0
26-50	0.0	0.0	0.0

Fig 12

Table 19

Table (20): Amino acids analytical data of *Bacillus firmus*-I-10104 purified polygalacturanase.

No.	Peak number	Amino acid name	R.T.	Concentration (μg/ml)
1	2	Glutamic acid	18.11	2.71
2	3	Proline	19.92	51.82
3	7	Cystine	28.87	2.46
4	8	Tyrosine	36.32	1.98
5	9	Phenylalanine	38.92	7.72
6	11	Lysine	48.94	2.99
8	14	Arginine	58.83	29.64
Total				100.00

R.T.: Retention time.

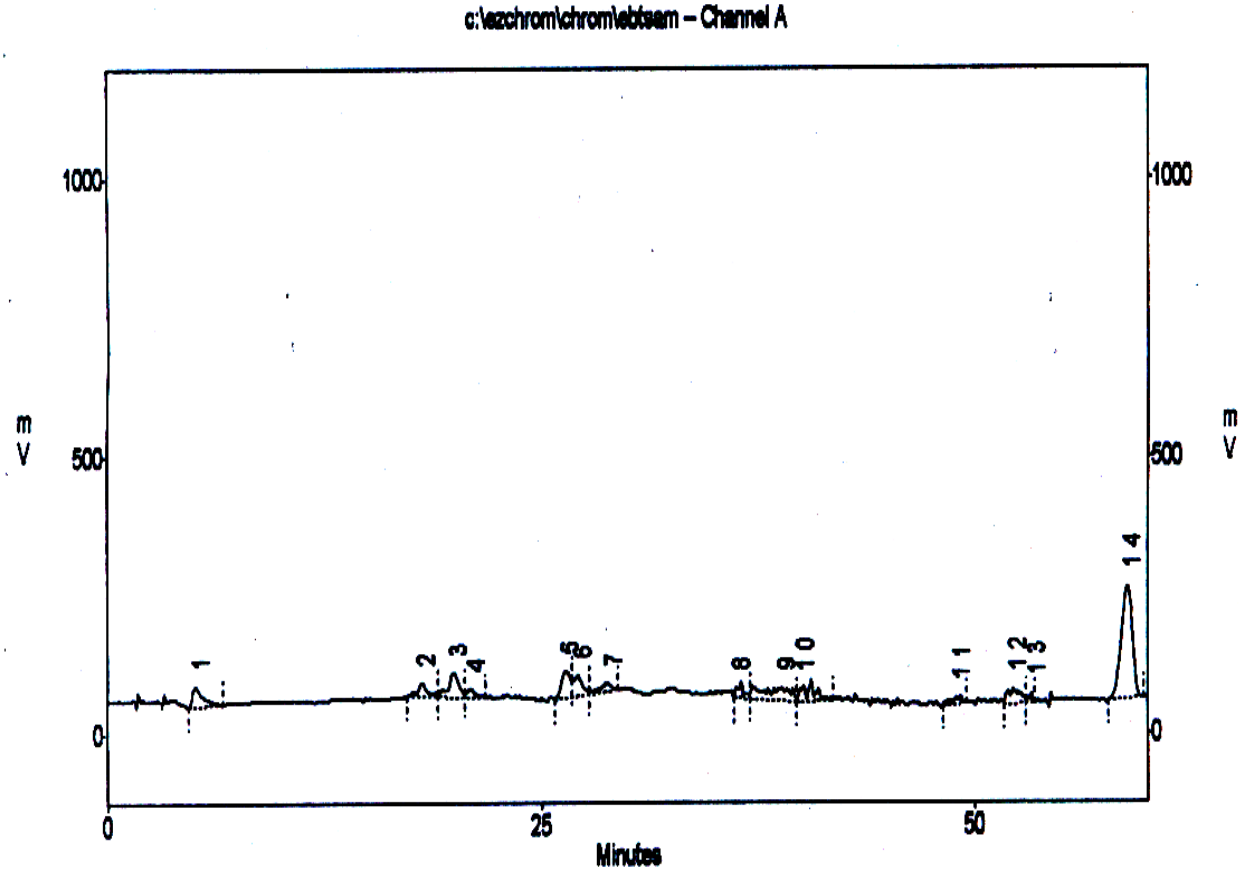


Fig. (13): Amino acids analytical data of *Bacillus firmus*-I-10104 purified polygalacturanase.

Amino acid analysis of the purified polygalacturanase produced by *B. firmus*-I-10104:

As it is obvious from the results given in Table (20) and analysis sheet (Fig. 13) seven amino acids were detected in addition to ammonium sulphate. Arginine and proline represented the highest value i.e. 29.64 and 51.82 µg/ml respectively.

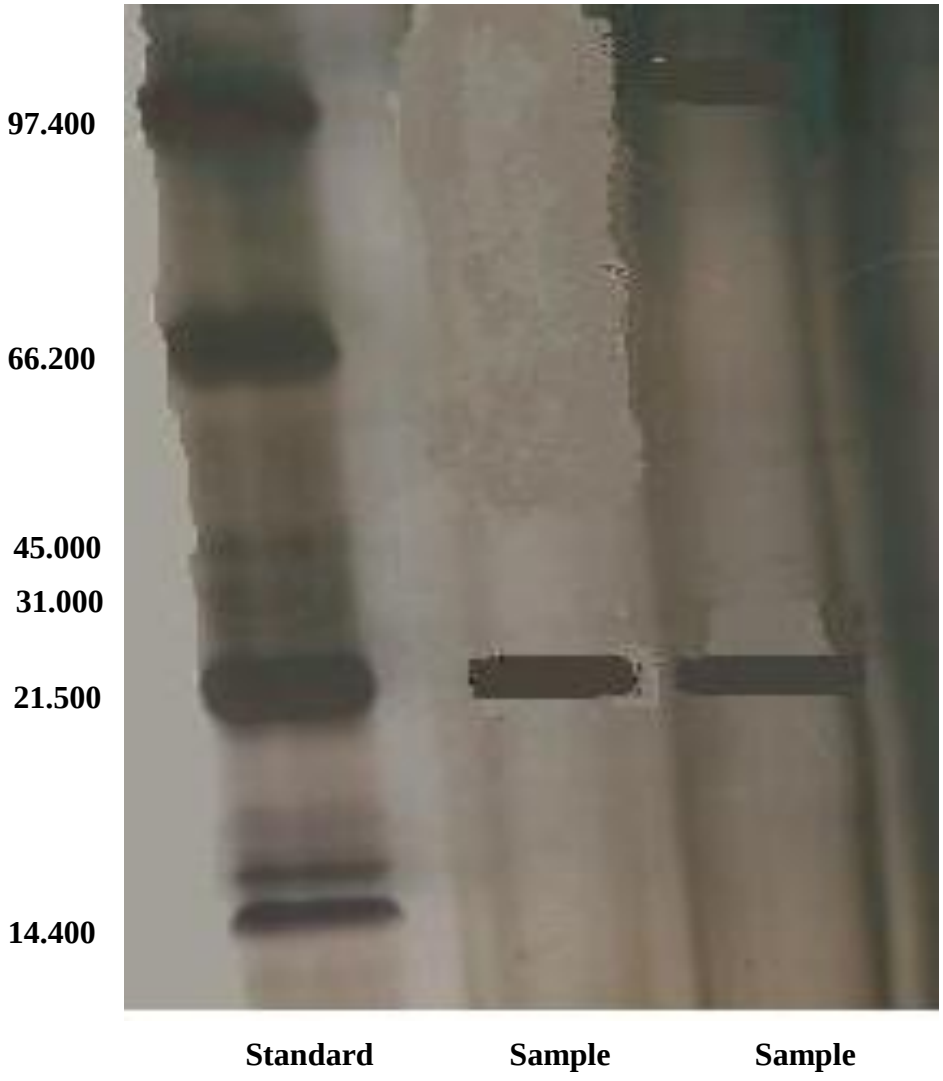


Plate (4): Nondenaturing SDS-PAGE of the purified *Bacillus firmus*-I-10104 polygalacturanase (PGase).

The purified produced polygalacturonase by Bacillus firmus-I-10104 was homogenous as judged by SDS-PAGE as shown in plate

(4). The molecular weight of the enzyme was estimated to be approximately 21.500 Dalton by SDS-PAGE. This indicates that the exo-PGase produced extracellularly is a monomeric protein.

Subsection II

Characterization of the purified enzyme produced by *B. firmus*-I-10104 at 37 °C.

The aim of the present series of experiments was to investigate some properties of the purified enzyme produced by *B. firmus*-I-10104, allowed to grow on *S. tuberosum* (ST) peels as best substrate and incubated under all optimal nutritional and environmental conditions. These properties include: effect of incubation temperature, thermostability, pH values, pH stability, different purified enzyme concentrations and substrate concentration.

1- Effect of incubation temperature:

As it is observed from the results in Table (21) and Fig. (14), the maximum activity of polygalacturnase was obtained at 50 °C, where it reached up to 375 U/ml. Below and above this optimal temperature, the enzyme activity decreased gradually.

2- Effect of different substrate concentrations on the purified enzyme activity:

The data represented in Table (22) and Fig. (15) revealed that, the maximum activity of polygalacturonase was attained at 2.0 % substrate pectin concentration, where it reached up to 472.5 U/ml. Below and above this optimal substrate concentrations, the enzyme activity decreased gradually.

Table (21): Effect of different incubation temperatures on the activity of purified polygalacturonase.

Incubation temperature (°C).	Polygalacturonase activity (U/ml).
10	102.5
20	115
25	147.5
35	237.5
45	252.5
50	375
55	322.5
65	227.5

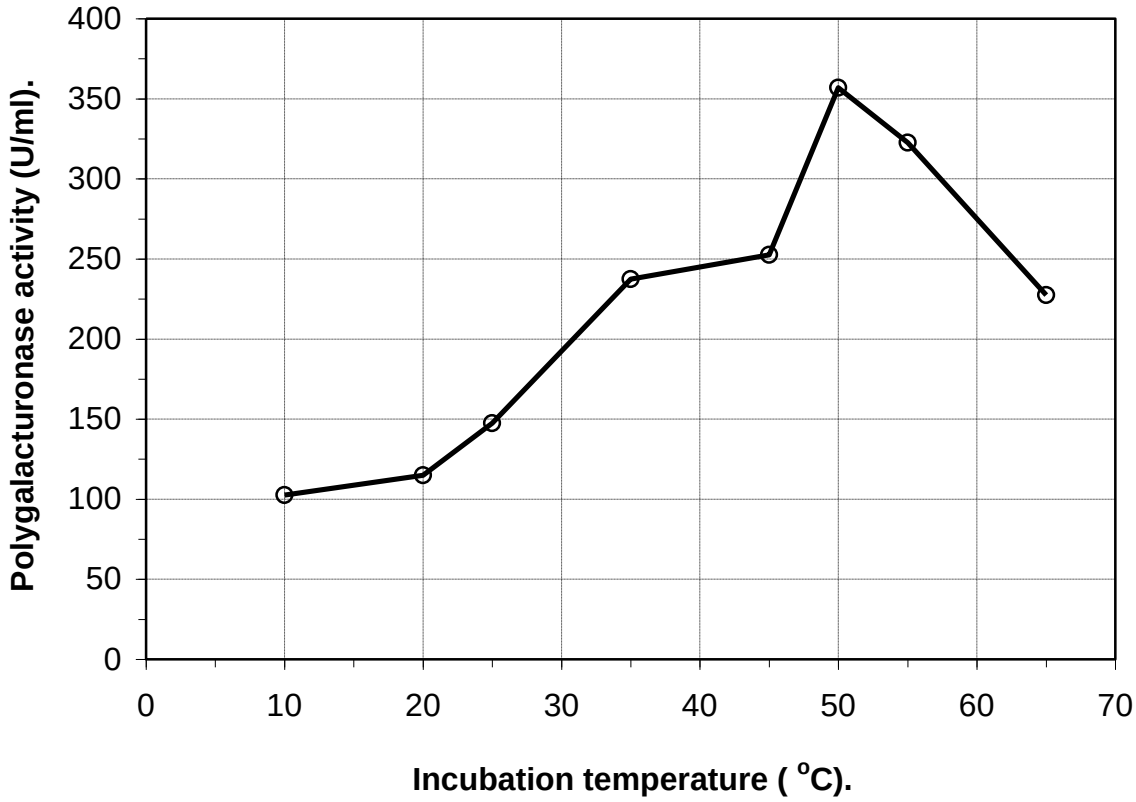
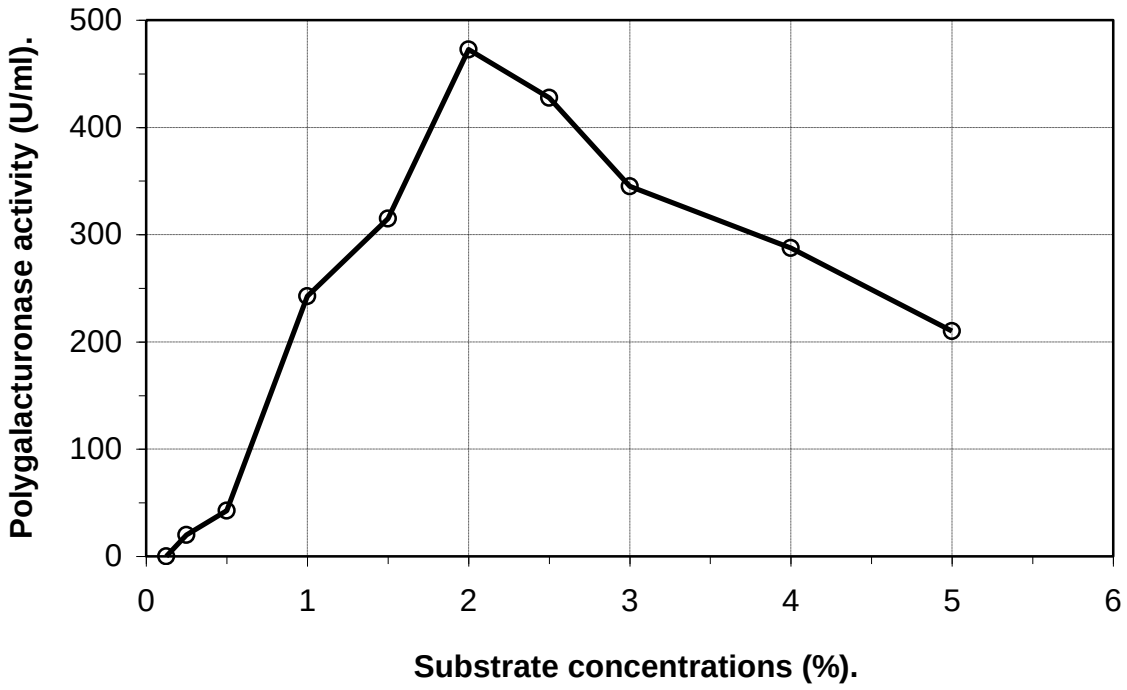


Figure (14): Effect of different incubation temperatures on the activity of purified polygalacturonase.

Table (22): Effect of different substrate concentrations on the activity of purified polygalacturonase.

Substrate concentration (%).	Polygalacturonase activity (U/ml).
0.125	0
0.25	20
0.5	42.5
1	242.5
1.5	315
2	472.5
2.5	427.5
3	345
4	287.5
5	210



Figure(15): Effect of different substrate concentrations on the activity of purified polygalacturonase.

3- Effect of different pH values on the activity of purified enzyme:

It was noticed that from the results in Table (23) and that represented in Fig. (16), the purified PG exhibited the maximum activity at pH 12 of phosphate buffer and gave 300 U/ml. Below this optimal pH value, the enzyme activity decreased gradually.

4- Different concentrations of the purified enzyme in relation to its activity:

From the results obtained from Table (24) and Fig. (17), it was found that there is a continuous increasing of enzyme activity due to the increase of enzyme concentration units, where it reached up to 227 U/ml for the purified polygalacturonase.

5- Thermostability of the purified enzyme:

The data presented in Table (25) and Fig. (18) showed that, PG activity reached its maximal value i.e. 275 U/ml at 80 °C.

6- pH stability of the purified enzyme activity:

The data given in Table (26) and Fig. (19), clear that, the purified pectinase still very active at alkaline pH and exhibited its maximal value 285 U/ml at pH 12.

Table (23): Effect of different pH values on the activity of the purified polygalacturonase.

pH value	Polygalacturonase activity (U/ml).
4	0
5	0
6	275
7	287.5
8	287.5
9	290
10	295
12	300

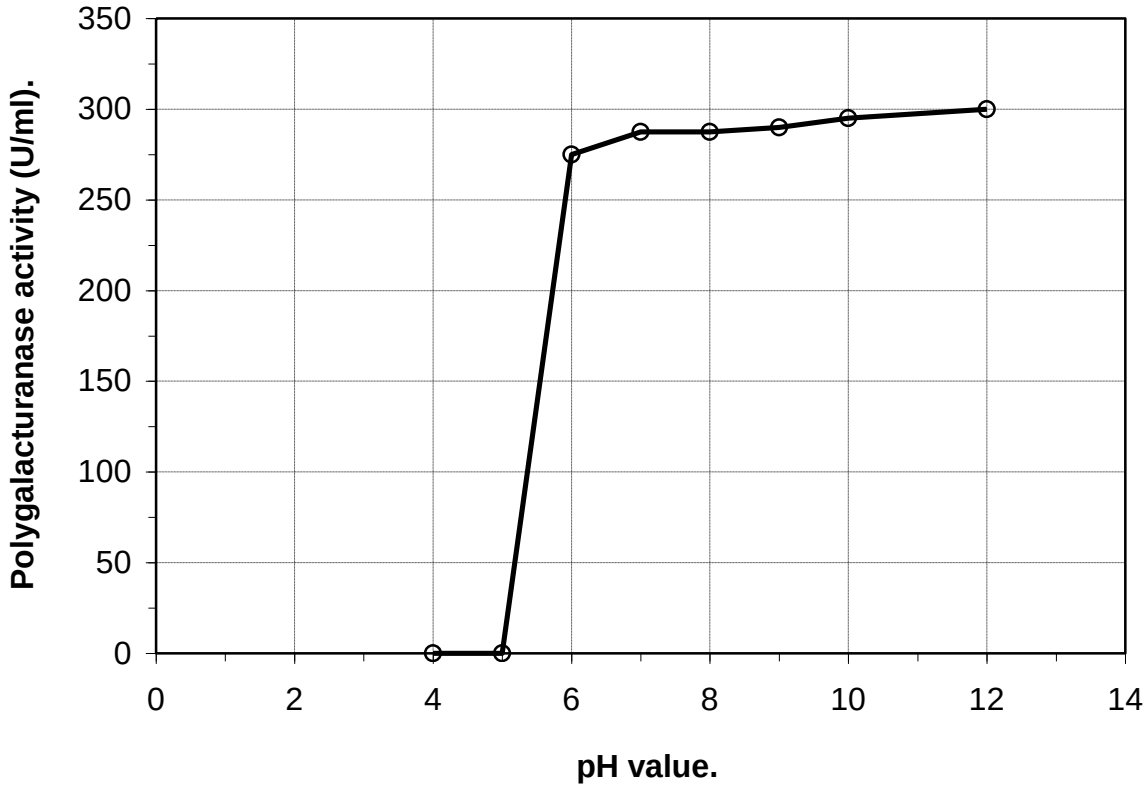


Figure (16): Effect of different pH values on the activity of the purified polygalacturonase.

Table (24): Effect of different enzyme concentrations on the activity of purified polygalacturonase.

Enzyme concentration (%).	Polygalacturonase activity (U/ml).
0.125	170
0.25	202.5
0.5	260
0.75	227.5

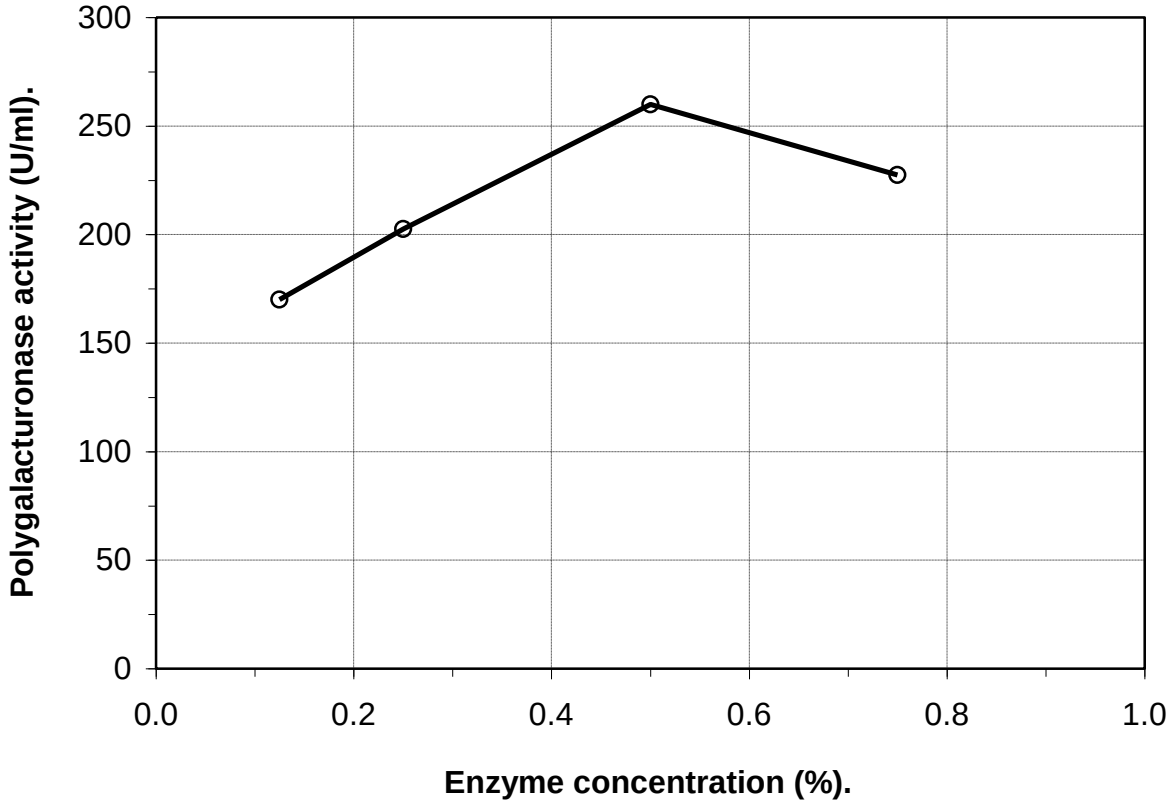


Figure (17): Effect of different enzyme concentrations on the activity of purified polygalacturonase.

Table (25): Effect of temperature stability on the activity of purified polygalacturonase, after in incubation period for 1 hour.

Incubation temperature (°C).	Polygalacturonase activity (U/ml).
30	115
35	142
40	175
50	187.5
60	215
70	270
80	275

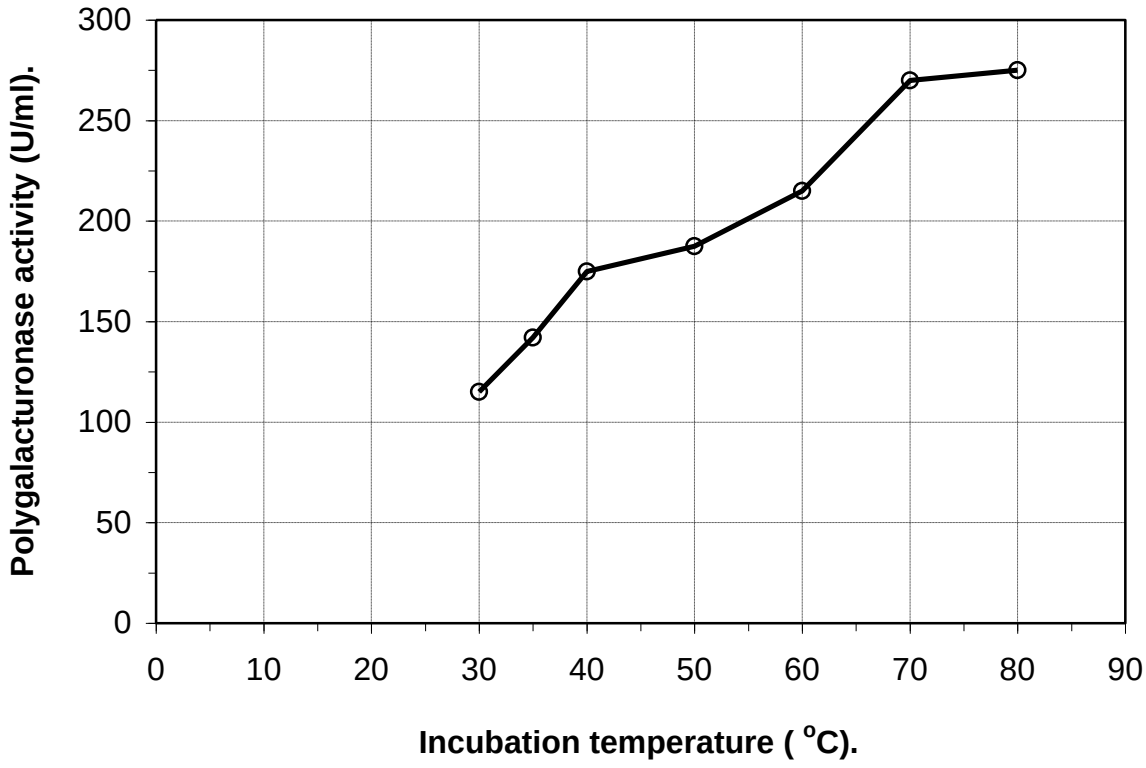


Figure (18): Effect of temperature stability on the activity of purified polygalacturonase.

Table (26): Effect of pH values stability on the activity of purified polygalacturonase.

pH value.	Polygalacturonase activity (U/ml).
3	0
4	0
5	0
6	25
7	47.5
8	105
9	195
10	245
12	285

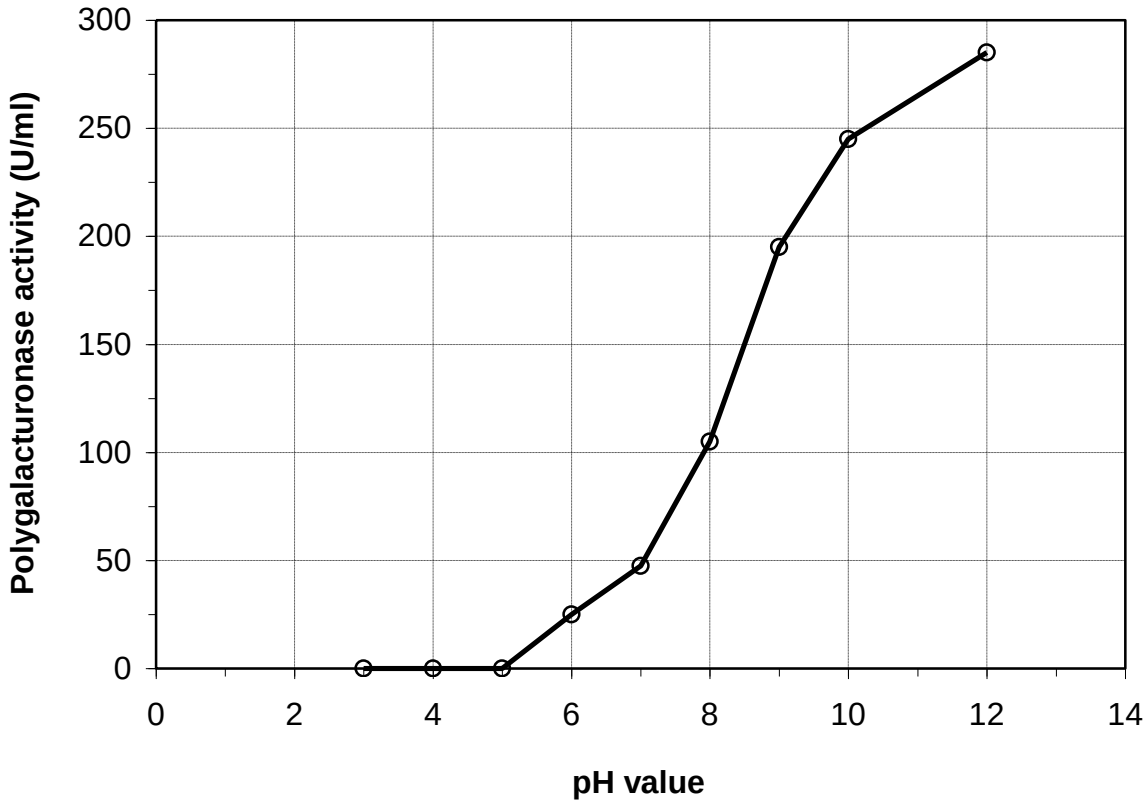


Figure (19): Effect of pH values stability on the activity of purified polygalacturonase.

Table (27): A summary of the optimal environmental parameters controlling the purified enzyme activity produced by *B. firmus*-I-10104 at 37 °C.

No	**Parameter**	**Purified enzyme activity**
1	Temperature (°C)	50
2	Substrate concentration (%)	2.0
3	pH value	12
4	Enzyme concentration (%)	0.75
5	Thermostability (°C)	80
6	pH stability	12

Section (C)

Biotechnological application of purified PG in clarification of Juava juice

Partially purified PG produced by *B. firmus*-I-10104 was subjected purposely to an application study included the following:

I- Study some parameters controlling the activity of purified PG in the reaction mixture of Nelson's technique e.g. commercial substrate concentration, PG concentration, pH values and effect of incubation temperature. the substrate (pectin) in the reaction mixture of Nelson's technique is replaced by the Juava juice.

II-Applying of PG in the clarification of commercial Juava juice.

Applying of the purified PG in the clarification of Juava juice using Nelson's technique:

1- Effect of different substrate (Juava juice) concentrations in the clarification of Juava juice:

This experiment was carried out in order to investigate the activity of purified PG in the clarification of Kaha Juava juice using Nelson's technique. The results given in Table (28) and Fig. (20) exhibited that, the maximum activity of purified PG was attained at (1.5) % substrate (Juava juice) concentration, where it reached up to 42.5 U/ml. Below and above this optimal substrate concentration and the enzyme activity gradually decreased.

Table (28): Effect of different substrate concentrations on the clarification of Juava juice.

Substrate concentration (%).	Polygalacturonase activity (U/ml).
0.5	20
1.5	42.5
2.5	32.5
3	25
4	5
5	0

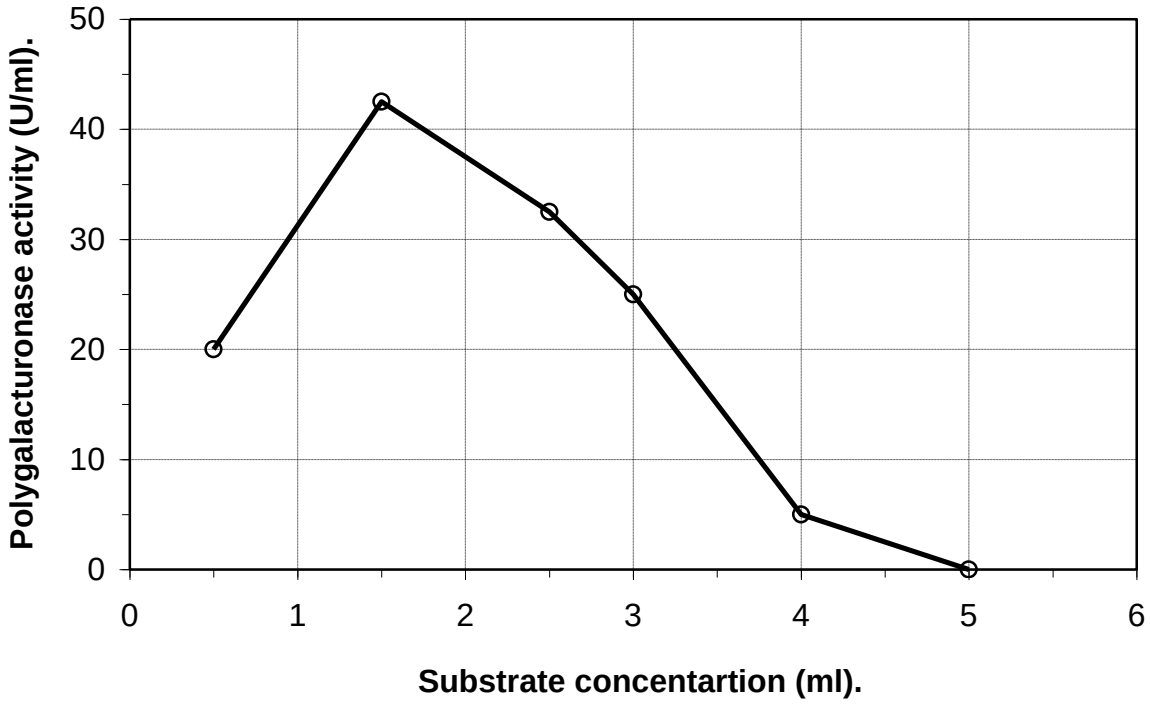


Figure (20): Effect of different substrate concentrations on the clarification of Juava juice.

2- Effect of different enzyme concentrations on the clarification of Juava juice

From the obtained results for the enzyme under study, it was found that there is a continuous increasing of enzyme activity due to the increase of enzyme concentration units, where it reached up to 147.5 U/ml, for the purified enzyme as shown in Table (29) and represented in Fig. (21).

3- Effect of different incubation temperatures on the clarification of Juava juice:

The results recorded in Table (30) and Fig. (22) illustrated that, the maximum activity of purified PG was obtained at 75 °C, where it reached up to 70 U/ml.

4- Effect of different pH values on the clarification of juava juice:

The data obtained from the Table (31) and Fig. (23) showed that, the purified PG exhibited the maximum activity at pH 5.0 of citrate phosphate buffer and gave 275 U/ml. Below and above this optimal pH value, the enzyme activity decreased gradually.

Table (29): Effect of different purified polygalacturonase concentrations on the clarification of Juava juice.

Enzyme concentration (%).	Polygalacturonase activity (U/ml).
0.5	20
1	7.5
1.5	52.5
2	72.5
3.5	130
4.5	147.5

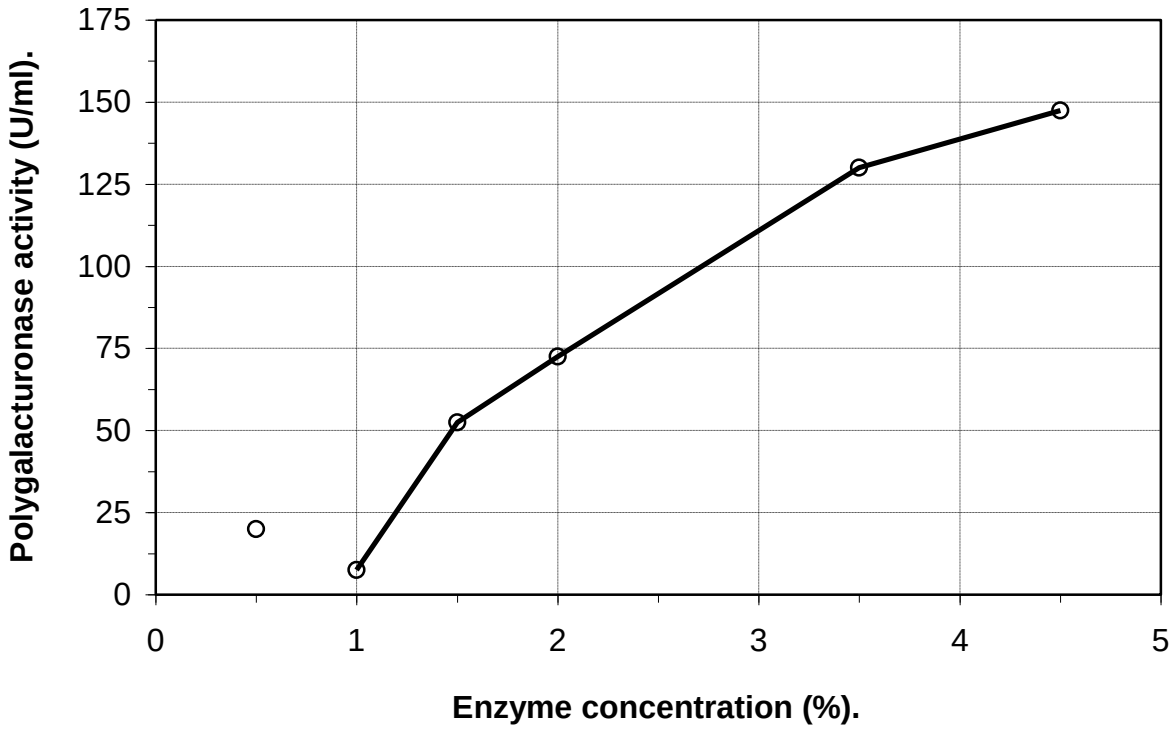


Figure (21): Effect of different purified polygalacturonase concentrations on the clarification of Juava juice.

Table (30): Effect of different incubation temperatures with purified PG on the clarification of Juava juice.

Incubation temperature (°C).	Polygalacturonase activity (U/ml).
15	0
25	40
35	20
45	60
75	70

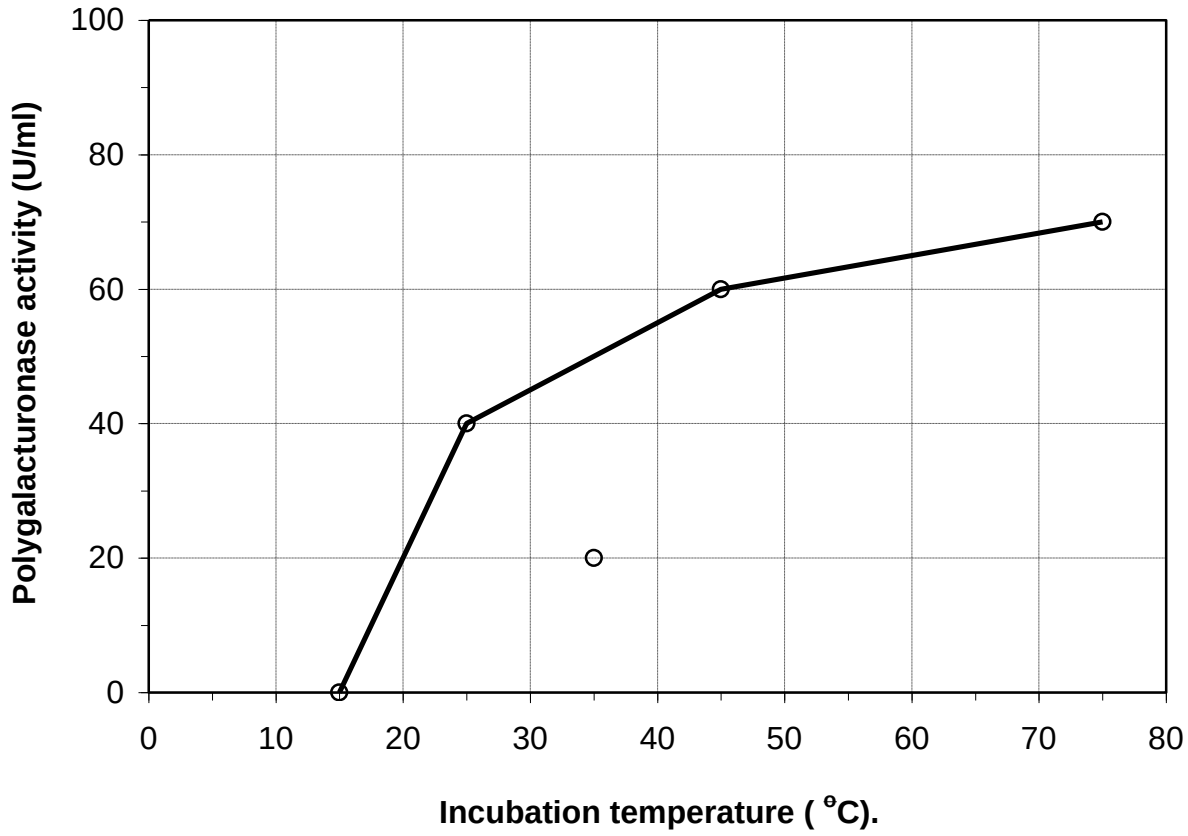


Figure (22): Effect of different incubation temperatures with purified PG on the clarification of Juava juice.

Table (31): Effect of different pH values with purified PG on the clarification of Juava juice.

pH values	Polygalacturonase activity (U/ml).
4	20.0
5	275
9	92.5
10	52.5

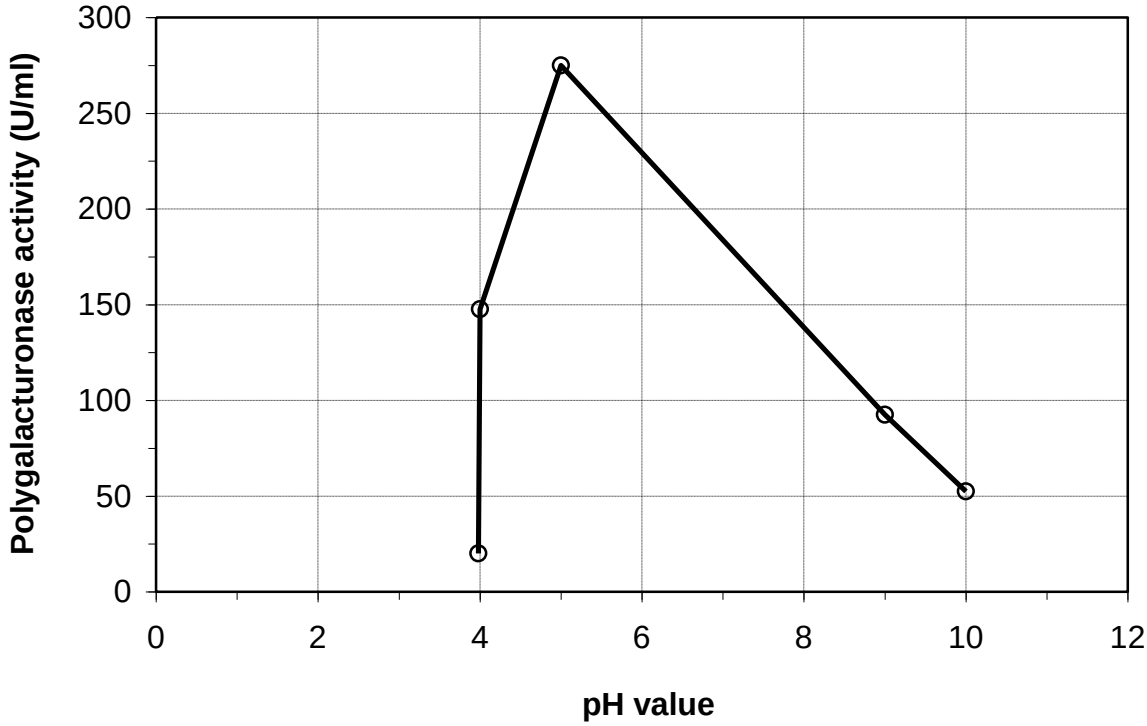


Figure (23): Effect of different pH values with purified PG on the clarification of Juava juice.

Table (1): Qualitative data of the pectinolytic productivities of the fifty one (51) bacterial isolates. Assay plates were incubated at 37 °C for 24 hours (First Survey).

No.	Code no. of bacterial isolates	Pectinolytic productivities Mean diameter of clearing zones (mm).	No.	Code no. of bacterial isolates	Pectinolytic productivities Mean diameter of clearing zones (mm).	No.	Code no. of bacterial isolates	Pectinolytic productivities Mean diameter of clearing zones (mm).
1	1046	2.5	18	3098	1.7	35	3034	2.5
2	3019	2.8	19	3021	2.2	36	30102	2.5
3	3066	2.4	20	4071	3.5	37	3062	2.6
4	3048	2.5	21	10404	3.5	38	40103	2.0
5	3035	1.7	22	3023	2.4	39	303	2.0
6	3012A	2.0	23	109	2.5	40	3065	2.0
7	3067	2.0	24	3011	2.5	41	30107	2.6
8	106	2.5	25	3074	2.0	42	1045	1.4
9	30101	2.2	26	4073	2.4	43	3029	2.3
10	1032B	2.5	27	3049	2.0	44	1043	2.4
11	1033	2.4	28	3022	2.4	45	4086	1.7
12	107	2.8	29	3050	2.6	46	30100	2.3
13	3052	2.2	30	40103	2.0	47	4087	2.6
14	4037	2.4	31	30106	0.7	48	3069	2.7
15	4072	2.0	32	3022	2.2	49	3080	2.0
16	3012B	2.0	33	30101	1.9	50	3084	2.7
17	3068	2.2	34	1032A	2.6	51	3056	2.5

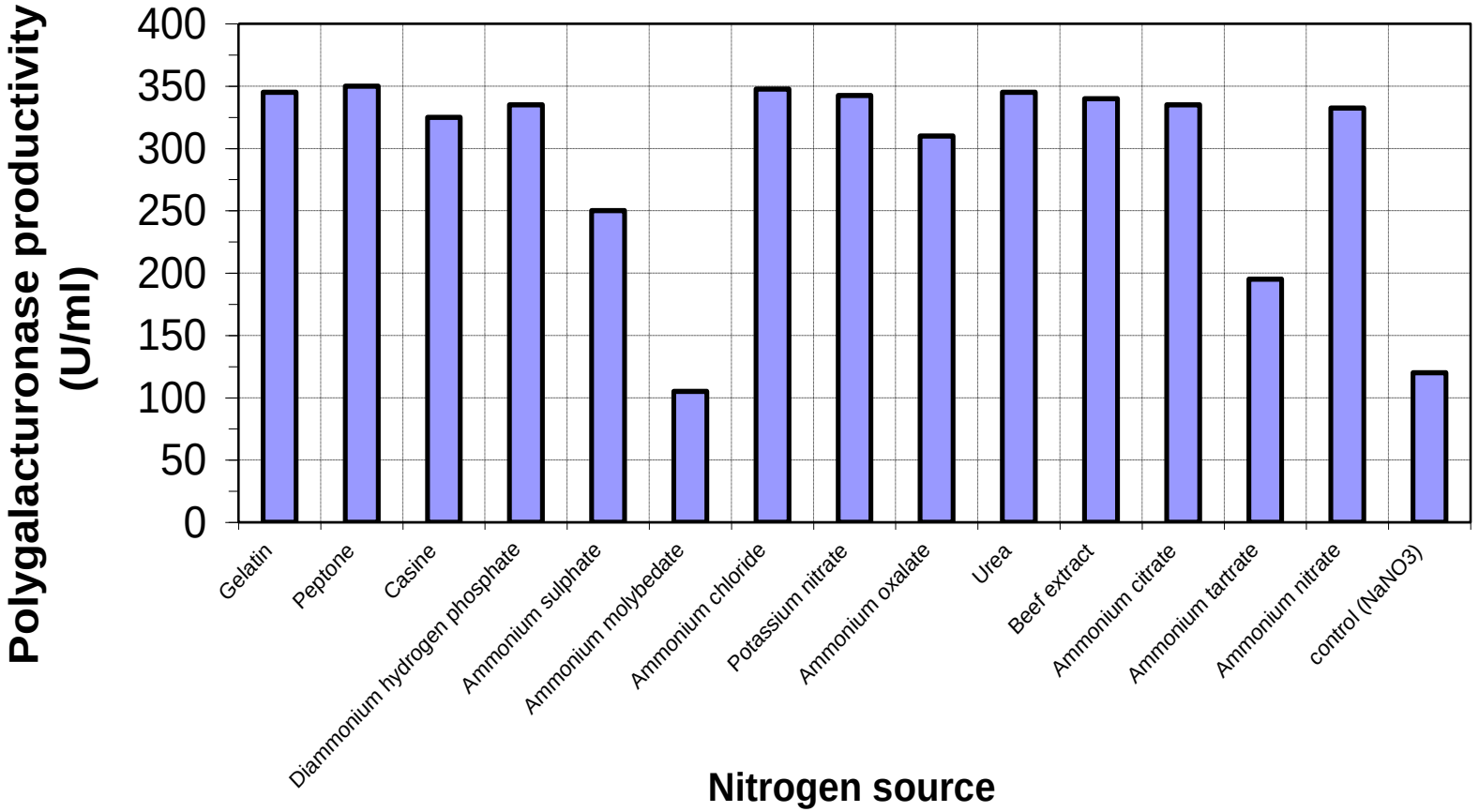


Figure (6): Relation among polygalacturonase productivity by *Bacillus firmus*-I-10104 with different nitrogen sources under solid state fermentation conditions.

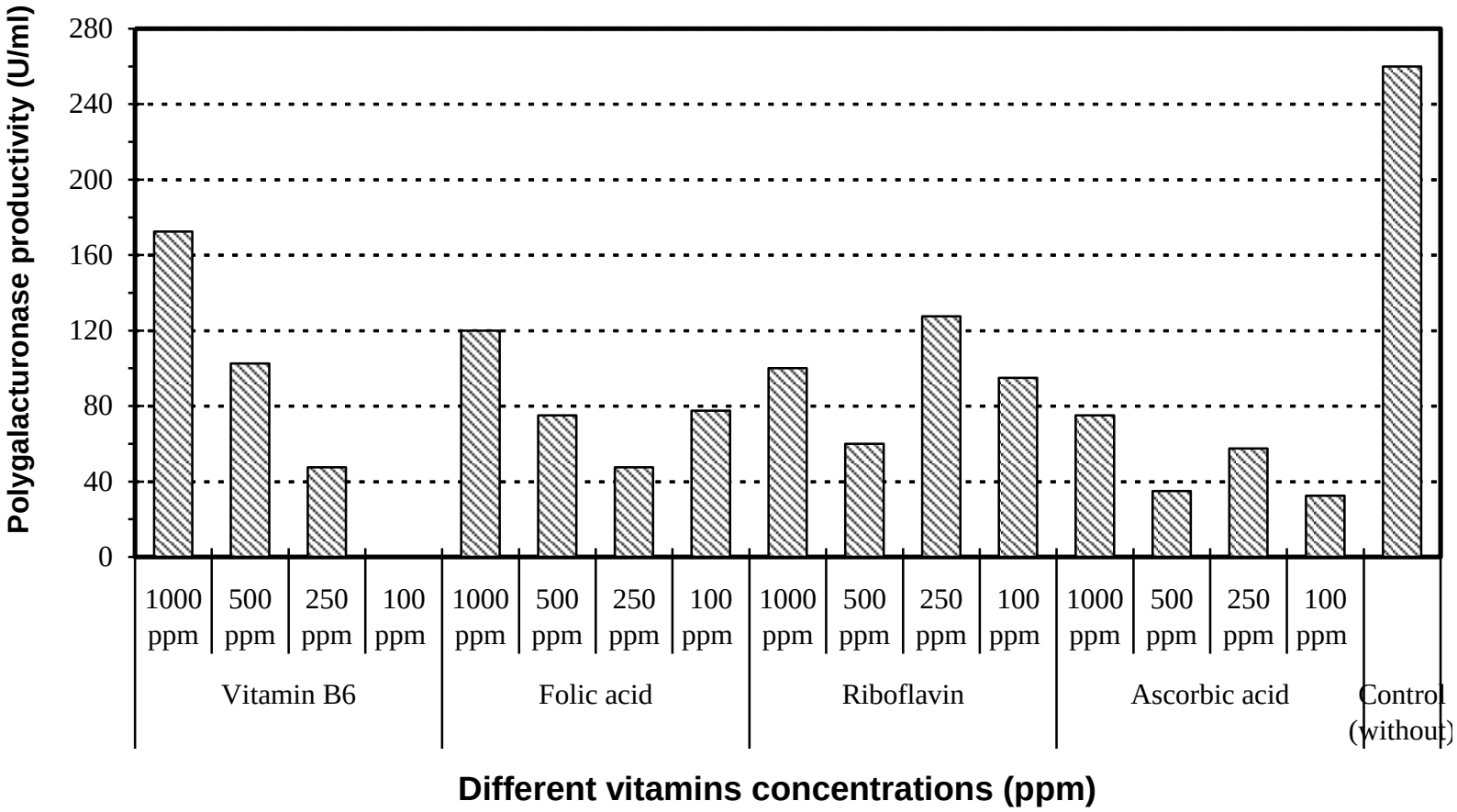


Figure (9): Relation between polygalacturonase productivity by *Bacillus firmus*-I-10104 with different vitamins concentrations under solid state fermentation (SSF) conditions.

Table (17): Ammonium sulfate saturation pattern of polygalacturonase produced by *Bacillus firmus-I*-10104.

Treatment		Volume (ml).	Polygalacturonase activity (U/ml).	Total activity (U/100ml).	Protein content (mg/ml).	Total protein content (U/100ml).	Specifiv activity (U/mg^{-1}) protein.
Ammonium sulfate concentration (%)	20	0.5	0.126	151.2	1.999	1199.4	0.126
	40	0.5	1.015	1218.0	1.648	988.8	1.231
	60	0.5	0.302	362.4	1.733	1039.8	0.348
	80	0.5	0.122	146.4	1.764	1058.4	0.138

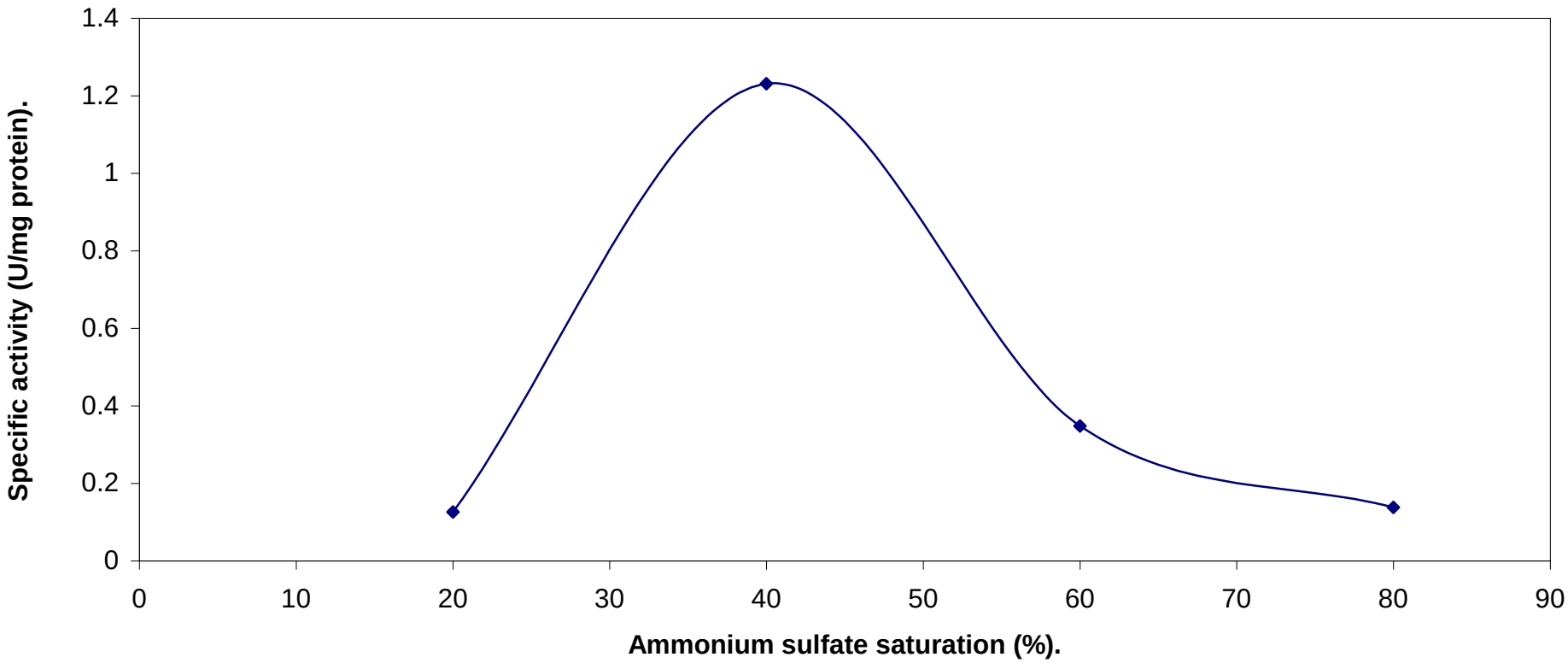


Figure (11): Ammonium sulfate saturation pattern of polygalacturonase produced by *Bacillus firmus*-I-10104.

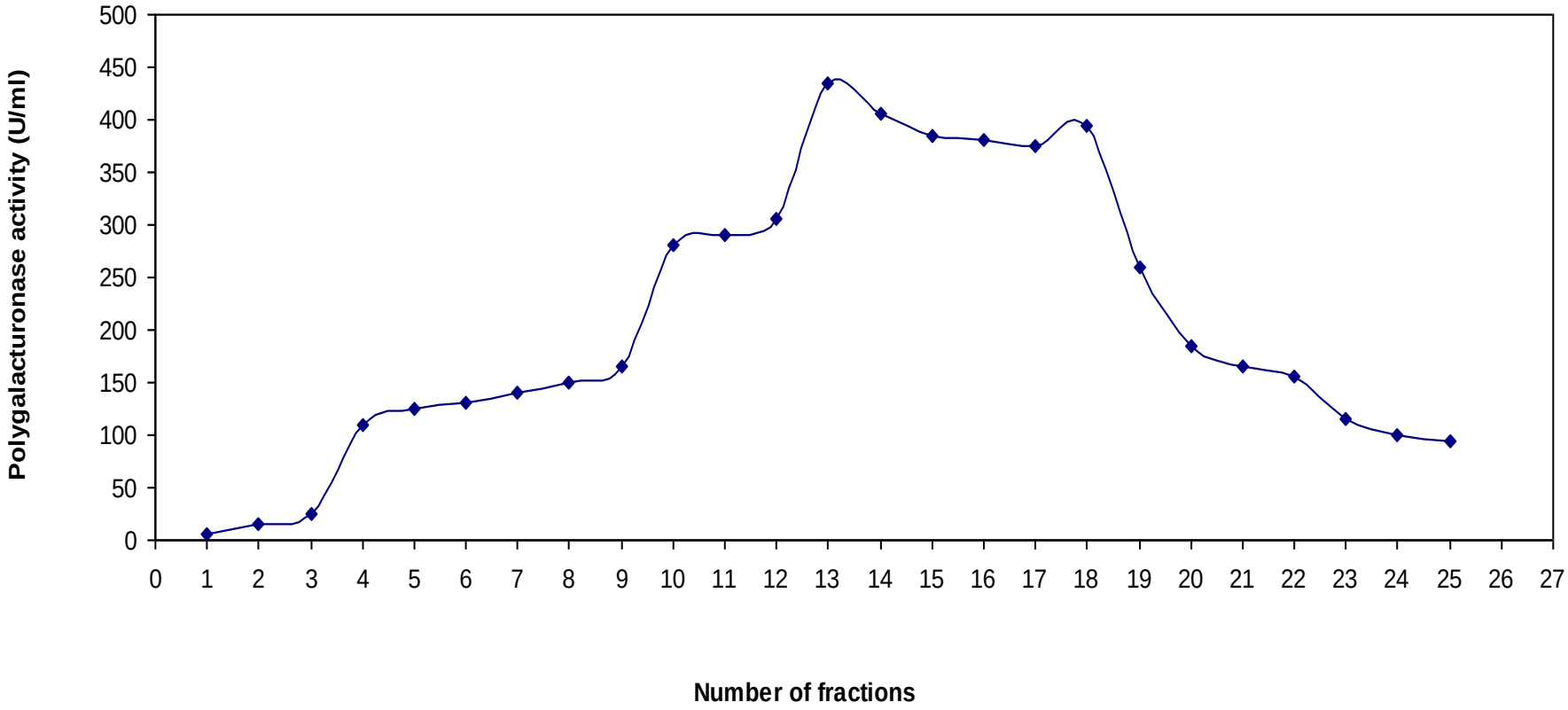


Fig. (12): Fractionation pattern of polygalacturonase productivity by *Bacillus firmus-I*-10104 on Sephadex G-200 column chromatography.

Table (19): Purification steps of polygalacturonase produced by *Bacillus firmus –I*-10104 allowed to grown on *S. tuberosum* substrate under solid state fermentation conditions.

Purification steps.	Total volume (ml).	Polygalacturonase activity (U/ml).	Protein content (mg/ml).	Total activity (U/ml).	Total protein content (mg/ml).	Specific activity (U/mg^{-1}) protein.	Purification fold.
Cell-free filtrate (CFF)	600	1.126	1.910	675.6	1146	0.589	1.0
$(NH_4)SO_4$ fraction (40%)	30	1.015	1.648	30.45	49.44	0.615	1.044
Dialysis against sucrose	5	1.287	0.156	6.435	0.78	8.25	14.00
Sephadex G-200 column chromatography (13-18)	30	1.182	0.097	5.91	0.485	12.18	20.670