PARTIAL PROPERTIES OF PECTIN METHYLESTERASE EXTRACTED FROM STREPTOMYCETE ISOLATE

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Abstract

Pectinases are one of the upcoming enzymes of fruit and textile industries. We are here reporting isolation and purification of pectin methyl esterase enzyme (PME) from an actinomycete isolate (StET). This isolate was identified and appeared to be close to *S. nigrifaciens*. Although there were some differences in the utilization of sucrose as sole carbon source for growth and producing faint yellow pigment. PME was purified *via* gel permeation chromatography on Sephacryl S_{200} column equilibrated in 0.1 M sodium acetate buffer at pH 4.2. Purification process of PME produced by *Streptomyces* sp. resulted in a pure protein preparation with specific activity of 1.70units/mg. A specific band with a molecular weight of about 110 KDa having a high specific activity 1.9 units/mg protein was obtained. PME was successfully purified by ammonium sulphate which increased the specific activity of PME 0.22 to 1.70. Results revealed that the optimum temperature range of the activity of purified PME was from 34 to 40˚C and the highest enzyme activity was recorded at pH 4.5. The effect of Zn^{+2}, Fe^{+2}, Mg^{+2}, Co^{+2}, Cu^{+2}, Cd^{+2} and Mn^{+2} at 50 µg/ml on PME activity was also determined.

INTRODUCTION

Pectin in native form is located in the cell wall and it may be interlined with other structural polysaccharides and proteins to form insoluble protopectin. Pectinases nowadays are upcoming enzymes of the commercial sector (Kashyap *et al*., 2001). Primarily, these enzymes are responsible for the degradation of the long and complex molecules called pectin that occur as structural polysaccharides in the middle lamella and the primary call walls of young plant cells. Pectin methyl-esterase (PME) is an enzyme that have been found in every plant tissue analyzed (Rexova-Benkova and Markovic, 1976), several fungi (Mendgen, *et al*., 1996), bacteria (Barras *et al*., 1994) and even insects (Shen *et al*.,1999). PME catalyzes the deesterification of galactosyluronate methylesters of pectins,releasing protons and methanol into the media (Frenkel *et al*., 1998). Despite the biochemical mode of action of PME is well known, it have been difficult to demonstrate any role for PME in the physiology of plants.

One of the main forms of tomato Pectin methylesterase (Pectin methyl-esterase E.C.3.1.1.11) that is applicable to the food industry was isolated from fresh tomato fruit. The extraction of the PME isoenzymes involved washing the fresh tomato flesh with water in order to remove sugars and than solubilizing the enzymes with a diluted HCl solution at pH 1.6 (Vovk *et al*., 2005). The effect of different metal ions on the activity of purified PME was proved to be promoters for the enzyme activity by *Erwinia chrysanthemi* (Abou Zeid and Tohamy, 2001).

Streptomyces species are found worldwide in soil and are important in soil ecology. Much of the characteristic earthy smell of soils arises from chemicals called

geosmens given off by *Streptomyces* species. Streptomycetes are metabolically diverse and can "eat" almost anything, including sugars, alcohols, amino acids, organic acids, and aromatic compounds. This is achieved by producing extracellular hydrolytic enzymes (Brühlmann *et al*., 1994).

Pectinases are now having various biotechnological applications; therefore, this study was designed to isolate and purify the PME from a Streptomycete isolate followed by partially characterization of the obtained PME and determination of some metallic salts effect on the enzyme activity.

Materials and methods

Source of bacterium isolate: The Streptomycete isolate under investi-gation was kindly provided by Dept. Agricultural Microbiol., Institute of Soil, Water and Environment Research (ISWER), Agricultural Research Center (ARC), Giza, Egypt.

Isolate identification: The cultural, morphological and physiological characteristics of the actinomycete isolates under investigation were determined as descrybed by Mohamed (1998). The key given by Pridham and Tresner (1974) was used for identification and the media used in these keys were described by Shirling and Gottlieb (1966).

Medium used: For production of PME, a medium consists of 20g/L citrus pectin, 2.0 $NaNO_3$, 1.0 K_2HPO_4, 0.5 KCl, 0.01 $FeSO_4.7H_2O$, 1.0 $CaCO_3$ and distilled water. The pH 6.4 was adjusted by using pH-meter before autoclaving.

Determination of PME activity: The PME activity was determined according to the method described by Ann and Paul (1986). The reaction was monitored at 620nm in a spectrophotometer.

Protein content: In this experiment, the method of Lineweaver and Jansen (1951) was applied to assay the protein content using bovine serum albumin as a standard and measuring the absorbance at 750 nm.

Purification of PME: On incubation period, the extracellular PME were separated by centrifugation at 2000 rpm for 20 min at 4°C and the supernatant was separated and used for PME purification as reported by Flurkey *et al.* (1978) at concentration of 66% saturations.

Filtration of PME *via* Sephacryl S_{200} column: The gel permeation chromatography on Sephacryl S_{200} column was used for the determination of the PME purity using the method of Harry and Jaap (1990). Elution was done by 0.1 M sodium acetate buffer, pH 4.2. The fractions (10 ml aliquots) were collected and dry tubes. The PME activity and protein content of each fraction were determined. The PME purity was checked using the gel electrophoresis method as reported by El Hawary and Ibrahim (1968).

Factors affecting PME activity: The effect of incubation temperature (Bongaerts *et al.,* 1978); pH values (3, 4, 5, 6, 7, 8) and some metallic salts (50µg/ml) on the activity of PME or PME stability were measured (Abou Zeid and Tohamy, 2001).

RESULTS AND DISCUSSION:

Description of *Streptomyces* isolates: The halotolerant *Streptomyces* isolate (**StSET)** which tolerates 7% salt, and obtained from sandy soil was identified according to the key proposed by Pridham and Tresner (1974). Data in Table-1 clearly indicate that the *Strepto-myces* isolate StSET belonged to the gray colour series and the substrate mycelium produced no pigments on the standard media used. The spores characterized by

rectiflexible and smooth surface. Melanoid pigment not formed on the standard media. This isolate characterized by moderate growth on Czapek's agar medium. The StSET isolate was able to utilize four out of the eight-used sugars as sole carbon source for growth. Moreover, the isolate was considered as halotolerant as it was able to grow in the presence of 7% NaCl in the growth medium. The isolate under investigation (isolate StET) appeared to be close to *S. nigrifaciens*. Although there were some differences in the utilization of sucrose as sole carbon source for growth and producing faint yellow pigment.

Pectinases are one of the upcoming enzymes of fruit and textile industries. These enzymes break down complex polysaccharides of plant tissues into simpler molecules like galacturonic acids. The role of acidic pectinases in bringing down the cloudiness and bitterness of fruit juices is well established. Recently, there have been a good number of reports on the application of alkaline pectinases in the textile industries (Kashyap *et al*., 2001). Pectinolytic enzymes are involved in the retting and degumming of jute, flax, hemp, ramie, kenaff (*Hibiscus sativa*), and coir from coconut husks (Chesson, 1980 and Bruhlmann *et al*., 1994).

In this study, PME was purified via gel permeation chromatography on Sephacryl S_{200} column equilibrated in 0.1 M sodium acetate buffer at pH 4.2. Results also showed that a specific band with a molecular weight of about 110KDa (Data not shown) had a high specific activity 1.7units/mg protein (Table- 1). This result agrees with that reported by Rao and Kembhavi (1996) and Abou Zeid and Tohamy (2001). It was also noted that the use of ammonium sulphate increased the specific activity of the enzyme from 0.22 to 1.7 and concen-tration increased up to 5.5 folds (units/ mg protein).

Table-1: Purification of PME of *Streptomyces* sp. by ammonium sulphate.

Protocols	Volume (ml)	PME activity (units)	Total protein (mg)	PME activity (μ/mg)	Purification folds
Crude extract	45	72.00	24.0	0.22	0.9
Ammonium sulphate	25	19.00	7.0	1.7	5.5

Data in Tables-2 and 3 showed that the relation between specific activity and protein content was only one sharp peak with highest activity (1.70μ/mg) protein which is corresponding to 70.1 folds.

Table-2: Purification of PME of *Streptomyces* sp. using gel permeation chromatography (Sephacryl S_{200}) (GPC).

Protocols	Volume (ml)	PME activity (units)	Total protein (mg)	PME activity (μ/mg)	Purification folds
Crude extract	50	72.00	24.0	0.22	0.9
GPC	10	11.50	0.91	1.70	70.1

Table-3: PME activity (μ/mg) and total protein (mg) of fractions of purified PME of *Streptomyces* sp. using gel permeation chromatography (Sephacryl S_{200}) (GPC).

Fractions No.	PME activity (μ/mg)	Total Protein (mg)	Fractions No.	PME activity	Total protein
1	0.22	0.17	24	0.32	0.41
4	0.20	0.16	28	0.80	0.62
8	0.21	0.15	32	1.70	0.91
12	0.21	0.20	36	0.75	0.73
16	0.22	0.18	40	0.32	0.34
20	0.20	0.16	44	0.16	0.16

Results in Table-4 revealed that the optimum temperature range of the activity of purified PME was found 37˚C, while it was denaturated at 48˚C and the activity declined.

Table-4: PME activity (μ/mg) of purified PME of *Streptomyces* sp. at different incubation temperatures.

Incubation temperature (°C)	% of relative PME activity
28	17.64
31	36.52
34	78.65
37	100
40	46.06
44	17.64
48	10.12

The experimental results showed that effect of pH on the activity of purified PME was increased gradually with increase in the pH value and the highest enzyme activity was recorded at pH 4.5 (Table 5).

Table-5: Effect of pH degrees on the activity of purified PME.

pH	% of relative PME activity
2.5	23.52
3.5	35.29
4.5	100
5.5	47.06
6.5	17.65
7.5	11.76

Data in Table-6 revealed that highest activity of the purified PME was obtained by adding Zn^{+2}, Fe^{+2}, Mg^{+2}, Co^{+2}, Cu^{+2}, Mn^{+2} (at concentration of 50 μg/ml), respectively, and less PME activity was observed with Cd^{+2} (50μg/ml). These results are in harmony with that found by Pinckard and Wang (1971) who showed that PME from culture filtrate of *Diplodia gossypina* was stimulated by Mn^{+2}, but inhibited by EDTA. Spalding and Abdul-Baki (1973) who reported that Mg^{+2} stimulated pectinlyase activity of *Penicillium expansum*. Abou Zeid and Tohamy (2001) studied the effect of different metal ions (Mg^{+2}, Zn^{+2}, Cu^{+2}, Mn^{+2} & Fe^{+2}), on the activity of purified PME produced by *Erwinia chrysanthemi*. They proved that these ions promoted the enzyme activity.

Table-6: Activity (units/mg protein) of PME of *Streptomyces* sp. affecting with some metallic salts (50 mg/ml).

Metallic salts	PME activities
$ZnSO_4$	1.75
$FeSO_4$	1.70
$MgSO_4$	1.64
$CoSO_4$	1.63
$CuSO_4$	1.60
$MnCl_2$	1.40
$CdSO_4$	0.75
Control (Metallic salts-free)	0.008

Table-1: Taxonomical characters of Streptomycete isolate named StSET producing PME enzyme**.**

I- Main taxonomical characters				
Color of aerial mycelium	Spore-chain	c-Melanoid pigments (C)		d-Spore surface
Gray	RA	C -		Smooth
e-Utilization of carbon compounds				
D-Glucose	+	Raffinose		±
D-Xylose	+	D-Mannitol		+
L-Arabinose	+	i-Inositol		±
L-Rhamnose	+	Sucrose		-
II- Other assisted characters				
Growth on Czapek's medium	Antagonistic activity	Color of substrate mycelium	Diffusible pigments	NaCl tolerance
Moderate	ND	No pigments	-	7%

C= not produced. RA= spore chain in the form of open loops, hooks or greatly extended coils of wide. - = not utilized or not produced. + = utilized. ND= Not determined.

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