

## Production and Characterization of Pectic Enzymes of

### *Xanthomonas campestris* pv. *sesami*

Nehal Osman Mohammed<sup>1</sup> Mai Abdalla A. Abdalla<sup>2</sup>, and Awad M. Abdel Rahim<sup>2</sup>

1. Univ. of Gezira, Faculty of Engineering and Technology, Center of Biosciences and Biotechnology
2. Univ. of Gezira, Fac. of Sciences, Department of Biochemistry & Molecular Biology

### ABSTRACT

The bacterium *Xanthomonas campestris* pv. *sesami*, is the causal organism of the leaf spot disease (Marad eldum) in sesame plants. Pectic enzymes which were reported to be produced by many plant pathogenic bacteria, were found to play an important role in pathogenesis. There are three main types of pectic enzymes (Polygalacturonase, PG, Polygalacturonate trans-eliminase PTE and Pectin methylesterase PME). The present study was conducted to investigate the capacity of the bacterium *X. campestris* pv. *sesami*, to produce the different types of pectic enzymes. A salt medium supplemented with different pectic materials as carbon sources were tested for the production of the enzymes. The produced enzymes were characterized using different selective tests, both the viscosity and reducing group methods were used to measure the enzyme activity. The results showed that no pectin methylestrease (PME) was produced and only the Polygalacturonase trans-eliminase (PTE) was produced by the bacterium. Sodium polypectate and polygalacturonic acid were found as the best substrates giving 26.9 and 19.8 viscosity enzyme units, respectively. The enzyme activity was maximum at the 6<sup>th</sup> day (9.3 viscosity units), then the activity was decreasing with further incubation time. The test of the effect of the pH indicated that the pH optimum of the enzyme activity was at pH 9.0. The effects of different metallic ions to the enzyme activity showed that,= a higher activity was found with Ca<sup>++</sup> which gave 12.2 viscosity units. When different concentrations of Ca<sup>++</sup> were tested, the maximum activity of the enzyme was found at 50 mg/ ml concentration, which gave 18.0 viscosity units. It could be recommended that the tested bacterium should be investigated for the production of the cellulases and hemicellulases which also have a role in the pathogenesis process.

**Key words:** Pectic enzymes production, *Xanthomonas campestris* pv *sesami*, Sesame leaf spot disease

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### INTRODUCTION

Enzymes are ubiquitous class of protein that acts as biological a catalyst, catalyzing all chemical reactions (Timoth, 2001). In the last few years, biotechnologists utilized the techniques for extraction and purification of proteins (enzymes) from microbial cultures (Scawen and Milling, 1985). An important application of enzymes in food processing is to increase the yield of product from the raw material, and to make it more amenable to treatment particularly in fruit and vegetable processing. Here the use of pectinases, cellulases and hemicellulases assists cell separation and weakening the cell walls so that the material can be more readily pulped and the yield of juice on pressing will be considerably enhanced (Birch *et al.*, 1981). Microorganisms produce enzymes which are involved primarily in the degradation of macro – molecules to units capable of being taken into the living cell (Fogarty and Kelly, 1979). Out of the 2000 various enzymes that have been described, only a few like cellulolytic, pectolytic and amylolytic enzymes have a wide industrial and biotechnological application (Bhat and Bhat, 1997). Fungi are the main micro-organisms reported to be enzymes producers, including species of the genera *Trichoderma*, *Penicillium* and *Aspergillus* (Coral *et al.*, 2001). Cellulases and pectinases are extra – cellular enzymes they are consist of more than one major compound which acting together to hydrolyze cellulose and pectin to glucose , respectively. Fungal cellulases and pectinases are widely used in industry for example in commercial food processing, pulp and paper industry, textile processing and they are even used in pharmaceutical applications (Chapin *et al.*, 2002). The present study was carried out to detect the ability of the bacterium *Xanthomonas campestris* pv *sesami* to produce pectic enzymes. The bacterium was reported as the causal organism of the leaf spot disease in sesame plants in different parts of the world (Sabit and Dowson, 1960, Abdel – Rahim, 1980, Azanaw, *et al.* 2018 and Golla, *et al.* 2020).

## MATERIALS and METHODS

### Plant Source

Infected sesame leaves (Fig 3) showing the leaf spot disease were obtained from the Research Station Wad – Medani.

### Isolation of the Bacterium

The bacterium *Xanthomonas campestris* pv *sesami* was isolated from the infected sesame plant leaves using the yeast extract glucose medium which is selective for *Xanthomonas* spp as was described by Abdel-Rahim (1980).. For further experiments the bacterium was grown in a nutrient agar or a nutrient both media, at 20-30 C. For the pectinase enzymes production, the bacterium was grown on a basic salt medium sublimated with a pectic substrate containing the following (g/L) as shown in Table (1).

**Table(1). The basic salt medium sublimated with different pectic**

#### Substrates in one liter of water

Substrates	G/ Liter
K <sub>2</sub> HPO <sub>4</sub>	1.0
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	0.5
MgSO <sub>4</sub> .7H <sub>2</sub> O	0.5
Pectic substrate	10.0
Distilled water	1000.0 ml

### Assays of Pectinases

#### Preparation of the pectinase enzymes:

After incubation for the required time, culture filtrates were centrifuged at 16,000 r.p.m for 15 minutes. The sterile filtrates were then aseptically collected in sterile McCartney bottles and stored in 10 ml portions at 20°C. Reaction mixture contained: 8.0

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ml w/v substrate in 0.1M buffer, 1.0 ml 0.01 CaCl<sub>2</sub> (0.001M), 1.0 ml enzyme. Both autoclaved enzyme and 0 times reaction mixtures containing active enzyme were employed as controls.

### **Pectinase activity measurement**

#### **Viscometric Method**

Pectinase was assayed by viscosity reducing method. Using U –tube viscometers (BS \ U, size G) containing 5 ml enzyme solution and 5 ml of a pectic substrate (1%) in citrate buffer (0.1 m) at 30°C. Reaction mixtures were as described above. Flow time through the tube recorded using a stop watch after each 4 minutes, up to 24 minute from the start of the reaction. The efflux time of substrates alone, without enzyme (zero time), and the efflux time for substrate – plus – enzyme treatment were measured enzyme activity is expressed as 100/t where t is the time\ minutes (Abdel – Rahim, 1980).

#### **Release of reducing groups:**

Reducing groups at reaction mixtures were estimated by the Nelson- Somogyi method (Nelson, 1944). Copper reagents were mixed on the day of use, 25 parts A to 1 part B. the test sample (0.2 ml) was added to 0.8 ml water and 1.0 ml of the mixed copper reagent and heated for 30minutes on a boiling water bath. After cooling 1.0 ml of arsenomolybdate reagent was added, the blue colour produce was measured at 660 nm against the control treatments .

#### **Cup-plate assay:**

This method was used to test for pectin methyl esterase (PME) and polygalacturonate trans-eliminase (PTE) activities , as was described by Abdel-Rahim (1980).

#### **Pectin methyl esterase activity:**

Washed pectin (2.5 g) was added to 245 ml water and the pH was adjusted to 6.0, methyl red solution (5 ml), salicylanilide (0.025 g) and agar (5 g) were added. The mixture was heated to dissolve the pectin and the agar, and autoclaved at 121°C for 15

minutes, 25 ml aliquots were then pipetted into 9.0 cm diameter plastic Petri dishes and when set, 7 mm diameter wells were cut into the medium and the bottoms of the wells were sealed with a film of agar to prevent leakage. 0.1 ml culture filtrates were pipette into the wells, plates were incubated at 25°C and observed after 24 hours intervals. PME activity will be detected as: red zone surrounding the wells (Abdel- Rahim, 1980)

### **Factors affecting the invitro activity of the enzyme:**

The tested factors include; the effect of temperature, The effect of cofactors (activators and inhibitors) and the effect of pH.

## **EXPERIMENTAL RESULTS**

### **Effect of different substrates on the Enzyme Production**

The ability of the bacterium *X. campestris* pv *sesami* to produce pectic enzymes was tested on a mineral salt medium supplemented with different substrates as carbon sources. Two methods were used, the viscosity method for the PTE enzyme and the cup plate method for the PME enzyme., After incubation for seven days at 28 °C for, culture filtrates were collected and tested the pectic enzyme activity. The results in Table (2) show that both sodium polypectate and polygalacturonic acid were giving high PTE enzyme activity. However pectin culture filtrates were showing very low enzyme activity. On the other hand, no PTE enzyme activity was detected with any of the other substrates tested. The results of the production of the PME enzyme on different substrates showed that no PME was produced with any of the substrates tested using the reducing group method. The substrates included; Sodium polypectate, Polygalacutonic acid Pectin and Pure cellulose.

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**Table (2). Pectinase enzyme production on different substrates using viscosity method after 8 days incubation time, pH (10) at 37 °C**

Substrate	Pectic enzyme activity(RUV)
Sodium polypectate	26.6
Polygalacturonic acid	19.8
Pectin	2.5
Pure cellulose	0.0
Xylan	0.0
Galactan	0.0
Arabinan	0.0
Starch	0.0
Glucose	0.0

Note: Values in the table are the mean values of three replicates

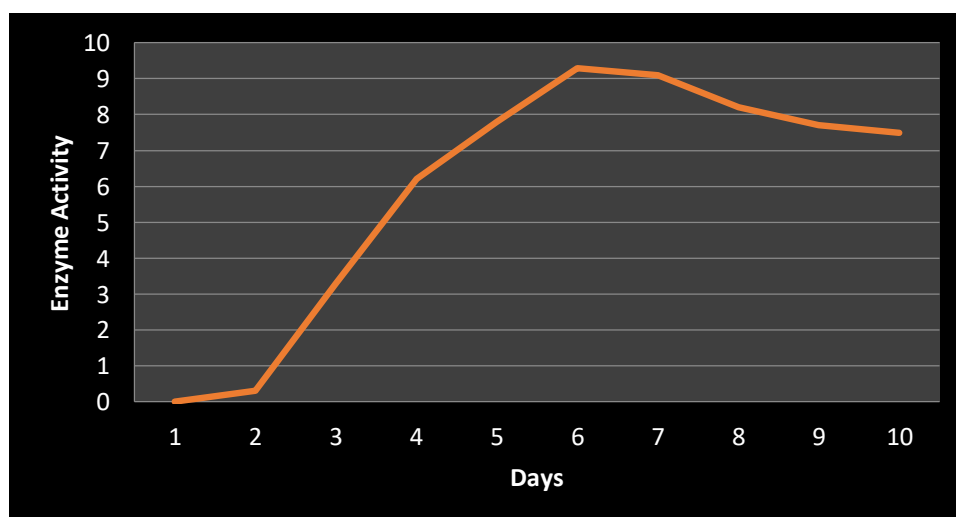
### **Effect of the Incubation Time on the Enzyme Production**

The effect of the incubation time of the pectic enzyme produced by the bacterium *X. campestris* pv. *sesami*, was investigated in the present using the viscosity reduction method. The activity of the produced enzyme was measured daily for ten days. The results are shown in Table (3) and Fig. (1), indicated that the enzyme activity was first detected on the second day. The activity was increased continuously reaching its maximum activity on 6<sup>th</sup> and the 7<sup>th</sup> days. However, the enzyme activity was then declined with further incubation time .

**Table (3): Effect of the incubation time on the production of the pectic enzyme by *X. campestris pv. sesami.* at 37 °C**

Culture age (days)	Activity(RUV)
1	0.0
2	0.3
3	3.3
4	6.2
5	7.8
6	9.3
7	9.1
8	8.2
9	7.7
10	7.5

Note: Values in the table are the mean values of three replicates



**Fig.1: Effect of the incubation time on the pectic enzyme (PTE) produced by *X. campestris p.v sesemi* using viscosity method at 37 °C.**  
**Effect of the pH value on the Pectic Enzyme Activity**

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The effect of the hydrogen ion concentration (pH) on the activity of the pectic enzyme produced by *X. campestris* pv. *sesami*, was studied in the present work, using the viscosity reduction method. The activity was measured every two days for ten days. The results (Table, 4 and Fig. 2) show that the optimum pH value for the enzyme activity was detected at pH 9.

### **Effect of different metal ions on the Pectic Enzyme activity**

The effect different metal ions ( $\text{Ca}^{++}$ ,  $\text{Ba}^{++}$ ,  $\text{Mg}^{++}$ ,  $\text{Cu}^{++}$ ,  $\text{Mn}^{++}$ , and  $\text{Fe}^{++}$ ) on the pectic enzyme activity were made using the reducing sugar method as described in chapter three. The results on Table (5) showed that the addition of calcium ions to the enzyme assay caused the highest increase in the activity. Barium and Magnesium ions were also effective. On the other hand, Manganese, Copper and ferric ions were less effective.

### **Effect of $\text{Ca Cl}_2$ concentration on the Pectic Enzyme Activity**

The effect of the calcium chloride concentration on the activity of the pectic enzyme produced by *X. campestris* pv. *sesami*, was investigated in the present work, using the viscosity reduction method. The followings concentrations were used 0.00, 0.0001, 0.0005, 0.001 and 0.005 M. However, EDTA at the concentration 0.005 M, also included in the test. The release of the reducing groups method was employed. The optimum rate of the enzyme activity was found at the 0.005  $\text{Ca Cl}_2$  concentration. On the other hand, the addition of the EDTA was resulting in an almost complete loss of the enzyme activity (Table, 6).

**Table (4): pH optimum of the pectic enzyme produced by *X. campestris p.v. sesami* at 37<sup>0</sup>C for 8 days**

<b>pH</b>	<b>Activity (RVU)</b>
<b>4</b>	<b>0.1</b>
<b>5</b>	<b>0.2</b>
<b>6</b>	<b>0.5</b>
<b>7</b>	<b>8.0</b>
<b>8</b>	<b>12.0</b>
<b>9</b>	<b>20.0</b>
<b>10</b>	<b>15.0</b>

Note: Values in the table are the mean values of three replicates  
RVU: Relative Viscosity Units

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**Table (5): Effect of different metal ions (mg/ml/glucose) on the pectin enzyme activity produced by *X. campestris* pv *sesami***

metal ions	enzyme activity (mg/ml glucose)
Ca <sup>++</sup>	122
B <sup>++</sup>	95
Mg <sup>++</sup>	82
Mn <sup>++</sup>	51
Cu <sup>++</sup>	33
Fe <sup>++</sup>	52
EDTA	0.0

Note: Values in the table are the mean values of three replicates

**Table (6). Effect of the calcium chloride Concentration on the activity of the Pectic enzyme produced by *X. Campestris* pv. *sesami***

CaCl <sub>2</sub> Concentration	Enzyme activity( mg/ml glucose)
0.0	0.0
0.5	2.0
1.0	4.0
5.0	6.0
10.0	8.0
50.0	18.0
100.0	15.0
500.0	0.0

Note: Values in the table are the mean values of three replicates

## DISCUSSIONS

Microorganisms produce enzymes which are involved in the degradation of macro-molecule to units capable of being taken into the living cell (Fogarty and Kelly 1979). Enzymes were defined by Braverman (1974) as organic, colloid catalysts mostly soluble in water. Their properties are determined by their structures. Pectin enzymes are a group of enzymes that degrade pectin substances by different mechanisms (Mirjana *et al.*, 2004). They are mixtures of enzymes which act together to degrade the pectin molecule (Ranveer *et al.*, 2005). The bacterium *Xanthomonas campestris pv. sesami* is the causal of the Leal spot disease of sesame plants (Sabet and Dowson, 1960). It was reported that pectin enzymes could have a major role in bacterial pathogenesis. Pectin substances are present in the primary cell wall and are the major components of the middle lamellae (Rowboats and Pinnik, 1980). Their degradation will pave the way for the penetration of the bacterium and attack the cells .

In the present study, the tested bacterium was grown in a basal salt medium containing different pectinase sources. The test showed that sodium polypectate and polygalacturonic and were the best inducers of the pectin enzyme. However, pectin gave a very low enzyme activity, while all the other compounds were found non-inducers- if has been reported that the increase in the rate of a specific enzyme is usually caused by the presence of substrate which acts as an inducer (Gupta and Ayyachamy, 2012). Sodium polypedate and polygala of uranic and were also reported as the best inducers of the pectin enzyme production by many fungi (Mustafa, 2008). The effect of the incubation time indicated that the enzyme activity was begun on the second day and then increased reaching its maximum activity at the 6th and 7th days, decreased. Similar results were reported by Mustafa (2008) for the pectin enzyme produced by three different fungi. Maximum activity of the pectin enzyme produced by *Aspergillums niger* was observed after 7 days of incubation. According, to the same author the maximum activity of both pectin and cellulase enzymes produced by *Penicillin erhordei* were at the 10th and 15th day, respectively. The present study was also investigated the effect of the pH values on the pectin enzymes produced by *X. campestris pv. sesami*. The results show that the enzyme activity was maximum the pH value 9.0. The different pectin

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enzymes were reported to have different pH optima. Pectin methylesterase obtained from the bacterium *Erwinia chrysanthemi* was found to have an optimum pH at the alkaline pH values (Laurent *et al.*, 2000). However, the same enzyme obtained from the fungus *Aspergillus japonicus* was reported to have its maximum activity at pH 3.8 (Hasunuma *et al.*, 2003). On the other hand the highest activity of both pectinase and polygalacturonase of *Aspergillus fumigatus* were found at pH 4.5 and pH 5.0, respectively (Urmila *et al.*, 2005). The pectinase enzyme obtained from the thermophilic fungus *Aerophialo phoranainlana* was more active at pH. 8.0 (Maria *et al.*, 2006). However, the optimum pH value for different pectinases has been reported to vary from 3.8 – 9.5, depending on the type of the enzyme and the source from which it was obtained (Acuna *et al.*, 1995).

## REFERENCES

- Abdel- Rahim, A. M. (1980).** Studies on Sesame Leaf Spot caused by *Xanthomonas sesame* (Sabet and Dowson). Ph.D. thesis, Department of Botany and Plant Technology, Imperial Collage of Science and Technology. London.
- Acuna, A. M.; Gutierrez, R. M.; Viniegra, G. G. and Favela, T. E. (1995).** Production and properties of three pectinolytic Activities produced by *Asperigillus niger* in submerged and solid state fermentation. *Journal of Applied Microbiology and Biotechnology*, 43:808-814.
- Azanaw, A., Belay, Y., Ebabuye, Y., & Ayizengaw, G. (2018).** Screening of Sesame Germplasm Against Bacterial Blight (*Xanthomonas campestris* pv. *sesami*) at Metema Ethiopia. *Academic Journal of Life Sciences*, 4(3), 15 -17.
- Bhat, M. K. and Bhat, S. (1997).** Cellulose degrading enzymes and their potential industrial application. *Biotechnology Advances*, 15: 583-620.
- Birch, G. G., Blakebrough, N. and Parker, J. (1981).** Enzyme and Food processing. Applied Science Publisher LTD. England. Pp 5- 13.
- Braverman, J. B. F. (1974).** Introduction to Biochemistry of food. 3<sup>rd</sup>. elsevier publishing company ed.. London. Pp 31.
- Chapin, F. S., Matson, P. A. and Mooney, H. A. (2002).** Principles of Terrestrial Ecosystem Ecology. Springer- Verlag, New York. Pp 150.
- Coral, G.; Arican, B. M. Nisa, O. and Hatice, G. (2001).** Some properties of crude carboxymethyl cellulase of *Asperigillus niger*. *Turkish Journal of Biology*. 26: 209 – 213.
- Fogarty, W. M. and Kelly, C. J. (1979).** Development of Microbial extracellular enzymes. in: enzyme and fermentation biotechnology. (Alan Wiseman.ed) Ellis Harwood LTD. Publishers. England, pp 239.
- Golla, W. N., Kebede, A. A., & Kindeya, Y. B. (2020).** Evaluation of sesame genotypes for seed yield and bacterial blight (*Xanthomonas campestris* pv. *sesami*) disease resistance in optimum moisture areas of Western Tigray, Ethiopia. *Cogent Food & Agriculture* , 6(1)
- Gupta, V. K. and Ayyachamy, M. (2012).** Biotechnology of Fungal Genes. Science publisher. England. Pp 53- 71.
- Hasunuma, T.; Fukusaki, E. and Kobayashi, A. (2003).** A Methanol Production is enhanced by expression of an *Asperigillus niger* Pectinmethylesterase in tobacco cells. *Journal of Biotechnology*. 106: 45-52.

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- Laurent, F.; Kotoujansky, A. and Bertheau, U. (2000).** Over Production in *Escherichia coli* of the Pectinmethylesterase. *Canadian Journal of Microbiology*. 46: 474-480.
- Maria, S.M.; Celestino, C.; Maria de Freitas, S.; Javier, F. M.; Valle desouse, M.; Ximenes, E. and Ferrira, F. (2006).** Purification and Characterization of a Novel Pectinase from *Acrophialophora nainiana* with Emphasis on Physiochemical Properties. *Journal of Biochemistry*, 123 (1): 33-42
- Mirjana, G. A., Antov, D. M. and Stana, N. P. (2004).** Pectinases partitioning in aqueous two- phase system: an integration of the systems poly (ethylene glycol)/ crude dextrin and poly (ethylene glycol) ammonium sulphate. *Journal of Chemical Society*. 69 (4): 299- 307.
- Mustafa, A.A. (2008).** Production and charactterazation of pectic enzymes. M. Sc. Thesis University of Gezeira
- Ranveer,S. J.; shivalika, S. and Reena, G. (2005).** Microbial Pectinolytic Enzymes. Elsevier publishing company. London and New York. Pp 34- 50.
- Rombouts, F. M. and Pinlnik, W. (1980).** Pectic enzymes In: microbial enzymes and bioconversion. ( Rose A. H.ed). Academic press, London. 5: 227- 270.
- Sabet , K ,A. and Dowson, W.T (1960).** Abacterial leaf spot disease of sesame (Sesamun orientel). *Phytobathological Z.* 37:252-256.
- Scawen, M. D. and Milling, J. (1985).** Practical Aspect of large –scale protein purification in: hand book of enzyme biotechnology ( A. A Wiseman.ed) John Willy and Sons. New York. Pp36 – 70.
- Timoth, Y. D. (2001).** Enzymes: General Properties. John Wiley and Sons. Ltd. New York and London. Pp 151- 167.
- Urmila, P.; Vikram, D.; Shobhna, S. and Chadha, B. S. (2005).** Pectinase and Polygalacturonase production by A thermophilic *A. fumigates* isolated from orange peels. *Brazilian Journal of Microbiology*, 36: 63 – 69.
- Vinoth ,B. Manivasagaerumal, R. and Belamurugan, S. (2012).** Phytochemical analysis and antibacterial activity of *Moringa oleifera* (lam), India. *International J. for research in Biological Sciences*, 2012; 2(3):98-102.