Journal of
Plant Sciences

ISSN 1816-4951

Academic
Journals Inc.

www.academicjournals.com

Journal of Plant Sciences 1 (4): 383-387, 2006
ISSN 1816-4951

Polygalacturonase from Tomato Fruits Infected with *Penicillium funiculosum* Thom.

A.O. Adejuwon, O.A. Oni and P.O. Olutiola
Department of Microbiology, Obafemi Awolowo University,
Ile-Ife, Osun State, Nigeria

Abstract: Tomato fruits infected with *Penicillium funiculosum* Thom. produced proteins which showed appreciable polygalacturonase activity within eight days. Uninfected tomato fruits showed only traces of polygalacturonase activity. The enzyme was partially purified by a combination of gel filtration and ion-exchange chromatography. Two components with molecular weight estimates of approximately 223,800 daltons and 89,100 daltons were expressed. Only the components of the lighter peak showed polygalacturonase activity. The enzyme showed optimum activity at pH 4.5 and 40°C. It possessed an apparent K_m of 0.05 mg mL^{-1} for the hydrolysis of pectin. Na^+ and Ca^H ions were stimulatory to the activity of the enzyme. EDTA and Hg^{++} were inhibitory.

Key words: polygalacturonase, tomato, *Lycopersion esculentum*, *Penicillium funiculosum* Thom.

Introduction

Tomato fruits are consumed worldwide. Tomato is an important vegetable accounting for approximately 18% of daily consumption of vegetables (Kateria and Mittal, 1984). Tomato fruits contain sugars at varying proportions. These sugars are mainly reducing sugars of approximately 1.3% glucose and 1.5% fructose (Stephens, 1978; Lamb, 1977). They also contain approximately 8% protein, 7% pectic substances and organic acids: mainly citric and malic acids (Crookes and Grierson, 1983). Although the nutritional value of tomato is rather low, the large amount of tomato consumed in any meal makes it quite valuable in standard and special diets (Moresi and Liverotti, 1982).

Fungi are found to be responsible for majority of tomato spoilage during storage (Adejuwon and Olutiola, 2005).

This study shows the degradation of the pectic portions of tomato fruits by Polygalacturonase produced by *Penicillium funiculosum* Thom. during infection. Studies carried out on the properties of this enzyme are described.

Materials and Methods

Organism and Culture Conditions

The isolate of *Penicillium funiculosum* Thom. used was obtained from the culture collection of the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Osun state. Nigeria. The organism was routinely grow and maintained on 1% malt yeast-extract glucose agar slants. Five day old cultures served as inoculum.

Corresponding Author: Adekunle Odunayo Adejuwon, Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria

Inoculation of Tomato Fruits

Apparently healthy, ripe tomato (*Lycopersicon esculentum* Mill.) fruits were obtained from the Ile-Ife local main market. They were surface sterilized in 3% sodium hypochlorite for 30 min and thoroughly rinsed with sterile distilled water. A cork borer (3 mm) was used to remove tissue disc from each tomato fruit prior to inoculation with tissue discs (3 mm) of 5-day old culture of *Penicillium funiculosum* in plates. The holes were sealed with paraffin wax. Tomato fruits inoculated with sterile malt yeast extract-glucose agar served as controls. Procedures were carried out in a sterile inoculating chamber. Experimental and control fruits were placed inside sterile petri dishes with bell jars inverted over them. The rims of the bell jars were sealed with Vaseline. Incubation was at room temperature (27°C) The fruits were observed daily for deterioration.

Extraction of Enzyme from Tomato Fruits

Within eight days of incubation, the inoculated tomato fruits had collapsed. The fruits were weighed, chilled in a refrigerator, homogenized with cold (4°C) liquid extractant (1:1w/v). The extractant was 0.5 M NaCl in 0.0l M citrate phosphate buffer (pH 5.0) containing 5 mM sodium azide to prevent microbial contamination. The homogenate was filtered through four layers of muslin cloth and further clarified by filtering through filter paper (Whatman No. 1). The filtrate served as the crude enzyme. The protein content of the filtrate was determined using the method of Lowry *et al*. (1951). Polygalacturonase activity was analyzed using a modified method of Miller (1959).

Preparation of Extract for Fractionation

The crude enzyme homogenate was concentrated to about one seventh of its original volume in a vacuum rotary evaporator (Quickfit, Rotavapor-R, Buchi, Switzerland) at 30°C. Frothing was avoided during this process by slow evaporation under low vacuum pressure (Whitaker *et al*., 1963).

Fractionation on Sephadex G-25 Column

The column (2.5×40 cm) of Sephadex G-25 (bead size 50-150 μ) was prepared in a column made up of a glass tube with water jacket supplied by Pharmacia Fine Chemicals, Uppsala, Sweden. This is as previously described by Olutiola and Cole (1980). It was equilibrated with 0.0IM citrate phosphate buffer pH 5.0 containing 5 mM NaN_3. Fractions were collected (5 mL/tube). Optical densities of fractions were measured at 280 nm. The fractions were analyzed for polygalacturonase activity.

Fractionation on Sephadex C-50 Column

Fractions (11-17) from Sephadex G-25 which showed appreciable polygalacturonase activity were pooled together. Six milliliter of the pooled enzyme was applied to Sephadex C-50 column (2.5 x 40 cm) (bead size 40-120 μ) with water jacket, supplied by Pharmacia fine Chemicals, Uppsala, Sweden. Fractions (5 mL/tube) were eluted with 0.0I M citrate phosphate buffer pH 5.0 containing a gradient (0.1-0.5 M) NaCl. Optical density of the fractions was measured at 280 nm. Fractions were analyzed for polygalacturonase activity.

Fractionation on Sephadex G-100 Column

Fractions (9-12) collected form Sephadex C-50 column which showed appreciable polygalacturonase activity were pooled together. Four milliliter of the mixture was applied to Sephadex G-100 column (2.5×70 cm) which had earlier been calibrated with proteins of known molecular weight as previously described by Andrews (1964), Olutiola and Cole (1976). Optical density of fractions (5 mL/tube) eluted with 0.0l M citrate phosphate buffer pH 5.0 was measured at 280 nm. The fractions were analyzed for polygalacturonase activity.

Table 1: Partial purification of polygalacturonase from tomato fruits infected by *Penicillium funiculosum* Thom.

Fraction	Total activity (U)	Total protein (mg)	Specific activity (u/mg protein)	Yield (%)	Purification (fold)
Crude enzyme	7950	169.5	46.9	100	1
Sephadex G-25	4290	12.8	335.2	54.0	7.1
Sephadex C-50	3300	3.8	868.4	41.5	18.5
Sephadex G-100	1916	1.6	1197.5	24.1	25.5

Assay Method

Polygalacturonase activity was analyzed by measuring the reducing sugars released in the reaction mixtures using a modified dinitro salicylic acid reagent method of Miller (1959). The substrate, 0.1% (w/v) pectin (Sigma) solution, was prepared in 0.01 M citrate phosphate buffer pH 5.0.

The reaction mixture was 0.5 mL of enzyme preparation added to 1 mL of the substrate. Incubation was at 35°C for 1h. The reaction mixture was terminated by adding 3 mL of dinitro salicylic acid (DNSA) reagent. Controls which initially contained only 1 mL of the substrate were incubated with the experimental tubes at 35°C for 1 h. Three milliliter of dinitro salicylic acid was added to each control tube after which 0.5 mL of enzyme was added. The reducing sugar released in the reaction mixture was then measured. One unit of polygalacturonase activity was defined as the amount of enzyme in 1 mL of the reaction mixture that liberated reducing sugars equivalent to 100 μg galacturonic acid per minute under assay conditions. Specific activity was calculated as enzyme unit per mg protein.

Results

Within eight days, tomato fruits infected with *Penicillium funiculosum* Thom., incubated at room temperature (27°C), had collapsed and extensively overgrown by mycelia and greenish spores. Extracts of the infected fruits exhibited appreciable amount of polygalacturonase activity. Uninfected fruits showed traces of polygalacturonase activity. Fractionation of the concentrated extract on Sephadex G-25 column gave two peaks of absorption. Only the components of the first peak showed polygalacturonase activity. Fractionation of the first peak on Sephadex C-50 column gave two peaks of absorption. Components of the first peak showed polygalacturonase activity. Further fractionation of the components of this peak on Sephadex G-100 column gave two peaks of absorption with molecular weight estimates of 223,800 daltons and 89,100 daltons. Only the components of the lighter peak showed appreciable polygalacturonase activity. The purification steps are shown in Table 1.

Properties of the partially purified polygalacturonase were investigated. Using 0.0IM citrate phosphate buffer with pH ranges of 3.0-7.0, optimum activity was observed at pH 4.5. With a temperature range of 20 to 50 °C optimum activity was observed at 40°C. The polygalacturonase was stimulated by Na^+ and Ca^H ions. Optimum activity was observed at 20 mM concentrations of Na^+ ions and Ca^Hions. With different concentrations of pectin as substrate (ranges of 0.05-6 mg mL^{-1}), optimum activity was recorded over a range of 4-6 mg mL^{-1}. The rate of enzyme reaction seemed to follow the Michalis-Menten Kinetics. From the Lineweaver Burk Plot, the apparent K_m for the hydrolysis of pectin was approximately 0.05 mg mL^{-1}. The polygalacturonase was inhibited by EDTA and Hg^H ions. There was total inhibition at 6 and 4 mM concentrations, respectively.

Discussion

Extensive deterioration and fruit rot occurred within a few days of infection of tomato fruits by *Penicillium funiculosum* Thom. Similar reports have been made of this fungi on pineapple fruits (Lim and Rohrbach, 1980). Polygalacturonase was detected in the extracts of infected tomato fruits. *Penicillium sclerotigenum* has been found to produce this enzyme in synthetic liquid medium with pectic substance as sole corbon source (Olutiola, 1982).

The enzyme showed optimum activity at pH 4.5. A similar result has been documented by Perez-artes and Tena (1989) for *Fusarium oxysporum*. The enzyme was affected by temperature changes. Optimum activity was observed at 40°C. However, Agarwal *et al.* (1979) reported a lower optimum temp of the range 30-35°C for polygalacturonase produced by *Curvularia lunata* and *Colletotrichum dematium*. The enzyme was able to degrade pectin with optimum activity at a range of 4-6 mg mL^{-1} concentrations. A similar report has been described by Aymerig *et al.* (1989). Activity of the enzyme was stimulated by Na^{+} and Ca^{H} ions. Popoola (1987) made an almost similar report on polygalacturonase produced by *Aspergillus niger* causing the black mould rot of yam.

Activity of the enzyme was inhibited by EDTA and Hg^{H} ions. Reports made by Yoichi *et al.* (1993) indicate that polygalacturonase from strawberry fruits are inhibited similarly. The results of this study show that polygalacturonase is produced by *Penicillium funiculosum* Thom. during infection to hydrolyse the pectic portion of tomato fruits into simple soluble forms such as polygalacturonic acid. These are then subsequently absorbed and metabolized by this phytopathogen for growth.

References

Adejuwon, A.O. and P.O. Olutiola, 2005. Protease in tomato fruits infected with *Fusanum oxysporum*. Plant Pathol. J., 4: 43-45.

Agarwal, G.P., D. Kulhara and P.S. Bisen, 1979. Factors affecting the in-vitro production of Pectolytic and Cellulolytic enzymes by bean leaf pathogens *Curvularia lunata* and *Colletotrichum dematium*. Biochem. Physio. J., 172: 277-284.

Andrews, P., 1964 . Estimation of the molecular weight of proteins by Sephadex gel filtration. J. Biochem., 91: 222-233.

Aymerig, J.L., Y. Bertheau, M. Boccara, S. Favery and A. Kotoujansky, 1989. Role of pectinases and cellulases in the pathogenicity of *Erwinia chrysanthemi* In: Abstracts of Papers and Posters. 7th International Conference on Plant Pathogenic Bacteria. Berlin Heidelberg 11-16 June, pp: 187.

Crookes, P.R. and D. Grierson, 1983. Ultra-structure of tomato in cell wall degradation. Plant Physiol., 72: 1088-1092.

Kateria, O.P. and J.P. Mittal, 1984. Vital vegetables. African Farming and Food Processing May/June, pp: 37-39.

Lamb, F.C., 1977. Tomato Products. Bulletin 27-L National Canners Association, Washington DC., pp: 2-3.

Lim, J.K. and K.G. Rohrbach, 1980. Role of *Penicillum funiculosum* strains in the development of pineapple fruit diseases. Phytopathology, 70: 663-665.

Lowry, O.H., N.J. Rosenbrough, A.L. Fare and N.J. Randel, 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem., 193: 265-275.

Miller, G.L., 1959. Use of dinitro salicylic acid reagent for determination of reducing sugar. Anal. Chem., 31: 426-482.

Moresi, M. and C. Liverotti, 1982. Economic study of tomato paste production. J. Food Technol., 17: 177-192.

Olutiola, P.O., 1982. Polygalacturonase and pectinlyase of *Penicillum sclerotigenum*. Nig. J. Microbiol., 2: 154-167.

Olutiola, P.O., 1983. Cellwall degrading enzyme associated with deterioration of cocoa beans by *Penicillium steckii*. Intl. Biodeter., 19: 27-36.

Olutiola, P.O. and O.O. Cole, 1976. Production of a cellulase complex in culture filtrates of *Aspergillus tamarii* associated with mouldy cocoa beans in Nigeria. Physiol. Plant., 37: 313-316.

Olutiola, P.O. and O.O. Cole, 1980. Extracellular invertase of *Aspergillus flavus*. Physiol. Plant., 50: 26-31.

Perez-artes, E. and M. Tena, 1989. Pectic enzymes from two races of *Fusarium oxysporum*. Enzyme production in culture and enzymatic activity on isolated chickpea cellwalls. Phytopathology, 24: 39-51.

Popoola, O.O., 1987. Studies on some carbohydrases associated with the black mould rot of yam (*Dioscorea rotunda* Linn.). M.Sc Thesis, Obafemi Awolowo University, Ile-Ife, Nigeria, pp: 95.

Stephens, M.A., 1978. Relationship between components contributing to quality variation among tomato fruits. J. Am. Soc. Hortic. Sci., 97: 70-73.

Whitaker, D.R., K.R. Hanson and P.K. Datta, 1963. Improved procedure for purification and characterization of *Myrothecium cellulase*. Can. J. Microbiol. Physiol., 41: 671-696.

Yoichi, N., H. Ohta and A.G.J. Voragen, 1993. Polygalacturonase in strawberry fruit. Phytochemistry, 34: 617-620.