



Journal of
Plant Sciences

ISSN 1816-4951



Academic
Journals Inc.

www.academicjournals.com

Pectin Lyase Activity in Culture Filtrate of *Penicillium species*

A.O. Adejuwon and P.O. Olutiola

Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria

Abstract: *Penicillium species* grew in a synthetic medium with pectin as the sole carbon source producing proteins which expressed alkaline pectin lyase activity. The enzyme was purified by a combination of ammonium sulphate precipitation, dialysis, gel filtration and ion-exchange chromatography. Optimum activity of the enzyme was at 35°C and at pH 8.0. The enzyme was stimulated by K⁺, Na⁺, Ca⁺⁺ and Mg⁺⁺ but inhibited by EDTA and Hg⁺⁺. The enzyme was able to degrade pectin with optimum activity expressed at 16 mg mL⁻¹.

Key words: *Penicillium*, pectin lyase, synthetic growth, carbon source, optimum activity

INTRODUCTION

Pectin lyase, also known as pectin transeliminase or pectinase is involved in the degradation of pectic substances (Gummadi and Kumar, 2005). The enzyme is a virulent factor, which degrades the pectic components of the plant cell wall (Mayans *et al.*, 1997). The enzyme acts to cleave certain activated glycosidic linkages present in acidic polysaccharides. It acts through an eliminase mechanism rather than through hydrolysis, resulting in unsaturated oligosaccharide products (Linhardt *et al.*, 1986).

Microorganisms have been reported to produce pectin lyase during plant and fruit infection (Schink *et al.*, 1981; Sakiyama *et al.*, 2001).

This study was designed to confirm whether an isolated species of *Penicillium* is capable of producing pectin lyase in a synthetic growth medium with pectin as sole carbon source. Attempts were made to purify the enzyme and determine some of its properties.

MATERIALS AND METHODS

Organism and Culture Conditions

An isolate of a *Penicillium species* used for this study was obtained from the culture collection of Professor P.O. Olutiola, Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Osun state Nigeria. It was grown and maintained on potato dextrose agar plates and slants.

The isolate was allowed to grow in a defined medium made up of K₂HPO₄, KH₂PO₄, MgSO₄, 7H₂O, pectin, thiamine, biotin, FeSO₄ and KNO₃. The carbon source, which is pectin, was autoclaved separately and aseptically added to the basal medium to give a final concentration of 1%. The growth medium (50 mL) in conical flasks was inoculated with 1 mL of spore suspension (5×10⁷ spores mL⁻¹) of the culture organism. The inoculated growth medium was incubated at 25°C and analysed daily for protein content (Lowry *et al.*, 1951) and pectin lyase activity (Olutiola and Akintunde, 1979; Alana *et al.*, 1990).

Extraction of Enzyme

The crude enzyme in each flask was clarified by filtering through filter paper (Whatman G/A) and analysed for pectin lyase activity.

Ammonium Sulphate Precipitation and Dialysis

The proteins of the extracted crude filtrate was precipitated using ammonium sulphate (analytical grade) to 90% saturation. Precipitation during salt saturation was at 4°C for 24 h. The precipitate was there after redissolved in 0.02 M citrate phosphate buffer pH 6.0. Analysis for pectin lyase activity was carried out.

Dialysis was carried out over night with three changes to 0.02 M citrate buffer pH 6.0.

Fractionation on Sephadex G-100 Column

Ten milliliter of the dialysed enzyme was applied to a column (2.5×70 cm) of Sephadex G-100 which had been calibrated earlier with proteins of known molecular weights (Andrews, 1964). Fractions (5 mL/tube) were collected and optical density, measured at 280 nm and analysed for pectin lyase activity. Fractions which showed appreciable activity were pooled and applied to a column of Sephadex C-50.

Fractionation on Sephadex C-50 Column

Ten milliliter of pooled fractions from Sephadex G-100 column which showed appreciable pectin lyase activity was applied to a column (2.5×40 cm) of Sephadex C-50. Fractions (5 mL/tube) were collected. Optical density of the fractions was measured at 280 nm and analysed for pectin lyase activity.

Pectin Lyase Assay

Pectin lyase activity was assayed by the pectin degradation method using the thiobarbituric acid technique of Olutiola and Akintunde (1979). The substrate 1.2% (w/v) pectin (Sigma) was prepared in 0.2 M Tris-HCL buffer (pH 8.5). The reaction mixture was 2 mL of enzyme added to 3 mL of substrate. Incubation was at 35°C for 1 h. The reaction mixture was terminated by adding 3 mL of 0.04 M Thiobarbituric Acid (TBA) and 1.5 mL of 1 N HCl. Controls which initially contained only 3 mL of substrate were incubated with the experimental tubes at 35°C for 1 h. Three milliliters thiobarbituric acid was added to the contents of each control tube followed by 2 mL of enzyme. Both experimental and control tubes with contents were boiled in a water bath for 20 min. Optical density measurements were taken at 540 nm.

One unit of pectin lyase activity was arbitrarily defined as the amount of enzyme in the reaction mixture, which caused an 0.01 increase in absorbance in 1 h under assay conditions.

RESULTS

Penicillium species grew in a synthetic growth medium with pectin as sole carbon source at 25°C within ten days of incubation. Pectin lyase activity was detected in the growth medium. Uninoculated medium lacked pectin lyase activity. The enzyme, purified by a combination of ammonium sulphate precipitation, dialysis, gel filtration (Sephadex G-100) and ion- exchange chromatography (Sephadex C-50) (Table 1), expressed optimum activity at 35°C within a temperature range of 10-60°C (Fig. 1). Within a pH range to 5.0 to 9.0, optimum activity was observed at 8.0 (Fig. 2).

Table 1: Purification of pectin lyase obtained from *Penicillium species*

Fraction	Total activity (Units)	Total protein (mg)	Specific activity (units/mg protein)	Yield (%)	Purification fold
Crude enzyme	4100	4500.0	0.9	100.0	1.0
(NH ₄) ₂ SO ₄	3502	625.0	5.6	85.8	6.2
Sephadex G-100	2956	102.5	28.8	72.3	32.0
Sephadex C-50	1950	26.9	72.5	47.8	80.6

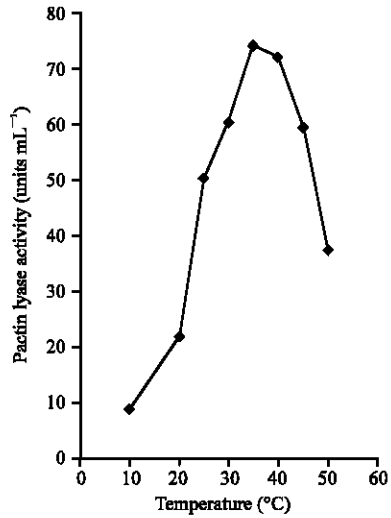


Fig. 1: Effect of temperature on the activity of partially purified (SP C-50 fraction) pectin lyase obtained from *Penicillium species*

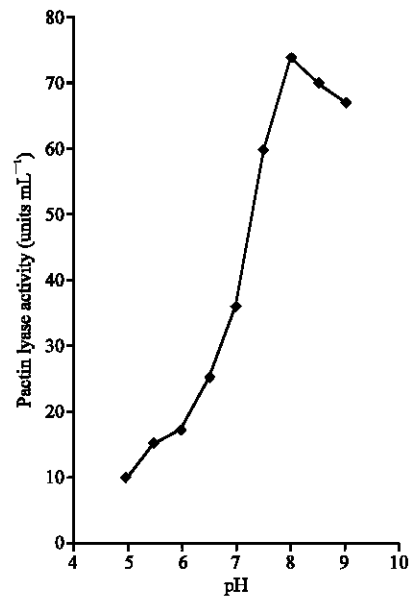


Fig. 2: Effect of pH on the activity of partially purified (SP C-50 fraction) pectin lyase obtained from *Penicillium species*

Activity of the enzyme was stimulated with increasing concentrations (within a range of 0-30 mM) of K⁺, Na⁺, Ca⁺⁺ and Mg⁺⁺. Optimum activities were observed at 15 mM concentration of Ca⁺⁺ 20 mM concentration of K⁺ and 25 mM concentration of Na⁺ and Mg⁺⁺. There after, there were declines for Ca⁺⁺, K⁺ and Mg⁺⁺. Na⁺ was stable at 30 mM concentration. The activity of the enzyme was inhibited by Hg⁺⁺ and EDTA within a range of 0-10 mM concentrations. Activity was completely

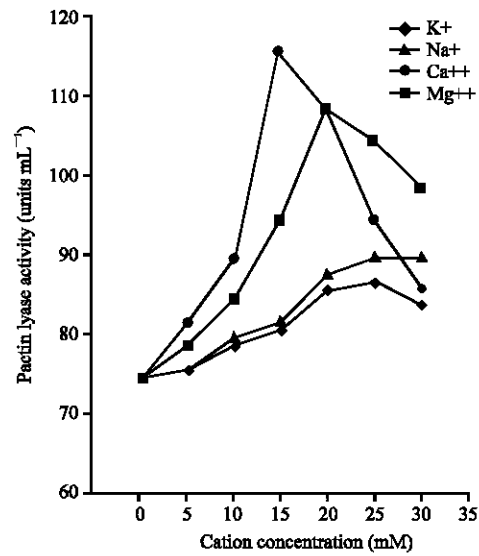


Fig. 3: Effect of cations on the activity of partially purified (SP C-50 fraction) pectin lyase obtained from *Penicillium* species

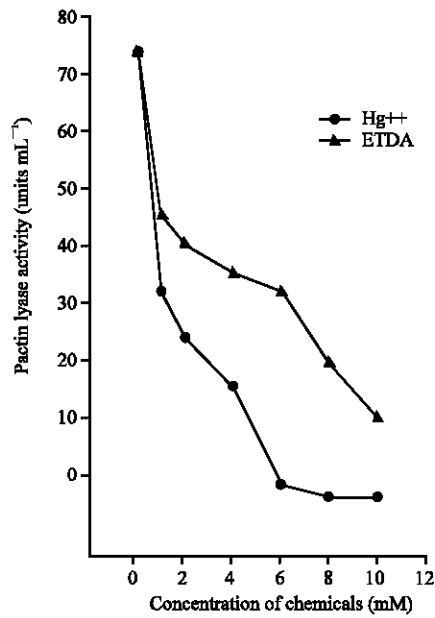


Fig. 4: Effect of chemicals on the activity of partially purified (SP C-50 fraction) pectin lyase obtained from *Penicillium* species

lost at 6 mM concentration of Hg⁺⁺. Within different concentrations of pectin as substrate (ranges of 5-20 mg mL⁻¹), optimum activity was observed at 16 mg mL⁻¹ (Fig. 5).

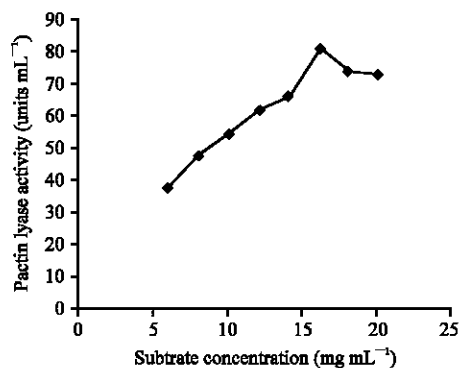


Fig. 5: Effect of substrate concentration on the activity of partially purified (SP C-50 fraction) pectin lyase obtained from *Penicillium species*

DISCUSSION

Penicillium species produced pectin lyase in a synthetic growth medium with pectin as sole carbon source. According to Gonzalez-Candelas (1992), *Fusarium solani* f.sp. pisi is capable of producing pectin lyase in growth medium with pectin as sole carbon source. Nakagawa *et al.* (2005) reported that the methylotropic yeast *Pichia methalonica* is able to grow on pectin compounds, pectin and polygalacturonate as sole carbon sources.

Temperature ranges affected pectin lyase activity from *Penicillium species*. Optimum activity was at 35°C. An almost similar temperature of 50°C for optimum activity of pectin lyase produced by *Bacillus subtilis* has been reported by Soriano (2000). Optimum activity of pectin lyase by *Penicillium species* was observed at an alkaline pH of 8.0 *Penicillium italicum* has been found exhibit optimum pectin lyase activity at alkaline pH range of 6.0-7.0 (Alana *et al.*, 1990).

K⁺, Na⁺, Ca⁺⁺ and Mg⁺⁺ enhanced activity of the Pectin lyase produced by *Penicillium species* with optimum activity at different concentrations. Mayans *et al.* (1997) observed that a species of *Aspergillus* is able to produce a Calcium- dependent pectin lyase. EDTA and Hg⁺⁺ were inhibitory to the activity of pectin lyase produced by *Penicillium species*. According to Soriano (2000), Ba⁺⁺ is inhibitory to a similar enzyme produced by *Bacillus subtilis*. Alana *et al.* (1989) reported that glycerol inhibits pectin lyase produced by *Penicillium italicum*.

Penicillium species was able to degrade pectin as substrate with optimum activity at 16 mg mL⁻¹ concentration. Strains of psychrophilic yeasts with taxonomic affiliations similar to *Cryptococcus cylindricus*, *Mrakia frigida* and *Cystofilobasidium capitatum* are able to grow on and degrade pectin at about 5°C (Nakagawa *et al.*, 2004). According to Alana *et al.* (1990), *Penicillium italicum* produces an extracellular pectin lyase in a liquid medium with K_m of 3.2 mg mL⁻¹ for pectin. Silva *et al.* (1993) reported that *Penicillium expansum* produces a monomeric pectin lyase with molecular mass of 36.5 kDa capable of degrading citrus and apple pectin, with a K_m for citrus pectin at 9 mg mL⁻¹.

Alkaline pectinases are among the most important industrial enzymes and are of great significance in textile processing, degumming of plant bast fibers, treatment of waste waters, paper making, coffee and tea fermentation (Hoondai *et al.*, 2002).

REFERENCES

- Alana, A., A. Gabilondo, F. Hernando, M.D. Moragues, J.B. Dominguez, M.J. Llama and J.L. Serra, 1989. Pectin lyase production by a *Penicillium strain*. Applied Environ. Microbiol., 55: 1612-1616.

- Alana, A., I. Alkorta, J.B. Dorminguez, M.J. Llama and J.L. Serra, 1990. Pectin lyase activity in *Penicillium italicum* strain. *Applied Environ. Microbiol.*, 56: 3755-3739.
- Andrews, P., 1964. Estimations of molecular weight of proteins by Sephadex gel filtration. *J. Biochem.*, 91: 222-233.
- Gonzalez-Candelas, L. and P.E. Kolattukudy, 1992. Isolation and analysis of a novel inducible pectate lyase gene from the phytopathogenic fungus *Fusarium solani* f. sp. pisi (*Nectria heamatococca*, mating population VI). *J. Bacteriol.*, 174: 6343-6349.
- Gummadi, S.N. and D.S. Kumar, 2005. Microbial pectic transeliminases. *Biotechnol. Lett.*, 27: 451-458.
- Hoondol, R., R. Tiwari, N. Tewari and Q.B. Dahiya, 2002. Microbial alkaline pectinases and their industrial application: A review. *Applied Microbiol. Biotechnol.*, 59: 409-418.
- Linhardt, R.J., P.M. Galliher and C.L. Cooney, 1986. Polysaccharide lyase. *Applied Biotechnol.*, 12: 135-176.
- 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.
- Mayans, O., M. Scott, I. Connerton, T. Gravesen, J. Bensen and J. Viser, 1997. Two crystal structures of pectin lyase A from *Aspergillus* reveal a pH driven conformational change and striking divergence in the substrate-binding clefts of pectin and pectate lyases. *Structure*, 5: 677-689.
- Nakagawa, T., T. Nagaoka, S. Taniguchi, T. Miyaji and N. Tomizuka, 2004. Isolation and characterization of psychrophilic yeasts producing cold-adapted pectinolytic enzymes. *Lett. Applied Microbiol.*, 38: 383.
- Nakagawa, T., K. Yamada, S. Fujimura, T. Ito, T. Miyaji and N. Tomizuka, 2005. Pectin utilization by the methylotrophic yeast *Pichia methanolica*. *Microbiology*, 151: 2047-2052.
- Olutiola, P.O. and A.O. Akintunde, 1979. Pectin lyase and pectin methylesterase production by *Penicillium citrinum*. *Tran. Br. Mycol. Soc.*, 72: 39-55.
- Sakiyama, C.C.H., E.M. Paula, P.C. Pereira, A.C. Borges and D.O. Silva, 2001. Characterization of pectin lyase produced by an endophytic strain isolated from coffee cherries. *Lett. Applied Microbiol.*, 33: 117.
- Schink, B., J.C. Ward and J.G. Zeikus, 1981. Microbiology of wetwood: Importance of pectin degradation and clostridium species in living trees. *Applied Environ. Microbiol.*, 42: 526-523.
- Silva, D.O., M.M. Attwood and D.W. Tempest, 1993. Partial purification and properties of pectin lyase from *Penicillium expansum*. *World J. Microbiol. Bio Technol.*, 9: 574-578.
- Soriano, M., A. Blanco, P. Diaz and F.I.J. Pastor, 2000. An unusual pectate lyase from a *Bacillus* sp. with high activity on pectin: Cloning and characterization. *Microbiology*, 146: 89-95.