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## **Pectin Lyase Activity in Culture Medium of *Lasidiopodia theobromae***

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**Abstract:** *Lasidiopodia theobromae* grew in a culture medium at 27°C producing proteins which showed pectin lyase activity. The enzyme was purified by a combination of ammonium sulphate precipitation, dialysis, gel permeation and ion-exchange chromatography. Optimum activity of the enzyme was at 40°C and at pH 8.5. The enzyme was stimulated by concentrations of K<sup>+</sup>, Na<sup>+</sup>, Ca<sup>++</sup> and Mg<sup>++</sup> but inhibited by EDTA and Hg<sup>++</sup>. When the enzyme was heated at 70°C, optimum activity had decreased by about 66.6% within 2 min but almost completely lost within 30 min. The enzyme was able to degrade pectin with optimum activity expressed at 18-20 mg mL<sup>-1</sup>.

**Key words:** Pectin lyase activity, *Lasidiopodia theobromae*

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### **INTRODUCTION**

Pectin lyase is associated with the breakdown of pectic substances (Gummadi and Kumar, 2005). It is a polysaccharide hydrolyzing enzyme produced by some fungi (Geimba and Brandelli, 2002; Masoud and Jespersen, 2006; Jayasinghe *et al.*, 2004). 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).

This study was designed to culture *Lasidiopodia theobromae* in synthetic growth medium with pectin as sole carbon source. Pectin lyase activity which was detected in the medium was partially purified. Attempts were made to determine some of its properties.

### **MATERIALS AND METHODS**

#### **Organism and Culture Conditions**

An isolate of *Lasidiopodia theobromae* used for this study was obtained from the International Institute for Tropical Agriculture (IITA), Ibadan, 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 K<sub>2</sub>HPO<sub>4</sub>, KH<sub>2</sub>PO<sub>4</sub>, MgSO<sub>4</sub>·7H<sub>2</sub>O, pectin, thiamine, biotin, FeSO<sub>4</sub> and KNO<sub>3</sub>. 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 (100 mL) in conical flasks was inoculated with 1 mL of spore suspension (6×10<sup>7</sup> spores mL<sup>-1</sup>) of the culture organism. The inoculated growth medium was incubated at 27°C and analysed daily for protein content (Lowry *et al.*, 1951) and pectin lyase activity (Olutiola and Akintunde, 1979).

#### **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.

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### **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 of 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**

*Lasidiopodia theobromae* grew in a synthetic culture medium with pectin as sole carbon source at 27°C within eight 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 permeation (Sephadex G- 100) and ion- exchange chromatography (Sephadex C- 50) (Table 1), expressed optimum activity at pH 8.5 within a pH range of 4 to 10 (Fig. 1). Optimum activity was observed at 40°C within a temperature range of 10-60°C

Table 1: Purification of pectin lyase obtained from *Lasidiopodia theobromae*

Fraction	Total activity (Units)	Total protein (mg)	Specific activity (units/mg protein)	Yield (%)	Purification fold
Crude enzyme	5200	5520.0	0.9	100.0	1.0
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	4696	811.8	5.8	85.4	6.4
Sephadex G-100	4150	137.6	30.2	75.5	33.0
Sephadex C-50	3244	37.2	87.2	59.0	96.9

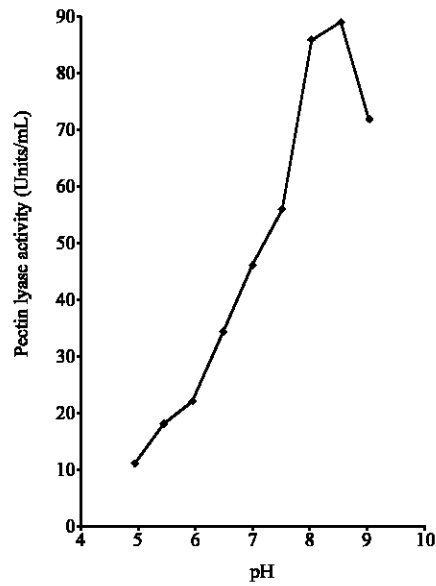


Fig. 1: Effect of pH on the activity of partially purified (SPC-50 fraction) pectin lyase obtained from *Lasidiopodia theobromae*

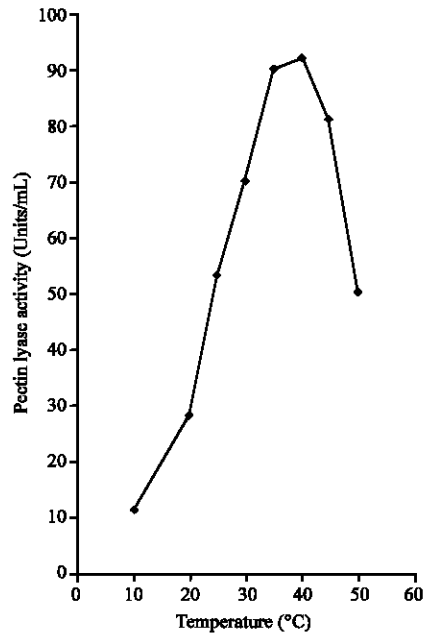


Fig. 2: Effect of temperature on the activity of partially purified (SPC-50 fraction) pectin lyase obtained from *Lasidiopodia theobromae*

(Fig. 2). Within different concentrations of pectin as substrate (ranges of 4-20 mg mL<sup>-1</sup>), optimum activity was observed at 18-20 mg mL<sup>-1</sup> (Fig. 3). When the enzyme was heated at 70°C within 0-30 min, optimum activity had decreased by about 66.6% within 2 min after which there was a gradual loss. Activity was almost completely lost within 30 min (Fig. 4).

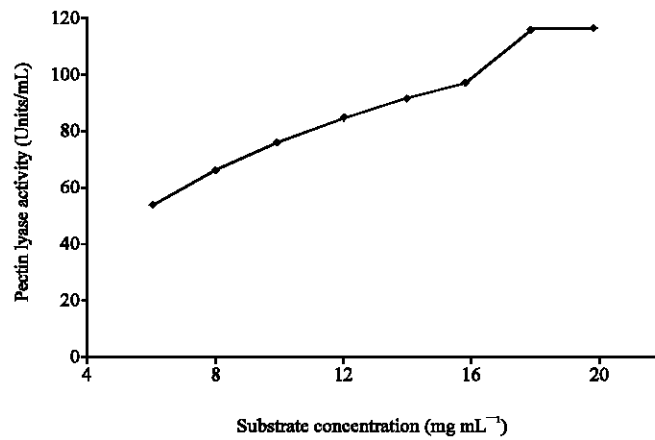


Fig. 3: Effect of substrate concentration on the activity of partially purified (SPC-50 fraction) pectin lyase obtained from *Lasidiopodia theobromae*

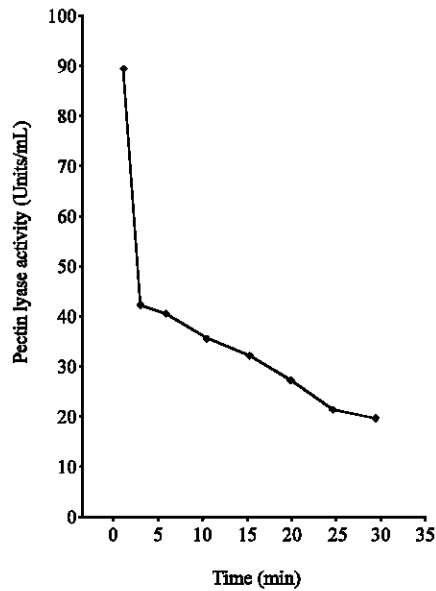


Fig. 4: Effect of duration of heating (70°C) on the activity of partially purified (SPC-50 fraction) pectin lyase obtained from *Lasidiopodia theobromae*

Activity of the enzyme was stimulated with increasing concentrations (within a range of 0-35 mM) of K<sup>+</sup>, Na<sup>+</sup>, Ca<sup>++</sup> and Mg<sup>++</sup>. Optimum activities were observed at 25 and 30 mM concentrations of K<sup>+</sup>, 30 mM concentration of Na<sup>+</sup>, 15 mM concentration of Ca<sup>++</sup> and 20 mM concentration of Mg<sup>++</sup> (Fig. 5). The activity of the enzyme was inhibited by Hg<sup>++</sup> and EDTA within 0-10 mM concentrations. At 10 mM concentration, activity was completely lost with Hg<sup>++</sup> but 61.1% loss was observed with EDTA (Fig. 6).

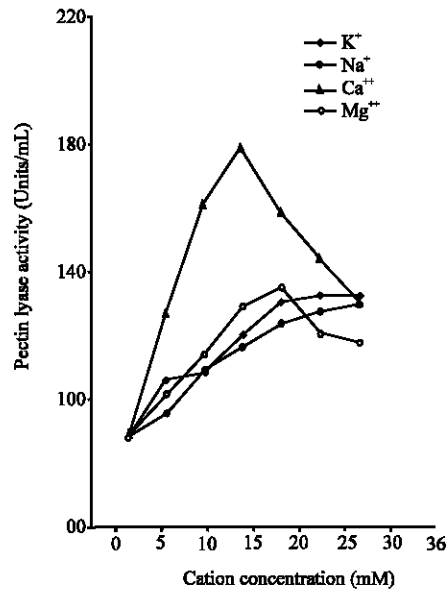


Fig. 5: Effect of cations on the activity of partially purified (SPC-50 fraction) pectin lyase obtained from *Lasidiopodia theobromae*

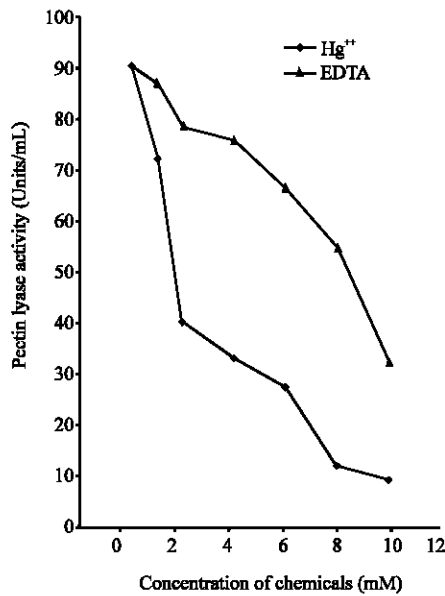


Fig. 6: Effect of chemicals on the activity of partially purified (SPC-50 fraction) pectin lyase obtained from *Lasidiopodia theobromae*

## DISCUSSION

*Lasidiopodia theobromae* produced pectin lyase in a synthetic culture medium with pectin as sole carbon source. According to Semenova *et al.* (2003), *Aspergillus japonicus* is capable of producing

pectin lyase in growth medium with pectin as carbon source. Celestino *et al.* (2006) reported that *Acrophialophora nainiana* is able to degrade pectin from citrus fruits. *Kluyveromyces wickerhamii* produces pectinase partially constitutively capable of degrading pectin (Moyo *et al.*, 2003).

Temperature ranges affected pectin lyase activity from *Lasidioplochia theobromae*. Optimum activity was at 40°C. An endophytic bacteria isolated from coffee produced an extracellular pectinase with maximal activity at 40°C and thermostable up to 45°C. Optimum activity of pectin lyase by *Lasidioplochia theobromae* was observed at an alkaline pH of 8.5 *Acrophialophora nainiana* produced an extracellular pectinase with maximum stability at pH 8.0 and 9.0 (Celestino *et al.*, 2006).

When the enzyme was heated at 70°C within 0-30 min, optimum activity had decreased by about 66.6% within 2 min after which there was a gradual loss. Activity was almost completely lost within 30 min. Moharib *et al.* (2000) reported that *Pichia pinus* is able to grow and produce pectin lyase on mango tree. The purified pectinase was stable up to 50°C for 60 min with 20% loss of activity.

K<sup>+</sup>, Na<sup>+</sup>, Ca<sup>++</sup> and Mg<sup>++</sup> stimulated the activity of the pectin lyase produced by *Lasidioplochia theobromae* with optimum activities at different concentrations. Omar and Abd-Alla (2000) reported that Cu<sup>+</sup>, Fe<sup>3+</sup> and Al<sup>3+</sup> were stimulatory to pectin lyase produced by some root nodule-colonizing fungi of faba bean (*Vicia faba*).

EDTA and Hg<sup>++</sup> were inhibitory to the activity of pectin lyase produced by *Lasidioplochia theobromae*. According to Famurewa *et al.* (1993), EDTA and DNP were inhibitory to Pectin Lyase produced by *Aspergillus flavus*. *Lasidioplochia theobromae* was able to degrade pectin as substrate with optimum activity at 18-20 mg mL<sup>-1</sup> concentrations. According to Nakagawa *et al.* (2004), some strains of psychrophilic yeasts are able to grow on and degrade pectin.

## REFERENCES

- Andrews, P., 1964. Estimations of molecular weight of proteins by Sephadex gel filtration. *J. Biochem.*, 91: 222-233.
- Celestino, S.M., S. Maria de Freitas, F. Javier Medrano, M. Valle de Sousa and E.X. Filho, 2006. Purification and characterization of a novel pectinase from *Acrophialophora nainiana* with emphasis on its physiochemical properties. *J. Biotechnol.*, 123: 33-42.
- Famurewa, O.O., M.A. Oyede and P.O. Olutiola, 1993. Pectin transeliminase complex from *Aspergillus flavus*. *Folia Microbiologia*, 38: 459-466.
- Giemba, M.P. and A. Brandelli, 2002. Extracellular enzymatic activities of *Bipolaris sorokiniana* isolates. *J. Basic Microbiol.*, 42: 246-253.
- Gummadi, S.N. and D.S. Kumar, 2005. Microbial pectic transeliminases. *Biotechnol. Lett.*, 27: 451-458.
- Jayasinghe, C.K., S.C. Wijayarathne and T.H. Fernando, 2004. Characterization of cell wall degrading enzyme of *Thanatephorus cucumeris*. *Mycopathologia*, 157: 73-79.
- 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. Biological Chem.*, 193: 265-275.
- Masoud, W. and L. Jespersen, 2006. Pectin degrading enzymes in yeasts involved in fermentation of *Coffea arabica* in East Africa. *Int. J. Food Microbiol.*, 110: 291-296.
- Moharib, S.A., S.T. El-Sayed and E.W. Jwamy, 2000. Evaluation of enzymes produced by yeast. *Nahrung*, 44: 47-51.

- Moyo, S., B.A. Gashe, E.K. Collison and S. Mpuchane, 2003. Optimizing growth conditions for the pectinolytic activity of *Kluyveromyces wickerhamii* by using response surface methodology. *Int. J. Food Microbiol.*, 85: 87-100.
- 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.
- Olutiola, P.O. and A.O. Akintunde, 1979. Pectin lyase and pectin methylesterase production by *Penicillium citrinum*. *Trans. Br. Mycol. Soc.*, 72: 39-55.
- Omar, S.A. and M.H. Abd-Alla, 2000. Physiological aspects of fungi isolated from root nodules of faba bean (*Vicia faba* L.). *Microbiol. Res.*, 154: 339-347.
- Semenova, M.V., S.G. Grishutin, A.V. Gusakov, O.N. Okunev and A.P. Sinitsyn, 2003. Isolation and properties of pectinases from the fungus *Aspergillus japonicus*. *Biochemistry*, 68: 559-569.