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EFFECT OF TEMPERATURE AND pH ON PECTIN LYASE ACTIVITY PRODUCED BY *PENICILLIUM DIGITATUM* ON ORANGE PEELS

Gawai D. U.*

Botany Research Lab.and Plant disease Clinic. PG Department of Botany, NES Science College, Nanded.

***Corresponding Author: Dr. Gawai D.U.**
Botany Research Lab.and Plant disease Clinic. PG Department of Botany, NES Science College, Nanded.

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ABSTRACT
Pectinolytic enzymes are one of the several extracellular enzymes produced by fungi that break down pectin. Pectin lyase extensively used in the clarification of fruit juices and wines. Currently, they are widely used in industry for retting of natural fibers and extraction of oils from vegetable and citrus peels. In the present study utilization of orange peels as an agro industrial waste for production of pectin lyase (PL) [E.C.4.2.2.10] by *Penicillium digitatum, Curvularia lunata and Aspergillus niger* was investigated using solid state culture (SSC). The highest level of extracellular pectin lyase was detected with this waste as an inducing substrate. The optimum pectin lyase activity was at pH 4.5,5.5 and 6.5 in case of *A.niger, P.digitatum C.lunata* respectively. The highest PL activity for all the three fungi was recorded at temperature 40 °C. and incubation time at 50 minutes.

KEYWORDS: Pectin lyase, Orange peels, *Penicillium digitatum, Curvularia lunata and Aspergillus niger*.

INTRODUCTION
Pectinases are group of enzymes that attack pectin and depolymerise it by ydrolysis and transelimination as well as by deesterification reactions, which hydrolyses the ester bond between carboxyl and methyl groups of pectin (Ceci and Loranzo, 1998).these enzymes act on pectin, a class of complex polysaccharides found in the cell wall of higher plants and cementing material for the cellulose network**.** Siessere and Said (1989) stated that pectin in its pure state has different characteristics from pectin extracted from the crude orange peel. Friedrich *et al.* 1989) showed that comparison of pectinases produced by different fungi is not easy because different culture conditions and different methods for enzyme activity have been used. Pectinases account for 10% of the global industrial enzymes produced **(**Stutzenberger, 1992) The enzymes that hydrolyse pectic substances are broadly known as pectinolytic enzymes or pectinases, which include polygalacturonase, pectin esterase, pectin lyase and pectate lyase on the basis of their mode of action (Alkorta *et al.*, 1998). Pectinolytic enzymes of fungal origin attract the most attention since they offer tremendous potential to the industry. Pectinases have widespread applications in retting of flax and vegetable fibres, de-pectinisation and clarification of fruit juices, extraction of oils from vegetables and citrus peels, manufacturing of paper and pulp and pre-treatment of pectic wastewater (Saito *et al.*, 2004). Pectin degradation plays an important role in plant diseases (Bateman and Miller, 1966; Ishii, 1976; Talboys, 1984; Collmer and Keen, 1986; Durrands and Cooper, 1988; Chen *et al.*, 1998; Omar and Abd-Alla, 2000). Pectin methyl esterase [E.C.3.1.1.11] and pectin lyase [E.C.4.2.2.10] (Wang and Keen, 1970). Pectin lyase [poly (methoxygalacturonide) lyase, PMGL, PNL or PL; E.C.4.2.2.10] seems to be the only pectic enzyme capable of breaking down pectin with high degree of esterification (like those found in fruits) into smaller molecules (Wijesundera *et al*., 1984; Alana *et al*., 1990; Serra *et al*., 1992). The production of extracellular pectinases is induced by agroindustrial wastes such as lemon or orange peels which contain appreciable quantities of pectin (Aguilar and Huitron, 1986; Maldonado, *et al*., 1986; Fonseca and Said, 1994; Grohmann *et al*., 1994; Alkorta *et al*., 1998; Castilho *et al*., 1999; Martins *et al*., 2002). High levels of pectinases were produced by *Talaromyces flavus*, *Tubercularia vulgaris* and *Penicillium charlesi* in solid state fermentation using citrus pulp pellets (Siessere and Said, 1989). In previous studies, we have shown that *Curvularia inaequalis* NRRL 13884 is capable of producing pectin methyl esterase in solid state culture using orange peels as an inducer. In the present study, the effect of temp. and pH on pectin lyase from *P. digitatum,C. lunata and A. niger* using orange peels was determined.

MATERIALS AND METHODS
Preparation of enzyme
Penicillium digitatum, Curvularia lunata and Aspergillus niger were isolated from peels of orange on Martins Rose Bengal Streptomycin agar medium. Pectin lyase (PL) was produced from these fungi using solid state

culture of washed and ground fresh orange peels. The medium used was composed of two constituents; Constituent A:: yeast extract 1 g/L and ammonium sulphate 2g/L,

Constituent B: washed and ground fresh orange peels.

Erlenmeyer conical flasks -250 ml capacity- were used, each containing 8 ml of solution A and 20 g of orange peels. The pH was adjusted to 5.0. Fifty flasks were used and autoclaved, inoculated with 2 ml of an evenly prepared spore suspension (~10^5 spores ml^{-1}) and incubated for 8 days at 30 °C. After incubation, the contents of the flasks were gathered, and thoroughly mixed with cooled distilled water (10 ml for each flask), rapidly filtered through Buchner's funnel and sucked into a sterilized Buchner's flask. The filtrate was stored at 4 °C prior to the purification of the enzyme.

Enzyme assay

PL activity was evaluated by the method of lbersheim and Killias (1962) as follows: 1.1% (wt/vol) solution of citrus pectin (Sigma Chemical Co., St Louis, Mo) was dissolved in 0.05 M citrate-phosphate buffer under magnetic stirring at 30 °C, then 3.0 ml of this solution was added to 2.0 ml of crude enzyme that had been previously adjusted to pH 7.5 with concentrated NaOH. After 60 min, the reaction was stopped by adding 3.5 ml of 0.5 M H_2SO_4. For the blank test, the order of the reagent was reversed (i.e., the acid was first added to the enzyme, followed by the pectin solution). The absorbency was read at 235 nm using a spectrocolorimeter (Spekol). One unit of pectin lyase (1 U) was defined as the amount of the enzyme that releases 1 µmol of 4,5-unsaturated digalacturonic acid per min.

Enzyme and protein assay

PL activity was evaluated by the method as follows:
1.1% (wt/vol) solution of citrus pectin (Sigma Chemical Co., St Louis, Mo) was issolved in 0.05 M citrate-phosphate buffer under magnetic stirring at 30 °C, then 3.0 ml of this solution was added to 2.0 ml of crude enzyme that had been previously adjusted to pH 7.5 with concentrated NaOH. After 60 min, the reaction was stopped by adding 3.5 ml of 0.5 M H_2SO_4. For the blank test, the order of the reagent was reversed (i.e., the acid was first added to the enzyme, followed by the pectin solution). The absorbency was read at 235 nm using a spectrophotometer. One unit of pectin lyase (1 U) was defined as the amount of the enzyme that releases 1 µmol of 4,5-unsaturated D-galacturonic acid per min. Protein was measured by UV absorption at 280 nm (Markwell *et al*., 1978) using bovine serum albumin as a standard.

Effect of various factors on pectin lyase activity

1. Effect of temperature:

The effect of temperature on enzyme activity was assessed by incubating 2.0 ml of the enzyme with 3.0 ml of 1.1% citrus pectin in previous buffer (0.05 M citrate-phosphate buffer) at various temperatures ranging from 25 to 55 °C.

2. Effect of pH

The effect of pH on enzyme activity was assessed by adding 2.0 ml of enzyme to 3.0 ml of 1.1% citrus pectin at different pH values (4.0 -7.5) using 0.05 M citrate-phosphate buffer and then incubating for 60 min. at 30 °C. After stopping the reaction, the enzyme activity was measured as previously described method.

3 Effect of incubation period

The effect of incubation time on enzyme activity was assessed by adding 2.0 ml of enzyme to 3.0 ml of 1.1% citrus pectin at various incubation times (20-60 min) using 0.05 M citrate-phosphate buffer.

RESULTS AND DISCUSSION

The highest activity of crude PLproduced by *A.niger*, *P.digitatum* and *C.lunata* were shown to be at pH 5.0, 5.5 and 6.5 respectively (Table 2). This result was in accordance with Obi and Moneke (1985) and Moharib *et al*. (2000). They stated that the enzyme of *A.niger* and yeast is highly active at pH 5.0 and 4.5, respectively. On the other hand, Chen *et al*. (1998) recorded the highest PL activity at pH 4.5 and 5.0 produced by *A.niger* and *P.digitatum* respectively. A rapid decline of PL activity of *all the three fungi* was recorded pH 5.5. This may be attributed to a decreased saturation of the enzyme with its substrate or decreased stability of the enzyme. The optimal temperature for highest activity of crude PLproduced by *A.niger*, *P.digitatum* and *C.lunata* were detected at 40°C (Table 1). Obi and Moneke (1985) stated that the maximum activity of their enzyme was observed at this degree. No activity was recorded after heating the enzyme over 55 °C.

The present investigation indicated that the crude PL showed its high activity after 50 minutes of incubation and its activity decreased by increasing the incubation time (Table3); this observation is in accordance with that reported by Obi and Moneke (1985).

Table 1: Effect of temperature on Pectin lyase enzyme activity of different fungi

S.No.	Temp	Pectin lyase enzyme activity		
		A.niger	***P.digitatum***	***C.lunata***
1	25^0C	16	20	12
2	30^0C	20	25	16
3	35^0C	25	30	20
4	40^0C	75	85	60
5	45^0C	70	75	45
6	50^0C	00	00	00
7	55^0C	00	00	00

Table 2: Effect of pH on Pectin lyase enzyme activity of different fungi

S.No.	pH	Pectin lyase enzyme activity		
		A.niger	***P.digitatum***	***C.lunata***
1	4.5	85	50	20
2	5.0	80	60	30
3	5.5	75	80	40
4	6.0	65	70	50
5	6.5	50	40	75
6	7.0	40	25	60
7	7.5	15	10	20
8	8.0	10	00	00

Table 3: Effect of incubation periods on Pectin lyase enzyme actjvity of different fungi

S.No.	Incubation periods(min)	Pectin lyase enzyme activity		
		A.niger	***P.digitatum***	***C.lunata***
1	20	*30*	*20*	*25*
2	25	35	25	30
3	30	40	30	35
4	35	50	25	45
5	40	60	40	50
6	45	65	60	55
7	50	80	70	75
8	55	60	40	50
9	60	40	20	30

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