

Citrus Peel as Substrate for Pectinase Production by *Aspergillus* spp.

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Abstract: Production of different pectinolytic enzymes was attempted using four fungal isolates (*Aspergillus foetidus*, *Aspergillus niger*, *Aspergillus flavus* and *Fusarium sp*) in submerged fermentation using citrus peel as substrate. The increased level on the production of pectinase was noticed when citrus peel was supplemented with additional carbon and nitrogen sources. Supplementation of sucrose and peptone at 1 per cent and 2 per cent respectively showed increased enzyme yield. The optimum pH and temperature for the production of pectinase (5.0/37°C) were also assessed.

Keywords: Pectinase, submerged fermentation, pH, temperature

1. Introduction

Pectin is a high value functional food ingredient widely used as gelling agent and stabilizer. It is also an abundant ubiquitous and multifunctional component of cell wall of all land plants¹. These polysaccharides are degraded by enzymes produced by saprophytic and phytopathogenic microorganisms including bacteria and fungi. Fungal pectinases are among the most important industrial enzymes and are of great significance with wide range of application for improving clarity of fruits and vegetable juices² in textile processing, paper making and coffee and tea fermentations³. Various agro-industrial wastes such as wheat bran⁴, sugarcane bagasse⁵, coffee pulp⁶, lemon peel⁷ and apple pomace⁸ have been explored for the microbial production of pectinase. Several authors have reported a wide range of pectin in citrus waste. This high content of pectin was a stimulant to explore citrus waste as a substrate for the microbial production of pectinase. The present study reports on the ideal conditions for pectinase production by fungal isolates in submerged conditions. The effect of various carbon sources, nitrogen sources, temperature, pH on production of pectinase by fungal isolates is compared with standard culture *Aspergillus foetidus* (MTCC).

2. Materials and Methods

Substrate

Citrus wastes were collected (5kgs) from different locations in and around the fruit juice manufacturing industries in Coimbatore, India. Collected citrus wastes were air dried ground and packed in polythene pouches for further studies.

Microorganisms and cultural conditions

Aspergillus niger, *Aspergillus flavus* and *Fusarium sp.* were isolated from citrus waste using modified czapecks medium. The isolated culture along with the standard culture

Aspergillus foetidus (MTCC 151) received from IMTECH, Chandigarh, India were maintained on czapecks agar slants at 4°C. The medium used for liquid cultures contained (g/l): NaNO₃(2); KH₂PO₄(1); MgSO₄.7H₂O(0.5); KCl(0.5); FeSO₄.7H₂O(0.01); Citrus peel powder(10). After inoculum (3×10⁶ spores/ml) the cultures were incubated at 30°C for five days. The mycelium was filtered through What man

No.1 filter paper and then centrifuged at 10,000 rpm and the supernatants were used as crude enzyme extract. To study the effect of supplementation of glucose and sucrose, they were added individually at 1 and 2 per cent concentration in the medium. To study the effect of supplementation of nitrogen source, yeast extract and peptone were added at 0.1 and 0.2 per cent concentration. For the determination of temperature optimum, the cultures were incubated at different temperatures viz., 25, 37 and 45°C and for pH optimum the culture medium were adjusted to pH 4.0, 5.0, and 6.0 with sodium citrate/HCL buffers.

3. Enzyme Assays

Pectin esterase

Pectin esterase activity can be measured either by measuring the amount of methanol release or increase in free carboxyl group by titration using pH meter⁹. For assaying pectin esterase activity, 20 ml of 1 per cent pectin dissolved in 0.15 M NaCl (pH 7.0) and 4 ml of crude enzyme extract were taken in a beaker and incubated for 1 h. After incubation, the solution was titrated against 0.02N NaOH to reach pH 7.0 using phenolphthalein as indicator. The heated crude enzyme extract was used as control. Pectin esterase was calculated by using the following formula.

Pectin esterase activity = $V_s - V_b \times (\text{Normality of NaOH} \times 100/V_t)$ Where,
 V_s = Volume of NaOH used to titrate sample (ml)
 V_b = Volume of NaOH used to titrate blank (ml)
 V = Volume of incubation mixture (ml)
 t = Reaction time (hrs.)

Pectin esterase activity is expressed as milli equivalents of NaOH consumed min⁻¹ ml⁻¹ of crude enzyme extract under assay conditions.

Polygalacturonase

Polygalacturonase activity was determined by measuring the release of reducing groups from citrus pectin using the 3,5 dinitrosalicylic acid reagent assay¹⁰. The reaction mixture containing 0.8 ml of 1 per cent citrus pectin (67 per cent methoxylated in 0.3M acetate buffer), pH 5.0 and 0.2ml of crude enzyme solution, was incubated at 50°C for 10 min. One unit of enzymatic activity (U) was defined as the

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amount of enzyme which releases one mmol of galacturonic acid per minute.

4. Results and Discussion

Citrus peel contains 8.5% reducing sugars, 17.3 % total sugars, 4.5% nitrogen, 0.18% phosphorous, 0.16% potassium, 28.2% organic carbon and 25.5% pectin. Pectin is present mainly in fruits of citrus peel, apple, mangoes, banana but commercially it is manufactured from citrus peel and apple pomace which are the waste products of citrus and apple processing industries Smock & Neubert¹¹. The pectic esterase activities of fungal isolates grown with glucose and sucrose along with pectic medium containing 1 per cent citrus peel powder is presented in Table 1. In general all the fungal isolates showed maximum activity at 2 per cent sucrose. The standard culture *Aspergillus foetidus* recorded higher pectin esterase activity of 0.350 (milli eq. of NaOH consumed $\text{min}^{-1} \text{ml}^{-1}$). Among the isolates *Aspergillus niger* recorded a higher enzyme activity of 0.330 (milli eq. of NaOH consumed $\text{min}^{-1} \text{ml}^{-1}$). Similar trends were observed in polygalacturonase activity also. Solis-Pereyra *et al*¹² reported that the addition of sugars to fermenting medium allows the organism to produce enzymes without catabolic repression. This result is in confirmation with findings of Talboys & Busch¹³ that when sucrose or galacturonic acid was added to pectin containing medium polygalacturonase activity could be increased.

All the fungal isolates recorded a higher pectin esterase and polygalacturonase activity when supplemented with 0.2 per cent peptone. The least enzyme activities were observed in *Fusarium* sp (Table 2). An increased pectin esterase production when apple pomace was supplemented with 0.2 per cent peptone was already reported by Taragano *et al*¹⁴.

The optimal temperature for the production of pectic enzymes was detected as 37⁰ C for all the four fungal isolates. The standard culture *Aspergillus foetidus* recorded increased pectic enzyme activity of 0.126 (milli eq. of NaOH consumed $\text{min}^{-1} \text{ml}^{-1}$) over isolated cultures. Among the isolates *Aspergillus niger* recorded higher enzyme activity of 0.460 (μm of galacturonic acid min^{-1}) while the standard culture recorded 0.553 (μm of galacturonic acid min^{-1}) at same temperature (Table 3). Similarly *Aspergillus niger* recorded higher polygalacturonase enzyme activity of 0.460 (μm of galacturonic acid min^{-1}) while the standard culture recorded 0.553 (μm of galacturonic acid min^{-1}) at same temperature. Temperature is yet another critical parameter that can affect fermentation processes. The present study on effect of temperature on pectinase production showed that optimum temperature for pectinase production was 37°C. Due to the production of large amount of metabolic heat, the fermented substrate temperature shoots up¹⁵.

At lower incubation temperature, practically all pectinolytic activities remain unchanged and at higher temperature all enzyme activities decreased. At higher temperature, there may be heat build up which causes evaporative water loss and reduction in vegetative growth, where as a controlled evaporation with continuous water replacement promote heat dissipation and thus assure productive vegetative

growth.¹⁶ Silva *et al.*¹⁷ also reported higher polygalacturonase activity at 40°C in *Penicillium viridicatum*. The optimum pH for pectin esterase and polygalacturonase enzymes was standardized and it was found that pH 5.0 was most suitable for maximum enzyme production for all the isolates tested (Fig 1 and 2). Gupta *et al.*¹⁸ also had same opinion and reported that polygalacturonase from fungi can occur at pH range of 4.0 to 6.0. Martin *et al.*¹⁹ also reported higher polygalacturonase activity of *Penicillium* sp at pH 5.0.

5. Conclusion

It has been found that agro industrial wastes such as citrus peel can be effectively used for pectinase enzyme extraction utilizing fungal cultures.

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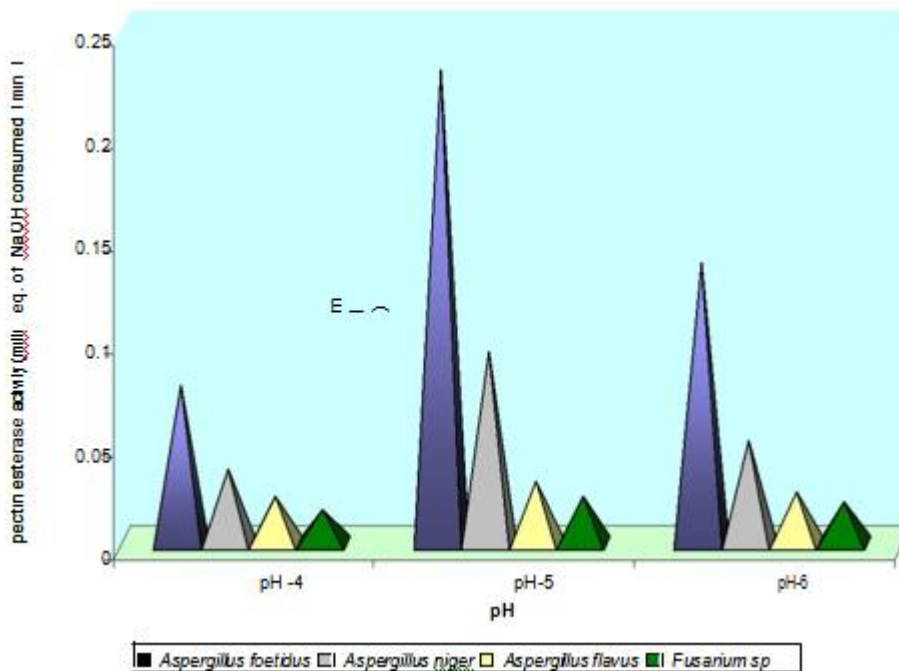


Figure 1: Effect of pH on pectin esterase activity in fungal isolates

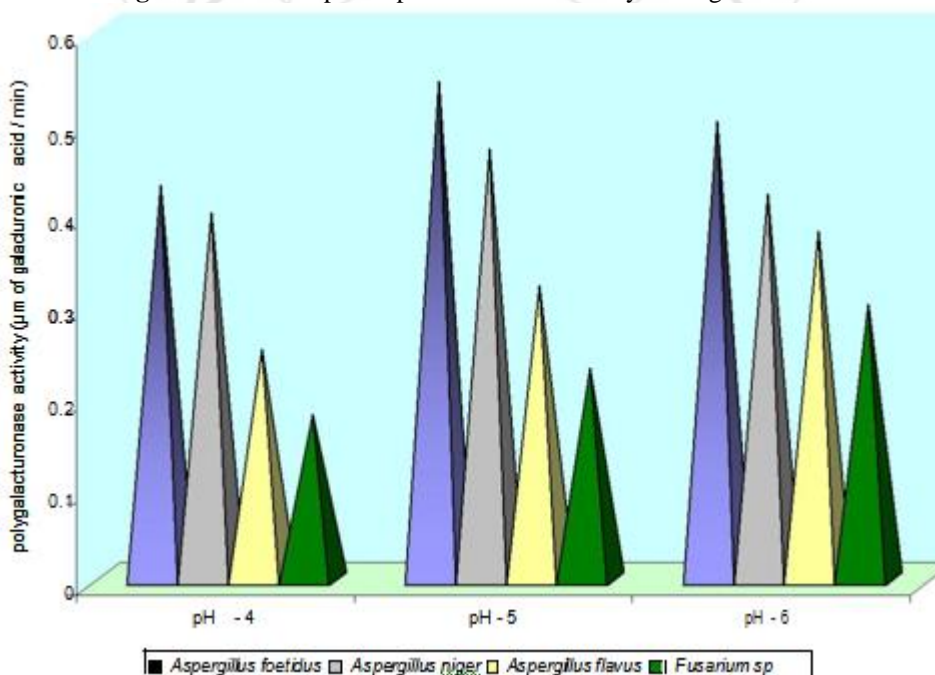


Figure 2: Effect of pH on polygalacturonase activity in fungal isolates

Table 1: Effect of carbon sources on pectinase activity

Organism	Pectin esterase activity (milli eq. of NaOH consumed min ⁻¹ ml ⁻¹)				Polygalacturonase activity (µm of galacturonic acid min ⁻¹)			
	Glucose		Sucrose		Glucose		Sucrose	
	1%	2%	1%	2%	1%	2%	1%	2%
<i>Aspergillus foetidus</i>	0.210	0.32	0.3	0.35	0.41	0.45	0.41	0.51
<i>Aspergillus niger</i>	0.18	0.23	0.25	0.33	0.39	0.4	0.36	0.5
<i>Aspergillus flavus</i>	0.132	0.18	0.2	0.25	0.32	0.36	0.3	0.45
<i>Fusarium sp</i>	0.11	0.15	0.2	0.22	0.25	0.33	0.3	0.4

Pectin esterase activity is expressed as milli equivalents of NaOH consumed min⁻¹ ml⁻¹ of crude enzyme extract under assay conditions.

One unit of enzymatic activity (U) was defined as the amount of enzyme which releases one mmol of galacturonic acid per minute.

Table 2: Effect of nitrogen sources on pectinase activity

Organism	Pectin esterase activity (milli eq. of NaOH consumed min ⁻¹ ml ⁻¹)				Polygalacturonase activity (µm of galacturonic acid min ⁻¹)			
	Yeast extract		Peptone		Yeast extract		Peptone	
	0.10%	0.20%	0.10%	0.20%	0.10%	0.20%	0.10%	0.20%
<i>Aspergillus foetidus</i>	0.150	0.23	0.21	0.3	0.55	0.65	0.62	0.71
<i>Aspergillus niger</i>	0.14	0.18	0.19	0.26	0.52	0.59	0.58	0.65
<i>Aspergillus flavus</i>	0.112	0.152	0.16	0.183	0.32	0.36	0.39	0.45
<i>Fusarium sp</i>	0.045	0.083	0.091	0.124	0.253	0.3	0.34	0.391

Pectin esterase activity is expressed as milli equivalents of NaOH consumed min⁻¹ ml⁻¹ of crude enzyme extract under assay conditions.

One unit of enzymatic activity (U) was defined as the amount of enzyme which releases one mmol of galacturonic acid per minute.

Table 3: Effect of temperature on pectinase activity

Organism	Pectin esterase activity (milli eq. of NaOH consumed min ⁻¹ ml ⁻¹)			Polygalacturonase activity (µm of galacturonic acid min ⁻¹)		
	25°C	37°C	45°C	25°C	37°C	45°C
	<i>Aspergillus foetidus</i>	0.057	0.126	0.11	0.42	0.553
<i>Aspergillus niger</i>	0.057	0.083	0.037	0.38	0.46	0.29
<i>Aspergillus flavus</i>	0.033	0.042	0.036	0.31	0.36	0.25
<i>Fusarium sp</i>	0.015	0.031	0.035	0.2	0.25	0.13

Pectin esterase activity is expressed as milli equivalents of NaOH consumed min⁻¹ ml⁻¹ of crude enzyme extract under assay conditions.

One unit of enzymatic activity (U) was defined as the amount of enzyme which releases one mmol of galacturonic acid per minute.