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RESEARCH ARTICLE

EFFECT OF CARBON AND NITROGEN SOURCES ON PECTINASE BY *PAECILOMYCES VARIOTII*

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A B S T R A C T

Microorganisms were isolated from fruit waste disposable area soil samples of Pazhamuthir Nilayam, Coimbatore, Tamil Nadu and screened in modified Czapek- Dox media with citrus pectin as sole carbon source for its pectinolytic activity. Among the isolates, only four fungal strains showed hydrolyzing zone, and was found predominant in *Paecilomyces variotii* with a clearance zone of 36 mm out of colony diameter of 57 mm was selected and hence, *Paecilomyces variotii* was selected as a candidate for the study. The effects of the various carbon and nitrogen sources on the pectinase activity by *Paecilomyces variotii* was carried out in surface culture fermentation. The commercial carbon and nitrogen sources included dextrose, maltose, cellulose, ammonium sulphate, potassium nitrate and urea respectively. The results of the investigation revealed that the maximum pectinase production of 9.66±0.17 Umg^{-1} protein and of 9.62±0.11 Umg^{-1} protein was obtained at both intracellular and extracellular level respectively were observed in 1% and 2% maltose as carbon sources. Among the nitrogen sources the maximum pectinase activity of 9.73±0.10Umg^{-1} protein and 7.44±0.12 Umg^{-1} protein was observed in 3% ammonium sulphate at both intracellular and extracellular level respectively.

INTRODUCTION

The pectinases are one of the important upcoming enzymes of the commercial sector especially for fruit juice industry as pre-requisites for obtaining well clarified and stable juices with higher yields (Sandri *et al*., 2011). These enzymes have useful applications in paper, fruit and textile industries. Almost 75% of the estimated sale value among industrial enzymes in 1995 has been contributed by pectinases. As a result, pectinase became one of the futuristic useful enzymes in commercial sector (Kashyap *et al.,* 2001).

Fruits, the gift of nature are an important constituent of human diet, which are widely distributed and are the vital source of nutrient to human beings. They give the necessary vitamins, fats, minerals and oil in the right proportion in the daily diet for their growth and development. But, fruits have serious challenges to their existence, which include changes in climate conditions, pests, inadequate rainfall and fungal attack. Enormous quantities of industries waste residues are also generated throughout the world from processing raw agricultural materials for foods and fruit processing industries. These waste materials impose high BOD burden on the environment when dumped, which ultimately leads to pollution. Thus, the utilization of renewable resources, particularly agricultural residues has captured world-wide attention and extraction of enzymes from bio-wastes, using the technology of fermentation is one of the many ways of exploiting them profitably. The large amount of waste from agricultural and fruit processing industries became a prominent section for biological utilization of this waste (Jose *et al*., 2008).

Identification and bioconversion of locally available agro-waste is advantageous as it not only leads to the value addition of these residues, but also helps to keep the environment clean. Agro industrial byproducts can be successfully utilized for the microbial pectinolytic enzyme production and as these residues are locally abundant low cost raw materials, they can be used for cost effective enzyme production. Thus, fruit waste generated from food industry can be utilized to biosynthesize pectinase enzyme at a cheaper rate which have numerous industrial applications (Patil *et al*., 2012).

The main source of the microorganisms that produce pectinolytic enzymes are yeast, bacteria and large varieties of fungi, insects, nematodes and protozoas (Jayani *et al*., 2010). Thus, by breaking down pectin polymer for nutritional purposes, microbial pectolytic enzymes play an important role in nature (Yadav *et al*., 2009). Microbes are rich source of enzymes and the exploration of extracellular enzymatic activity from them has formed the basis of industrial enzymes of which, 50 per cent is from fungi and yeast, 35 per cent from bacteria and the remaining 15 per cent are from either plant or animal origin. The aim of the present investigation is to assess the enhancement of enzyme production by *Paecilomyces variotii* in different carbon and nitrogen sources as substrates.

MATERIALS AND METHODS

Isolation of Fungal Species

Soil samples were collected from fruit waste disposal areas of Pazhamuthir Nilayam, Coimbatore, Tamil Nadu. The soil samples were serially diluted and plated on potato dextrose agar medium and incubated for seven days at 30°C. After incubation, the plates were observed for fungal growth and were sub-cultured and maintained on PDA slants at 4°C. The fungal isolates were identified based on their morphology, mycelia structure and spore formation (Domsche and Gams, 1972).

Screening of Soil Fungal Isolates for Pectinolytic Activity

The isolates, were cultured on modified Czapek-dox Agar medium (Nwodo-chinedu *et al*., 2010) with commercial citrus pectin as the sole carbon source and screened by modified plate method of Phutela *et al*. (2005). The clearance zone of pectinolysis formed around the colonies was determined using potassium iodide – Iodine solution (5.0 g potassium iodide and 1.0 g iodine in 330 ml of distilled water) and incubated at 30°C for 24 hours. The culture showing high clearance zone was selected for the enzyme study.

Enzyme production

Erlenmeyer flasks containing 100ml of Czapek-dox Liquid Medium (Raper and Thom, 1949) was sterilized at 1 atm for 15 minutes. After cooling, one ml of streptomycin sulphate (10,000 ppm) was added and incubated for 5, 7 and 9 days at 30°C under static conditions. The mycelium was filtered through Whatman No. 40 filter paper using a Buchner funnel under suction and the clear filtrate was used as a source of extracellular enzyme. A quantity of 5 g of the washed mycelia mat was macerated in five ml of acetate buffer of pH 4.8 in a prechilled mortar and pestle with a pinch of acid washed sand. The homogenate was centrifuged and the supernatant was used as crude source of intracellular enzyme.

Pectinase Assay

Pectinase activity was determined by the production of reducing groups from citrus pectin using DNS method. The reaction mixture consisted of acetate buffer (2ml of 1% citrus pectin, pH 4.8) and enzyme solution (0.5 ml). The mixture was kept in water bath at 45°C for 30 minutes. After cooling, 2.5ml of DNS reagent was added and again heated for 5 minutes. Finally, the content was cooled and 10 ml of distilled water was added and the absorbance was read at 540 nm. Protein content was determined by the method of Lowry *et al.,* (1951).

Effect of Carbon and Nitrogen Sources on Enzyme Production

The isolated fungal strain was incubated in the production medium containing various concentrations (1%, 2% and 3%) of carbon sources (dextrose, maltose and cellulose) and nitrogen (ammonium sulphate, potassium nitrate and urea) sources. The enzyme activity for each trial was estimated at 5th, 7th and 9th day of incubation in triplicates.

RESULT

Screening of Soil fungal isolates for Pectinolytic Activity

Among the number of mycoflora isolated from the fruit waste disposable area soils, only four fungal strains (*Aspergillus flavus, Penicillium chrysogenum, Trichoderma harzianum* and *Paecilomyces variotii)* showed maximum hydrolyzing zone (Figure-1). Out of the four isolates, *P. variotii* showed highest hydrolyzing zone (clearance zone) of 36 mm out of colony diameter of 57 mm (Plate 1) and so it was selected as a potential candidate for pectinolytic enzyme production. The present result is in accordance with the findings of Banakar and Thippeswamy (2012) who observed the maximum clearance zone of pectinolytic activity in *Syncephalastrum recemosum* of 17.0 mm followed by *Trichosporiella cerebriformis* (14.0mm) and *Paecilomyce smarquandii* (13.0 mm). Similar results of exhibiting highest zone of clearance of 4.0mm (pectinolytic activity) around the colonies of *Aspergillus, Penicillium jenseni* and P. *citrinum* was reported by Priya and Sashi (2014).

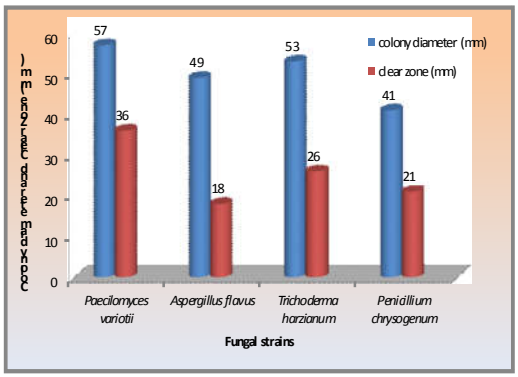

Figure 1 Colony diameter and hydrolytic zone

Figure 2 Plate 1 *P. Variotii* Showing Maximum

Optimization of Culture Conditions for Enzyme Production

Effect of Carbon Source on Enzyme Production

Pectinase activity showed an increasing trend in different carbon sources at different concentrations (1%, 2% and 3%) upto 7 days, after that, its activity declined gradually at an intra and extra cellular level by *Paecilomyces variotii*. Among the different carbon sources (dextrose, maltose and cellulose), a significantly enhanced activity observed was as 9.71±0.07 Umg^{-1} protein in 1% maltose followed by 9.62±0.11 Umg^{-1} protein in 3% dextrose on the 7th day of incubation at an

intracellular level over the controls (1.73±0.18 Umg^{-1} protein). At an intracellular level, pectinase activity was highly pronounced (9.66±0.17 Umg^{-1}protein) in 2% maltose as carbon source on the 7th day of incubation compared to control (Table 1) the present result supported the work of Arotupin *et al*. (2011). They reported the ability of maltose in maximum polygalacturonase activity of 3033 Uml^{-1} by Trichoderma viride (BITRS – 1001) in submerged cultivation. Similar result was expressed by Khan *et al.* (2012) who observed maximum pectinase activity in dextrose (1.04 I Uml^{-1}) by *Aspergillus niger* ATCC 16404.

Table 1 Colony Diameter and Hydrolytic Zone

S. No.	Fungal strains	Colony diameter (mm)	Hydrolytic zone (mm)
1.	*Paecilomyces variotii*	57	36
2.	*Aspergillus flavus*	49	18
3.	*Trichoderma harzianum*	53	26
4.	*Penicillium chrysogenum*	41	21

Effect of Nitrogen Source on Enzyme Production

An increasing trend in enzyme activity at both inter and extracellular level was observed in different nitrogen sources (ammonium sulphate, potassium nitrate and urea) in different concentrations (1%, 2% and 3%) upto 7 days of incubation and after that, a decreasing trend was observed (Table –2).

An enhanced activity of 9.73±0.10 Umg^{-1}protein was observed in 3% ammonium sulphate followed by 9.63±0.09 Umg^{-1} protein in 2% potassium nitrate on the 7th day of incubation at an intracellular level than the control. Pectinase activity showed a significantly highest level of 8.65±0.13 Umg^{-1} protein in 1% ammonium sulphate followed by 7.06±0.16 Umg^{-1} protein in 2% potassium nitrate on the 7th day of incubation at an extracellular level by *Paecilomyces variotii*.

Similar view was expressed by Tariq and Reyaz (2012). They have obtained enhanced pectinase activity of 65.0 U/ml in ammonium sulphate by *Pencillium chrysogenum*. Vasanthi and Meenakshisundaram (2012) also reported similar finding that ammonium sulphate had greater influence on the production of pectinase (15.74IU/ml) by *Aspergillus Niger*. The present view is on par with the result of Sarkar et al. (2014). They have observed enhanced activity in ammonium sulphate (0.936mg/ml) by *Pencillium chrysogenum*.

CONCLUSION

It can be inferred from the present investigation that the pectinase production by Paecilimyces variotii was maximum in maltose and ammonium sulphate as carbon and nitrogen

Table 2 Pectinase activity (Umg^{-1} protein) of *Paecilomyces variotii* on carbon source

	Carbon sources	1%			2%			3%		
		5th day	7th day	9th day	5th day	7th day	9th day	5th day	7th day	9th day
Intracellular	Control	0.67±0.13	1.73±0.18	0.85±0.11	0.67±0.13	1.73±0.18	0.85±0.11	0.67±0.13	1.73±0.18	0.85±0.11
	Dextrose	1.45±0.08	2.12±0.09	1.92±0.10	1.04±0.08	1.38±0.09	1.30±0.10	2.32±0.09	9.62±0.11	6.08±0.10
	Maltose	3.55±0.07	9.71±0.10	6.18±0.16	1.14±0.09	3.83±0.09	1.42±0.13	2.68±0.09	3.84±0.11	1.15±0.13
	Cellulose	1.36±0.13	1.52±0.11	1.56±0.12	1.32±0.11	1.77±0.12	0.93±0.15	1.24±0.17	1.94±0.09	1.20±0.15
	SEd					0.090				
	CD (1%)					0.240				
	Carbon sources	1%			2%			3%		
		5th day	7th day	9th day	5th day	7th day	9th day	5th day	7th day	9th day
Extracellular	Control	0.27±0.12	0.93±0.10	0.31±0.14	0.27±0.12	0.93±0.10	0.31±0.14	0.27±0.12	0.93±0.10	0.31±0.14
	Dextrose	0.93±0.10	2.03±0.14	1.35±0.14	1.74±0.17	3.37±0.11	1.67±0.12	2.85±0.13	3.56±0.14	1.87±0.12
	Maltose	4.09±0.12	8.44±0.16	6.53±0.11	5.92±0.09	9.66±0.17	4.93±0.10	3.62±0.11	5.08±0.19	2.52±.009
	Cellulose	2.15±0.12	4.27±0.09	1.38±.016	1.34±0.08	4.77±0.08	1.53±0.14	4.12±0.12	6.52±0.10	3.91±0.14
	SEd					0.098				
	CD (1%)					0.260				

Results are the mean ± standard deviation of triplicates
Umg^{-1} = μ mol polygalacturonic acid released min^{-1} mg^{-1} protein

Table 3 Pectinase activity (Umg^{-1} protein) of *Paecilomyces variotii* on nitrogen sources

	Nitrogen sources	1%			2%			3%		
		5th day	7th day	9th day	5th day	7th day	9th day	5th day	7th day	9th day
Intracellular	Control	0.67±0.13	1.73±0.18	0.93±0.15	0.67±0.13	1.73±0.18	0.93±0.15	0.67±0.13	1.73±0.18	0.93±0.15
	Ammonium sulphate	2.62±0.11	5.54±0.09	1.97±0.13	2.42±0.12	4.77±0.09	1.55±0.08	2.06±0.13	9.73±0.10	4.86±0.13
	Potassium nitrate	1.53±0.13	4.04±0.12	2.38±0.11	3.76±0.11	9.63±0.10	6.84±0.10	3.24±0.15	6.17±0.10	4.35±0.14
	Urea	2.26±0.12	5.48±0.11	3.28±0.11	2.66±0.12	6.04±0.13	3.65±0.11	2.83±0.08	5.86±0.12	2.24±0.12
	SEd					0.092				
	CD (1%)					0.245				
Extracellular	Control	0.27±0.12	1.17±0.10	0.91±0.14	0.27±0.12	1.17±0.10	0.91±0.14	0.27±0.12	1.17±0.10	0.91±0.14
	Ammonium sulphate	6.52±0.11	8.65±0.13	4.37±0.11	4.54±0.13	6.12±0.11	1.44±0.13	1.28±0.12	7.44±0.12	4.63±0.12
	Potassium nitrate	2.26±0.13	4.26±0.15	3.85±0.08	2.25±0.12	7.06±0.16	3.62±0.19	2.83±0.14	3.83±0.09	2.17±0.11
	Urea	3.51±0.12	5.14±0.11	2.36±0.12	1.77±0.13	3.12±0.15	2.04±0.16	2.36±0.10	3.74±0.15	2.85±0.17
	SEd					0.090				
	CD (1%)					0.240				

Results are the mean ± standard deviation of triplicates
Umg-1 = □ mol polygalacturonic acid released min-1 mg-1 protein

Sources. Thus, it can be concluded that, for effective pectinase production from *P. variotii* maltose at 1% and ammonium sulphate at 3% concentration acts as a potent carbon and nitogen sources.

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