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RESEARCHARTICLE

OPTIMIZATION OF PECTINASE ACTIVITY BY *PAECILOMYCES VARIOTII*

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ABSTRACT

Pectinase producing microorganisms were isolated from fruit waste disposable area soil samples of Pazhamuthir Nilayam, Coimbatore, Tamilnadu and screened in modified Czapek- Dox media with citrus pectin as sole carbon source. Among the isolates, only four fungal strains showed hydrolyzing zone of pectinolytic activity. Since *Paecilomyces variotii* showed maximum clearance zone of 36 mm (out of colony diameter of 57 mm), it was selected as acandidate for the study. The optimum conditions of pectinase production was carried out by growing *Paecilomyces variotii* in different fruit waste substrate at varying pH (4, 45, 5, 5.5, 6, 6.5, 7, 7.5 and 8) and temperatures (30°C, 35°C, 40°C, 45°C and 50°C). The results of the investigation revealed that maximum pectinase production of 9.05 U mg^{-1}protein and of 9.03 U mg^{-1}protein respectively were observed in 3% banana peel as substrateat both extracellular and intracellular level. A significantly pectinase activity of 6.19 Umg^{-1} protein was observed at an optimum pH of 6.5, and at a temperature of 40°C (4.73 Umg^{-1} protein) at an intracellular level on the 7th day of incubation.

INTRODUCTION

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 industrial waste residues are generated throughout the world from processing raw agricultural materials for foods and fruit processing industries, which are highly perishable and their disposal is a problem. Thus, the utilization of renewable resources, particularly agricultural residues has focused world-wide attention for the extraction of industrially important enzymes from bio-waste and fermentation technology is one of the many ways of exploiting them profitably. Pectins are complex polysaccharides consisting of partially methyl esterified α- (1,4) linked homogalactronic acid backbone and branched neutral sugar side chains. They are important components of middle lamella and primary cell wall of higher plants and also found in fruits and vegetables. Pectinase is an enzyme of high molecular weight with negatively charged acidic glycosidic macromolecules that breakdown complex polysaccharides in plant tissues into simpler molecules with extraordinary specificity, catalytic power and substrate specificity (Approvi and Vuppu, 2012). Pectinases produced from food and food bio products processed waste, alone account to a total of one-third quarter of world's food enzyme production and are today's one of the upcoming enzymes of the commercial sector. Byproducts or waste obtained from orange, apple, grapes, pine apple, papaya, lemon juice manufacturing industries are used as source for the enzyme production (Prathyusha *et al*., 2011).

Vast varieties of micro-organisms are present in the environment which can be exploited for the utilization of waste material. Microbes are rich source of enzymes. 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 explore the production of pectinase by *Paecilomyces* from cheap fruit waste in an ecofriendly manner and to solve the problem of waste disposal.

MATERIALS AND METHODS

Isolation of fungal species

Soil samples were collected from fruit waste disposal area of Pazhamuthir Nilayam, Coimbatore, Tamilnadu. The samples were serially diluted and plated on potato dextrose agar medium and incubated for seven days at 30°C.

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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 (Domsch and Gams 1972).

Screening of soil fungal isolates for pectinolytic activity

The isolates were cultivated 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 used for the enzyme study. The size of the clearance zone formed around the colonies corresponds to the enzymatic activity of a particular culture.

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. The clear filtrate obtained was used as a source of extracellular enzyme. A quantity of 5.0 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 2ml of 1% citrus pectin, acetate buffer (pH 4.8) and 0.5 ml enzyme solution. 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. The absorbance was read at 540 nm and the protein content was determined by the method of Lowry *et al.*(1951).

Optimization of culture conditions for enzyme production

The parameters selected for optimization were different concentrations (1%, 2% and 3%) offruit waste substrates (orange peel, pomegranate peel, banana peel and grape peel), varying pH (4-8) and temperatures (30-50°C)

Effect of fruit wastes as substrate on enzyme production

The fungal strain selected as a candidate for enzyme production was incubated in a medium containing various concentrations (1 %, 2% and 3%) of fruit waste powders (orange peel, pomegranate peel, banana peel and grape peel] as substrates. All sets have been performed in triplicates and the enzyme activity was estimated at 5th, 7th, and 9th day of incubation.

Effect of temperature and pH

The fungal strain was inoculated in 100ml of production medium which was prominently adjusted by using 1 N HCl or 1 N NaOH to various pH ranges (4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8) and varying temperature ranges (30°C, 35°C, 40°C, 45°C and 50°C) under static condition. The experimental set up was done in triplicates and the enzyme activity was estimated on 5th, 7th and 9th days of incubation.

RESULTS

Screening of isolatesforpectinolytic activity.

Among the number of mycoflora isolated from the fruit waste disposable area soil, 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* highest hydrolyzing zone (clearance zone) of 36 mm out of colony diameter of 57 mm and so, it was selected as a potential candidate of pectinolytic enzyme.

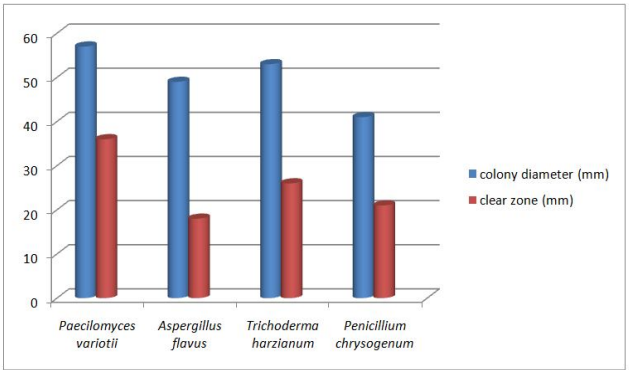

Figure 1. A Graph showing diameter of colony and clear zone of Pectinolytic activity

Optimization of parameters for pectinase production by *P.variotii*

To assess the optimal values, different parameters were optimized at different concentrations (1%, 2% and 3%) fruit waste powder substrates(orange peel, pomegranate peel, banana peel and grape peel), pH ranges from 4.0 to 8.0 and temperature ranges from 30-50°C

Effect of different fruit waste as substrate on enzyme production (Table – 1)

In the present study,3 per cent banana peel as the fruit waste substrate was found to be the most significant substrate for the pectinase enzyme activity (9.05 Umg^{-1} protein and 9.03 Umg^{-1} protein) followed by orange peel of 8.34 Umg^{-1} protein and 3.66 Umg^{-1} protein at both extracellular and intracellular level by *Paecilomyces variotii* when compared to the control (1.44

Umg^{-1} protein and 1.85 Umg^{-1} protein) at intracellular and extracellular level respectively.

Effect of pH on enzyme production

An increase in the production was observed between the pH ranges of 4 and 6.5 respectively (Table – 2). Beyond this range there was a considerable deterioration in the enzyme production by *Paecilomyces variotii*. A significantly higher pectinase activity of 6.19 Umg^{-1}protein was registered at an intracellular level when compared to extracellular level of 6.17 Umg^{-1} protein at pH6.5. The least production of 1.84 Umg^{-1}protein at intracellular level and 1.44 Umg^{-1} protein at extracellular level was recorded at pH 8.0.

Effect of temperature on enzyme production (Table –3)

Temperature plays an important role in the enzyme production. Pectinase enzyme production at an intracellular level by *Paecilomyces variotii* was found to be higher (4.73 Umg^{-1} protein) when compared to extracellular level of 3.17 Umg^{-1} protein at 40°C. The least production of 1.42Umg^{-1}protein at intracellular level and 0.64 Umg^{-1}protein at extracellular level was recorded at 50°C.

DISCUSSION

Screening of isolates for pectinolytic activity

The present result is in accordance with the findings of Anuradha *et al*. (2010) who observed a maximum hydrolyzing

Table 1. Pectinase activity (Umg^{-1} protein*) of *Paecilomyces variotii* on different fruit wastes as substrate

	Fruit Wastes	Control			1.0 Conc.			2.0 Conc.			3.0 Conc.			CD (P < 0.05)	SED
		5 Day	7 Day	9 Day	5 Day	7 Day	9 Day	5 Day	7 Day	9 Day	5 Day	7 Day	9 Day		
Intracellular	Orange peel				5.18	6.66	3.83	5.84	7.69	3.85	5.53	8.34	3.62		
	Pomegranate peel	1.35	1.85	1.74	3.18	5.54	2.25	3.66	4.13	2.55	3.61	4.73	2.42	0.197	0.098
	Banana peel				4.36	8.66	3.90	6.77	6.96	4.17	3.97	9.03	3.47		
	Grape peel				2.94	3.61	3.43	3.45	4.74	2.65	2.60	4.47	2.45		
Extracellular	Orange peel				2.83	3.75	1.34	3.52	5.97	3.47	3.36	3.66	2.96		
	Pomegranate peel	1.24	1.44	1.14	1.54	3.44	1.35	1.56	3.56	2.17	1.36	2.65	2.05	0.206	0.102
	Banana peel				2.52	6.97	1.91	2.37	8.43	2.12	2.37	9.05	1.93		
	Grape peel				1.46	1.86	1.58	1.64	1.92	1.65	2.43	2.75	1.62		

*Umg^{-1} = μ mol polygalacturonic acid released min^{-1} mg^{-1} protein

Table 2. Pectinase activity (Umg^{-1} Protein*) Of *Paecilomycesvariotii*at different pH

Level	Days	pH									CD(P < 0.05)	SED
		4	4.5	5	5.5	6	6.5	7	7.5	8		
Intracellular	5	2.44	2.50	2.64	3.00	3.14	3.35	1.94	1.91	1.84		
	7	4.45	4.61	5.01	5.05	5.73	6.19	5.03	4.73	4.56	0.238	0.118
	9	2.16	2.17	2.31	2.67	2.81	3.37	2.55	2.34	2.25		
Extracellular	5	1.84	1.93	2.23	2.34	2.45	2.66	2.06	2.02	1.44		
	7	3.50	3.54	4.07	5.15	5.77	6.17	4.96	4.16	3.63	0.226	0.113
	9	1.80	1.88	1.94	1.95	2.15	2.55	2.48	2.14	1.31		

*Umg^{-1} = μ mol polygalacturonic acid released min^{-1} mg^{-1} protein

Table 3. Pectinase activity (Umg^{-1} protein*) of *Paecilomycesvariotii*at different temperatures

Level	Days	Temperature (°C)					CD(P < 0.05)	SED
		30	35	40	45	50		
Intracellular	5	1.53	1.96	2.57	1.53	1.42		
	7	2.04	3.06	4.73	3.87	3.23	0.225	0.110
	9	1.93	2.44	2.75	1.96	1.48		
Extracellular	5	1.45	1.75	1.96	1.86	1.18		
	7	1.66	2.32	3.17	2.83	2.05	0.207	0.101
	9	1.31	1.55	2.04	1.44	0.64		

*Umg^{-1} = μ mol polygalacturonic acid released min^{-1} mg^{-1} protein

zone of pectinolytic activity by *Aspergillus awamori*. The present finding is also on par with the finding of Hannan *et al*. (2009) who observed a maximum hydrolyzing zone formation in *Aspergillus niger* among 52 strains of *Aspergillus niger* and *A. flavus*.

Effect of different fruit waste as substrate on enzyme production

Similar view was expressed by Adeleke *et al.* (2012) who reported that the mixture of orange bagasse and wheat bran was the best substrate for the production of pectinase in solid state fermentation by *Penicillium* sp. Phutela *et al.* (2005) also observed maximum activity of 264 Ug^{-1} of pectinase in orange peel as substrate.

Effect of pH on enzyme production

Similar result was obtained by Banu *et al*. (2010) with a maximum pectinase activity of 27.07 Uml^{-1} by *Penicillium chrysogenum* at a pH of 6.5. The present finding is in accordance with the result of Patil *et al*. (2012) who reported maximum polygalacturonase activity by *Paecilomyces variotii* at pH 6.0.

Effect of temperature on enzyme production

The present result coincides the work of Banu *et al*. (2010) who reported that *Penicillium chrysogenum* showed enhanced enzyme productivity of 27.21 U ml^{-1} at optimum temperature of 37°C. The present findings were on par with Patel *et al*. (2012) who obtained the highest polygalacturonase activity of 47.83 Uml^{-1} at a temperature of 35°C by *Paecilomyces variotii* NFCCI 1769.

Conclusion

It can be inferred from the present investigation that, the pectinase production by *Paecilomyces variotii* was maximum in banana peel as substrate. The optimum pH and temperature for pectinase activity were 6.5 and 40°C. Thus, fruit waste can be effectively utilized as substrate for pectinase production which will not only reduce the production cost of the enzyme but also found to be environmentally friendly (ecofriendly). Thus, the thousands of tons of fruit waste discarded from Pazhamuthir Nilayam can be effectively harnessed to enhance the pectinase production by exploiting the potential candidate, *Paecilomyces variotii*.

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