Available online at www.elixirpublishers.com (Elixir International Journal)

Applied Botany

Elixir Appl. Botany 79 (2015) 30699-30701

ISSN: 2229-712X

Effect of carbon and nitrogen sources on pectinase production by *Paecilomyces variotii*

Nisha M. K.* and C.K. Padmaja

Department of Botany, Avinashilingam Institute for Home Science and Higher Education for Women (University), Coimbatore, Tamilnadu, India.

ARTICLE INFO

Article history:

Received: 17 September 2014;

Received in revised form:

15 February 2015;

Accepted: 25 February 2015;

Keywords

Pectinase, Paecilomyces variotii, Extracellular, Intracellular.

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. The fungal strain showing maximum clearance zone of 36 mm (out of colony diameter of 57 mm) was selected and it was identified as *Paecilomyces variotii*. The effects of the various carbon sources like dextrose, maltose and cellulose and nitrogen sources like ammonium sulphate, potassium nitrate and urea on the pectinase activity by *Paecilomyces variotii* were carried out in surface culture fermentation. The results of the investigation revealed that the maximum pectinase production of 9.71 Umg^{-1}protein and of 9.66 Umg^{-1}protein were obtained at both intracellular and extracellular level in 3% dextrose and 3% maltose as carbon sources. Among the nitrogen sources, the maximum pectinase activity of 9.73 Umg^{-1}proteinand 8.65 Umg^{-1}protein was observed in 3% and 2% ammonium sulphate at both intracellular and extracellular level.

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. Fruit processing industries produce a large amount of waste material in the form of peel, pulp, seeds, etc. 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 focused world-wide attention for the extraction of industrially important enzymes from bio-wastes. For this purpose, the technology of fermentation can be exploited profitably in many ways.

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 [1]. Pectinases 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 cheap source for the enzyme [2].

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 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 explore the production of pectinase from cheap fruit waste as substrates 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 areas of Pazhamuthir Nilayam, Coimbatore, TamilNadu. 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 [3].

Screening of soil fungal isolates for pectinolytic activity.

The isolates, were cultured on modified Czapek-dox Agar medium [4] with commercial citrus pectin as the sole carbon source and screened by modified plate method [5]. 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 identified and selected for the enzyme study.

Enzyme production:

Erlenmeyer flasks containing 100ml of Czapek-dox Liquid Medium [6] 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 30C under static conditions. The mycelium was filtered through Whatman No. 40 filter paper using a Buchner funnel under suction and the clear filtrate obtained was used as a source of extracellular

Tele:

E-mail addresses: nishamurarii@gmail.com

enzyme. A quantity of 5.0 g of the washed mycelia mat was macerated in five ml of acetate buffer (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 and the protein content was determined [7].

Effect of carbon and nitrogen sources on pectinase 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 on 5^{th}, 7^{th} and 9^{th} 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 (*Aspergillusflavus*, *Penicilliumchrysogenum*, *Trichodermaharzianum* 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 and so, it was selected as a potential candidate for pectinase production. Similar result showing a maximum hydrolyzing zone of pectinolytic activity by *Aspergillusawamori* is in accordance with the present study [8]. A maximum hydrolyzing zone formation in *Aspergillusniger* among 52 strains of *Aspergillusniger* and *A. flavus* was observed [9] which is similar to our results.

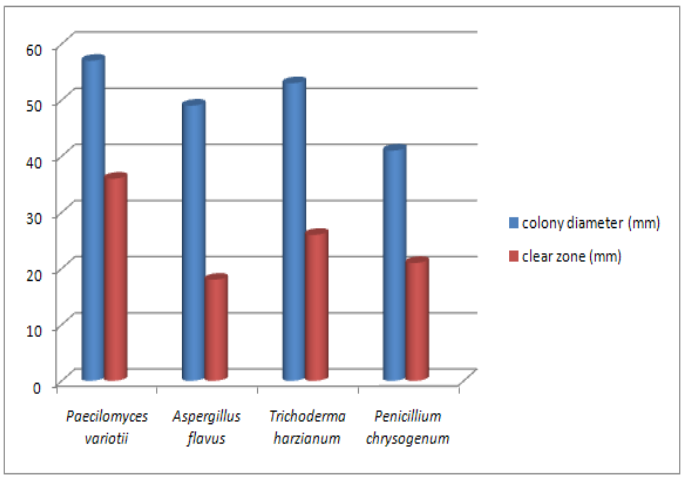

Figure 1: A Graph showing diameter of colony and clear zone of Pectinolytic activity

Optimization of culture conditions for pectinase production

Effect of carbon source on enzyme production by *Paecilomyces variotii*

The extracellular and intracellular enzyme activity showed an increasing trend in carbon sources (dextrose, maltose and cellulose) up to 7 days of incubation and after that it's activity declined gradually at both extra and intracellular level (Table – 1). Among the different carbon sources (dextrose, maltose, cellulose) in different concentration (1%, 2% and 3%), maximum pectinase production of 9.71 Umg^{-1}protein and 9.66 Umg^{-1} protein were obtained at both intracellular and extracellular level in 1 per cent dextrose and 2 per cent maltose respectively when compared to control.

The ability of maltose in maximizing the polygalacturonase activity (3033 Uml^{-1}) by *Trichodermaviride* (BITRS – 1001) in submerged cultivation have been reported earlier, which supported our work [10]. **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).

Among different concentrations (1%, 2% and 3%) of nitrogen sources (ammonium sulphate, potassium nitrate and urea), highest pectinase activity of 9.73 Umg^{-1} protein and 8.65 Umg^{-1} protein were obtained in 3 and 2 per cent ammonium sulphate at an intracellular and extracellular level respectively when compared to the control. Ammonium sulphate was the most optimal nitrogen source for polygalacturonase activity of 181 per cent in the optimal concentration [11]. Ammonium sulphate stimulated pectinase synthesis and in its absence, the fungus displayed a slight proteolytic activity [12].

Sodium nitrate and ammonium sulphate influenced the production of polygalacturonase activity and the increase was much higher with ammonium sulphate (1.6 Uml^{-1}) in comparison with sodium nitrate (1.2 Uml^{-1}) [13].

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 sources. The effective utilization of thousands of tons of fruit waste discarded from Pazhamuthir Nilayam as a cheap source for the production of industrially important enzymes like pectinase, will not only solve the disposal of waste but reduce the production cost of the enzyme. For this purpose, *P. variotii* can be exploited as a potential candidate.

References:

[1]Apoorvi, C. and Vuppu, S., 2012. Microbially derived pectinases: A review, JOSR, **Journal of Pharmacy and Biological Science (IOSRJPBS), 2 (2)**: 01-05.

[2] Prathyusha, K., Suneetha, V. and Bishwambhar, M., 2011. Bacterial pectinases and their potent biotechnological application fruit processing / Juice production industry: A Review, **J. Phytolog., 3** : 16-19.

[3] Domsch, K.H. and Gams, W., 1972. Fungi in agricultural soils, Longman Group Ltd., London, P. 290.

[4] Nwodu-Chinedu, S., Eni,A.O., Adeniyi, A.I. and Ayangbemi, J.A., 2010. Assessment of growth and cellulase production of wild-type microfungi isolated from Ota, Nigeria, **Asian Journal of Plant Science, 9 (3)** : 118-125.

[5] Phutela, U., Dhuna, V., Sandhu, S. and Chadha, B.S., 2005. Pectinase and polygalacturonase production by a thermophilic*Aspergillusfumigatus* isolated from decomposting orange peel, **Branz. J. Microbiol., 36**: 63-69.

[6] Raper, K.B. and Thom, C., 1949. Manual of the penicillia, Williams and wikins Baltimore, USA, Mycology present Penicillin, **Mycologia, 37**: 460-475.

[7] Lowry, O.H., Rosenbrough, N.J., Farr, A.L., Randell, 1951. Protein measurement with R.J. Folin phenol reagent, **J. Biol. Chem., 193**: 265-275.

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

Carbon Sources		Control			Concentrations									CD ($P < 0.05$)	SED
					1%			2%			3%				
		5 Days	7 Days	9 Days	5 Days	7 Days	9 Days	5 Days	7 Days	9 Days	5 Days	7 Days	9 Days		
Intracellular	Dextrose	1.35	1.85	1.74	1.95	2.12	1.92	1.94	4.93	1.97	2.32	9.62	1.86	0.180	0.089
	Maltose				3.55	9.71	2.18	2.14	3.83	2.42	2.68	3.84	2.15		
	Cellulose				2.36	2.52	2.56	2.32	2.77	1.85	2.24	2.94	2.26		
Extracellular	Dextrose	1.27	1.44	1.14	1.49	2.03	1.35	1.74	3.37	1.67	2.85	3.56	1.87	0.195	0.097
	Maltose				4.09	8.44	6.53	5.92	9.66	1.93	3.62	5.08	1.52		
	Cellulose				2.15	4.27	1.58	1.54	4.77	1.53	8.02	8.56	1.91		

*Umg^{-1} = µmolpolygalacturonic acid released min^{-1} mg^{-1} protein

Table 2. Pectinase activity (*Umg^{-1} protein) of *Paecilomyces variotii* on nitrogen sources

Nitrogen Sources		Control			Concentrations									CD ($P < 0.05$)	SED
					1%			2%			3%				
		5 Days	7 Days	9 Days	5 Days	7 Days	9 Days	5 Days	7 Days	9 Days	5 Days	7 Days	9 Days		
Intracellular	Ammonium sulphate	1.35	1.85	1.74	2.62	5.54	1.97	1.92	4.77	1.95	2.06	9.73	3.86	0.183	0.179
	Potassium nitrate				1.93	4.04	2.38	3.76	9.73	2.84	3.24	6.17	3.035		
	Urea				2.26	5.48	3.28	2.66	6.04	2.65	2.83	5.86	4.24		
Extracellular	Ammonium sulphate	1.27	1.44	1.14	8.52	8.65	4.37	4.57	6.12	1.44	1.28	7.44	3.63	0.091	0.089
	Potassium nitrate				2.26	4.26	3.85	1.45	5.06	3.62	2.72	3.83	1.47		
	Urea				3.51	5.14	2.36	1.77	3.12	2.04	2.36	3.74	1.85		

*Umg^{-1} = µmolpolygalacturonic acid released min^{-1} mg^{-1} protein

[8] Anuradha, K., Padma, P.N., Venkateshwar, S. and Reddy, G., 2010. Fungal isolates from natural pectic substrates for polygalacturonase and multi enzyme production, **Indian J.Microbiol., 50** : 339-344**.**

[9] Hannan, A., Bajwa, R. and Latif, Z., 2009. Status of *Aspergillusniger* strains for pectinases production potential, **Pak. J. Phytopathol., 21 (1)**: 77-82.

[10] Arotupin, D. and Ogunmolu, F., Emmanuel, F., 2011. Effect of carbon and nitrogen sources on polygalacturonase production by *Trichodermaviride* (BITRS – 1001) isolated from tar sand in Ondo state, Nigeria, **Malaysian Journal of Microbiology, 7 (3)** : 153-158.

[11] Reda, A.B., M.Y. Hesham, A.S. Mahmoud and Z.A. Ebtsam, 2008. Production of bacterial pectinase(s) from agro-industrial wastes under solid state fermentation conditions. **J. Applied Sci. Res., 4**: 1708-1721.

[12] Sapunova, L.I., Lobanok, G. and Mickhailora, R.V., 1990. Condition of synthesis pectinases and proteases by *Aspergillusniger* and production of a complex macerating preparation, **Appl. Biotechnol. Microbial, 33**: 257-260.

[13] Abbasi, H., Shafighzadeh, H. and Rahimi, A., 2011. Continuous production of polygalacturonases (PGases) by *Aspergillusawamori* using wheat flour in surface culture fermentation, **Iranian Journal of Biotechnology, 9 (1)** : 50-56.