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RESEARCH ARTICLE

SCREENING, PRODUCTION AND OPTIMIZATION OF PECTIN LYASE PRODUCING FUNGI FROM AGRO WASTES

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ABSTRACT

Pectins are polysaccharides, ubiquitous in the plant kingdom and constitute the important major component of middle lamellae of plant cell walls. Pectin lyases are widely used in the food industry in the production of juice, fruit drinks and wines. In present study, pectin lyase producing fungi was isolated from fruit, vegetable, and dead organic waste soil. Totally 25 fungal organisms were isolated and identified. Screening of pectin lyase producing fungi were isolated using pectin screening agar medium. Out of 25 organisms, only two fungi namely *Aspergillus niger* and *Penicillium citrinum* were able to produce pectin lyase activity. The pH, temperature, carbon sources, nitrogen sources and trace elements were also optimized. Enzyme assay was determined by Thiobarbituric method (TBA). There are a lot of industrial processes can be applied to improve the quality and the yield of the final product.

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INTRODUCTION

Pectins are polysaccharides, ubiquitous in the plant kingdom and constitute the important major component of middle lamellae of plant cell walls (Fogarty and Kelly 1983). Pectinases are produced by many organisms such as bacteria (Horikoshi 1972; Karbassi and Vaughn, 1980), fungi (Aguilar and Huitron, 1990) and yeasts (Gainvors and Belarbi, 1993). In the industrial sector, acidic pectinases are used in the extraction and clarification of fruit juices (Rombouts and Pilnik, 1986), whereas alkalophilic pectinases are finding immense use in the degumming of ramie fibers (Cao *et al.*, 1992), retting of flax (Sharma, 1987), plant protoplast formation and treatment of effluents discharged from fruit processing units (Tanabe *et al.,* 1987). In view of the above, the present study was focused in pectinase production by isolated fungi of *A.niger* and *P.citrinum* using different agrowastes. The aim of this study was to observe growth in liquid medium and the concomitant release of pectin lyase from group of 25 fungi of which *A.niger and P.citrinum* seemed to be the best producer.

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The presence of other extracellular pectinolyic activities in these two strains were also reported.

MATERIALS AND METHODS

Fungal organisms

Samples were collected from different places (fruit waste soil,organic waste soil and vegetable waste soil) and fungi were isolated and identified using standard manuals, organisms were maintained in potato dextrose agar medium for further studies.

Screening

Screening of pectin lyase producing fungi using pectin screening agar medium containing.

Pectin – 1g, Diammonium orthophosphate – 0.3g, KH_2Po_4-0.2g, K_2HPo_4 – 0.03g, $MgSo_4$ – 0.01g,
Agar- 2.5g(100 ml), pH – 4.5

Optimization: (Miller, 1959)

Pectin lyase activities were carried out using different parameters as follows. pH: The importance of pH on enzyme activity was studied by adjusting the pH to acquire desired ranges from 5-9 using 1N NaOH and 1N Hcl.

Temperature

The enzyme reaction was carried out in different temperatures at 15,20,25,30 and 35^{o}C the maximum activity was checked.

Carbon sources

The fungi was grown on the different carbon sources such as glucose, lactose, maltose, sucrose and mannitol. To check the best carbon source at 540 nm.

Nitrogen sources

The fungi was given on the different nitrogen sources like peptone, malt extract, sodium nitrate, potassium nitrate, beef extract to determine the best nitrogen source at 540nm.

Trace elements

The fungi was grown on the different trace elements such as boric acid, zinc sulphate, mercuric chloride, magnesium sulphate, lead acetate and chloride check to determine the best trace elements at 540 nm.

Enzyme Assay

Pectin lyase activity was checked by Thiobarbituric method (TBA)(Ayers *et al*.,1966). Took 0.5ml fungal sample added 1N NaOH and adjusted the pH 8 using PO_4 buffer. Heated the above mixture for 80 ^{o}C and cooled it for 2-3 minutes. After cooling, added 0.6ml of Hcl (1N) and 0.04m TBA (in aqueous solution) heated the above mixture for 80^{o}C for 5 minutes and placed in ice cubes and after cooling the readings were observed at 550 nm.

Production and estimation of pectin lyase

Production of pectin lyase using different substrates such as paddy husk, groundnut shell, corn molasses and jack fruit were tested. The collected substrates were powdered and sterilized at 121^{o}C for 15 mins. After sterilization 1g substrates and 1% of *A.niger and P.citrinum* were added in PDA broth. 15 days of incubation the production of pectin lyase enzyme was estimated, (Ayers *et al*., 1966). 5ml of pectin solution was added with 1ml of calcium chloride and 1ml of enzyme solution made up to 100 ml with distilled water, and incubated at 2 hours. After incubation 0.6 ml of zinc sulphate and 0.6 ml of sodium hydroxide centrifuged at 3000 rpm for 10 mins. After centrifugation, 5ml of supernatant was added 0.3ml with of thiobarbituric acid, 1.5ml of Hcl and 0.5ml of distilled water, heated at boiling waterbath for 30 mins. After cooling, the readings were observed in spectrophotometer at 550 nm.

RESULTS

In the present study, fungal species were isolated from the agrowaste dumped soil. From the 25 isolates, two fungal colonies were identified using routine morphological tests. The observed characteristics were compared with manual of soil fungi. The identified fungal species were *A.niger* and *Penicillium citrinum* (Table 1). The selected fungal isolates were further screened for pectinolytic activity by pectin screening agar medium (PSAM). The two isolates had a zone of clearance around the colonies (Fig-1). The optimization of the different factors pH, temperature, carbon source, nitrogen source and trace elements. Maximum activity at temperature 35^{o}C and pH-6. Carbon source is boric acid, Nitrogen source is malt extract, and trace element is magnesium sulphate (Table 2, 3 and 4).

Table 1. Isolation of fungi from agrowastes

S.No	Name of the organisms
1	*Aspergillus awamori*
2	*A.candidus*
3	*A.clavatus*
4	*A.flavus*
5	*A.fumigatus*
6	*A.luchuensis*
7	*A.nidulans*
8	*A.niger*
9	*A.sydowi*
10	*A.sulphureus*
11	*A.versicolor*
12	*A.terreus*
13	*A.terricola*
14	*A.variecolor*
15	*Bipolaris oryzae*
16	*Colletotrichum falcatum*
17	*Curvularia lunata*
18	*Chaetomium globosum*
19	*Fusarium chlamydosporum*
20	*F.oxysporum*
21	*Penicillium citrinum*
22	*P.chrysogenum*
23	*Rhizoctonia solani*
24	*Trichoderma harzianum*
25	*T.viride*

Screening of Pectin lyase enzymes:

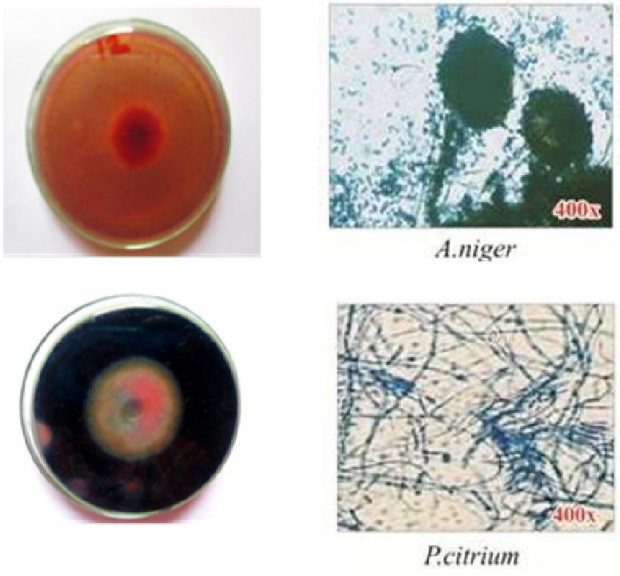

Fig.1.Screening of Pectin lyase enzymes

Table 2. Optimization of pH and temperature

Organisms	pH					Temperature (°C)				
	5	6	7	8	9	15	20	25	30	35
A.niger	0.2	1.69	1.62	1.14	0.88	1.15	1.50	1.74	1.79	1.49
P.citrinum	0.1	0.74	0.71	0.14	0.7	0.09	0.17	0.7	0.72	0.9

Table 3. Optimization of carbon sources and trace elements

Organism	Carbon sources (mg/g)					Trace elements(mg/g)				
	G	L	Mann	Ma	S	B	ZnS	Hg	Mg	Pb
A.niger	1.70	0.71	0.81	0.82	0.80	0.82	0.89	0.85	0.90	0.90
P.citrinum	1.72	0.73	0.83	0.87	0.83	0.87	0.90	0.91	0.93	0.97

G-Glucose, L- Lactose, Mann- Mannitol, Ma- Maltose, S- Sucrose, B- Boric acid, ZnS- Zinc Sulphide, Hg- Mercuric Chloride, Mg-Magnesium sulphate and Pb- Lead acetate

Table 4. Optimization of nitrogen sources

Organisms	Nitrogen sources(mg/g)				
	P	Sn	M	Pn	B
A.niger	0.01	0.81	0.93	0.03	0.52
P.citrinum	0.21	0.20	0.53	0.01	0.05

P-Peptone, Sn- Sodium nitrate, M- Malt extract, Pn- Potassium nitrate, B-Beef extract.

Table 5. Quantification assay of pectinolytic enzymes from fungi

S.No	Name of the organisms	Activity (IU/ml)
1	*Aspergillus awamori*	1.5
2	*A. candidus*	0.7
3	*A. clavatus*	0.9
4	*A. flavus*	0.4
5	*A. fumigatus*	0.6
6	*A. luchuensis*	0.4
7	*A. nidulans*	0.4
8	*A. niger*	4.8
9	*A. sydowi*	0.1
10	*A. sulphureus*	0.2
11	*A. versicolor*	0.9
12	*A. terreus*	0.6
13	*A. terricola*	1.1
14	*A. variecolor*	1.4
15	*Bipolaris oryzae*	0.9
16	*Collectotrichum falcatum*	0.8
17	*Curvularia lunata*	0.3
18	*Chaetomium globosum*	0.5
19	*Fusarium chlamydosporum*	1.2
20	*F.oxysporum*	1.4
21	*Penicillum citrinum*	4.6
22	*P.chrysogenum*	1.2
23	*Rhizotonia solani*	0.3
24	*Trichoderma harzianum*	0.5
25	*T.viride*	0.8

Table 6. Production of enzyme activity in different substrates

S.No.	Substrates	Percentage %	*Aspergillus niger*		*Penicillium citrinum*	
			Protein (IU/ml)	Pectinlyase (IU/ml)	Protein (IU/ml)	Pectinlyase (IU/ml)
1	Corn molasses	1	1.2	0.2	0.9	0.8
2	Groundnut shell		1.0	0.4	0.6	0.2
3	Paddy husk		0.8	0.6	0.3	0.4
4	Jack fruit		2.1	1.0	1.6	0.9
5	Corn molasses	2	3.1	0.9	3.8	1.0
6	Paddy husk		2.2	0.2	5.3	0.2
7	Groundnut shell		3.8	0.2	4.4	0.2
8	Jack fruit		4.2	1.5	4.8	1.2

In enzyme assay method, pectin lyase activity was maximum in *A. niger* (4.8 IU/ml) and *Penicillium citrinum* (4.6 IU/ml) (Table - 5). The selected fungal isolates were tested for its pectinase activity by using different substrates viz ground nut shell, corn molasses, jack fruit and paddy husk. The obtained results indicated that enzyme activity was higher in jack fruit (Table-6).

DISCUSSION

In the present study, isolation, screening, optimization and production of extracellular pectin lyase (EC 4.2.2.10) produced by *A.niger and P.citrinum* was tested. In optimum condition, the production of pectin lyase was detected in 15 days at temp 30 ^{o}C and pH-6. Similar results were also reported in pectin lyase production by Penicillium spp. (Lizu *et al*., 2008). The carbon sources, nitrogen sources and trace elements are also important part in the production of enzymes, where boric acid , malt extract and magnesium sulphate showed increased activity of pectin lyase and obtained results were compared with the findings of Vries and Visser, (2001). The production of pectin lyase by using different substrates were tested in two different concentration. Below and above the optimal concentration showed increased productivity (Reda *et al*., 2008).Thus in the present study among four substrates screened, jack fruit was found to be the best and most significant one for pectin lyase production only by *A.niger* (1.2 IU/ml).

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