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Production of Polygalacturonase and Pectin Methyl Esterase from Agrowaste by using Various Isolates of *Aspergillus niger*

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Abstract: Background: Search for new enzymes for use in commercial applications with desirable biochemical and physico-chemical characteristics and a low cost of production have been the focus of much research. Major impediments to the production of industrially important enzymes are their yield, stability, specificity and the cost of production. The objective of this study was to isolate a strain of *Aspergillus niger* for better and economical production of thermostable pectinolytic enzymes using cheap agrowaste by submerged fermentation. **Results:** The orange peel isolate showed the maximum activity of PG (2.29±0.05 μ moles/mL/min) and PME (0.73±0.08 μmoles/mL/min) followed by those of tamarind isolate (1.76±0.03 and 0.37±0.05 μmoles/mL/min). Their activities were significantly higher ($p<0.01$) than those of the standard strain *A. niger* MTCC 281 which gave PG and PME activities of 0.70±0.06 and 0.41±0.11 μmoles/mL/min, respectively. Both PG and PME of isolates obtained from orange peel (Tm-48 and 48.5°C) and tamarind (Tm- 47and 48°C) were found to be slightly better thermostable than those of the standard strain *A. niger* MTCC 281 (Tm- 46.5 and 47.5°C). The maximum yield of enzymes was achieved on 11th day of fermentation with 3% wheat bran as a carbon source and peptone as a nitrogen source. The thermostability of both PG and PME activities of enzyme preparations from these isolates could be significantly ($p<0.01$) improved by addition of polyhydric alcohols such as glycerol, sucrose and xylose at 20% level. **Conclusion:** The present study shows that it is feasible to exploit various isolates of *Aspergillus niger* to produce commercial pectinolytic enzymes by using agriwaste and submerged fermentation. The potential implications of pectinases are in fruit industry where they are widely used to help extract, clarify and modify fruit juices.

Key words: Microbial pectinases production, *Aspergillus niger*, thermostability, submerged fermentation, agribiomass

INTRODUCTION

There is considerable interest in different fiber hydrolytic enzymes such as cellulases, xylanases and pectinases for their potential applications in the paper, food and feed industries (Saadoun *et al*., 2007). Pectinases are one of the most important groups of enzymes used in fruit and vegetable industry for increasing juice yield and juice clarification. These enzymes act on pectin, a class of complex polysaccharides found in the middle lamellae or primary cell walls of higher plants, where they function as a hydrating agent and cementing material for the cellulose network in the tissue (Thakur *et al*., 1997). Pectinolytic enzymes are classified according to their mode of attack on the galacturonan part of the pectin molecules. They can be distinguished from pectin methylesterases, that de-esterify pectins to low methoxyl pectins or pectic acid and from pectin depolymerases, that split the glycosidic linkages between galacturonosyl (methyl ester) residues. Polygalacturonases split glycosidic linkages next to free carboxyl groups by hydrolysis while pectate lyase split glycosidic linkages next to free carboxyl groups by β-elimination. Both endo types of PGs and PLS are known to randomly split the pectin chain. Exo-PGs release monomers or dimers from the non reducing end of the chain, whereas exo-PLs release unsaturated dimers from the reducing end (Martin *et al*., 2004). Highly methylated pectins are degraded by endo-pectin lyases and by a combination of pectin esterses with PG (Sarkenen, 1991). It has been estimated that microbial pectinases account for 25% of the global food enzymes sales. Microbial pectinases can be produced from bacteria including actinomycetes, yeast and fungi. *Aspergillus niger* is the

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most commonly used fungal species for industrial production of pectinolytic enzymes. Among industrial applications of pectinases are using these enzymes as an animal feed supplementation. This usage of pectinases for ruminant's feed production can reduces the feed viscosity, which increases absorption of nutrients, liberates nutrients, either by hydrolysis of non-biodegradable fibers or by liberating nutrients blocked by these fibers and reduces the amount of faeces (Murad *et al*., 2011). Preparation containing pectin-degrading enzymes has been extensively used to improve the stability of fruit and vegetable nectars and in the clarification of fruit juices and wines. Tochi *et al*. (2009) ascertained influence of pectinase and pectinase/ hemicellulases enzyme preparations on pineapple juice and reported that the ready to serve pineapple juice (RTS) was rated for acceptance on a 5 point hedonic scale. The biotechnological potential of pectinolytic enzymes from microorganisms has drawn a great deal of attention from various researchers worldwide as likely biological catalysts in a variety of industrial processes. Alkaline pectinases are among the most important industrial enzymes and are of great significance in the current biotechnological arena with wide-ranging applications in textile processing, treatment of pectic wastewaters, paper making, and coffee and tea fermentations (Hoondal *et al*., 2002). The enzymes preparation used in the food industry are of fungal origin because fungi are potent producers of pectic enzymes and the optimal pH of fungal enzymes is very close to the pH of many fruit juices, which range from pH 3.0 to 5.5. Rogaia Al- Gashgari (2002) studied occurrence of fungi and their pectolytic activity in fruit juices from Saudi Arabia and reported highest pectinase production related to *A. flavus*, *A. fumigatus*, *A. niger*, *A. parasiticus* and *A. terreus*. Pectic enzymes are available in dry powdered form as well as in liquid form. Now-a-days interest in thermostable liquid enzymes has developed as they are associated with many benefits. They are non-polluting biochemical catalysts which are safe and easy to use. Moreover, enzymatic liquids go into process medium instantly and disperse evenly through the application process, as opposed to powder products. Moreover, enzymes are susceptible to proteolytic attack by contaminating microorganisms or extracellular proteases coming as contaminants. With population growth, massive pressure has been put on natural resources to produce more agricultural products, whilst million tons of agricultural products go to waste during agricultural process (Gholifar *et al*., 2010). Considering the utilization of agriwaste and industrial importance of pectinases, the present investigation was conducted with the objective of increased production of microbial pectinases by making use of different agro and domestic wastes. An effort has also been made to improve their thermostability.

MATERIALS AND METHODS

The present investigation was started in the month of December 2009 at Department of Biotechnology, Lovely School of Sciences, Lovely Professional University, Phagwara, Punjab, India and accomplished in July 2010.

Organism: Different isolates of *Aspergillus niger* were isolated from various sources such as orange peel, tamarind, apple baggasse, pomegranate peels, carrot baggasse, groundnut, soil, waste water from starch industry and bread. These samples were especially rotten fruits collected from the grocery market of Jalandhar, Phagwara and campus of Lovely Professional University Punjab. These cultures isolated were maintained on Potato Dextrose Agar (PDA) slants and stored at 4°C and were sub-cultured fortnightly.

Fermentation of substrates for pectinase production

Preparation of inoculums: The fungal cultures were grown on PDA at 30°C for 5 days. A spore inoculum was prepared by adding a loopful of spores from the slants to 5 mL of sterile normal saline and shaken vigorously, 0.5 mL of the inoculum was used to inoculate fermentation medium.

Screening of fungal cultures by submerged culture fermentation technique: The substrate medium was prepared by adding the soluble pectin at the rate of 1%, in the mineral solution and 30 mL of it was taken in each 100 mL Erlenmeyer flask. The cotton plugged flasks were autoclaved at 121°C for 20 min., cooled to room temperature and inoculated with 0.5 mL of inoculum, mixed thoroughly by gentle tapping and were incubated at 30°C for different incubation periods. After completion of the incubation period, the contents of the flasks were filtered through a perforated funnel and centrifuged to get the clear culture filtrate. The filtrate was employed for the estimation of the activities of pectinases.

Determination of enzyme characteristics

Optimum temperature and pH: In order to determine the optimum temperature the enzyme assays were performed at temperatures ranging from 35-75°C at fixed pH of 5.0 and to find optimum pH the enzyme assays were

prerformed at different pH ranging from 4.0 to 6.0 at optimum temperature observed.

Thermostability: The thermal stability of enzyme preprations was determined by determining the residual activity after thermal treatment of these preparations.

The thermal treatments were carried out at different temperatures ranging from 35-70°C for 15 min. After cooling, the residual enzymatic activities were determined at preset conditions of optimum pH and temperature. Tm, the temperature at which 50% activity was lost, was determined by plotting residual activity vs exposure temperature.

Optimization of cultural parameters for pectinase production: The effect of various parameters such as medium pH, incubation period, incubation temperature, nitrogen and carbon supplements were studied.

Effect of incubation period: To monitor the effect of incubation period on the production of pectinases, it was varied from 3-15 days. The fermentation was carried out at 30°C in submerged fermentation.

Effect of carbon and nitrogen sources: Different carbon sources viz. wheat bran, citrus bagasse, sugarcane bagasse, apple bagasse and banana peel at 3% (w/v) and nitrogen sources namely sodium nitrate, ammonium nitrate, ammonium chloride, ammonium sulfate and peptone at the rate of 0.2% N (w/v), were evaluated for production of pectinases. The production experiments were carried out in triplicates and the enzyme assays were performed in duplicates. The enzyme activities are presented as Mean±SE of all the values. The results of enzyme production were analysed statistically using student's t-test.

Assay of pectinases

Polygalacturonase (EC 3.2.1.15) activity: Was determined by measuring the amount of reducing sugars (galacturonic acid) realeased from non esterified pectin hydrolysed in the reaction mixture (Singh *et al.*, 2002). The reaction mixture consisting of 2 mL of 1% buffered solution of poly-galacturonate in phosphate citrate buffer (pH 5.5) and 0.5 mL of enzyme preparation was incubated at 30°C. Samples were drawn at 0 and 30 min. intervals and the amount of reducing sugars was estimated using Nelson-Somoygi (Somogyi, 1952) method. The enzyme activity was expressed as micromoles of the reducing sugars liberated/min/mL of enzyme preparation.

Estimation of pectin methyl esterase activity: Pectin Methyl Esterase activity was determined by measuring the increase in acidity after the hydrolysis of methyl esters of pectin by the enzyme preparation. For this purpose, 5 mL of buffered pectin substrate was incubated with 1 mL of enzyme and 2 mL of samples drawn at 0 and 60 min. And were titrated against 0.01 N sodium hydroxide to determine the amount of acid released. The enzyme activity was expressed as the micro-equivalents of acid released/min/mL of enzyme preparation.

RESULTS AND DISCUSSION

Screening of different isolates for pectinolytic enzymes: For screening of different isolates the fermentation was carried out under submerged condition using Czapek's medium containing 1% pectin (apple grade) as carbon source. It is evident from the results (Table 1) that maximum PG activity was shown by *A.niger* from orange peel (2.29±0.05 µmoles/mL/min) followed by *A. niger* from soil (2.19±0.06 µmoles/mL/min) tamarind (1.76±0.03 µmoles/mL/min) and carrot (1.65±0.02 µmoles/mL/min) on 11th day of fermentation. These activities were significantly higher ($p<0.01$) than that of the standard strain *A. niger* MTCC 281 (0.70±0.06 µmoles/mL/min) which was maximum on 7th day. Other isolates of *A. niger* from pomegranate (0.65±0.03 µmoles/mL/min), wast water (0.62±0.03 moles/mL/min), apple (0.27±0.11 µmoles/mL/min) and bread (0.76±0.06 µmoles/mL/min) showed relatively lower PG activity and were not significantly different from that of the standard strain. Likewise the PME activity was also maximum (0.73±0.08 µmoles/mL/min) with *A. niger* isolate from orange peel on 11th day of fermentation whereas it was (0.45±0.06 µmoles/mL/min) with *A. niger* MTCC 281 on 7th day of fermentation. PME activity of tamarind (0.37±0.05 µmoles/mL/min,) carrot (0.36±0.09 µmoles/mL/min) and pomegranate (0.38±0.02 µmoles/mL/min) isolates were similar to that of standard strain (0.41±0.11 µmoles/mL/min,) whereas the PME activities of all other isolates were significantly lower than that of the standard strain (Table 1). Silva *et al.* (2005) investigated that pectin lyase and polygalacturonase production by newly isolated *Penicillium viridicatum* strain Rfc3 was carried out by means of solid state fermentation using orange bagasse, corn tegument, wheat bran and mango and banana peels as carbon sources. They reported polygalacturonase (Pg) (30U g-1) using wheat bran as carbon source while maximal pectin lyase (Pl) (2000 U g-1) activity value was obtained in medium composed of orange bagasse. Yield

Table 1: Screening of various fungal isolates for Polygalacturonase (PG) and Pectin Methyl Esterase (PME) activity

	PG (µmoles/mL/min)			PME (µmoles/mL/min)		
A. niger isolate	7 Days	9 Days	11 Days	7 Days	9 Days	11 Days
Orange peel	1.51±0.10[a]	1.64±0.08[a]	2.29±0.05[a]	0.04±0.01	0.62±0.07	0.73±0.08[a]
Tamarind seed powder	1.19±0.16[a]	1.71±0.14[a]	1.76±0.03[a]	0.16±0.03	0.36±0.13	0.37±0.05
Groundnut	0.80±0.13	0.65±0.07	0.95±0.06	0.44±0.04	0.26±0.04	0.2±0.05
Carrot	1.07±0.02	1.25±0.03	1.65±0.20	0.36±0.07	0.33±0.08	0.36±0.09
Bread	0.44±0.08	0.53±0.03	0.76±0.06	0.07±0.05	0.23±0.08	0.24±0.06
Pomegranate peel	0.26±0.09	1.18±0.04	0.65±0.03	0.13±0.04	0.32±0.02	0.38±0.02
Apple baggasse	0.51±0.02	0.62±0.07	0.27±0.11	0.09±0.02	0.07±0.04	0.16±0.03
Soil sample	0.54±0.09	0.94±0.10	2.19±0.06	0.09±0.04	0.14±0.09	0.11±0.06
Water	0.14±0.01	0.64±0.02	0.62±0.03	0.17±0.04	0.28±0.04	0.18±0.05
A.niger MTCC281	0.70±0.06	0.65±0.09	0.58±0.11	0.45±0.06	0.43±0.09	0.41±0.11

[a]Values are significant at $p<0.01$

Table 2: Enzyme characteristics with reference to optimum pH, optimum temperature and Thermostability of Polygalacturonase (PG) and Pectin Methyl Esterase (PME)

	Enzyme characteristics					
	Optimum pH		Optimum temperature (°C)		Thermostability (°C)	
A. niger isolate	PG	PME	PG	PME	PG	PME
Orange peel	5.0	5.4	50	50	48.0	48.5
Tamarind seed	5.4	5.0	50	50	47.0	47.0
MTCC 281	5.4	5.0	50	50	46.5°	47.5

of PG and PME increased upto 7th day of incubation in submerged fermentation for *A. niger* MTCC 281 and up to 11th day for *A. niger* from orange peel as well as for *A. niger* from tamarind seed powder (Table 1). Incubation period beyond 7th day for *A. niger*. MTCC 281 and beyond 11th day for *A. niger* from orange peel and *A. niger* from tamarind seed powder resulted in decline in enzyme activity. Phutela *et al*. (2005) also reported decline in PG activity upon prolonged incubation using *Aspergillus fumigatus* isolated from decomposting orange peels.

The PG activity of soil isolate was better than that of tamarind isolate but its PME activity was very poor. Based on this initial screening further work on the enzyme characteristics and optimization of enzyme production was carried out only with isolates from orange peel and tamarind.

Enzyme characteristics: Enzyme characteristics from two best producing sources viz. *A. niger* from orange peel and *A. niger* from tamarind seed powder were determined and compared against standard strain *A. niger* MTCC 281.

Optimum pH: It's evident from the result that the optimum pH for PG activity of orange peel isolates was 5.0 where as that of tamarind and MTCC was 5.4. However, optimum pH for PME activity for these isolates was 5.4 for orange peel, 5.0 for tamarind and MTCC 281 (Table 2). The differences in pH optima could be due to the differences in the source of their isolates. The optimum pH of PG produced by *Moniliella* was reported to be pH 4.5. In contrast, the optimum pH of PG produced from *Penicillium* sp. was 4.5-5.0. Optimum pH of PG II activity has also been reported by Singh *et al*. (2002) between 3.8-4.3 and that of PG IV between 3 and 4.6. As per the study, conducted by Delcheva *et al*. (2007), the immobilized enzyme showed a shift in pH optimum which was at pH 5.0 for the enzyme immobilized on not activated membrane and at pH 5.5 for the enzyme immobilized on membrane activated with glutaraldehyde.

Optimum temperature: The optimum temperature for PG and PME activities of both these isolates was same as that of MTCC standard strain 50°C (Table 2). Silva *et al.* (2005) reported the optimum temperature for PG activity by *Moniliella* to be 55°C. However, this enzyme from *Penicillium* sp. presented optimum activity at 40°C. Thermophilic fungi have been reported to optimally produce enzymes at 50°C (Rubinder *et al*., 2000; Rubinder *et al*., 2002). The observed difference of optimum temperature for PG and PME activities could therefore be due to the difference in fungal species.

Thermostability: The thermostability of PG and PME were assessed by determining the temperature required for 50% loss of activity. It is evident from the result that the thermostability of PG and PME of both these isolates was almost similar to that of the standard strain (Table 4). Pectin lyase (Pl) and polygalacturonase (Pg) production by *Thermoascus aurantiacus* was carried out by means of solid-state fermentation using orange bagasse, sugar cane bagasse and wheat bran as a carbon sources. Pg and Pl had optimum activity at pH 5.0 and 10.5-11.0, respectively. Maximal activity of the enzymes was

Table 3: Effect of Substrate concentration on polygalacturonase (PG) and pectin methyl esterase (PME) activity

	PG			PME		
Pectin concentration (%)	Orange peel	Tamarind seed	MTCC 281	Orange peel	Tamarind seed	MTCC 281
1	1.91±0.04	1.62±0.06	0.51±0.02	0.70±0.02	0.36±0.02	0.44±0.02
2	2.25±0.05	1.81±0.04	1.00±0.09	0.81±0.03	0.44±0.02	0.47±0.004
3	2.6±0.04	2.04±0.06	1.27±0.04	1.51±0.03	0.53±0.01	0.52±0.01
4	2.5±0.06	1.89±0.01	0.87±0.04	0.94±0.04	0.43±0.01	0.42±0.03

Table 4: Effect of different carbon sources on polygalacturonase (PG) and pectin methyl esterase (PME) activity at 30°C on 11th day

	Carbon sources (0.2% w/v)					
	Wheat bran		Citrus bagasse		Banana peel	
A. niger isolate	PG	PME	PG	PME	PG	PME
Orange peel	2.32±0.11	0.64±0.05	2.03±0.08	0.52±0.05	0.78±0.07	0.47±0.04
Tamarind seed powder	2.34±0.07	0.61±0.04	1.71±0.08	0.46±0.09	0.47±0.06	0.20±0.02
MTCC281	1.93±0.08	0.61±0.03	1.82±0.03	0.53±0.02	0.65±0.10	0.38±0.008

Table 5: Effect of different nitrogen sources on polygalacturonase (PG) and pectin methyl esterase (PME) ativity at 30°C on 11th day

	Nitrogen sources (0.2% w/v)							
	Sodium nitrate		Urea		Peptone		Ammonium sulphate	
A. niger isolate	PG	PME	PG	PME	PG	PME	PG	PME
Orange peel	1.41±0.02	0.94±0.11	1.26±0.07	1.02±0.03	2.72±0.04	1.211.02±0.02	0.74±0.008	0.62±0.40
Tamarind seed powder	1.32±0.04	0.59±0.03	1.24±0.02	0.89±0.02	1.60±0.06	1.13±0.07	0.53±0.01	0.11±0.04
MTCC 281	1.43±0.06	0.75±0.08	1.38±0.13	0.84±0.01	1.69±0.04	1.19±0.05	0.82±0.008	0.33±0.03

Table 6: Effect of additives on thermostability of polygalacturonase (PG) and pectin methyl esterase (PME) preparation*

	Orange peel isolate		Tamarind seed isolate		MTCC 281	
	PG	PME	PG	PME	PG	PME
Additives (%)	(µmoles/min/mL)					
None (Control)	0.85±0.04	0.38±0.02	0.67±0.03	0.18±0.02	0.34±0.02	0.11±0.01
Glycerol						
20	1.52±0.08	0.77±0.06	1.23±0.07	0.25±0.04	1.23±0.02	0.13±0.04
30	1.84±0.05	0.84±0.05	1.51±0.08	0.45±0.07	1.32±0.04	0.46±0.04
Sorbitol						
20	1.14±0.04	0.38±0.09	0.95±0.09	0.27±0.03	1.20±0.07	0.24±0.02
30	1.23±0.08	0.50±0.03	1.03±0.13	0.27±0.02	1.17±0.02	0.36±0.03
Sucrose						
20	1.06±0.04	0.48±0.0	0.84±0.04	0.23±0.05	0.74±0.03	0.35±0.04
30	1.37±0.33	0.84±0.0	0.90±0.20	0.33±0.03	0.93±0.05	0.30±0.04
Xylose						
20	1.23±0.06	0.43±0.0	1.06±0.06	0.31±0.05	0.88±0.04	0.44±0.02
30	1.16±0.08	0.76±0.0	0.92±0.08	0.37±0.02	0.84±0.04	0.39±0.01

*Residual activity after exposure to Tm for 15 min

determined at 65°C. Pg was stable in the acidic to neutral pH range and at 60°C for 1 h, whereas Pl was stable at acidic pH and at 60°C for 5 h (Martin *et al*., 2004).

Optimization of pectinase production

Effect of different pectin concentration: It is evident from the results that production of pectinolytic enzymes (PG and PME) increased with the increase in concentration of pectin up to 3% for both the isolates (Table 3). Further, increase in substrate concentration rather suppressed the production of these enzymes. Similar results were obtained for standard strain MTCC 281.

Effects of different Carbon sources: In order to study the effect of carbon sources on the production of pectinolytic enzymes, the fermentation was carried out using carbon source at 3% level. Wheat bran was the found the best carbon source for the production PG and PME by both the isolates and the standard stain MTCC 281 followed by citrus bagasse and the production of these pectinolytic enzymes was least with banana peel (Table 4). Silva *et al.* (2005) using solid state fermentation has also shown wheat bran to be the best carbon source for the production of PG by *Penicillium viridicatum* Rfc 3.

Effect of different Nitrogen sources: It is evident from the results that peptone was the best nitrogen source for

PG production followed by sodium nitrate with either of the isolates. Similarly for PME production the best N source was also peptone followed by urea and sodium nitrate with either of the strains/isolates (Table 5). However, the PME production using urea or sodium nitrate by both the isolates was not significantly different from each other and standard strain. Phutela *et al.* 2005 reported that the presence of yeast extract and $(NH_4)_2SO_4$ supported maximal production of pectinase (925 Ug^{-1}) followed by malt sprouts and $(NH_4)_2SO_4$ (785 Ug^{-1}). A study on optimization of pectinase production from *Manihot utilissima* by *Aspergillus niger* (NCIM 548) was carried out by Yugandhar *et al.* (2008) which reflected maximal productivity of pectinases (22.87 U mL^{-1}) under optimum conditions such as tapioca starch concentration 3.71% w/v, C/N ratio 5.94 and salt concentration 0.25% w/v.

Stabilization of enzyme preparation: In order to improve the thermostability, various polyhydric alcohols such as glycerol, sorbitol, sucrose and xylose at varied concentrations were tried as additives. The residual activities of these preparations were recorded after subjecting these to a selected Tm. (Table 6) clearly indicate that glycerol at 20 or 30%, sucrose 30% and Xylose 20% had a reasonable degree of thermo stabilizing effect on the enzyme preparation (Table 6). Also there was no initial loss in the enzyme activity on addition of these additives at the tried concentrations. These results are supported by the studies of Devi *et al.* (1998) studied the stabilizing effect of various additives on Polygalacturonase II from *A. carbonarius* was investigated and found that except for ethylene glycol, the other polyhydric alcohols enhanced thermal stability in a concentration dependent manner the stabilizing effect of polyhydric alcohols increased with increase in hydroxyl content of the polyol and stabilization of enzyme by sucrose was by preventing unfolding of the enzyme. Back *et al.* (1979) studied the stabilizing effect of sugars and polyols against heat denaturation. They found that the thermostability of endoxylanase was enhanced through addition of polyhydric alcohols, mainly 2 M xylitol and 2 M sorbitol solutions. The present investigation revealed that orange peel and tamarind isolates are promising producers of pectinases. Maximum production of pectinases by either of these isolates can be achieved using wheat bran and peptone as a carbon and nitrogen sources, respectively. Moreover, the pectinases from these sources were equally stable and their stability could be further improved significantly by polyhydric alcohols as additives.

CONCLUSION

Thus from the present study it can be concluded that the enzyme isolates from orange peel and tamarind isolated in our laboratory were better producer of pectinolytic enzymes as compared to the standard MTCC strain. The maximum production of these enzymes could be achieved using wheat bran as carbon source and peptone as nitrogen source by these isolates. Moreover, the pH and temperature optima and thermostability of enzymes from these sources were similar to those of the standard strain. Thermostable enzyme preparations could however be improved reasonably by addition of polyhydric alcohols such as glycerol (20%), sucrose (30%) and xylose (20%). Based on the present study, it appears worthwhile to explore the production of these enzymes using solid state fermentation on wheat bran supplemented with peptone and minerals to get economical production of commercial enzymes.

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