International Journal of Biosciences (IJB)
ISSN: 2220-6655 (Print)
Vol. 1, No. 1, p. 33-42, 2011
http://www.innspub.net

RESEARCH PAPER

Production of Pectinase by *Aspergillus niger* Cultured in Solid State Media

Nazneen Akhter[1], M. Alam Morshed[1,3*], Azim Uddin[3], Feroza Begum[2], Tipu Sultan[1], Abul Kalam Azad[1]

[1] Department of Biotechnology and Genetic Engineering, Faculty of Applied Science and Technology, Islamic University, Kushtia-7003, Bangladesh.
[2] Bangladesh Industrial Microbiology Laboratory, IFST, BCSIR, Dhaka, Bangladesh.
[3] Department of Pharmacy, North South University, Bashundhara, Dhaka-1229, Bangladesh.
*Corresponding author: morshedbt@gmail.com

Received: 10 January 2011, Revised: 20 January 2011, Accepted: 21 January 2011

Abstract

Solid state fermentation was carried out with 7 fungal strains, obtained from different sources. Among 7 isolates *Aspergillus niger,* IM-6 was found as effective pectinase producer. Maximum enzymatic activity (142.44U/gm) was observed after 7 days incubation at 40˚C temperature in 750 ml conical flask. In this study 1.69% $(NH_4)_2SO_4$ was used as nitrogen source, although peptone as a nitrogen source showed better result but use of peptone was not cost effective. As a substrate, wheat bran and potato starch showed good result (85.54U/gm) in solid state culture. Addition of 9.68% pectin was found to increase the enzyme production as 116.57U/gm. Pectinase production was optimum in 60% moisture (98.34U/gm). Aeration showed positive effects on pectinase production (136.86U/gm) at 750 ml flask than 1000 ml flask. Thus the wild strain *Aspergillus niger* IM-6 has outstanding pectinase producing

capability at 40°C in 60% initial moisture content for 7 days of incubation in solid state fermentation.

Key words: Pectinase, Pectin, *Aspergillus* sp. Solid state fermentation.

Introduction

Pectinase is a well known term for commercial enzyme preparation that break down pectin; a polysaccharide substrate, found in the cell wall of plants. This enzyme splits polygalacturonic acid into monogalacturonic acid by opening glycosidic linkages. Through this process, it softens the cell wall and increase the yield of juice extract from the fruits. The two major sources of the enzyme pectinase are plant and microorganism. But for both technical and economic point of view microbial source of pectinase has become increasingly important. A great variety of strains of bacteria (Itoh et al., 1982) yeast (Sakai et al., 1982) and mold (Arima et al., 1964) are capable of producing pectic enzymes. The composition of pectic enzymes varies among species of microorganisms. Many studies have been reported that the enzyme preparations used in the food industry are of fungal origin because fungi are the potent producers of pectic enzymes (Marie K. W. Sin, et al., 2002; Abe, J. et al., 1988; Aguilar and Huitron, 1987). Many useful enzymes are produced using industrial fermentation of *Aspergillus niger* (Perrone G. et al., 2006; Tjamos SE. et al., 2004; Abe, J. et al., 1988). Now a day's pectinase is one of the most important enzymes in food processing industries mainly for extraction and clarification of fruit juices and wines. Solid state fermentations (SSF) were used for pectinase production because of the potential advantages such as simplicity, high productivity and concentrated products over submerged fermentations (Lonsane and Ramesh 1992; Trejo-Hernandez et al., 1991).

In this study, heat tolerant filamentous fungus *A. niger* was used for the optimization of pectinase production parameters in solid state fermentations and also to clarify the specific fungal strain with the best enzyme (pectinase) production activity. The optimization was carried out by experimental designing and surface analysis methodology.

Materials and methods

Microorganisms

The pectinase production was carried out with seven different strains of fungi namely P1, P2, P3, P4, P5, P6, P7. Five fungal strains (P1–P5) were supplied from the Industrial Microbiology Section, IFST, BCSIR, Dhaka, Bangladesh. Two strains (P6 and P7) were collected respectively from spoilage wood apple (*Aegle marmelos*) and bread. Pure cultures were maintained in PDA media at 4°C and were subcultured at 30 days interval.

Screening of best isolates

Seven strains of fungi were used for the production of pectinase. The enzyme activity was measured by the method of *Stiles* et al., (1926). Extensive screening was carried out by measuring glucose and pectinase activity. On the basis of screening program, *Aspergillus niger* IM-6 was selected for further experiments.

Culture condition for pectinase production

For the production of pectinase solid state fermentation was performed. To select the optimum growth condition for maximum enzyme production, following parameters was studied stepwise using 14 gm wheat bran and 6 gm rice husk for the solid state fermentation process. Fungus, *Aspergillus niger* IM-6 was inoculated in PDA media in test tube and these inoculums were inoculated at 30°C for 4 days to produce enough mature spore. The fungul spores from 4 days old culture were suspended in 4 ml of sterile water. The fungal suspension was then scrubbed with loop and shaken gently to make homogeneous suspension. This suspension was used as inoculum and was inoculated in solid state medium. To determine the effect of temperature on enzyme production, solid substrates with inoculum were incubated at temperature ranging from 30°C to 50°C. To find the moisture content suitable for the production of maximum enzyme, 50, 60, 70 and 80% tap water were added with solid substrate. The cultures were incubated at different days (up to 9^{th} days) to identify the correct incubation period for the maximum enzyme production. Various concentration of pectin was used as carbon sources. The cultures were incubated

with different kinds of nitrogen sources such as urea, peptone yeast extract, $(NH_4)_2SO_4$ for the production of pectinase.

Extraction of culture filtrate

Ten gram koji (fermented ingredient of solid medium is called koji) and 0.5 gm NaCl were in 100 ml of distilled water. It was stirred for 10 minutes and then kept stand for one night in refrigerator. It was then centrifuged at 4000 rpm for 15 minutes and filtered through whatman filter paper and volume was adjusted. This filtrate was used as crude enzyme for assay of pectinase activity and reducing sugar. Extracted solution was taken in an Erlenmeyer flask plagued with cotton and preserved at 4°C with one drop of toluene.

Glucose estimation and pectinase assay

The reducing sugar of the extra cellular enzyme was determined according to Stiles *et al.,* 1926. pectinase was measured as follows: 2ml pectin solution, 1 ml distilled water, 1 ml acetate buffer (0.05 M, P^H 4.0) was incubated at 40°C in water bath for 10 minutes then 1 ml enzyme solution was added and kept it for 60 minutes and the increase of reducing sugar was estimated by the usual method. One unite of pectinase is defined as1µ mol reducing sugar liberated per minute under assay condition.

Results and discussion

Among 7 isolates *Aspergillus niger,* IM-6 shows relatively higher pectinase activity than that of other isolates (Table 1). Therefore *Aspergillus niger* IM-6 was selected finally for further experiments.

Table 1. Screening for potential isolates for pectinase activity.

Fungal Isolates	Pectinase activity (U/gm) (1^{st} time)	Pectinase activity (U/gm) (2^{nd} time)	Pectinase activity (U/gm) (3^{rd} time)
P1	68	64	64
P2	40	44	46
P3	56	60	64
P4	94	96	96
P5	50	56	50
P6	110	106	112
P7	90	90	92

Table 2. Different substrates for pectinase production.

Days	Pectinase activity (U/gm) W.B +C.S	Pectinase activity (U/gm) W.B +P.S	Pectinase activity (U/gm) R.B +P.S
2	36.50	37.54	32.14
3	52.02	56.78	42.28
4	55.16	66.80	47.16
5	57.54	85.54	51.35

W.B = Wheat bran; C.S = Cassava starch; P.S = Potato starch; R.B = Rice bran.

To find out the effect of different substrates on enzyme activity, Wheat Bran, Rice Bran, Cassava Starch, Potato Starch, Rice husk were used. The results indicated that enzyme activity was higher in wheat bran + potato starch media (Table 2.) which is a cheap and readily available carbon source, and similar findings were also reported by Fujio et al., *(*1993) in *Rhizopus* sp.

To determine the optimum incubation period for the highest enzyme production *Aspergillus niger* IM-6 was incubated up to 9 days. The maximum pectinase activity 130.4 U/g was found at 7th day of incubation (Fig. 1). It means that pectinase production activity is correlated with the incubation time, which was also found from other investigations (C. Venugopal et al*.,* 2007; Pereira et al., 1992) Different concentrations of commercial pectin were added to find out the maximum pectinase production. 9.68% pectin was better for maximum enzyme activity (Fig. 2).

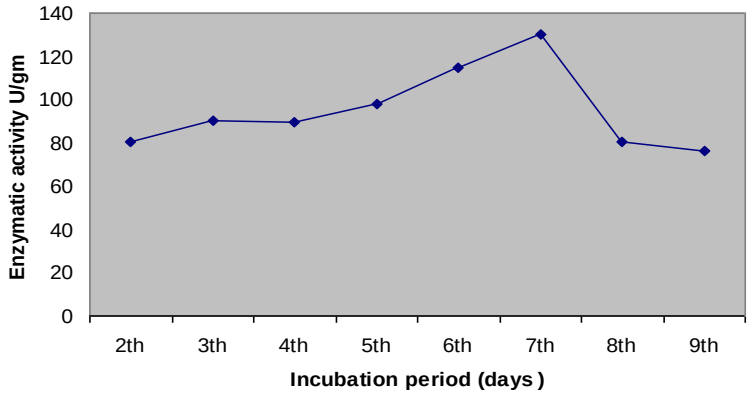

Fig. 1. Effect of incubation period on pectinase production.

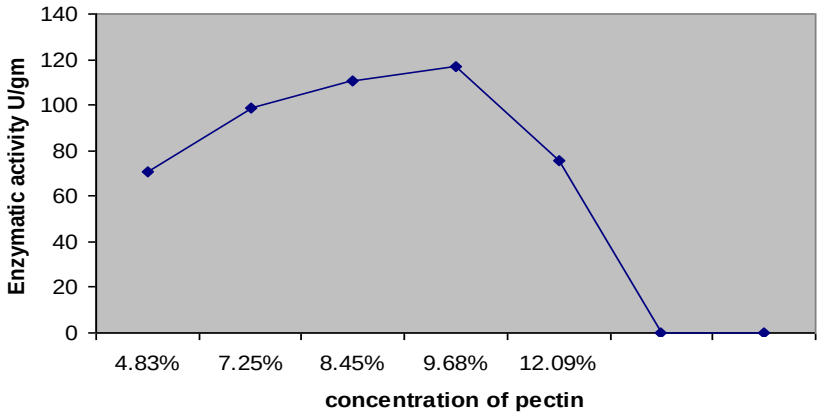

Fig. 2. Effect of pectin on pectinase production.

To investigate the effect of different nitrogen sources on the production of pectinase by *Aspergillus niger* IM-6, the nitrogen sources in the media was replaced by peptone, yeast extract, urea and $(NH_4)_2SO_4$. Peptone was found to support maximal production of pectinase activity (113.68 U/gm) (fig. 3). Peptone was not cost effective, so this work was done with $(NH_4)_2SO_4$. Again when different concentrations of $(NH_4)_2SO_4$ were used, the enzyme activity showed a large variation. Among various concentrations, 1.69 % showed the best result (90.64 U/gm) (Fig. 4).

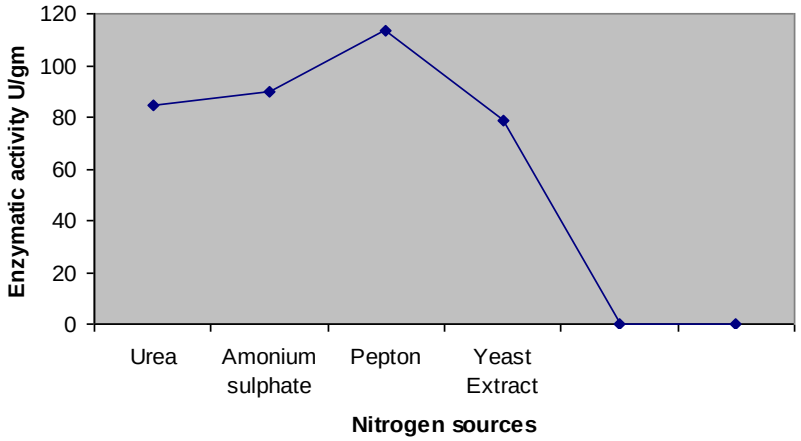

Fig. 3. Effect of Nitrogen sources on pectinase production.

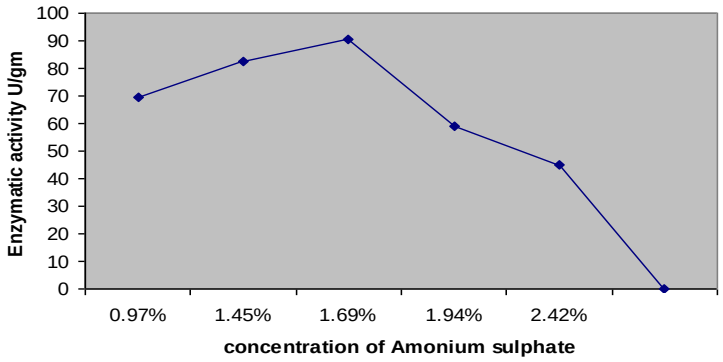

Fig. 4. Effect of concentration of $(NH_4)_2SO_4$ on pectinase production.

Incubation temperature has been found to be a significant controlling factor for enzyme production (Kitpreechavanich et al., 1984). Fig. 5 showed that the temperature has great influence for the production pectinase. Maximum enzyme activity 142.44 U/gm was found at 40˚C and lower activity 98.78 U/gm was showed at 30˚C

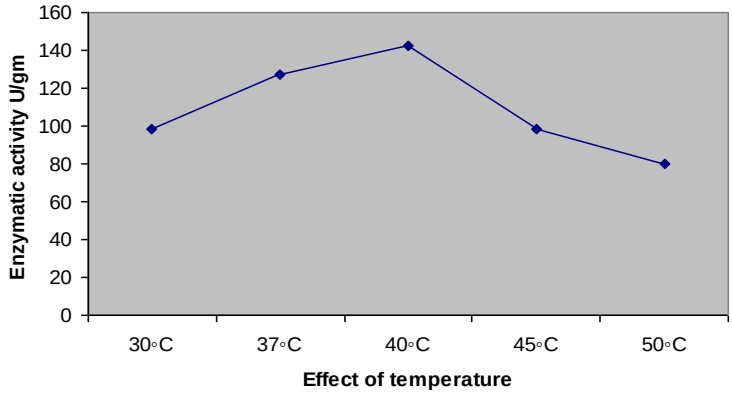

Fig. 5. Effect of temperature on pectinase production.

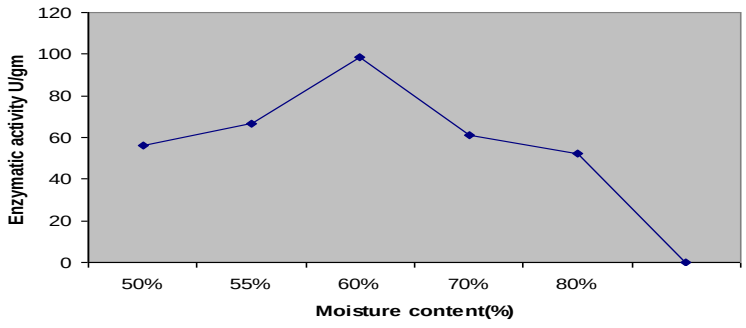

Fig. 6. Effect of initial moisture content on pectinase production.

Moisture adjustment is another important controlling factor in the solid-state fermentation (SSF). Initial moisture was adjusted at various amounts (50%-80%) for optimization of enzymatic activity at 40°C. Fig. 6 Showed that pectinase activities were gradually increased till 60% of moisture adjustment but 70% and 80% showed very poor result. This result is consistent with other investigation where it was found that both low and high moisture content abated the growth rate and fermentation rate of *Aspergillus niger* in solid state fermentation of cassava starch (Eric Oriol et al., 1988). So an optimum moisture content is the critical factor in case of solid state fermentation.

Aeration (oxygenation) affected fungal growth (Dekker and Barbosa 2001). Optimum aeration for maximum pectinase production was examined having different sizes of conical flasks (250 ml-1000 ml). Fig. 7 showed that maximum pectinase activity 136.86 U/gm was found in 750 ml conical flask. Present study depicted that fermentation in 1000 ml flask decreased the enzymatic activity. In another study, it was also found that aeration increases the production of industrial enzymes by maintaining an optimum aeration level. (Venugopal *et al*., 2007).

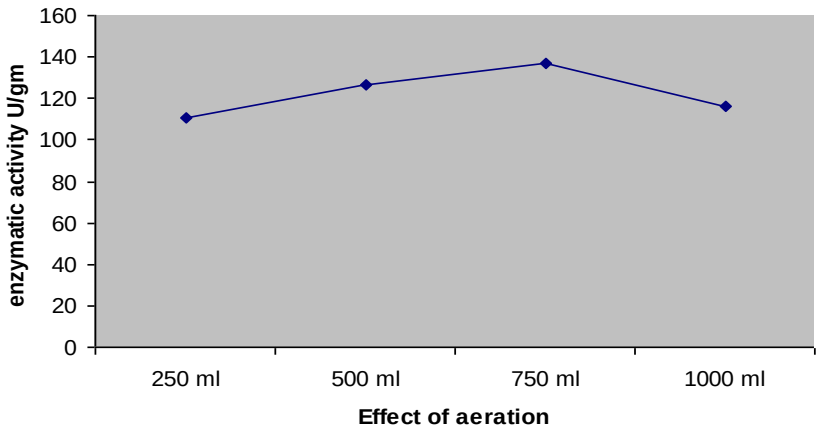

Fig. 7. Effect of aeration on pectinase production.

It can be concluded that the wild strain *Aspergillus niger* IM-6 has outstanding pectinase producing capability at 40°C in 60% initial moisture content for 7 days of incubation in solid state fermentation. This could be highly beneficial for the production of microbial enzymes, pectinase, from lignocellulosic materials in the food and beverage based biotechnological industries

Acknowledgements

We are thankful to all of the stuffs of the Industrial Microbiology Laboratory, IFST, BCSIR, Dhaka, Bangladesh.

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