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Production and optimization of Pectinase enzyme using *Aspergillus niger* strains in Solid State fermentation

Ashfaq Khan*[1], Sanjay Sahay[1] and Neha Rai[2]

[1]Department of Botany, Govt. Science and Commerce College, Benazeer, Bhopal, M.P 462001
[2]Department of Research, Jawaharlal Nehru Cancer Hospital and Research Center, Bhopal M.P 462001
Corresponding author email: k.ashfaq@rediffmail.com

Solid state fermentation was carried out with 4 fungal strains, obtained from different sources. Among 4 isolates *Aspergillus niger*, ATCC 16404 was found as effective pectinase producer. Maximum enzymatic activity (1.62 IU ml^{-1}) was observed after 7 days incubation at 30˚C temperature in 250 ml Erlenmeyer conical flask. In this study 1% dextrose was used as carbon source, although citric acid as a carbon source showed better result (2.73 IU ml^{-1}) but starch was not cost effective. As a substrate, combination of wheat bran and fresh mosambi, orange and lemon peel in ratio of 9:1:1:1 showed good result (5.38 IU ml^{-1}) in solid state culture. Addition of 5% pectin was found to increase the enzyme production as (3.38 IU ml^{-1}) Pectinase production was optimum in 65% moisture thus the wild strain *Aspergillus niger* ATCC16404 has outstanding pectinase producing capability at 30°C in 65% moisture content for 7 days of incubation in solid state fermentation.

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

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 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 (Sin et al., 2002; Abe et al., 1988; Aguilar and Huitron, 1987). Many useful enzymes are produced using industrial fermentation of *Aspergillus niger* (Perrone et al., 2006; Tjamos et al., 2004; Abe 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

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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.

Materials and Method

Microorganism

The pectinase production was carried out with four different strains of fungi namely ATCC 16404, MTCC 872 MTCC 1344 and MTCC 2733 these all fungal strains were supplied from the National chemical laboratory Pune and Institute for microbial technology Chandigarh respectively. Pure cultures were maintained in PDA media at 4°C in refrigerator and were sub cultured at 30 days interval.

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 were studied stepwise using 9 gm wheat bran and 1 gm mosambi peel for the solid state fermentation process. These substrates were used as sole constituent of media or were used as main carbon source, to which was added 45 ml of a micro nutrient solutions containing (% w/v) KH_2PO_4, 0.2; $MgSO_4$, 0.1; $(NH_4)_2SO4$, 0.4; $FeSo_4$, 6.3 x 10^{-4}; MnSO4, 1.0 x 10^{-4} and ZnSO4, 6.2 x 10^{-4} for each 100 g of solid substrate Fungus, *A.niger ATCC 16404* was inoculated in PDA media in test tube and these inoculums were inoculated at 30°C for 5 days to produce enough mature spore. The fungal spores from 5 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 inoculums and was inoculated in solid state medium. To determine the effect of temperature on enzyme production, solid substrates with inoculums were incubated at temperature ranging from 30°C to 50°C. Moisture content was maintained uptyo 65% in solid state medium for suitable growth of fungus for the production of maximum enzyme, tap water were added with solid substrate. Various concentration of pectin was used as carbon sources. The cultures were incubated with different kinds of Carbon source such as dextrose, citric acid and starch 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.

Protein 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, 1ml distilled water, 1ml acetate buffer (0.05 M, PH 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. Protein content measured according to Lowry method (Lowry et al 1951).

Result & Discussion

Among 4 isolates *Aspergillus niger, ATCC 16404* shows relatively higher pectinase activity than that of other isolates (Table 1). Therefore *Aspergillus niger ATCC 16404* was selected finally for further experiment The wild type strain ATCC16404 showed highest activity (1.12 IU ml^{-1}) as well as specific activity (0.49 IU ml^{-1} mg^{-1}) followed by MTCC 1344 (0.30 IU ml^{-1}) and MTCC 2733 (0.20 IU ml^{-1}) however MTCC 872 was shown least activity (0.11 IU ml-1).

Table 1. Screening for potential isolates for pectinase activity

Strain	Protein Conc. (mg/ml)	Specific activity (IU ml^{-1} mg^{-1})	Enzyme unit (IU ml^{-1})
ATCC 16404	0.23	0.49	1.12
MTCC 1344	0.10	0.30	0.30
MTCC 872	0.12	0.09	0.11
MTCC 2733	0.18	0.11	0.20

To find out the effect of different substrates on enzyme activity, Wheat Bran, mosambi peel, lemon peel, mosambi peel extract, mosambi baggase ans its combination with wheat straw was taken in 250 ml Erlenmeyer conical flask moisture content was maintained up to 65%. The results indicated that enzyme activity was higher in Wheat straw + Mosambi peel ext + Orange +Lemon ext (5.38 IU ml^{-1}) with low protein concentration (0.16mg/ml) followed by use of mosambi peel extract with wheat straw (2.12 IU ml^{-1}) which is a cheap and readily available carbon source, and similar findings were also reported by several workers. Lemon peel extract and mosambi peel extract was also shown promising result for the production of pectinolytic enzyme both are having approx equal protein concentration 0.28 mg/ml and 0.21 mg/ml respectively while mosambi bagasse was shown lowest activity (0.66 IU ml^{-1})with lowest protein concentration.

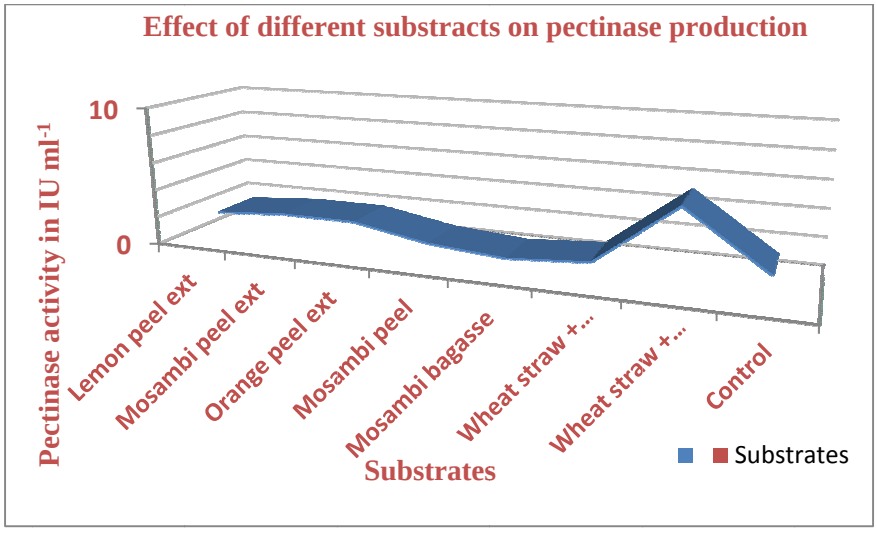

Fig 1: Production of pecinase by using different subtract in solid state fermentation

The productions of primary metabolites by microorganisms are highly influenced by their growth, which is determined by the availability of the nutrients in the substrates. Therefore, it is expected that the improvement of the nutritional value of solid medium by the supplementation of carbon sources was also improve the growth of fungus and subsequently the enzyme production(fig. 2) shows the supplementation of sugars, which might act either as carbon sources or inducers. As shown in the fig 2 the addition of citric acid resulted in pectinases production (2.73 IU ml^{-1}) two fold higher as compared to addition of dextrose (1.04 IU ml^{-1}). However the protein content was found higher in presence of citric acid while with the addition of starch protein content was found less than that of other additives. The effect of starch in 1% conc. was found to reduce three fold pectinase productions (0.47 IU ml^{-1}) which were lesser than that obtained by addition of dextrose and citric acid.

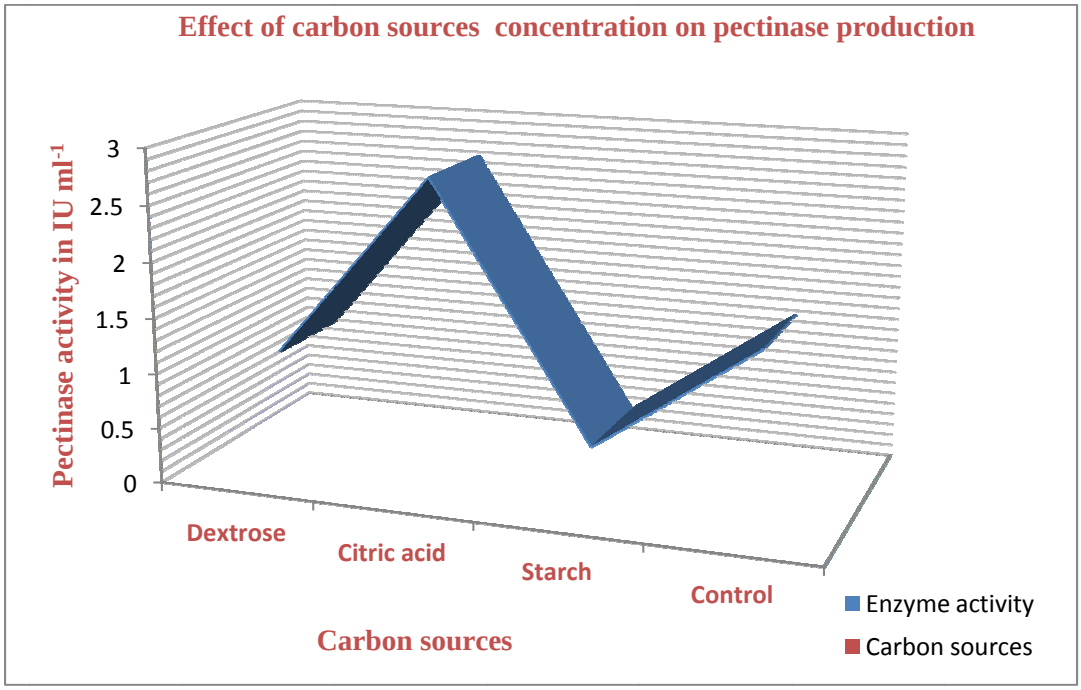

Fig 2: Effect of different carbon source on the production of pectinase

Temperature is one of the important parameters that determine the success of SSF system therefore, the effect of temperature on pecitnase production by ATCC 16404 was examined and the results obtained are shown in fig 3 the production of pectinase was maximum at the ambient temp. 30°C (1.62 IU ml^{-1}**)** followed by at 37°C (0.52 IU ml^{-1}**).** A lower activity in the range of (0.33 IU ml^{-1}) to (0.40 IU ml^{-1}) exhibited at 4°C and 45°C respectively. The results indicated that the enzyme production corresponded closely to the growth of the fungus. The optimum temp for pectinase production was similar to the optimum temp for the growth of the fungus.

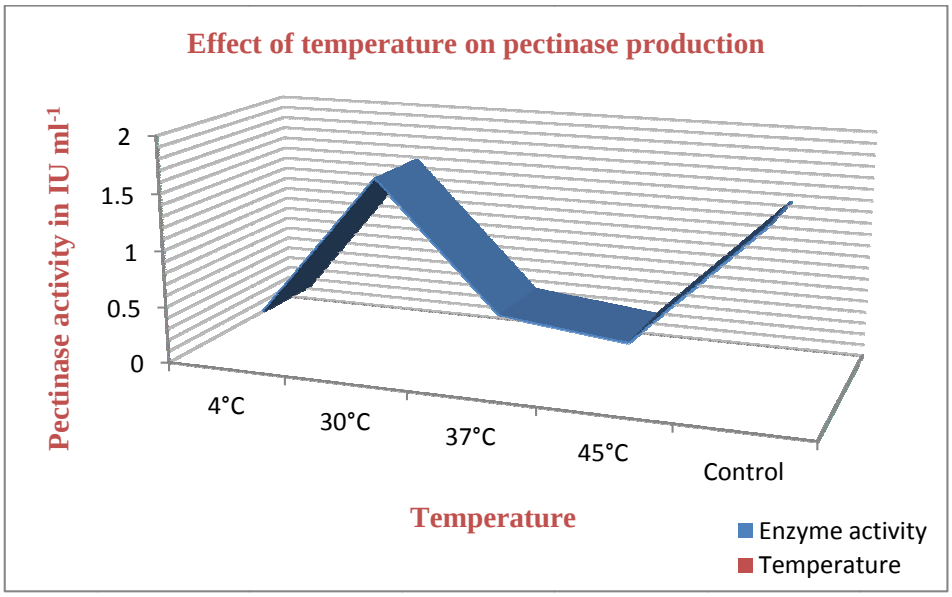

Fig 3: Pectinase production under different temperature in solid state fermentation

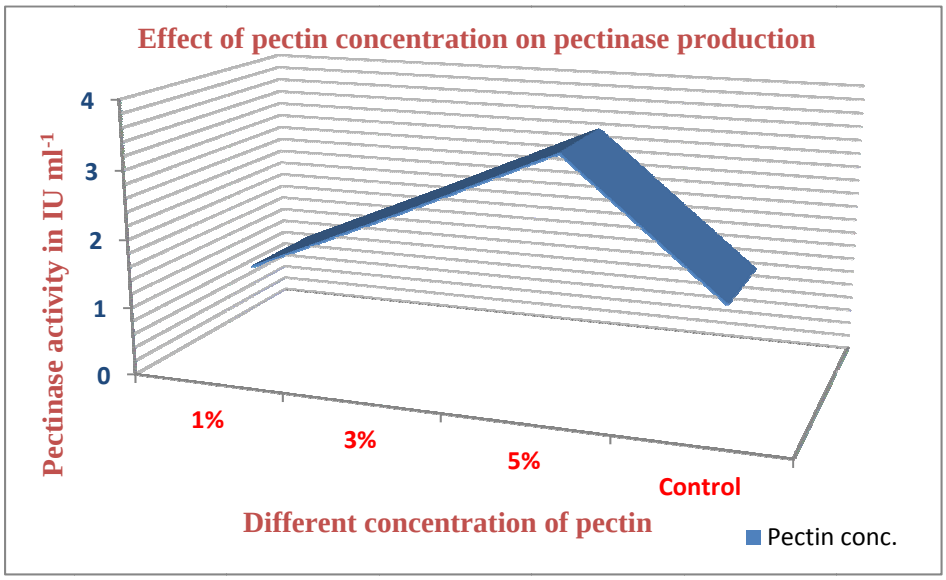

Fig 4: Effect of different concentration of Pectin as inducer for the production of pectinase enzyme

The data given in fig 4 indicated that 5% pectin gave maximum enzyme production (3.38 IU ml^{-1}) however, a drop in the pectinase production (2.35 IU ml^{-1}) obtained in presence of 3% pectin, while 1% showed least yield (1.35 IU ml^{-1}) with low protein content 0.22 mg/ml which indicates a state of pectin inadequacy for growth /enzyme expression. In other hand highest protein content was found in presence of 5% pectin which is approx two fold s compare to control. The results obtained so far suggested

that the existence of the saturation phenomenon on the induction of pectinase.

Discussion

Almost all the commercial preparations of Pectinases are produced from fungal sources and *A. niger* is the most commonly used fungal species for the industrial production of Pectinases (Gummadi and Panda, 2003). Enzyme production is a growing field of biotechnology and the world marked for enzyme is over 1.5 billion (Lowe *et al*., 2002). The majority of the industrial enzymes are of microbial origin (Bai *et al*., 2004 and Barrow *et al*., 1993). In the present study, total four fungal isolates were used. These fungal isolates were grown at 30 °C and at pH 4.5 to be able to produce a polygalacturonase to be used as additive for clarification of the juices. A screening of pectinolytic productivities of the four fungal isolates showed that, three two isolates gave a good pectinolytic productivities. The most promising wild type strain among all used is ATCC16404. The combination of wheat straw and mosambi bagasse are most suitable substrates for ensuring highest yield among all the substrates used during present study. Supplementation of starch and 5% pectin is also found to marginally improve the production level.

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