EVALUATION OF FERMENATION CONDITION FOR PRODUCTION OF ENDOPOLYGALACTURONASE BY FUNGAL STRAIN IN SOLID STATE FERMENATION

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ABSTRACT

The production of enzymes by bioprocesses is a good alternative to add value to agro industry residues. Endo PG's enzyme production was attempted using *Aspergillus niger* (ATUV08), from the mixture of wheat straw and mosambi peel extract (9:1) under Solid state fermentation. Among the various carbon source and heavy metals were tested, highest yield of pectinase production was observed for starch 39.0 IU ml^{-1} mg^{-1} as the carbon source in solid state fermentation. The Endo PG's production (37.4 IU ml^{-1} mg^{-1}) was found to be higher in the pH 5.0. A maximum endo PG's production of 37.8 IU ml^{-1} mg^{-1} was obtained in the presence of Zinc sulphate followed by ferric chloride 37.4 IU ml^{-1} mg^{-1} at 1 M concentration in solid state medium. The present study showed that the fungal strain *Aspergillus niger* has high potential for industrial productions of Endo PG's.

Key words: Endo PG's, Solid state fermentation, *Aspergillus niger*, Mosambi peel extract

INTRODUCTION

Currently, the fundamental exploitation of agricultural and food wastes, which participate in pollution, is the controlled biological degradation of the wastes by microorganisms for the production of valuable compounds such as proteins, polysaccharides, oligosaccharides, vitamins, hormones, enzymes and other raw materials for medicinal and industrial uses. Now

a day's endopectinase 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 the ability to synthesize pectinolytic enzymes is very common in groups of microorganisms, but fungus is preferred on an industrial scale. This is because about 90% of the enzymes produced may be secreted in the culture medium (Blandino et al., 2001). *Aspergillus niger* is the most commonly used fungal species for industrial production of pectinolytic enzymes (Naidu and Panda, 1998; Dinu et al., 2007). The most abundant and extensively studied of the pectinolytic enzymes, typically exist in multi-gene families and may have both endo and exo activities (Sakamoto et al., 2002)..Solid-state fermentation is traditionally defined as those processes in which microbial growth and products formation occur on the surfaces of solid substrates in the near absence of free water due to this low amount of water available in solid-state bioprocessing, the class of microorganisms that are most commonly used is fungi Several agro-industrial waste and by-products such as orange bagasse, sugar cane bagasse wheat bran and other food processing waste are effective substrates for depolymerizing enzyme production by solid-state fermentation In this study, filamentous fungus *A. niger* was used for the optimization of endopectinase production parameters in solid state fermentations and also to clarify the specific fungal strain with the best enzyme (endopectinase) production activity. The optimization was carried out using different pH, carbon sources and heavy metals.

MATERIALS & METHODS

Isolation and identification of fungal microorganism: The fungal strains were recently isolated from fruit peel and soil Sample (2 g) from local market of Bhopal and agricultural farm respectively. After the screening of fungal isolates, ATUV08 were selected for the enzyme production which is isolated from agricultural soil sample were pooled and homogenized in sterile medium containing 1% of citrus pectin; 0.14% of (NH4)2SO4; 0.20% of K2HPO4; 0.02% of MgSO4.7H2O; 0.10% of nutrient solution (5 mg/l FeSO4.7H2O; 1.6 mg/l MnSO4.H2O; 1.4 mg/l ZnSO4.7H2O; 2.0 mg/l CoCl2), pH 5.0. to get pure colony of desired fungal isolated for identification. The fungal spp were identified according to their

morphological, cultural characteristics. They were preliminarily identified according to their morphological physiological, biochemical cultural characteristics (Barnett and Hunters, 1998; Davis,1969). Microscopic identification was on the basis of the structures bearing the spores. The fresh sub-cultured actively grown fungal isolates were picked with a sterile needle into a clean glass slide and observed under microscope. The stock cultures of the isolates were maintained on PDA slants at 4°C. For sub culturing the fungal isolates were maintained on agar slants for five days at 30°C and stored in a refrigerator. Among the isolates, isolate were identified as *A. niger* (ATUV08) was selected based on their protein production capability for this fermentation study. The cultures of *A. niger* (ATUV08) was stored on PDA slants for further evaluation and for fermentation studies on the production of protein enriched biomass from orange wastes.

Preparation of seed culture spore suspension: The petrish containing matured (4 days incubated) spores was added with 5 ml water containing 1% Tween 80. All the spores were thoroughly scrapped out using spatula to scrap all spores which is stick to the agar medium. The resulting spore suspension (approx 10^6 spores/ml) was transferred in to a sterile test tube and cotton plugged. All these preparations were carried out aseptically in a laminar chamber.

Agro-industrial Wastes: The mosambi peel were collected from local market in Bhopal, India. They washed to remove the clay for using, dried in open air, and then grounded than filtered using muslin cloth to collect fine powder which support the fermentation process, wheat straw were collected from the local farm of Bhopal, dried and prepared in the form of ground preparations.

Production of Endo PG's Under SSF: All the above substrates were air dried, ground or cut into small pieces and autoclaved. These substrates were used separately in flasks for Endo PG's production. 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 salts (MS) solution 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. 1ml of conidial suspension ($1x10^7$conidia/ml) was inoculated and incubated statically at 30^OC for 96 h. 60 ml of distilled water was added to the flasks, shaked for 1 h. and filtered through muslin cloth. The filtrate

was used to assay enzyme. The enzyme unit was defined in terms of substrate released/mg protein in a specified time.

Analytical method

Construction of Galacturonic Acid Standard Curves: A stock solution (10.000 mg/ml) of the standard galacturonic acid supplied by Loba chemicals Mumbai was prepared in acetate buffer (0.2 M) at pH 5. The stock solution was used for making of different concentrations. After preparing of the required dilutions, only 1.0 ml of each dilution was transferred to determine of the amount of reducing sugar according to Nelson et al 1944. A standard curve was constructed relating all different sugar concentrations applied against their corresponding to optical density at 510 nm. The obtained standard curve was used for estimating the polygalacturonase activities in terms of mg/ml and then units (U). One unit is defined as the amount of enzyme protein (mg) required to exert free

galacturonic acid, from pectin of time under 35 °C for 1hour in acetate buffer (0.2 M) at pH 5.0

Protein Determination: Protein of all enzymatic preparations was determined by the method of **Lowry (lowry et al 1951)**

Endo PG's Assay: The culture sample was centrifuged at 10,000 rpm for 10 minutes. The culture supernatant was used for Endo PG's assay. Endo-PG action was assayed by determining the percentage decrease in apparent viscosity of a mixture of 12 ml of enzyme and 12 ml of 3% pectin solution in 0.2 M citrate-phosphate buffer, pH 6.5 at 40 °C, using a Fenske-Ostwald viscosimeter as described by (Fellows et al 1984).The results were subtracted from experimental controls when denatured enzyme was used, according to the following formula: Apparent viscosity reduction (%) = (Vc - Vr) x (Vc - Vs))-1 x 100 where: Vc = flow time of control, Vr = flow time of sample; Vs = flow time of water. One unit of endo- PG activity was defined as the amount of enzyme which reduced the initial viscosity of pectin solution by 50% in 10 min.

RESULT & DISCUSSION

The capacity of microorganisms to produce extracellular enzymes is influenced by environmental conditions such as temperature, pH, aeration, inoculums age and the presence of inducer or repressor substrates (El-Refai et al., 1984). Initially, the influence of the different carbon sources, heavy metals and initial pH of the cultivation medium of *Aspergillus niger* was investigated. All other process parameters such as inoculation, mixing, aeration, temperature, fermentation time were same. Almost all the commercial preparations of Pectinasess are produced from fungal sources. *A. niger* is the most commonly used fungal species for the industrial production of Pectinasess (Gummadi and Panda, 2003).

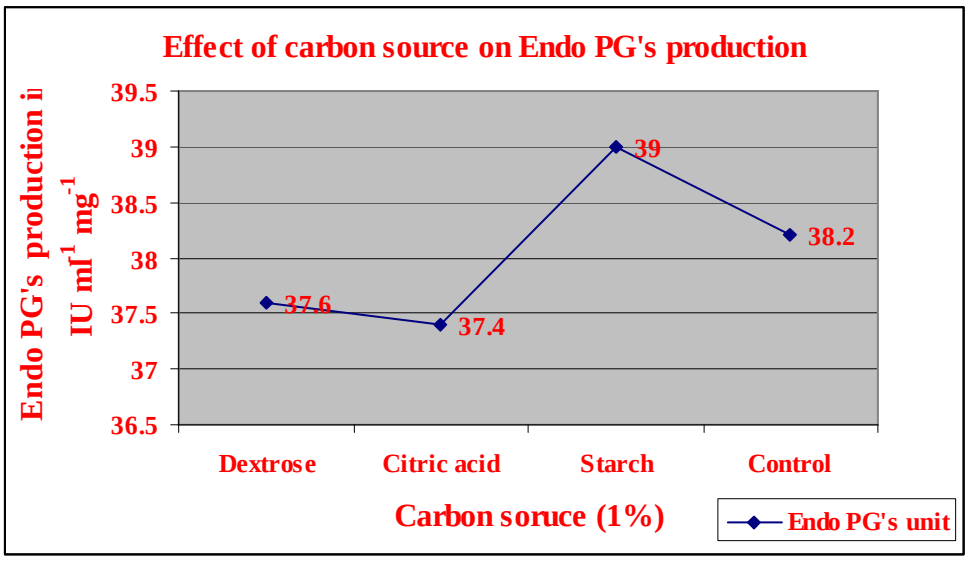

Fig 1 Effect of different carbon source at 1% concentration on the Endopolygalacturonase productivity using *Aspergillus niger* ATUV08 under solid state fermentation conditions in 250 ml Erlenmeyer conical flask having 5 g of wheat straw + mosambi peel extract as solid medium pH 5.0

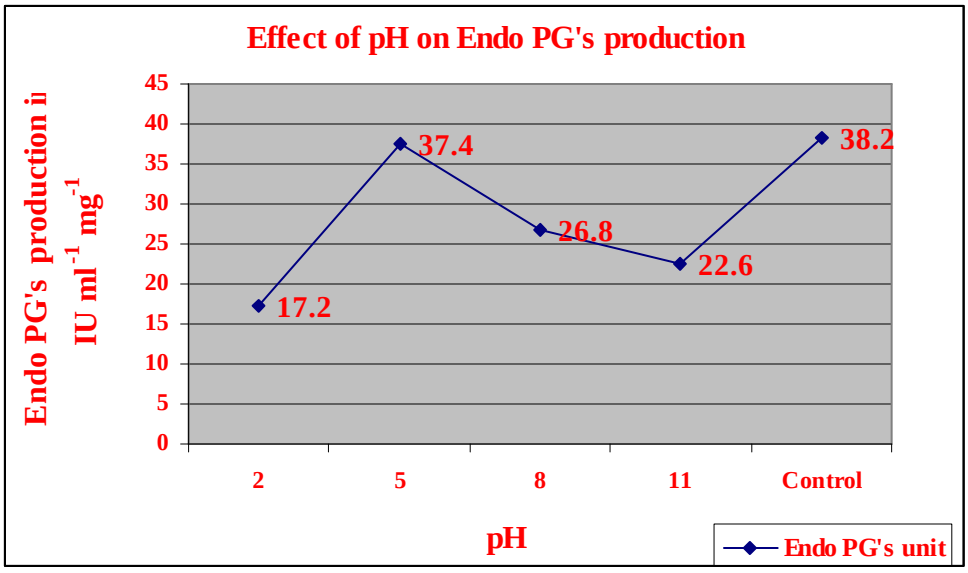

Fig 02 Effect of different pH on the Endopolygalacturonase productivity using *Aspergillus niger* ATUV08 under solid state fermentation conditions in 250 ml Erlenmeyer conical flask having 5 g of wheat straw+ mosambi peel extract as solid medium

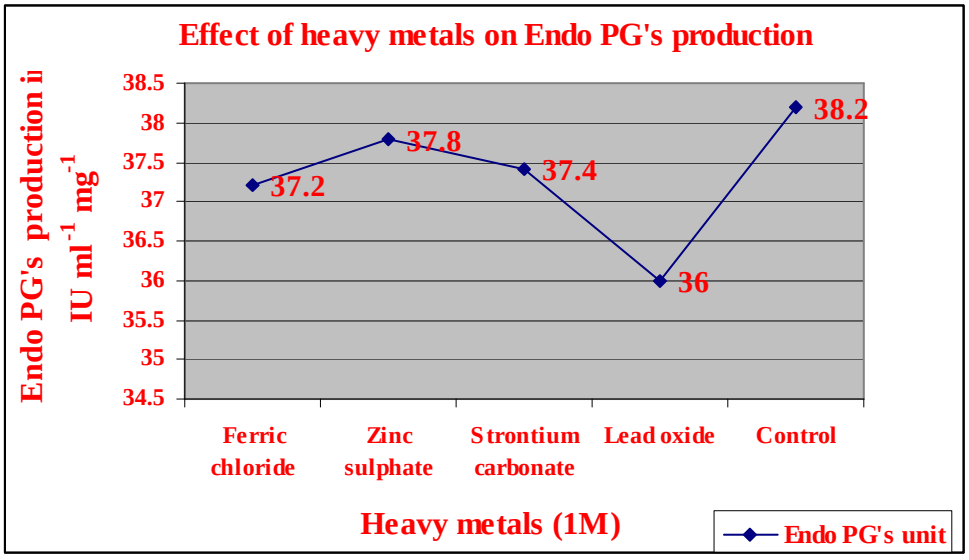

Fig 03 Effect of different heavy metals at 1M concentration on the Endopolygalacturonase productivity using *Aspergillus niger* ATUV08 under solid state fermentation conditions in

250 ml Erlenmeyer conical flask having 5 g of wheat straw + mosambi peel extract as solid medium pH 5.0

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 microorganism and subsequently the enzyme production. The effect of three different carbon sources (Citric acid, starch and Dextrose) were introduced into the applied production medium of polygalacturonase productivity by *Aspergillus niger* ATUV08 under SSF condition were studied. It was clear that all the different carbon sources exhibited various degrees lower than control Endo PG's production where the productivity reached up to 39 IU $ml^{-1}mg^{-1}$ Table 1 show the effect of supplementation of sugars, which might act either as carbon source or inducer. As shown in the figure 1 the supplementation of starch resulted in a higher production of Endo PG (39 IU $ml^{-1}mg^{-1}$) followed by Dextrose (37.6 IU $ml^{-1}mg^{-1}$) and least production ((37.4 IU $ml^{-1}mg^{-1}$) was obtained by the addition of 1 % Citric acid in medium. An effect of pH of the medium on endo-PG biosynthesis and growth of the culture *Aspergillus niger* was studied at four pH-values in a range of 2.0-11.0 (Table 2). The maximal endo-PG production was observed in the medium with the acidic initial pH-values within a range of 4.0 to 5.0. Maximal endo-PG's production, 37.4 IU $ml^{-1}mg^{-1}$ from results in Table 3 be can see biosynthesis of endo PG's and growth of the culture *Aspergillus niger* in relation to the heavy metals. The results of the study on the effect of heavy metals on the production of EndoPG by *A. niger* are given in the Table 3 The enzyme production was found to be the maximum (38.2 IU $ml^{-1}mg^{-1}$) in absence of any heavy metal (control). The presence of heavy metals in the growth medium caused slight to considerably reduction in enzyme yield for instance, presence of zinc sulphate in the growth medium at 7 days in SSF yielded 37.8 IU $ml^{-1}mg^{-1}$. Strontium carbonate (37.4 IU $ml^{-1}mg^{-1}$) and lead oxide (36 IU $ml^{-1}mg^{-1}$) caused slightly more reduction in enzyme production. Many authors who described the use of different inexpensive carbon sources for better production of pectinolytic enzymes (Aguilar and Huitron, 1986; Maldonado and Navarro, 1986; Hours et al., 1988; Larious et al.,

1989;Leuchtenberger et al., 1989; Pericin et al., 1992; Shevchik et al., 1992; Hang and Woodams, 1994; Berovic and Ostroversnik, 1997; Alkorta et al., 1998; Zheng et al., 2000;Kaur and Satyanarayana, 2004; Joshi et al., 2006; Zhong-Tao et al., 2009; Tsereteli et al.,2009).

CONCLUSION

The present work has been taken up with a view of exploring the possibilities of using wheat straw and mosambi peel extract *as* a substrate and *A.niger* (ATUV08) as a microbial source for the production of Endopolygalaturonase which can galaturonic acid This strain is able to produce Endo PG's in the medium containing pectin as the sole carbon source. Endo PG's is an industrially important enzyme that is mainly used in the food industry. As the range of applications of this enzyme is very wide there is always a scope for novel endo PG's with better characteristics, which may be suitable in the diverse fields of applications. Solid state fermentation technology using non pathogenic microorganisms which can produce hydrolytic enzymes such as Endo PG's will be advantageous for the proper utilization of these residues. Since microbial activity especially fungal activity is the key aspect in this area, there is enormous opportunity for the cost effective production of Endo PG's, which is an important enzyme in the food industry.

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