Production and characterization of pectinase enzyme from _Aspergillus ustus_ BL5 using submerged fermentation

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Abstract

Pectinase enzymes catalyzing the degradation of pectic substances are one of the most important enzyme for industry. They contribute approximately 25% of in the global sales of food enzymes. In this study, we looked for potential microbe producing pectinase and characterized the enzyme. A microbe strain producing pectinase was isolated after primary screening of 81 different indigenous isolates from BFLC (Biocatalyst and Fermentation Laboratory Collection) & BTCC (Biotechnology Culture Collection). Based on ITS1, 5.8S, ITS2 of rDNA analysis, the selected microbe was identified as _Aspergillus ustus_ BL5. Using submerged fermentation, the optimum levels of time incubation was determined. The optimum of pH and temperature of pectinase producing by _Aspergillus ustus_ BL5 were also determined.

Keywords : _Aspergillus ustus_ BL5, submerged fermentation, pectinase, enzyme characterization.

Introduction

Pectinases are a big group of enzymes that degrade pectins of plant tissues into simpler molecules like galacturonic acid (Pedrolli et al., 2009). This enzymes plays a vital role in food processing industries, for example, in the production of fruit juices, soft drinks, liquors (Panda et al., 1999) and alcoholic beverages industries (Naidu & Panda, 1998). Pectinase is used also for improvement of tea leaves fermentation (Angayarkanni et al., 2002). The importance of pectinase in many industries conducted of looking for the best source to produce the enzyme.

Microorganism are widely used as the best sources for pectinases production. Almost of the commercial preparations of pectinases are produced from fungal sources, mainly from _Aspergillus niger_ (Tari et al., 2008). There are many microbes have been reported in ability of pectinase production, _Bacillus sp_ (Ouattara et al., 2008), _Aspergillus fumigatus_ (Phutella et al., 2005), _Aspergillus sojae_ (Tari et al., 2008), _Aspergillus clavatus_, _Fusarium sp_, _Penicillium chrysogenum_, and _Trichoderma sp_ (Okafor et al., 2010; Banu et al., 2010). _Aspergillus sp_ is one of fungi that often identified as source microbe in pectinase activity. The utilization of microorganism in producing enzyme have a number of advantages; through the application of selection methods increase of biosynthesis via the conditions of cultivation, in-depth interaction on various substrate, wide spectrum of enzyme complex and their application in gene engineering via gene cloning (Kutateladze, 2009)

Submerged fermentation has been extensively employed for the production enzymes and to understand physiological aspects of the enzyme synthesis (Patil & Dayanand, 2006). In this object, we determined optimization factor of pectinase enzyme production using submerged fermentation.

In this research, the objective of the present study was to produce pectinase enzymes by a newly isolated strain of _Aspergillus ustus_ BL5 (as selected microbe) by submerged fermentation and to determine the optimum of time incubation. Furthermore, the characteristics of pH and temperature of the enzymes was also determined.

Materials and Methods

Microorganism for screening

Eighty-one microorganisms for assay was obtained from BFLC (Biocatalyst and Fermentation Laboratory Collection) & BTCC (Biotechnology Culture Collection), consist of actinomycetes and fungi.

Screening for pectinase activity

Screening methodology for 81 isolates was used in this study. It was formulated the pectinase screening agar medium (PSAM): 1 g pectin, 0.3 g Diammonium orthophosphate, 0.2 g KH₂PO₄, 0.3 g K₂HPO₄, 0.01 g MgSO₄ and 2.5 g agar (for 100 ml). This medium was sterilized and distributed aseptically in petri dishes. The petri dishes containing PSAM were inoculated and incubated in room temperature for 24 hours. The clearance zone...