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Alkaline protease production by Bacillus sp. MTCC 511 from cost effective substrate

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ABSTRACT

The objective of this work is to synthesis extracellular alkaline protease by Bacillus sp. using low cost agro waste in solid state fermentation (SSF). The cost effective feed stocks like rice bran, wheat bran, cassava, saw dust and oil cake were taken for alkaline protease production. Among this, rice bran was found to give maximum enzyme production. Various nitrogen compounds like peptone, urea, yeast extract, ammonium sulfate and ammonium chloride were evaluated as nitrogen source and among them peptone gave maximum enzyme production. Maximum production of alkaline protease was achieved when initial moisture content and initial pH were 70 % and 10 respectively.

KEY WORDS: Rice bran, Bacillus sp., Solid State Fermentation, alkaline protease.

1. INTRODUCTION

Alkaline protease, proteolytic enzyme is intensively used in various field namely tanning, detergent, waste treatment, pharmaceutical industry and photography etc. (Gupta, 2002). It has been reported for about 60 – 65% of world enzyme's sales (Mukherjee, 2008). Among three types of proteases (acid, neutral and alkaline) alkaline protease is commercially important.

Various alkalophilic microorganisms have been screened for alkaline protease the production. Bacillus sp. is predominant among them (Joo, 2004). Apart from this, various yeast cells like *Candida lipolytica, Auerobasidium pullulans* etc., and fungi such as *Aspergillus flavus, Aspergillus niger, Aspergillus melleu, Fusarium graminarum, Chrysosporium keratinophilum, Scedosporium apiosermum, Penicillium griseofulvin* also produce protease enzyme in submerged and solid state fermentations (Madhuri, 2012; Sugumaran, 2012).

Cost of production medium itself accounts for more than 30% cost of the product (Sugumaran and Ponnusami, 2015). Therefore, identification of low cost substrates and development of process to improve the enzyme yield are very much essential to cut down the cost of enzyme production.

However, solid state fermentation is superior to submerged fermentation as it requires a smaller amount of water, less energy requirement, less amount of effluent generation and high quality product formation (Sugumaran, 2014; Pandey, 2000). For these reasons, SSF is widely adopted for several industrial applications including production of commercially important enzymes, bio insecticides, secondary metabolites etc. As a result, many researchers have focused on solid-state fermentation for alkaline protease production to cut down the cost of production. The various cost effective substrates namely green gram husk, wheat bran, castor husk, *Pongamia pinnata* seed cake, red gram husk, potato peel and rice bran been tried to enhance alkaline protease production during fermentation (Naidu and Lakshmi Devi, 2005; Prakasham, 2006; Mukherjee, 2008; Reddy, 2009; Naga Jyothi and Suryanarayana Raju, 2010; Madhuri, 2012; Sangeetha, 2011).

In this work, investigation on production of alkaline protease was performed with cost effective substrate by Bacillus sp. under SSF, since limited protease production studies were investigated in SSF using this strain.

Produce protease enzyme in submerged and solid state fermentations (Madhuri, 2012; Sugumaran, 2012). Cost of production medium itself accounts for more than 30% cost of the product (Sugumaran and Ponnusami, 2015). Therefore, identification of low cost substrates and development of process to improve the enzyme yield are very much essential to cut down the cost of enzyme production.

2. MATERIALS AND METHODS

Microbial Cultivation: For this study, *Bacillus* sp. was procured from MTCC, Chandigarh, India which as designated by MTCC 511. The stock was preserved in nutrient broth with following composition (g/L): beef extract: 1, yeast extract: 2, peptone: 5 and NaCl: 5 at pH 7.8.

Fermentation: The mineral salt medium containing the following composition (g/L): yeast extract: 0.1, NH_4Cl : 0.5, KH_2PO_4 :0.4, K_2HPO_4 : 0.3, NaCl: 0.5 and $MgCl_2$: 0.1 (Ganesh Kumar and Parrack, 2003) was prepared and added with twenty gram of solid substrate according to desired moisture content, mixed well and sterilized. Then, cooled and sterilized heterogeneous production medium was inoculated with 10% (V/V) inoculum having 0.6 optical density at 650 nm under aseptic condition. Fermentation medium was thoroughly mixed and incubated at room temperature.

Factors affecting alkaline protease production: Agro wastes namely rice bran, wheat bran, cassava bagasse, saw dust and oil cake were examined for their potential to serve as a cost effective solid substrate for alkaline protease

production. Based on maximum activity of enzyme, suitable solid substrate was screened. Then, screening nitrogen sources namely ammonium sulfate, yeast extract, peptone, urea and ammonium chloride, initial pH in the fermentation medium (6-11) and moisture content (10 – 90%) on enzyme activity were investigated. Enzyme sample was extracted from the fermentation medium as explained by Nagamine (2003). Enzyme activity is one µmol of tyrosine elaborated per unit time per g dry solid substrate.

3. RESULT AND DISCUSSION

Identification of suitable solid substrate: In this work, cost effective substrates such as oil cake, cassava bagasse, rice bran, saw dust and wheat bran were considered for the production of alkaline protease. Maximum activity was exhibited using rice bran as solid substrate (Fig.1). Therefore, the production of alkaline protease from rice bran is so good compared with previous findings.

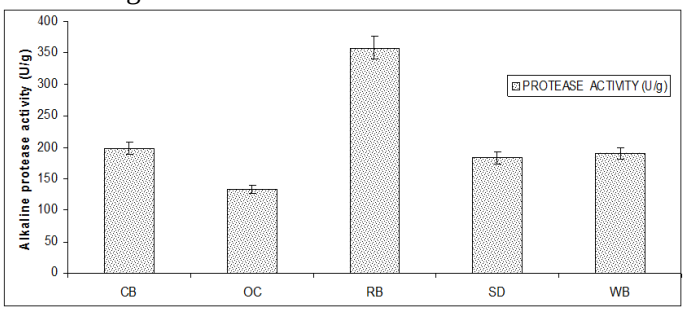

Figure.1. Screening of cost effective substrate on protease production (CB-Cassava bagasse; OC-Oil cake; RB-Rice bran; SD- Saw dust; WB- Wheat bran)

Effect of N- Source on protease production: Nitrogen source is another important factor influencing cell metabolism and enzyme production. Based on the type of cells and metabolites production, the usage of nitrogen source was varied (Shivakumar, 2012). In this study, sources of nitrogen namely yeast extract, ammonium sulfate, urea, ammonium chloride and peptone were taken in solid medium. Out of all nitrogen sources tested, peptone and urea have resulted in increased alkaline protease activity compared to control. While, yeast extract did not affect the enzyme activity, ammonium sulfate and ammonium chloride have decreased enzyme activity (Fig.2). These observations suggest that inorganic nitrogen sources repress protease production and media supplemented with organic nitrogen sources yield improved enzyme production. Similarly, Prakasham, 2006 had observed lower activity of alkaline protease production than control. Maximum alkaline protease activity was established with peptone in this study. This is consistent with previous observations.

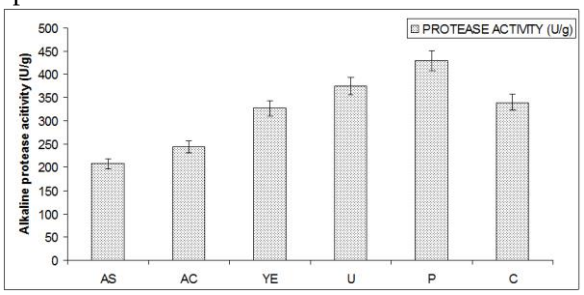

Figure.2. Effect of N-source on protease production (Error bar±5%) (AS-Ammonium sulfate; YE-Yeast extract; P-Peptone; U-Urea; C-Control; AC-Ammonium chloride)

Effect of initial pH on protease production: Alkaline protease activity was maximum with initial pH 10 in the heterogeneous medium containing rice bran as solid substrate with peptone (Fig.3). The results are consistent with previous reports (Prakasham, 2006; Rathakrishnan and Nagarajan, 2011).

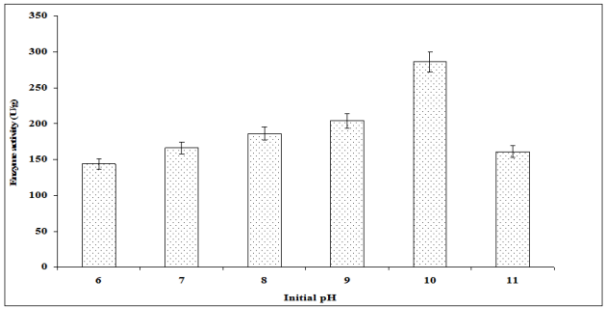

Figure.3. Effect of initial pH on protease production (Error bar±5%)

Effect of Moisture Content: Optimum moisture content on enzyme production depends on the type of microorganism with cost effective solid substrates agro waste in solid state fermentation (Pandey, 2000; Sugumaran, 2013; 2014; Sugumaran and Ponnusami, 2015). Influence of moisture content (10 – 90 %) on the production of alkaline protease was carried out with rice bran as carbon source. At 70% moisture content, maximum activity (150 U/g) was observed (Fig. 4). Similarly, High concentration of exo-glucanase was obtained from *B. subtilis* using banana stalk at 70% moisture content (Pandey, 2000). Madhuri, 2012 observed maximum enzyme activity with 80% moisture content in presence of castor husk as a solid substrate. Uyar and Baysal, 2004 reported optimum moisture level of 40% using lentil husk as substrate by *Bacillus* sp. However, maximum production was obtained at 100% moisture with *Bacillus subtilis* (Mukherjee, 2008).

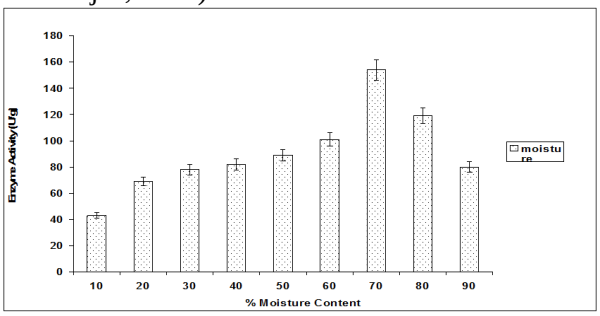

Figure.4. Effect of moisture content on alkaline protease production (Error bar±5%)

4. CONCLUSION

Five different agro wastes were examined for their potential to act as inexpensive solid substrate for alkaline protease enzyme production. Rice bran was selected as the best substrate among the five. Similarly, among various nitrogen source examined, peptone was found to show maximum alkaline protease activity. Influences of initial pH, temperature and moisture content on alkaline protease enzymatic reaction were studied. It was found that 50°C, pH 10 and 70% moisture content were optimum conditions for the enzyme production.

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