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PRODUCTION AND CHARACTERIZATION OF ENDOGLUCANASE FROM BACILLUS SUBSTILLS BY USING WHEAT BRAN AS SUBSTRATE

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ABSTRACT

Microbial cellulases have great significance due to their wide range of applications from industrial sector to household products and comprise 60% of worldwide enzyme market. cellulase are used for a variety of industrial applications, such as commerical food processing in coffee, cellulases are widely used in textile industry and laundry industry, pulp and paper industry, Pharmaceutical industryalso used in fermantation of biomass. Ideally, Endoglucanase used in industries should have good activity over a wide range of pH and temperatures .Because, most of the industrial practices are carried out at higher temperature and broad range of pH most of the enzymes are unstable. Therefore, there is a great demand and scope for screening thermostable and alkaliphilic endoglucanase that can tolerate the adverse industrial condition This has attracted the attention of many researchers throughout the world for screening of potential Endoglucanase from various *microbial strains viz., Aspergillus sp., Bacillus sp., Clostridium sp., Mucor sp., Pseudomonas sp.,* etc. These microbes were successful in producing endoglucanase and their yields can also be enhanced by optimizing the media components and various physical parameters such as pH, temperature and agitation or both. It was also found that simple sugars have catabolic repression whereas complex carbon and nitrogen substrate like casein, chicken feather, shrimp shell powder etc., might act as inducers for endoglucanase production. Therefore the current study is focused on Production and characterization of endoglucanase from Bacillus substills by using wheat bran as substrate.

KEYWORDS: Cellulase, Endoglucanase, Wheat Bran, Bacilus Substils.

INTRODUCTION

The global interest in cellulose conversion to energy and chemicals is not surprising since cellulose is the most abundant carbohydrate produced in the biosphere.[1] Cellulose can be converted to useful products by hydrolyzing with chemical, enzymatic or physical methods. It has been shown that the rate and extent of cellulose hydrolysis are influenced by the structural features of the cellulose substrate.[2] Cellulose in the form of cereal, grain residue, stalks, bagasse and saw dust is produced by photosynthesis. Such agricultural wastes are either thrown away or burnt which causes pollution in the environment. Conversion of agricultural residues to useful products is also an attractive option as a remedy for air pollution, energy production and other environmental concerns.[3] Furthermore, fast growing population of the world is becoming a permanent threat to the natural resources.[4][5]

Under such conditions we have to look for alternative strategies to meet our future energy demands. Therefore, efficient methods of recycling of waste materials into useful products must be found out. Cellulose biodegradation is mediated by several enzymes which have been extensively studied because they are secreted in large quantities. The development of microbial strains, media composition and process control has contributed in the achievement of high level of extracellular accumulation of cellulases for subsequent application in industry.[6]

Cellulases are also used in the manufacturing of pharmaceuticals, beverages, paper and textiles. Bacterial and fungal cellulases now days are used in animal feed industry, grain alcohol fermentation, brewing, malting and extraction of fruit and vegetable juices.[7]

MATERIALS AND METHODS

Microorganism's growth conditions and Inoculum preparation: ***Bacillus substilis*** was isolated from soil in Bioprocess laboratory,Global institute of biotechnology,Hyderabad.and wheat bran was collected from agriculture.Nutrient agar medium was used for the cultivation and maintainance of strains and incubated at 27 ± 1ºC for 48 hours,,0.25g of peptone was dissloved in 50 ml of distilled water.1gm of agar,015g of beef

extact,was added to the above solution and PH was adjusted to 7 and autoclave at 121ºc for 15 minutes,Pretreatment of the substrate was done as previously described [36].Air dried and milled wheat bran samples (10g) were soaked in 2.5 % (w/v) KOH, 1% (v/v) H_2SO_4, 3% (v/v) H_2O_2 solutions at the ratio of 1: 10 (solid: liquid) for 2hr at room temperature. After then the samples were autoclaved at 121°C for 15 min. Then samples were filtered and solid residues were washed up to neutrality. The production medim contained (g/l of distilled water): agar (carboxymethyl cellulose or CMC: 0.5g Beef extract: 0.05g, K_2HPO_4:0.1, $MgSO_4$:0.05 and NaCl: 0.1%, glucose: 0.1g; kcl: 0.1 g 100ml distil water pH9) and plates were incubated at 37^0 C for 24h. After 48hrs suitable volume of fermentation broth was centrifuged at 10000 x g for 5 min at 4^0C. Supernatant was collected in a sterile beaker and used as crude enzyme which is used for further studies.

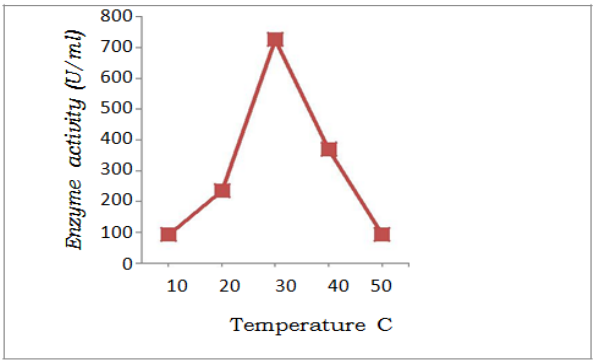
ANANLAYSIS OF RESULTS

Total protein content in supernatant was measured by the method of Lowry et al with bovine serum albumin (BSA) as the standard. The concentration of protein during purification studies was calculated from the absorbance at 594-596 nm.Enzyme extraction has performed by using Ammonium precipitation method,Dialysis, Sephadex G-100 Gel Filtration Chromatography.for the determiation of molecular weight of the compound Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) has performed

RESULTS AND DISCUSSION

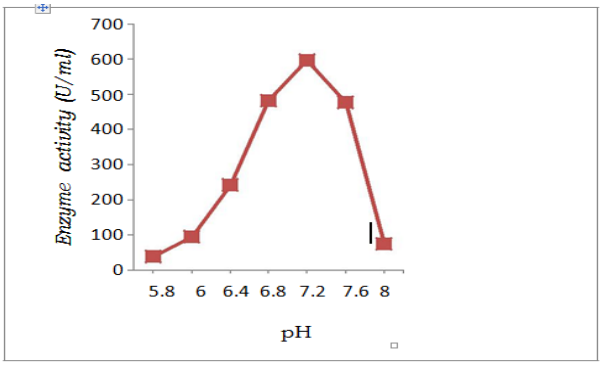
Effect of different parameters like Temperature, pH, substrate concentration, Metal ions concentration on endoglucnase production was determined.

Effect of temperature: The effect of different incubation temperatures of the reaction mixture on the purified Endoglucanase enzyme activity. This was performed by incubating the enzyme reaction mixtures at different temperatures viz: 10, 20, 30, 35, 40, 50 and 55^0C respectively.



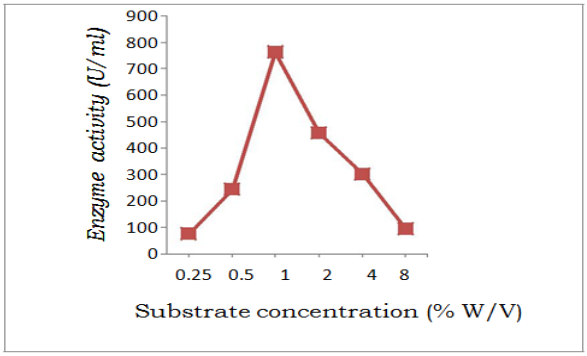
The results showed that the activity of Endoglucanase was increased up to 30^0C which represented the optimum temperature (specific activity was 726.1 for Endoglucanase). Beyond 30^0C the activity decreased by the increase of the temperature. However, the Endoglucanase was showed a responsible activity within the temperature range of 10-50^0C

Effect of pH: This experiment was performed in order to investigate the effect of different pH values on the purified Endoglucanase enzyme activities. The purified enzyme reaction mixture was incubated at different pH values via: 5.8, 6.0, 6.2, 6.4, 6.6, 6.8, 7.0, 7.2, 7.4, 7.6, 7.8 and 8.0 using 0.2M phosphate buffer. The enzyme activities for each case were determined.



The results revealed that the optimum pH for activity of Endoglucanase was attained at pH7.2 with enzyme activity of 597.3 U/ml. Beyond pH7.2 the activity decreased by the increase of the pH. However, the Endoglucanase was showed a responsible activity within the pH values ranged from 6.8 to 7.6.

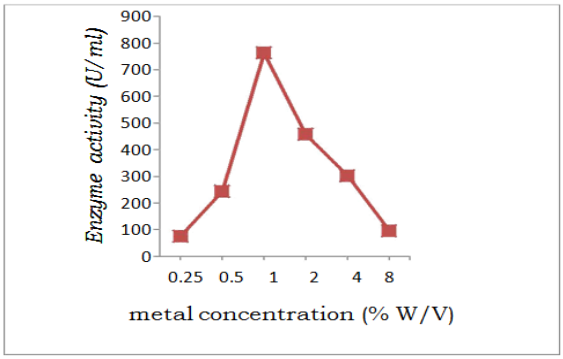
Effect of substrate concentration: This experiment was undertaken to investigate the effect of different substrate concentrations on the activity of the purified Endoglucanase. Carboxymethyl Cellulose was applied at the concentrations viz. (W/V, %) 0.25, 0.5, 1.0, 2.0, 5.0 and 10.0 respectively. Enzyme activities were determined



It is obvious from the results recorded in Table (6); the maximum Endoglucanase activity was obtained at 1% concentration of Carboxymethyl Cellulose (763.8 U/ml). A higher concentration of gelatin (more than 1%) showed a dramatically decrease in Endoglucanase activity while a lower concentrations e.g. 0.25 and 0.5% (W/V), the activity of Endoglucanase attained their maximum activities, beyond which no increase of

enzyme activity was recorded.

Effect of Metal ions concentration: This experiment was undertaken to investigate the effect of different metal ions on the activity of the purified Endoglucanase. Carboxymethyl Cellulose was applied at the concentrations viz. (W/V, %) 0.25, 0.5, 1.0, 2.0, 5.0 and 10.0 respectively. Enzyme activities were determined.



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CONCLUSION

It may be concluded from the results that ***Bacillus substilis*** a highly alkali tolerant and moderately thermo tolerant organism, is capable of successfully growing at highly alkaline pH (8–10) and at moderately elevated Temperature (45–55 °C). The organism produced a highly thermo active endoglucanase (80–100 °C), which has got activity over a broad range of pH (5–10) and temperature. Furthermore, the organism successfully utilized crude carbon sources such as wheat bran and produced much higher titer of endoglucanase than that reported on pure substrate. This reflects the future Potential of the organism for successful and economic Production of thermo stable and alkali stable endoglucanase. The relatively high thermal stability displayed by the endoglucanase is a valuable characteristic for its application in processes such as enzymatic hydrolysis of cellulose and lignocellulose for the production of glucose syrup. There is possibility that the organism has a potential to produce diverse types of cellulases with varying thermo stability and alkali stability. Further study of this enzyme may provide information about the molecular basis of stability and activity of cellulases at elevated temperatures and extremes of PH.

Based on the discussion above, the following conclusions are made: the optimum pH ofpurified enzyme was the 30^{o}C. The purified enzyme has optimum pH of 7.2 and this enzyme has ability to work in the pH ranges of 6.4-8.0. The purified enzyme has molecular weight of 56 kDa. Finally the maximum production of endoglucanase was at 1% Carboxymethyl Cellulose concentration.

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