ENZYMATIC KINETICS OF CELLULASES ISOLATED FROM SOIL BACTERIA OF DOON VALLEY, UTTARAKHAND

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ABSTRACT: Cellulases refers to a suite of enzymes produced chiefly by fungi, bacteria, and protozoans that catalyze cellulolysis which is the hydrolysis of cellulose. Cellulose is the most abundant natural polymer on earth. It is the structural component of the plant cell walls which helps in the hydrolysis of 1, 4-beta-D-glycosidic linkages in cellulose, lichenin and cereal beta-D-glucans. Cellulases are used for clarification of fruit juice, vegetable juice, roots, treatment of wine, extraction of oils and improving the quality of the bakery products. Eight soil samples were collected for cellulose preliminary screening from Gullarghati, Doon valley at different pH and temperatures, because maximum diversity was possible there as there was no interference by the humans. 110 colonies were isolated by the activity zone plate method containing CMC as a substrate using Congo red dye. Best twelve colonies were selected and checked using DNS method at 540 A°. Four strains BR-1, BR-2, BR-3 and BR-4 were used on the basis of spectrophotometrically and characterized with the study of substrate. Maximum velocity (V_{max}) was observed for BR-2 i.e. 170 units per mg protein with Km of 49.50mg/ml. Strain BR-1 gave to pH optima at 4.5 and 6.5, strain BR-2 gave maximum activity at 4.5 and 7.0 pH, BR-3 strain gave maximum activity at pH 5.0 and 6.5 with the highest yield of cellulases were obtained at pH 4.5, 5.5 and 7.0 in bacterial strain BR-4. The results also shows the effect of temperature bacterial strain BR-1, BR-2 and BR-4 with maximum cellulases activity at 45°C and bacterial strain BR-3 maximum activity at 25°C.

KEYWORDS: Cellulases, DNS, Enzyme, CMC.

INTRODUCTION: Cellulases an enzyme that specificity cleaves the internal b-1, 4-glycosydic bonds of cellulose and release glucose, cellobiose and cello-oligosaccharides depending on the characteristic of the enzyme.^(1,2) The endoglucanases in contrast to cellobiohydrolases can also hydrolyze substituted cellulose such as carboxy-methyl cellulose (CMC), hydroxy ethyl cellulose (HEC).

The best producing micro-organisms have been isolated and genetically improved.^(3,4,5,6) In nature, lignocellulose containing organic matter is converted in different phyco-chemical and biochemical degradation process into simple compounds.^(7,8,9) Cellulose is probably the most abundant biological compound on terrestrial earth.⁽¹⁰⁾

Cellulases are being studied increasingly due to their application in the hydrolysis of cellulose, the most abundant biopolymer and potential source of utilizable sugars, which serve as raw materials in the microbial production for a wide variety of chemicals, food and fuel.⁽¹¹⁾

Among thermophilic bacteria, all isolates showed proteolytic activity and 77% produced amylases, but no one produced cellulases or chitinases.⁽¹²⁾

So in this study we exclude thermophilic bacteria and rest strains were studied for further cellulase activity.

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MATERIALS AND METHODS: Eight soil samples were collected after making the serial dilution 10^{-4} & 10^{-5} . The dilution streaked on NAM (Nutrient Agar medium) and found 110 separate colonies and the culture were maintained on nutrient agar medium at 40°C . Active biomass for different strains were generated using with inoculums medium (Nutrient Broth) and this active biomass were checked for cellulose production using with production medium (NAM+CMC) & pH was adjusted at 7.0. Enzyme activity (Cellulase) was determined by using DNS method,⁽¹³⁾ as follows; 1ml enzyme solution was mixed with 1ml DNS reagent and boil the mixture for five minutes, then cooled. Optical Density was taken at 540 nm against the 1ml DNS mixed with 1ml distilled water as a blank.

Although a large number of microorganisms are capable of degrading cellulose, only a few of these produce significant quantities of cell free enzymes capable of completely hydrolyzing crystalline cellulose.

Based on enzyme activity using Spectrophotometrically screening was done and four best strains were selected i.e. BR-1, BR-2, BR-3, BR-4 and then characterization of cellulase enzyme activity was done for substrate concentration, effect of pH and effect of temperature.

RESULTS AND DISCUSSIONS:

Effect of Substrate Concentration: Initially the enzyme activity increased with increasing substrate concentration than the activity become independent of substrate concentration. The “Kinetic study of enzyme showed that the enzyme followed michaelian behaviour”. $V_{\max}$ for strain BR-1 was determined to be 94 unit/mg protein and K_m was 50mg/ml (Fig. 1), $V_{\max}$ for strain BR-2 was determined to be 170 unit/mg protein and K_m was 49.50 mg/ml (Fig. 2), $V_{\max}$ for strain BR-3 was determined to be 166 unit/mg protein and K_m was 50.50 mg/ml (Fig. 3) and $V_{\max}$ for strain BR-4 was determined to be 114 unit/mg protein and K_m was 49 mg/ml (Fig. 4).

Effect of pH: For determining optimum pH, a range of initial pH 4 to 9 of the medium tested, keeping the incubation temperature constant of 37°C . An acidic pH favored cellulase production while the yield was markedly reduced at pH 7.

Strain BR-1 gives two pH optima at 4.5 and 6.5 (Fig. 5), strain BR-2 gives maximum activity at 4.5 and 7.0 pH (Fig. 6). BR-3 strain gives highest yield of cellulases at pH 5.0, 6.5 (Fig. 7). The highest yield of cellulase was obtained at pH 4.5, 5.5 and 7 in bacterial strain BR-4 (Fig. 8).

Effect of temperature: The medium was adjusted to an initial optimum pH during optimization of incubation temperature. It was observed that three out of four bacterial strains (BR-1, BR-2 and BR-4) give maximum cellulase activity at 45°C (Fig. 9, 10 & 12) and bacterial strain BR-3 gives maximum activity at 25°C (Fig. 11).

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6

Fig. 7

Fig. 8

Fig. 9

Fig. 10

Fig. 11

Fig. 12

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