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ISOLATION AND KINETIC STUDY OF XYLANASE ENZYME

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ABSTRACT

Endo 1, 4- β -D-xylan xylanohydrolases (EC 3.2.1.8) are glycosyl hydrolases and are generally secreted by microorganisms that live by degrading plant biomass and are able to break down xylan from plant cell walls. Bacteria samples were collected from agriculture waste soil showing advantageous of short period of growth. The maximum enzyme activity found 480 µ mol / ml. min. and enzyme kinetic were done. The K_m and V_{max} values of xylanase were 2.6 (µ mol /mL) and 515 (µ mol / ml. min.) respectively.

Keywords: Xylanases, Isolation and Kinetics

I INTRODUCTION

Xylanases belongs to the group of hemicellulolytic enzymes which are required for the hydrolysis of ß-1,4-xylans present in lignocellulosic materials like pulps of various kinds. Commercial applications of xylanases especially in biobleaching of pulps require high specific activity, alkalophilic pH range of working and wide substrate specificity. Therefore, it is necessary to find a robust xylanase which can hydrolyze the biomass under extreme industrial processing conditions. Biobleaching involves the use of alkalophilic xylanase for removal of lignin during the process of paper making. It is an environment-friendly and economical alternative to the use of chlorine and chlorine compounds which generate carcinogenic, toxic and recalcitrant byproducts posing a threat to already dwindling ecosystems.

Biological bleaching of pulp with enzymes has shown immense potential in minimizing the use of chlorine-based bleaching chemicals and is mainly performed using hemicellulolytic and ligninolytic enzymes (Azeri et al., 2010). Bacteria provide many advantages over fungal systems for biobleaching of pulps. Xylanases (E.C.3.2.1.8) produced by bacteria are stable at alkaline pH and generally thermotolerant. On the other hand bacteria are fast growing as compared to fungi, which reduces the operation time and cost. In proposed study, alkalophilic xylanase producing bacterium will isolate from environment and later will apply on wheat straw rich soda pulp for delignification of wheat straw rich soda pulp.

Xylan, the major renewable hemicellulosic polysaccharide of plant cell walls, forms an interphase between lignin and other polysaccharides. It is a heteropolymer consisting of b-1,4-linked xylopyranose backbone with side-linked

groups namely arabinofuranosyl, acetyl and glucuronosyl residues. Its complete hydrolysis requires the cumulative action of endo-b- 1, 4-xylanase (EC 3.2.1.8), b-xylosidase (EC 3.2.1.37), and a series of enzymes that degrade side chain groups. Among these, the most important enzyme is endo-b-1, 4-xylanase, which cleaves the glycosidic bonds in the xylan backbone to produce xylo-oligosaccharides of various lengths and xylose (Polizeli et al. 2005). Xylanase has gained immense interest due to its biotechnological potential in xylitol and ethanol production, paper industry, production of xylo-oligosaccharides, food industry, textile industry, animal feed industry, etc. (Dhiman et al. 2008; Kuhad and Singh 1993; Sharma and Kumar 2013). Xylanases used in industry are produced mainly from bacteria and fungi. The wide-scale industrial applications of xylanase require cost-effective production of the enzyme to make the process economical. This can partly be achieved by using cheaply available agro-industrial residues such as wheat bran (Azeri et al. 2010; Mittal et al., 2012). The optimization of culture medium is a pre-requisite for successful use of microorganisms in industrial biotechnology because its composition can significantly affect the product yield. Wheat straw, an abundant low value by-product of wheat production worldwide, is an attractive lignocellulosic material for paper production. Its main constituents are 35–45 % cellulose, 20–30 % hemicelluloses and 8–15 % lignin (Saha et al. 2005) and its composition may vary depending on the wheat species, soil, climate conditions, etc.

II MATERIALS AND METHODS

Media composition for isolation of xylanase producing bacteria

The minimal media composition will use as Na_2HPO_4 0.7%, KH_2PO_4 0.3%, NH_4Cl 0.1%, NaCl 0.5%, 0.1% yeast extract, 0.1% tryptone and the pH will be adjusted to 8.0 with 10% Na_2CO_3.

Screening of xylanase producing bacteria

Soil sample (1g) will suspended in 10 mL of sterile saline ($9gL^{-1}$), mixed uniformly, and allowed to settle. The serially diluted samples will be steraking on agar plates contained minimal media constituted with 1% Birchwood xylan. Single colonies will be chosen among those, which will produce clear zone and will be purified.

Xylanase enzyme activity by 3, 5-dinitrosalicylic acid (DNS) method

Xylanase activity was determined using xylan as a substrate. 50 µl of xylan solution (10 mg/ml) was added to 100 µl of enzyme solution and 150 µl of 50 mM sodium acetate buffer (pH 4.5). After incubating for 30 min at 70°C, 200 µl of samples or xylan standards was added to a test tube containing 1 ml of DNS reagent; 800 µl of deionized water was added and left in boiling water bath for 10 min, cooled down at room temperature and 1 ml of 40% potassium sodium tartarate and 1 ml of deionized water was added and the absorbance was read at 540 nm and standard curve drawn. One unit of enzyme activity was defined as the amount of enzyme required to liberate 1 µmole of the reducing sugar as xylose from xylan per min.

Ammonium sulphate precipitation of xylanase

Cell-free supernatant will saturate with 50% ammonium sulphate at 5ºC and will left for 3h to assure a proper precipitation of protein. Precipitates of protein will separated by cold centrifugation at 10000 rpm for 30 min and

later dissolved in a minimal amount of phosphate buffer of optimum pH. The dissolved protein precipitates will dialyze against same buffer for 15-24h.

III RESULTS

a) Isolation of xylanase producing bacteria

For the preliminary experiment of this study, bacteria samples were collected from agriculture waste soil. Xylanolytic clear and transparent zone (Fig. 1: Xylan agar plate) producing bacterial strains were collected and incubated at 37°C for 48 hr.

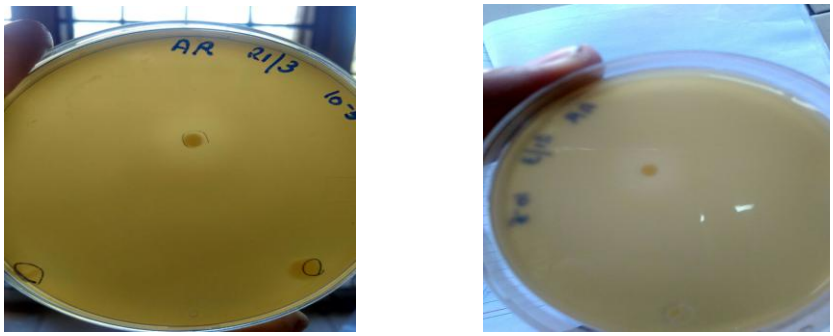

Fig. (1): Plate showing zone of clearance around colony by isolate from agriculture waste soil

b) Enzyme activity of xylanases (crude) enzyme

Xylanase from isolate from agriculture waste soil inoculated at optimized media, after 48 hr it is centrifuged at 10000 RPM for 15 min. The supernatant crude enzyme was partially purified with ammonium sulphate method. It was found to be maximum at optimum condition (Temperature 40 °C and pH 7.5) is 480 (µ mol / ml. min.).

c) Determination of Vmax and Km for xylanase enzyme

The kinetic parameters K_m and V_{max} of the enzyme were determined from lineweaver-Burk double - reciprocal plots of xylanase activity at 45°C using various concentrations of xylan as substrate. The K_m and V_{max} values of xylanase were 2.6 (µ mol /mL) and 515 (µ mol / ml. min.) respectively.

IV CONCLUSION

In this study bacterial strains were used form soil xylanase enzyme production which has advantage of short period of growth and produce at optimum condition and kinetic of enzyme were done.

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