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RESEARCH ARTICLE

ROLE OF NITROGEN SOURCE FOR THE PRODUCTION OF XYLANASE FROM *ASPERGILLUS* SP

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ABSTRACT

Recently, xylanases have expanded their use in many processing industries, such as pulp and paper, food and textile to newer needs such as biofuel production. .This study were taken up to the enhance the biosynthesis of xylanase by supplementation of organic nitrogen and inorganic nitrogen sources were employed in range of 0.25% to 1.0%. The organic nitrogen source were supplemented are peptone, yeast extract and beef extract and inorganic nitrogen sources are ammonium Sulphate and ammonium chloride. The beef extract and ammonium nitrate were yielded higher xylnase production and showed 7.5 IU and 8.46 IU

Key words: Xylanase, submerged fermentation, xylose, fermentation kinetics and inoculums size

INTRODUCTION

Xylanases show great potential for industrial applications mainly for the bioconversion of lignocelluloses to sugar, ethanol, and other useful substances, clarification of juices and wines, improving the nutritional quality of silage and green feed and the de-inking processes of waste papers [1]. The interest in xylan degrading enzyme and its application in the pulp and paper industries had advanced significantly over the past few years [2-3]. Haltrich et al.[4] gave an overview of fungal xylanases and showed that the enzyme can be produced by a number of micro-organisms including bacteria, yeasts and filamentous fungi including *Trichoderma*, *Bacillus*, *Cryptococcus*, *Aspergillus*, *Penicillium*, *Aureobasidium*, *Fusarium*, *Chaetomium*, *Phanerochaete*, *Rhizomucor*, *Humicola*, *Talaromyces* etc. These enzymes havebeen widely detected in fungi and bacteria [5-7].

Filamentous fungi are attracting greater attention than bacteria as potential sources of plant cell wall hydrolyzing enzymes such as xylanases because they secrete high levels of the enzymes into the culture medium [8]. Filamentous fungi are useful producer of xylanases from the industrial point of view. The reasons are many fold - they are non pathogenic, capable of producing high levels of extra cellular enzymes and they can be cultivated very easily.

The aim of the present study involves the process should economize by using carbon nitrogen and metal ion sources. The enhanced production of xylanase by using *Aspergillus* sp through submerged fermentation by using defined medium.

MATERIALS AND METHODS

Fungal strain

The *Aspergillus* strains were isolated from different soils. Soils are taken from different regions from in and around Bangalore and tentatively identified in the laboratory.

Screening of xylanase producers and Fermentation Medium

Aspergillus strains were screened for their xylanase activity by plate assay [9] and among the thirty isolates, *Aspergillu* sp 5 were used for further studies. The selected *Aspergillus*sp5were cultured on production medium. The production medium consists (mg/100 ml) of sucrose 3, di potassium hydrogen phosphate0.1, $MgSO_4$,0.05g, KCl 0.05g, NaCl, 0.01%, $FeSO_4$.The condition of the fermentation medium is as fallows .pH,6 temperature 30^0C and inoculums size is of 0.5 ml.

Influence of Organic and inorganic nitrogen source for the biosynthesis of Xylanase

The organic and inorganic nitrogen sources were supplement in the range of 0.25% to 1.0% with an increment of 0.25%.The organic nitrogen source are yeast extract, peptone and inorganic nitrogen source were supplemented to the production medium with optimized conditions. Peptone, Yeast extract and Beef

extract were used as organic nitrogen sources and inorganic nitrogen sources are.

Extraction of Xylanase

The samples were withdrawn periodically at 24 hrs in aseptic condition. The extract was filtered through Whatman filter No.1. The clear extract was centrifuged at 2000-3000 rpm for 15 min, supernatant were used as enzyme preparation. Thus prepared crude enzyme was used for assay of xylanase.

Assay of Xylanase

The xylanase activity was determined by measuring the release of reduced sugars from oat spelt Xylan (1% w/v) by dinitrosalicylic acid method (Miller, 1959). The enzyme solution (0.5 ml) and 0.5 substrate (xylan 1% w/v) along with 1 ml of buffer were taken in a test tube, the tubes were then allowed to stand at room temperature for 10 mins, 3ml of dinitrosalicylic acid was added to arrest the reaction. After the addition of dinitro-salicylic acid, the tubes were placed in boiling water bath for 10 min. The color which had developed was read colorimetrically at 540nm. A blank test tube was prepared by adding dinitrosalicylic acid prior to the addition of enzyme to the test tubes.

International unit (IU)

One unit of xylanase was defined as the amount of enzyme required to release 1µmol of xylose from oat spelt xylan in one minute under standard assay conditions.

RESULTS AND DISCUSSION

Thirty *Aspergillus* isolates were isolated from different soil samples from Bangalore. All thirty isolates were named serially *Aspergillus* KSN1-KSN30and used for screening of xylanase production by plate assay method. Out of thirty isolates *Aspergillus* Sp KSN 5were showed maximum enzyme hydrolytic zone were observed. It showed around 0.07cm zone of clearance observed.

Fungal isolates were identified as *Aspergillus* Sp identified in the laboratory. All thirty strains of *Aspergillus* sp produced enzyme hydrolytic zone on xylan plate medium; those were selected from the soil sample. Of the thirty isolates *Aspergillus* sp 5was considered to be the best and high xylanase producing strain. It showed 0.77 cm of hydrolytic zone around the colony.

The organic and inorganic nitrogens were used for the production xylanase through submerged fermentation. Among the organic nitrogen source peptone, yeast extract and beef and inorganic nitrogen ammonium Sulphate and ammonium chloride were used. The concentration was used in the range of 0.25 to 1.0%.Among the organic nitrogen source beef extract showed
the better source for the synthesis of xyalanase and it sho wed 7.5 IU as highest xylanase and 2.8IU is the lowest biosynthesis of xylanase at 72 hrs of fermentation. The peptone and yeast extract were showed 6.6 IU and 5.8IU at 0.75% at 72 hrs of fermentation periods and graphical representation were showed in Fig 1- 3 for organic sources.

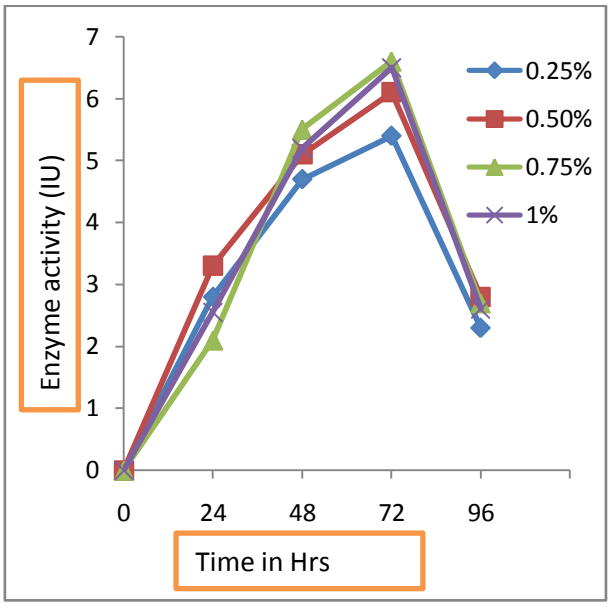

Figure 1: Effect of peptone on biosynthesis of Xylanase

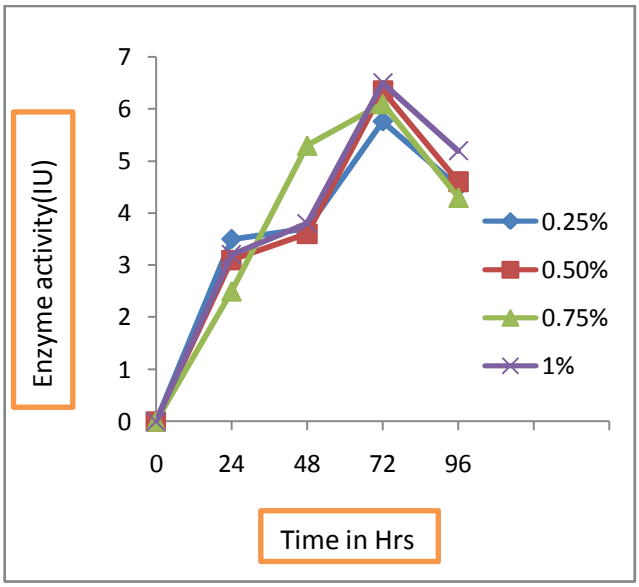

Figure 2: Effect of yeast extract on biosynthesis of Xylanase

The inorganic nitrogen sources are Ammonium Sulphate and Ammonium chloride were used and Fig 4 and 5 represented the xylanase production. Ammonium chloride showed 8.46 IU showed maximum xylanase at 0.5% and lowest xylanase at 5.12IU at 72 hr fermentation period. The ammonium Sulphate also showed 5.6IU xylanase synthesis was found and 2.20 IU is the lowest enzyme production at 72 hr of fermentation period..

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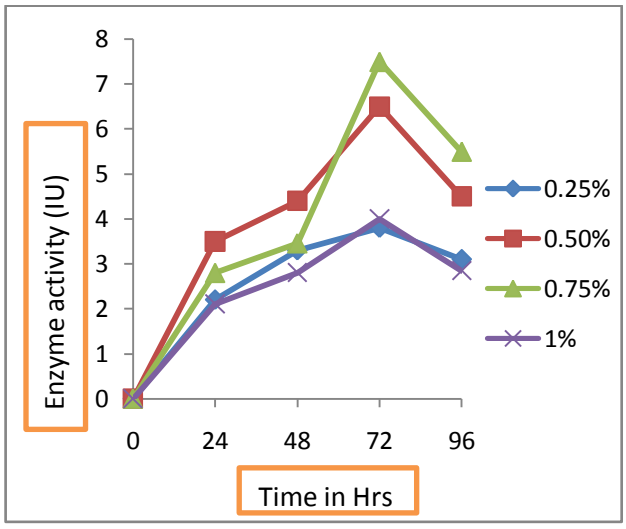

Figure 3: Effect of beef extract on biosynthesis of Xylanase

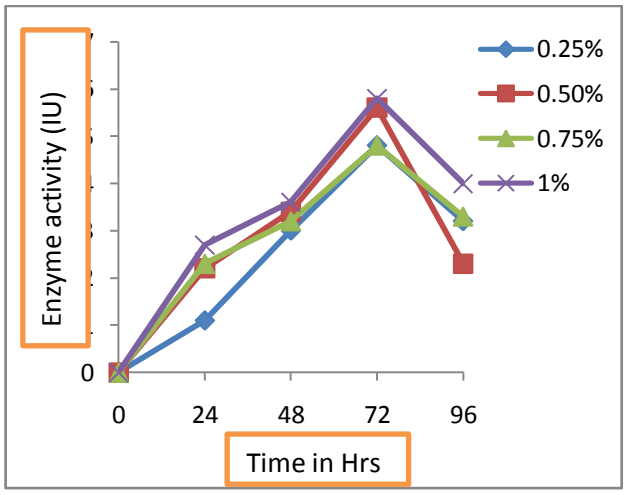

Figure 4: Effect of Ammonium sulphate on biosynthesis of Xylanase

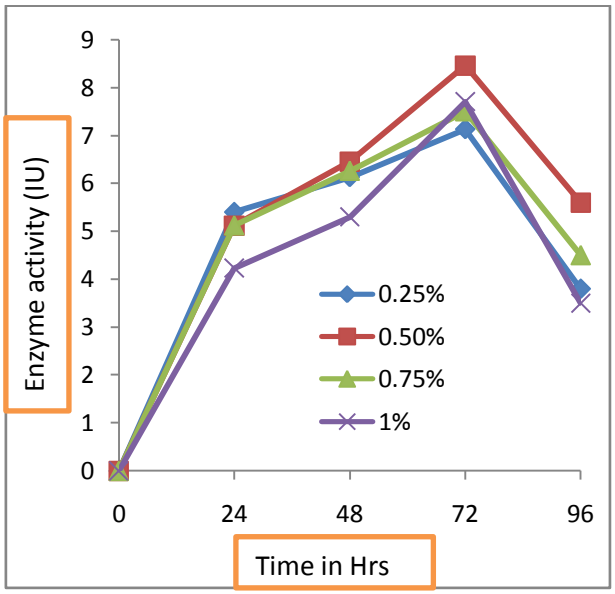

Figure 5: Effect of Ammonium chloride on biosynthesis of Xylanase

Kanimozhi and Nagalakshmi[11]were studied the organic and inorganic nitrogen sources for xylnase synthesis by using *Aspergillus niger* through solid state fermentation and concentration is 0.075%. Xiuting Li et al.,[12] were highlighted organic and inorganic nitrogen source at the rate of 1.5% by using *Streptomyces chartreusis*. Here our results were stimulated on the biosynthesis of xylanase were found and our results were coincides the results of Kanimozhi and Nagalakshmi[11].

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