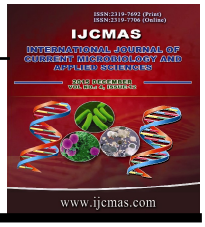
Int.J.Curr.Microbiol.App.Sci (2015) 4(12): 815-819

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 12 (2015) pp. 815-819**
http://www.ijcmas.com



Original Research Article

Influence of Metal Source for the production of Xylanase from *Penicillium citrinum*

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A B S T R A C T

K e y w o r d s

Xylanase, Submerged fermentation, Xylose, fermentation kinetics and Metal ions

Xylan is the major constituent of hemicellulose and is the second most abundant polysaccharide in the cell walls of land plants, representing up to 30–35% of the total dry weight. Biotechnological uses and potential applications of xylanases include bioconversion of lignocelluloses material to fermentative products in the Kraft process for the removal of the lignin-carbohydrate complexes This study were taken up to the enhance the biosynthesis of xylanase by supplementation of metal sources were employed in range of 0.01% to 0.03%. The metal source were supplemented are Magnesium sulphate, manganous sulphate, copper sulphate and zinc sulphate. The Magnesium sulphate, manganous sulphate were yielded higher xylnase production and showed 8.79 IU and 7.23 IU.

Introduction

The enzyme, xylanase are the upcoming enzyme of commercial sector and are widely used in paper and pulp industry, animal feed, textile industry, coffee and tea fermentation, oil extraction, waste paper recycling and in the fruit juice industries**.** This enzyme are the tools of nature that help us in providing everyday products in an environmentally conscious manner.

Paper and pulp industry is one of the major sources of pollution, generating large volumes of intensely colored effluent for each metric ton of paper produced (Ali and Sreekrishnan, 2001). In the paper production process, pulping is a step where cellulose fibers are broken apart and lignin is removed by using chlorine. This step even though necessary, is the prime cause of pollution as elemental chlorine reacts with lignin to form chlorinated lignin derivatives such as chlorolignols, dioxins and sulfur compounds. In addition, other organic matter in the pulp reacts with chlorine to form adsorbable organic halides which are carcinogenic, toxic and recalcitrant to degradation (Ali and Sreekrishnan, 2001). Owing to this problem and strict governmental regulation, more companies are investigating alternative methods such as biobleaching, hydrogen peroxide or oxygen based delignification.

Xylanases are hydrolases depolymerising the plant cell wall component-xylan, the second most abundant polysaccharide. The Molecular structure and hydrolytic pattern of the xylanases have been reported extensively and mechanism of hydrolysis has also been proposed (Subramaniyan and Prema, 2002).

Many different microbial genera, ranging from bacteria to fungi, have been found to produce one or several xylanases (Balaa *et al.,* 2006). Fungal species known to produce xylanase include *Aspergillus, Disporotrichum, Pencillium, Neurospora, Fusarium, Trichoderma,* etc. (Kulkarni *et al.,* 1999). Filamentus fungi have been used for more than 50 years in the production of industrial enzymes (Dalboge, 1997). They are particularly interesting producers of xylanases and excrete much higher xylanolytic enzymes into the medium than bacteria or yeast (Li *et al.,* 2006).

The importance of xylanase production, we made an effort to produce xylanase from *Penicillium citrinum* through submerged fermentation, achieved an enhanced level production of xylanase by supplementation metal ion source.

Materials and Methods

Fungal Strain

The *Penicillium citrinum* 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

Penicillium citrinum strains were screened for their xylanase activity by plate assay (Dhulappa & Lingappa, 2013) and among the thirty isolates, *Penicillium citrinum* KGSN 05 were used for further studies. The selected *Penicillium citrinum* KGSN 05 were confirmed at molecular level in next steps.

Influence of Carbon Source for the Biosynthesis of Xylanase

A set of conical flasks with 100 ml of production medium supplemented with a particular carbon source with concentrations ranging from 0.01% to 0.03% with increments of 0.01%. The different metal sources like, magnesium sulphate, manganous sulphate, copper sulphate were used under the present study.

The production medium consists (mg/100 ml) of sucrose 3, di potassium hydrogen phosphate 0.1, $MgSO_4$,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.

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 and Gail Lorenz. 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, and 3ml of dinitrosalicylic acid was added to arrest the reaction. After the addition of dinitrosalicylic acid, the tubes were placed in boiling water bath for 10 min. The color which had developed was read 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 *Penicillium citrinum* isolates were isolated from different soil samples from Bangalore. All thirty isolates were named serially *Aspergillus* KSN1-KSN30 and used for screening of xylanase production by plate assay method. Out of thirty isolates *Penicillium citrinum* KGSN 05 were showed maximum enzyme hydrolytic zone were observed.

Fig.1 Effect of MgSO4 on Xylanase Production

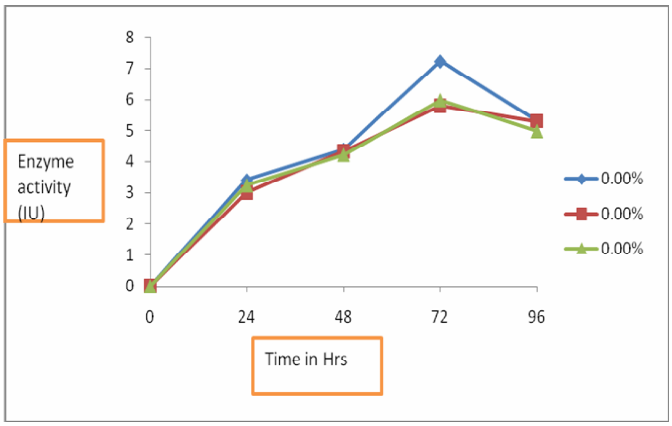


Fig.2 Effect of MnSO4 on Xylanase Production

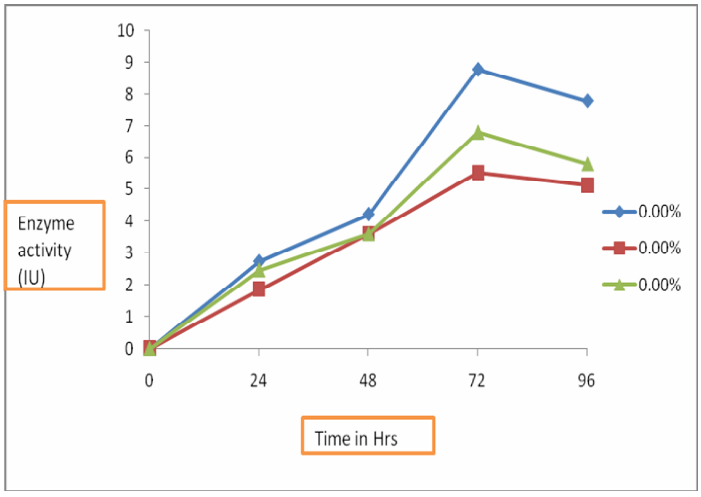
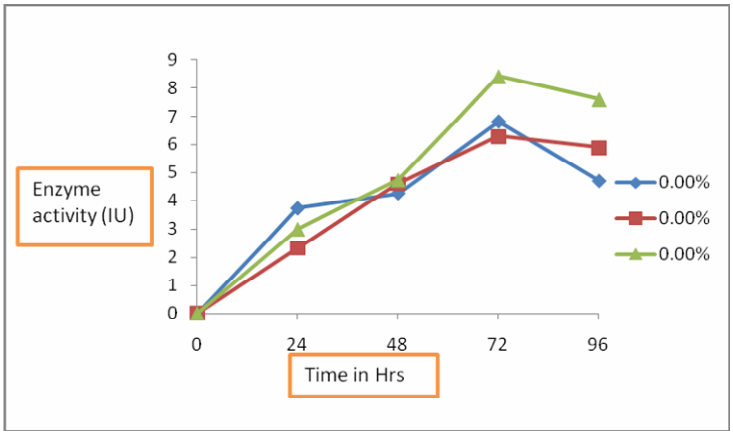


Fig.3 Effect of CuSO4 on Xylanase Production



The results on the studies pertaining to the production of xylanase by *Penicillium citrinum* KGSN 05 on synthetic medium supplemented with different concentrations of various metal sources like magnesium sulphate, manganous sulphate, copper sulphate are presented in Fig-1, Fig-2 and Fig-3 respectively.

The process economization for xylanase production with metal sources supplemented to the production medium were carried out with concentration of 0.01%, 0.02% and 00.03. The additions of metal ions were ions were done for Manganous sulphate and Magnesium sulphate acted as best source of metal ions for the production of xylanase at 0.01% at 72hrs of fermentation period, the enzyme production observed was 8.79 IU and 7.23 IU respectively. Copper sulphate were less inducers of Xylanase production and the enzyme production observed was 6.82 IU respectively at 0.01% for 72 hrs of fermentation period.

Asish Manda.(2015) suggest that extracellular xylanase activity in this *Bacillus cereus* BSA1 is induced by xylan and xylose (up to 0.5%). The presence of NaCl in culture media mostly stimulated enzyme production and this might develop a particular membrane potential that favoured enzyme release from the cell. Studies have shown interest on the unique structural and biochemical characteristics of exoenzyme regarding their relation with salt (Lee et al., 2006) and their potentialities in many industrial applications (Ventosa and Nieto, 1995). Decrease in xylanase production in presence of Hg++ ion may be nonspecific binding or aggregation of this ion with some essential enzymes. They may also cause a reduction in catalytic activity due to partial denaturation of enzyme (Tunga et al., 1999). Our results are coincides with the Ana Asish Manda. (2015).

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