INTERNATIONAL JOURNAL OF SCIENCE AND NATURE

www.scienceandnature.org

ISOLATION, SCREENING AND PRODUCTION OF XYLANASE FROM *ASPERGILLUS* SP.

[1]Naveen, M., [1]Saroj Yadav & [2]Siddalingeshwara, K. G.
[1]Research and Development Centre, Bharathiar University, Coimbatore.
[2]Department of Studies in Microbiology, Padmshree Institute of Information Science, Nagarabhavi Circle, Bangalore.

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 investigates the production of extracellular xylanase synthesis were carried out by using *Aspergillus sp.* were evaluated under different fermentation parameters by employing submerged fermentation method. The xylanase producers detected by the enzyme hydrolytic zone around the colony by simple plate assay method. *Aspergillus sp.* 05 is the potential strain among the fungal isolates. The xylanase synthesis were increased their yield after the optimization of fermentation parameters. The optimum pH 6.0, temperature 30°C and inoculum size 0.75 ml and it showed 5.53 IU.

KEY WORDS: Xylanase, submerged fermentation, xylose, fermentation kinetics and inoculums size.

INTRODUCTION

There are several applications of xylanases in industry (Subramaniyan and Prema 2002). Currently, the major applications of xylanases are in pulp and paper, feed, and baking industries. Xylanases are used in the prebleaching of kraft pulp to reduce the use of harsh chemicals in the subsequent chemical bleaching stages. The enzymatic treatments improve the chemical liberation of lignin by hydrolyzing residual xylan. This reduces the need for chlorine-based bleaching chemicals, which is beneficial for the environment (Beg *et al*., 2001). In feed formulations, cooperation of xylanases, glucanases, proteinases and amylases reduces viscosity of the feed and increases the adsorption of nutrients. Enzymes liberate nutrients either by hydrolysis of non-degradable fibers or by liberating nutrients blocked by these fibers. In the food industry, xylanases are used to improve the dough properties and baking quality of bread and other baked goods by breaking down the polysaccharides in the dough. The enzyme treatment has favorable effects on dough handing, bread volume, texture and stability (Bhat and Hazlewood, 2001). Filamentous fungi are particularly useful producers of xylanases from the industrial point of view, due to the high production level and extra cellular secretion of enzymes, as well as relative ease of cultivation. In general, xylanase activity levels from fungal cultures are typically much higher than those from yeasts or bacteria (Paloheimo *et al*., 2003). The aim of the present study was to describe the extracellular xylanase produced by *Aspergillus sp.* were used *to* screen and optimization of fermentation kinetics through submerged fermentation.

MATERIALS & METHODS

Chemicals

Xylan used in the study was procured from Hi-Media Laboratories, Bombay, India; the other ingredients used for the preparation of CzapekDox's media were also products of Hi-Media Laboratories, Bombay.

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 and Fermentation Medium

Aspergillus strains were screened for their xylanase activity by plate assay (Dhulappa and Lingppa, 2013) (Plate-1) and among the thirty isolates, *Aspergillu* sp. 5 were used for further studies. The selected *Aspergillus* sp. 5were cultured on production medium. 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%, $FeSO_4$

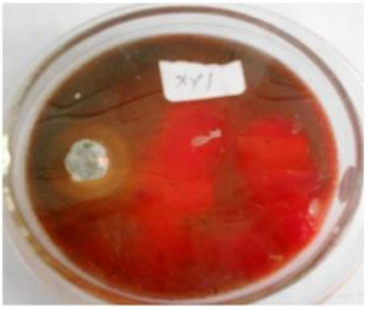

Plate-1. Xylanase hydrolytic Zone

Optimization Studies

The 250 ml Erlenmeyer flasks containing 100 ml of production medium were prepared by mixed with acid/alkali solution to obtain required pH. The pH was adjusted in the range of 3-7 with increments of 1.0. Thus prepared flasks were cotton plugged and autoclaved at 121ºC for 15 min. The flasks were inoculated and incubated. The 100ml of the production medium was separately taken in 250 ml Erlenmeyer flasks and prepared for submerged fermentation. Thus prepared flasks were incubated at different temperatures like 25-40ºC with in increments of 5º C. The inoculum was prepared separately by reviving the 168 h old culture of *Aspergillus* sp 05 at different levels *i.e*., 0.25, 0.50, 0.75, 1.0 and 1.25 ml and then fermentation studies were carried out.

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 dinitrosalicylic 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 & DISCUSSION

Thirty *Aspergillus* 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 *Aspergillus* Sp KSN 5 were 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 5 was considered to be the best and high xylanase producing strain. It showed 0.77 cm of hydrolytic zone around the colony. The data obtained in the present study on the effect of pH and temperature on submerged fermentation is shown in (Fig. 1 and 2) which reveals that the production of xylanase increased with the increase in the pH of the medium up to pH 6.0 temperatures 30ºC and thereafter the decrease of xylanase was observed.

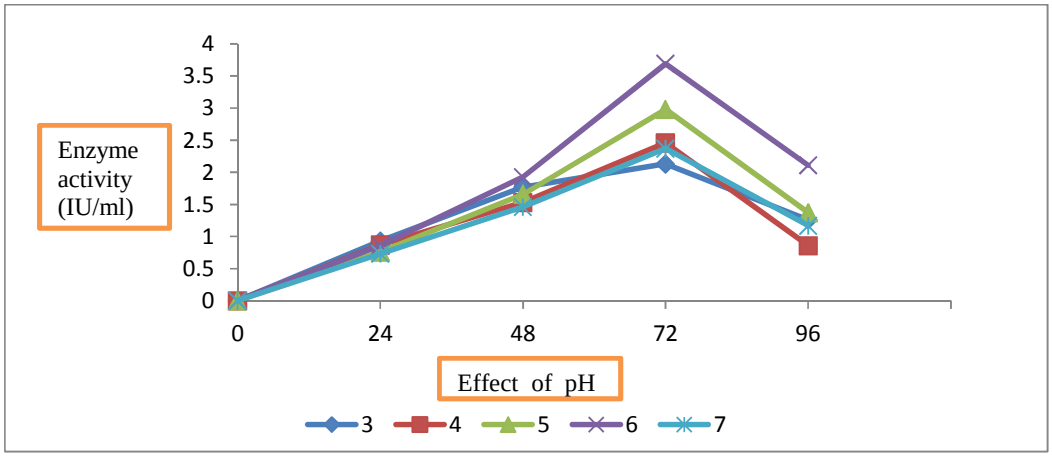

FIGURE1. Effect of pH on xylanase production

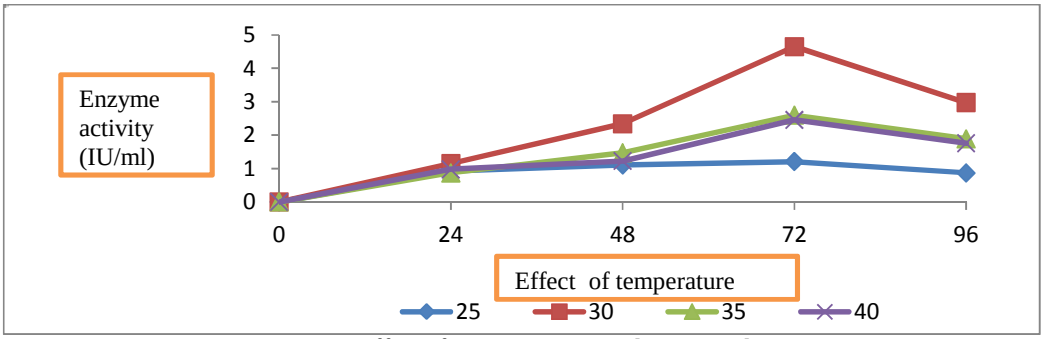

FIGURE 2. Effect of temperature on xylanase production

The maximum production of xylanase is 3.69 IU was obtained at pH 5.0 and the minimum production of xylanase 1.13 IU was observed at pH 7.0. The production of xylanae increased significantly with the increase in fermentation temperature from 25-350ºC and decreased above 30ºC. The maximum xylanase production obtained at 30ºC was 4.65 IU and the least production was observed at 25ºC resulted only 1.21 IU of xylanase at 72 hrs of fermentation period. Any temperature beyond the optimum range is found to have some adverse effect on the metabolic activities of the microorganisms and it is also reported by various scientists that the metabolic activities of the microbes become slow at lower or higher temperature (Okolo *et al*., 1995). The pH of the medium is one of the most critical environmental parameter affecting the mycelial growth, enzyme production and the transport of various components across the cell membrane (Kapoor *et al*., 2008). In our study, the data revealed that the pH of 6.0 was found as suitable for maximum production of xylanase with *Aspergillus* sp. KSN 5 strain under submerged fermentation. Fungal strains are noted for their best performance in the range of 3.5-7.0 and also low pH avoids the contamination by other microbes (Pandey et al., 2001). Muthezhilan *et al*. (2007) reported that pH 8 is the optimum for maximum xylanase production. Padmavathi and kavya (2011) were reported pH 8 is the optimum pH for maximum production of xylanase. Our findings are in close agreement with the earlier findings of Muthezhilan *et al*. (2007) they showed that pH 8 was the suitable for maximum xylanase production. Incubation temperature dependent variation in xylanase production was reported by Muthezhilan *et al*. (2007) Keeping this in view, experiments were conducted to understand the effect of temperature on xylanase production by *Aspergillus* sp. KSN 5. The present study revealed that the 30 ºC is suitable and maximum production of xylanase with *Aspergillus* sp. KSN 5. Muthezhilan *et al*. (2007) reported that the maximum production of xylanase was observed at temperature 45 °C by using *Penicillim. Oxalicum* and it showed 3.79 U/ ml, similar observations were reported for xylanase from thermophilic fungi, such as *Thermomyces lanuginosus* (Purkarthofer *et al*., 1993). As such our findings are close agreement with Muthezhilan *et al*. (2007). Importance of inoculum size on microbial fermentation process is widely accepted. Out of five inoculum size tested (0.25, 0.50, 0.75, 1.0 and 1.25 ml) and 0.75 ml inoculum was found to be the most suitable for high production of xylanase by *Aspergillus* sp. KSN 5 in submerged fermentation at 72 hrs of fermentation. From Fig. 3, it is clear that the xylanase production steadily increased with the increasing in the size of the inoculum until it reaches to the magnitude

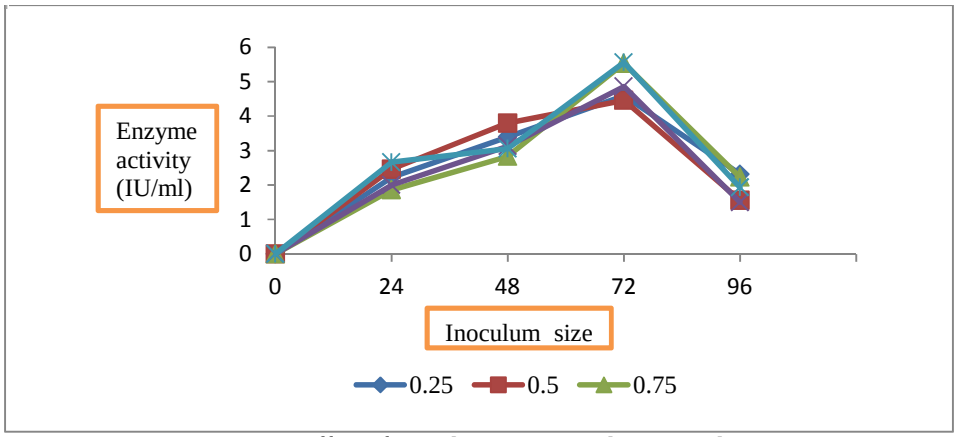

FIGURE 3. Effect of inoculums size on xylanase production

When enzyme productivity became maximum, thereafter no appreciable change in production of xylanase with high inoculum size could be observed. The maximum enzyme activity was showed at 5.53 IU. at 0.75 ml inoculum size and least enzyme activity 2.31 IU was showed at 0.25 ml of inoculum size. Muthezhilan *et al.* (2007) reported that 2ml of fungal spores as an inoculums from one week old culture were inoculated for the maximum production of xylanase. Suprabha *et al*. (2008) were showed 1 ml of spore suspension containing $1x10^6$ spores/ ml was the inoculums used for the production of fungal xylanase.

REFERENCES

Beg, Q.K., Kappor, M., and Mahajan, L. (2001) Applied Microbiology and Biotechnology. 56, 326- 338.

Bhat, M. K. and Hazlewood, G. P. (2001) Enzymes in Farm Animal Nutrition, In: Bedford M.R. and Partridge CC (eds.), CAB International, Wallingford, UK.

Damaso, M.C.T., Almeida, M. S. and Kurtenbach, E. (2003) Applied Environmental Microbiology. 69, 6064-6072.

Dhulappa, A. and Lingappa, K. (2013) Xylanase screening and biosynthesis from *Aspergillus tamari*. *Int. J. Curr. Microbiol. App. Sci*. 2(7), 79-83.

Kapoor, M., Nair, L.M., and Kuhad, R.C. (2008) Cost effective xylanase production from free and immobilized *Bacillus pumilus* strain MK001 and its application in saccharification of *Prosopis juliflora*. Biochem.Eng. J.38, 88–97.

Muthezhilan, R., Ashok, and Jayalakshmi, S. (2007) Production and optimization of thermostable alkaline xylanase by *Penicillium oxalicum* in solid state fermentation. African Journal of Microbiology Research pp. 020-028,

Miller, and Gail Lorenz, (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar". Anal. Chem. 31 (3), 426-428.

Okolo, B.N., Ezeogu, L.I., Mba, C.N. (1995) Production of raw starch digestive amylase by *Aspergillus niger* grown on native starch sources. Journal of Science of Food and Agriculture. 69, 109–115.

Purkarthofer, H., Sinner, M., Steiner, W. (1993) Cellulase-free xylanase from *Thermomyces lanuginosus*: optimization of production in submerged and solid-state culture. Enzyme Microbiol. Technol. 15, 677-682.

Paloheimo, M., A. Mäntyla; A. Kallio, J. (2003) Applied Environmental Microbiology. 69, 7073-7082.

Pandey, A., Soccol, C.R., and Roudriguez- Leon, J. A., Nigam, P. (2001) In Solid State Fermentation in Biotechnology Fundamentals and Applications. Asiatech Publishers. New Delhi.

Subramaniyan, S. and Prema, P. (2002) Critical Review of Biotechnology. 22, 33–64.