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Screening studies for efficient xylanolytic isolates from diverse sources

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ABSTRACT: Xylanase (EC 3.2.1.8) enzyme degrades the xylan backbone into small oligomers. Xylanases have been reported mainly from Bacteria, Actinomycetes, Yeasts and Fungi. Xylanase is an important commercial enzyme which can be used for bleaching the kraft pulp, in bread making, in clarification of fruit juices and conversion of xylan rich lignocellulosic materials to D-xylose, which can be used further to produce a number of bioproducts with a great aggregate value. The microbial xylanases are also used in improving the nutritional quality of animal (poultry/pig/fish) feed and so is mostly added either in isolation or in multi-enzyme complexes for use in pre-processing of diet as food additives to animal feed. Plant ingredients have so far been the most cost effective alternative for fish in aquafeed industry but they have an increased dietary content of indigestible carbohydrates called non starch polysaccharides (NSP) that dilute the energy concentration of feeds by reducing digestibility of nutrients. Xylanase acts as NSP enzyme which hydrolyses cell wall components in plants thus efficiently reducing NSP content in plant ingredients. In present study xylanase producing Bacteria and Fungi belonging to *Bacillus sp.*,and *Aspergillus sp.*,*Trichoderma sp.*, *Saccharomyces sp.*,respectively were isolated from various xylan rich sources using enrichment culture technique. From the different isolates efficient xylanolytic primary soil isolates were screened using chromogenic plate assay method with Congo red as chromogen. Two potential isolates from different sources identified morphologically and biochemically as belonging to *Aspergillus sp* were selected for further study. The xylanase production ability for these two selected *Aspergillus sp* was checked using different xylan rich agro wastes like saw dust, sugar cane bagasse, hay, corn cob and cotton seed extract. This feature holds great promise as low cost cheap raw materials could be used for commercial production of xylanase enzyme.

KEYWORDS: Xylanases, Xylose, Raw xylan Source, Chromogen, *Aspergillus sps.*

I. INTRODUCTION

Xylan is widely distributed in plant cells and forms main part of the hemi cellulose fraction next to cellulose which displays a large polydiversity. Most higher plants and agricultural wastes contain xylan in variable proportion ranging from 20-40% of the dry weight. It has been estimated that 500 million tons of such materials could be annually available from the residues of major crops and as leaf litter. Effective extraction by enzymatic and microbio- logical processes of these materials is of great interest not only for the utilization of wastes for the production of value added products [1] but also degradation of wastes to minimize pollution. Enzymatic hydrolysis of xylan to xylose is catalysed by endo-1, 4-β-xylanase and β-xylosidase, the former randomly hydrolyzing xylan to xylo-oligomers and the latter producing xylose from xylo-oligomers [2,3]. Interest in xylanase has grown markedly during recent years because of their potential applications in the bleaching of paper pulp, in food processing, pre-treatment of poultry feeds, pretreatment of cereal and millet flours for use in the baking industry [4,5]. These enzymes are also used in degumming of plant fiber sources such as flax, hemp, jute, ramie [6,7,8]. Relatively high cost of enzyme, use of complex costly procedure for screening and isolation of organisms has hindered the industrial production. The aim of this study is to isolate efficient xylanase producers from different source samples using enrichment isolation technique. The study would concentrate on selection of an efficient xylanase producer from among the primary isolates. The selected isolates would be further tested for their cheaper and raw xylan source utilization capacity for xylanase production. Utilization of cheaper xylan rich agro-wastes for enzyme production would be a commercially significant process.

II. MATERIALS AND METHODS

A. Collection of Source Samples

Different soil samples were collected aseptically from various xylan rich sources like soil from saw mill, sugar cane dump yard, decaying wood, wood waste & spoiled corn cob and oil extraction units and screened for xylanase producing isolates.

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B. Primary Screening

Xylanolytic primary isolates were screened using nutrient agar (NA) and malt extract agar (MEA) containing 0.1% oat spelt xylan. The plates were incubated for 1 day for bacterial and 3 days for fungal isolation at 37^0C and 30^0C respectively. Positive xylanolytic bacterial and fungal isolates were detected based on clear zones of hydrolysis on xylan plates. The colonies showing a clear zone on agar plates were further confirmed for their xylanolytic ability by chromogenic plate assay method using Congo red. For this the xylan plates with colonies were flooded with 0.1% Congo red solution and kept for 15min, then these were washed with 1M Nacl, dried and observed for hydrolysis zones [9].

C. Secondary Screening

Primary isolates were further screened for efficient xylanase production using commercial oat spelt xylan. Efficient xylanolytic isolates were tested for their utilization of different raw and cheaper xylan sources like saw dust, sugar cane bagasse, hay, corn cob and cotton seed extract. The isolates were identified based on cultural, microscopic and biochemical studies. The amount of xylanase produced was quantified under submerged fermentation condition.

D. Enzyme Production

For xylanase production under submerged conditions, Mandels and Sternburg's basal medium supplemented with 10% oat spelt xylan was used [10]. *Aspergillus* inoculated flasks were incubated at 28 ± 2°C under static conditions for 1 to 15 days [11]. The culture medium was filtered using Whatmann no.5 filter paper, the filtrate was centrifuged at 5000 rpm for 10 min. The clear supernatant was used as the crude extracellular enzyme source.

E. Enzyme Assay

The xylanase activity was assayed by DNS method [12]. Xylanase activity was expressed as the amount (units) of enzyme required to produce one micromole per minute under the assay conditions [14].

III RESULTS AND DISCUSSION

Diverse xylanolytic organisms were primarily screened from diverse sources (Figure-1) using 0.1% oat spelt xylan as substrate. Screening of xylanolytic organisms was done by observing the zone of hydrolysis (Figure-2) when the plates were flooded with 0.1% Congo red. Primary screening yielded nearly 50 isolates which tested positive for xylanase activity and most of the selected isolates belonged to different genera namely *Bacillus sp.,Aspergillus sp.* and *Trichoderma sp.*(Figure-3) based on cultural and microscopic characters. Xylanolytic isolates degrade xylan as it is used as their carbon and energy source and so degradation of xylan results in a halo or hydrolytic zones seen in many xylanolytic isolates like those of *Bacillus sp* [15] Secondary isolates were selected based on highest zone of hydrolysis. Fungal cultures showed maximum activity (Figure-4) compared to Bacterial cultures and they were further tested for their utilization of raw xylan source after 24 hrs of incubation. The selected xylanolytic isolates i.e *Aspergillus sp.*1 and 2 were tested further for their utilization of raw xylan sources like saw dust, sugar cane bagasse, hay, corn cob and cotton seed extract. (Figure-5). The isolates showed good response for enzyme production in sugar cane baggase and cotton seed extract with a maximum production of 45 U/ml and 22 U/ml enzyme units respectively. Thus this isolate has potential to degrade raw xylan sources for xylanase enzyme production which can further be exploited for various applications.

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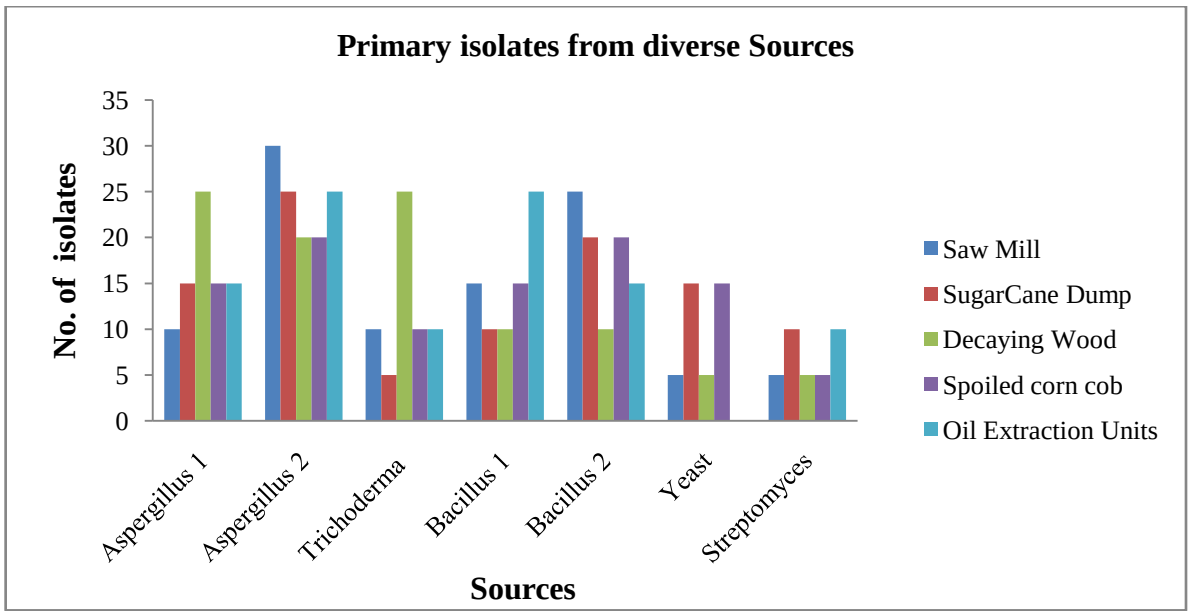

Fig. 1 Xylanolytic primary isolates from diverse sources

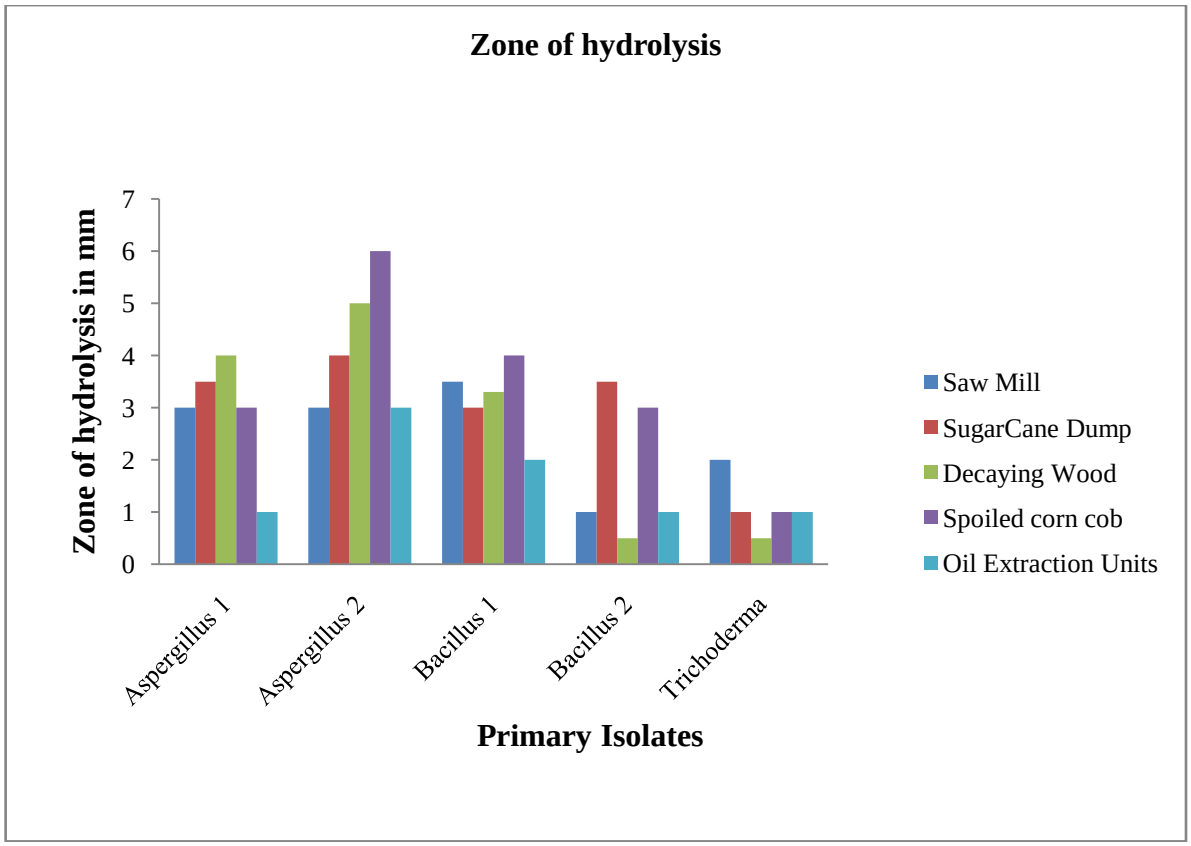

Fig.2 Zone of hydrolysis of secondary isolates

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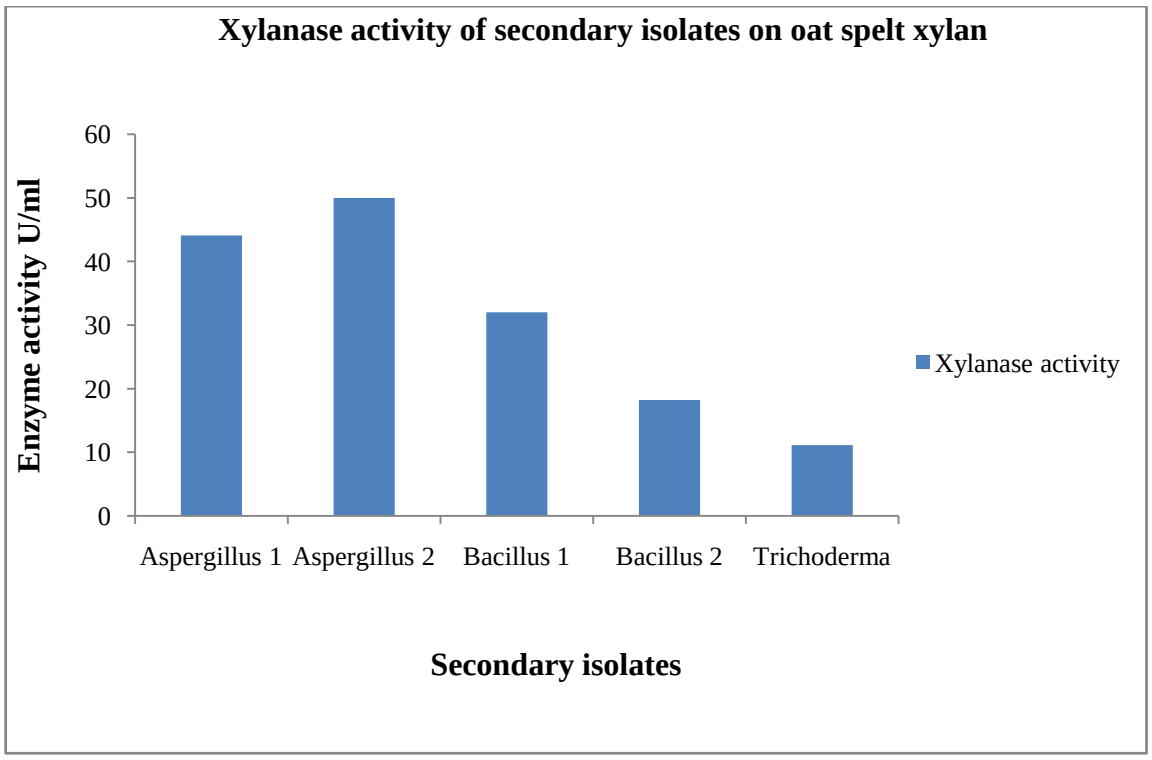

Figure 3: Xylanase activity of secondary isolates in U/ml using oat spelt xylan.

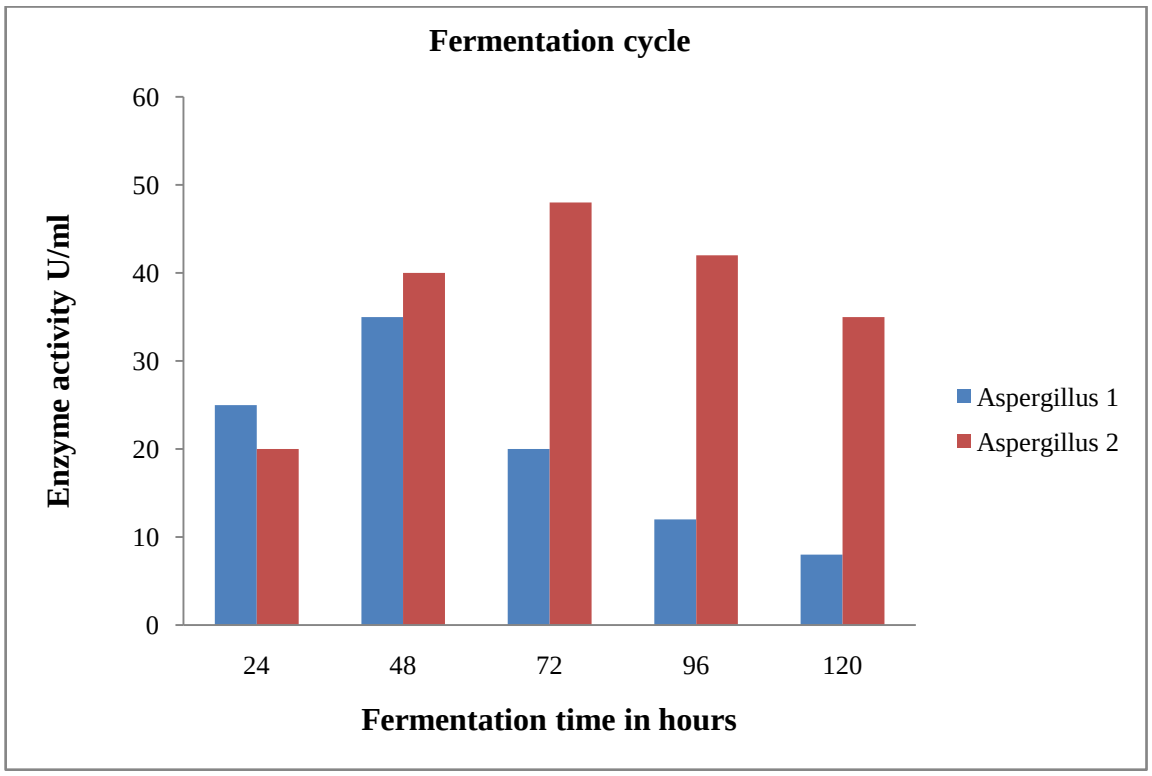

Fig.4 Fermentation cycle of two selected *Aspergillus* isolates.

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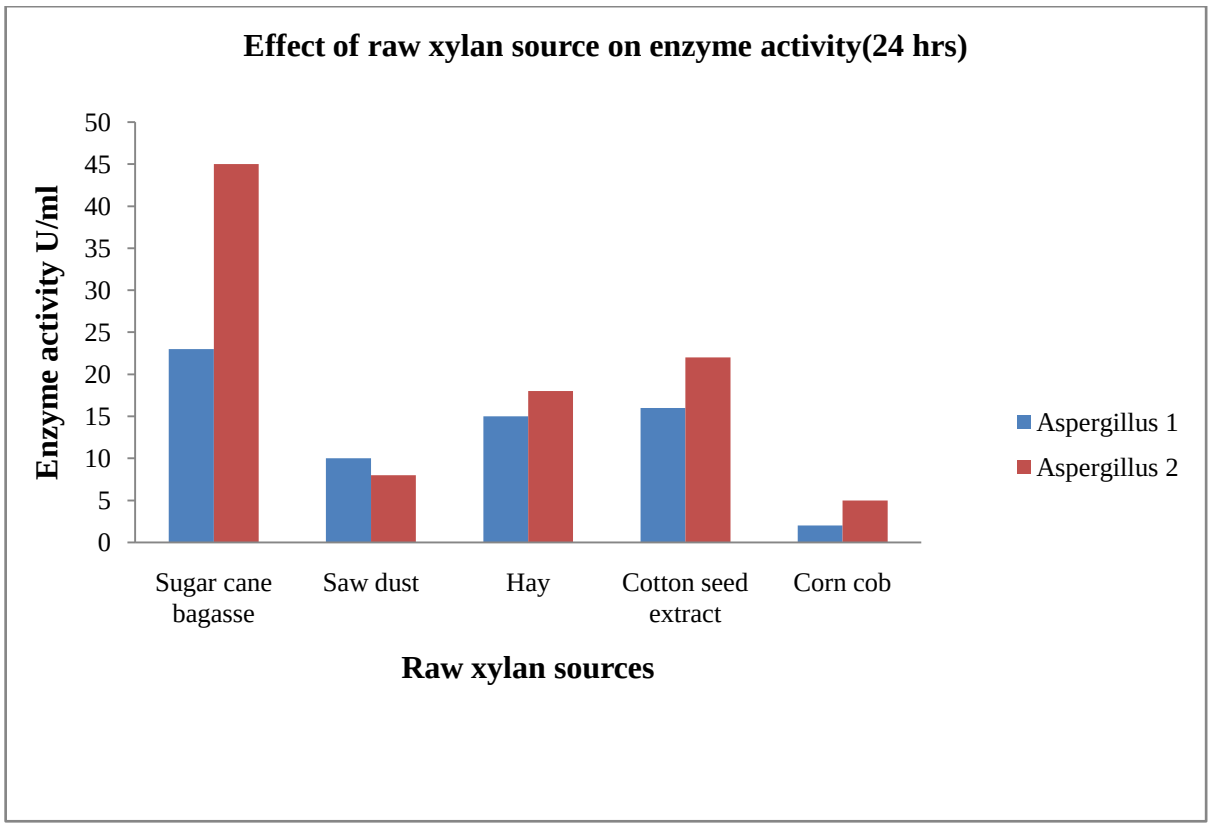

Fig.5 Effect of raw xylan source on enzyme activity at 24 hrs.

IV CONCLUSION

Different types of Xylanolytic isolates were screened from various sources. Two fungal isolates identified as belonging to genus *Aspergillus sp.* from sugar cane bagasse dumped soil showed higher xylanase production. One isolate *Aspergillus sp* 2 showed highest xylanase production in oat spelt xylan and also raw xylan sources. Sugar cane bagasse yielded highest enzyme production and so can be effectively exploited for enzyme production using cheaper raw source at low cost and later used for various applications.

V ACKNOWLEGMENTS

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