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ISOLATION AND SCREENING OF XYLANASE PRODUCING ASPERGILLUS SP FROM SOIL.

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ABSTRACT:

Present investigation deals with the isolation and screening of *Aspergillus sp.* for the biosynthesis of xylanase. Isolation of fungi was carried out on Potato Dextrose Agar (PDA) followed by serial dilution method using the samples obtained from the soil collected from different areas of Meerut. The culture was incubated at 30 ^{0}C for 3-5 days. The fungal isolates were subculture to purity and examine for xylanolytic activities. Screening for xylanolytic activities was also performed on potato dextrose agar (PDA) containing 0.1% (w/v) of xylan from oat spelt. Positive xylanolytic isolates were detected based on the clear zones of hydrolysis on the xylan. The fungi were considered forxylanase production.

Keywords— *Aspergillus*, xylanase, xylan.

INTRODUCTION

Fungi are an important component of the soil micro biota. (Ainsworth & Bisby, 1995). The role of fungi in the soil is an extremely complex one and is fundamental to the soil ecosystem (Diana, 1994). *Aspergilli* are ubiquitous in nature. They are geographically widely distributed and have been observed in a broad range of habitats principally in soils and decaying vegetation. Species of *Aspergillus* are an important microorganism, both medically and commercially. Some of these fungi are important pathogens of plants and animals (Gregory *et al*., 1997).

Xylanases have been well characterized by their properties and mode of action towards xylan hydrolysis. They have industrial applications in jute mill, paper and pulp industry, processing of coffee and poultry feed (Wu *et al.,* 2000). Xylanase enhance the availability of feed components to the animal and eliminate some of their natural occurring nutritional effects(Chen *et al.,*. 2001). Owing to these novel applications interest has increased in microbial production of Xylanse in the recent years. Production of xylanase by *Aspergillus* sp. may be carried out by solid state fermentation. Isolation and screening of a hyper producer strain plays a key role in the production of enzyme (Haq *et al.,* 2002).

There has been increased usage of xylanase preparations having an optimum pH < 5.5 produced invariably from fungi (Subramaniyan and Prema, 2000). The optimum pH for xylan hydrolysis is around 5 for most of the fungal xylanases although they are normally stable at pH 3 - 8. Most of the fungi produce xylanases, which tolerate temperatures below 50ºC. In general, with rare exceptions, fungi reported to be producing xylanases have an initial cultivation pH lower than 7. Nevertheless it is different in the

case of bacteria. The pH optima of bacterial xylanases are in general slightly higher than the pH optima of fungal xylanases (Khasin *et al*., 1993). In most of the industrial applications, especially paper and pulp industries, the low pH required for the optimal growth and activity of xylanase necessitates additional steps in the subsequent stages which make fungal xylanases less suitable. Although high xylanase activities were reported for several fungi, the presence of considerable amount of cellulase activities and lower pH optima make the enzyme less suitable for pulp and paper industries.

In this regards, different cultures of Aspergillus sp were isolated from soil collected from different areas of Meerut. The cultures of Aspergillus sp. were identified and screened out for the maximum production of xylanases.

MATERIALS AND METHODS

Isolation of fungal isolates:

The different cultures of *Aspergillus* sp. were isolated from the agricultural soils agricultural waste materials (Clark *et al.,* 1958). The samples were processed using the soil plate method (Warcup, 1950) and Soil dilution plate Method (Waksman, 1922).

Soil plate method:

About 1g of soil was scattered on the bottom of a sterile Petri dish and molten cooled (40-45°C) agar medium (PDA) was added, which was then rotated gently to disperse the soil particles in the medium. The plates were then incubated at 28°C for three days.

Soil dilution plate method:

The soil samples were mixed with sterile distilled water and a series of dilutions were made. From the dilutions, 1ml volumes were pippeted onto Potato Dextrose agar and Czapek Dox agar and incubated at 28°C for three days. Fungal were then screened for *Aspergillus* species.

Screening of soil fungal isolates for Xylanolytic activity:

A total of eight isolates were assayed for xylanase activity. Screening for xylanolytic activities was performed on malt extract agar (PDA) containing 0.1% (w/v) of xylan from oat spelt, pH 5.0. Culture plates with xylan containing agar medium were inoculated with each isolate and incubated for 3-5 days at 30°C. Positive xylanolytic isolates were detected based on the clear zones of hydrolysis on the xylan.

Identification of *Aspergillus* species:

Identification was done on the basis of clear zone of hydrolysis on culture plates containing xylan. Identification was performed according to Raper & Thom (1945) and Gilman (1957).

RESULTS

The isolated and screened *Aspergillus* sp. from agriculture soil and waste material from different places of Meerut were purified and identified on the basis of their morphological characteristics Raper & Thom (1945). Out of eight isolates a greater number of species and colonies were isolated on soil plates than on dilution plates. Isolate 2 and Isolate 4 cultures had a highest growth (6.5 and 5.25 mm) on PDA (Table 1). at temperature 25-35 °C. Isolate 2 had highest zone of clearance 3.5 mm (Table 2).

Table 1. Per day growth in mm at room temperature.

S. NO.	Name of Isolates	PDA medium	Temperature °C
1	Isolate 1	5.0	25-35
2	Isolate 2	6.5	25-35
3	Isolate 3	4.8	25-35
4	Isolate 4	5.25	25-35
5	Isolate 5	2.8	25-35
6	Isolate 6	4.7	25-35
7	Isolate 7	4.9	25-35
8	Isolate 8	4.8	25-35

Table 2. Clear zone of hydrolysis in mm by all isolates.

S. NO.	Name of Isolates	Clear zone of hydrolysis
1	Isolate 1	1.5
2	Isolate 2	3.5
3	Isolate 3	1
4	Isolate 4	2
5	Isolate 5	3
6	Isolate 6	2.4
7	Isolate 7	1.25
8	Isolate 8	2.25

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