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A comparitive xylanase production by two *Aspergillus* species

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SUMMARY

The present investigation was undertaken with the objective to find out the suitable micro organism for the maximum xylanase production using two *Aspergillus* species namely *Aspergilus fumigatus* and *Aspergillus terreus* using Czapek's dox wheat bran xylan medium. The effect of pH, temperature, agitation, incubation period, nitrogen sources, bivalent ions, substrate concentration, stability, inhibitors on xylanase production was studied. It was inferred that *Aspergillus fumigatus* enhanced the maximum production of xylanase than *Aspergillus terreus in* all the parameters studied.

Key words : Xylanase, *Aspergilus fumigatus* and *Aspergillus terreus*

Xylan is the main hemicellulose's present in plant cell walls. It is a linear polysaccharide consists of D-xylose residues linked by β-1, 4 bonds with a variety of substituents in carbon 2 and 3 of the xylose units. Due to its complex structure, the biodegradation of xylan requires the synergistic action of several hydrolytic enzymes for efficient and complete breakdown (Biely, 1985) .The enzyme that degrade the complex polysaccharide xylan into its monomers are constitutively known as xylanases. The xylanases are of two types - extracellular xylanase and intra cellular xylanases. Various micro organisms like bacteria, fungi possess the ability to secrete these enzymes.

The products of hemicellulose hydrolysis *i.e.* xylose and arabinose can be used as substrates in the production of different antibiotics, alcohol, feeds, chemicals and fuels (Thompson, 1983).Hence these enzymes which are of commercial interest should ideally be produced quickly and in high quantities from simple and inexpensive substrates.

The production of enzymes is highly dependable on the cultural conditions that favour fermentation. Thus the present study aims in selecting a suitable pH, temperature and other cultural conditions which favours the enzyme production by two *Aspergillus* species namely *Aspergilus fumigatus* and *Aspergillus terreus*

MATERIALS AND METHODS

Glasswares :

All the glassware's used are of Borosil brand.

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Chemicals:

All the chemicals used were of analytical grade. Oats spelt xylan from Sigma Chemicals. Co. / USA were used as a substrate for xylanase assay.

Micro organism:

The fungi used for the study namely *Aspergillus terreus and Aspergillus fumigatus* are obtained from the laboratory of PSGR Krishnammal college for Women, Coimbatore. The cultures were maintained at 4^0 C on Potato Dextrose Agar (PDA) slants.

Media used:

Czapek's dox medium was used with sucrose replaced by 3% wheat bran xylan.

The media and the distilled water used were sterilized in an autoclave at 15 lbs p.s.i. for 15 minutes.

Xylan extraction:

Xylan was extracted from wheat bran by the method of Panbangred *et al*., 1983

Preparation of inoculum and cultivation conditions:

The culture broth consists of 50 ml of czapek-dox medium with wheat bran xylan as carbon source in a 250 ml conical flask. Each flask was inoculated by an actively sporulating mycelial disc. Cultures were incubated for 5 days at 40^o C.Fungal mat grown in the liquid medium was filtered through Whatmann No. 41 filter paper and the filtrate were centrifuged at 3000rpm for 20 minutes. The supernatant was collected and used as a crude enzyme.

Substrate preparation:

1gm of oat spelt xylan was homogenized in 50 ml of 0.05 m phosphate buffer of pH 7.0. It was heated to boiling

point and cooled with continued stirring. The volume was made upto 100 ml with the same buffer and stored at 4^0C, for further use (Bailey *et al*., 1992).

Determination of protein concentration:

The protein of the samples was estimated using the method of Bradford, 1976 with Bovine serum albumin as the protein standard.

Xylanase assay:

The xylanase activity was determined using Di Nitro Salicylic acid (DNS) method, Miller (1959) by measuring the amount of reducing sugars released during 10 minutes in a reaction mixture containing 1%w/v oat spelt xylan and 0.05m potassium phosphate buffer at 50^0 C. One unit of enzyme activity was defined as the amount of enzyme required to release 1µ mol of D xylose per min per ml.

RESULTS AND DISCUSSION

The two species taken for study namely *Aspergillus terreus* and *Aspergillus fumigatus* were grown on the medium containing different pH ranges – 5.4, 5.6, 5.8, 6.0, 6.2, 6.4 and 7.0.Each fungus prefer its own pH for the maximum growth and enzyme production. Both the fungus showed maximum enzyme production in pH 5.8. (Mukhopadhyay *et al.,* 1997). No growth was recorded at pH 5.4 by *A.terreus* where as *A.fumigatus* showed an activity of 120 IU/ml. *A.fumigatus* produced maximum xylanase at all pH ranges than *A.terreus*.(Fig. 1). Incubation period also plays an important role in the

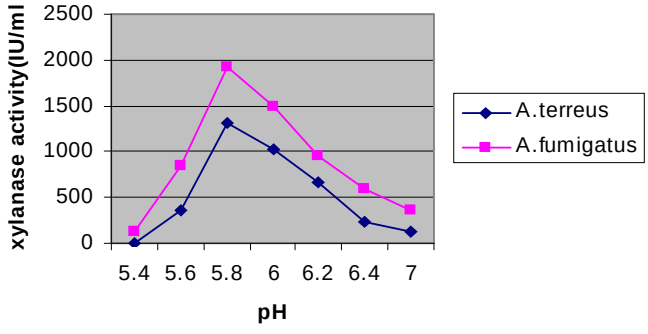

Fig.1 : Effect of different pH ranges on xylanase production

enzyme production. Each fungus requires a specific time period for its growth and to produce enzyme. Hence varied incubation periods like 96 hrs, 120 hrs, and 144 hrs were studied. Though both the fungus recorded maximum production at 120 hrs of incubation (Poutanen *et al*., 1987), *A.fumigatus* produce more activity in all the three periods (Fig. 2).

A.terreus and *A.fumigatus*, when grown in czapek's dox wheat bran xylan medium under different temperature conditions -30^0 C, 35^0 C, 40^0 C, 45^0 C, 50^0 C, both the species recorded maximum production at 40^0 C (Dubey and Johri, 1987). But at 35^0 C, *A.terreus* recorded maximum activity than *A.fumigatus*. Drastically both the

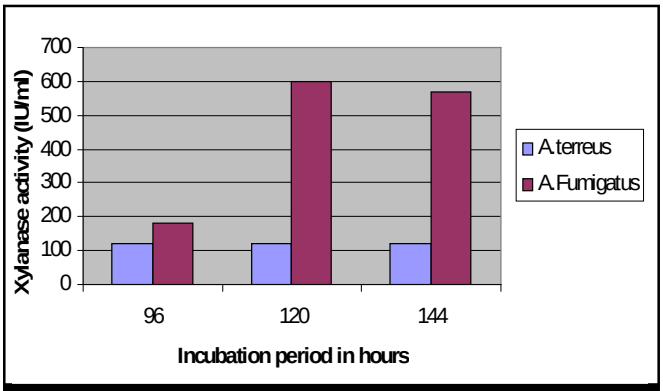

Fig.2 : Productionj of xylanase by two fungal species during various incubation period

species showed no activity at 45^0 C, 50^0 C (Fig. 3). The two fungus, when grown under agitation at different temperature conditions -30^0 C, 35^0 C, 40^0 C, 45^0 C, 50^0 C showed no growth and activity. Agitation has a negative impact on the enzyme production, since intensive agitation alters the morphology of the fungus (Palma *et al.,* 1996; Panda, 1989).

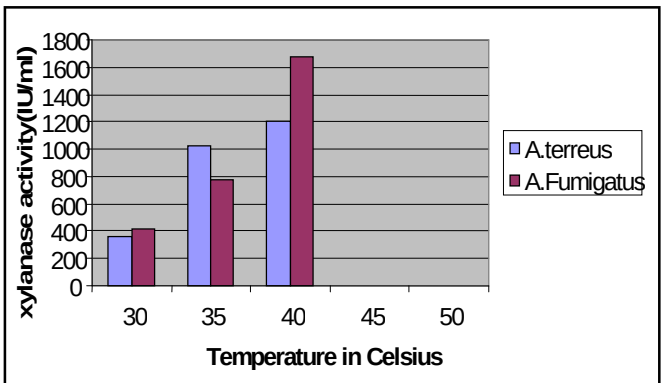

Fig.3 : Effect of temperature on xylanase activity

In order to find out, the effect of time intervals for enzyme hydrolysis the reaction mixture (crude enzyme + substrate) was subjected to different incubation period like 30 min, 40 min, 50 min and 60 min. It was noted that there was a gradual increase in the activity from 30 min to 50 min. (Fig. 4). At 50 min and 60 min, *A.Fumigatus* showed a sharp increase in the activity than *A.terreus*

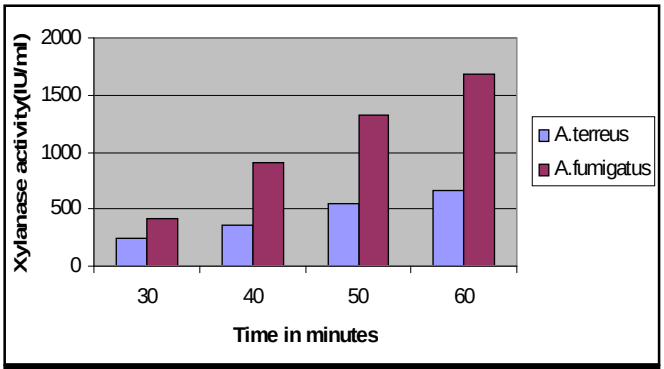

Fig. 4 : The effect of time intervals on enzyme hydrolysis

(Maheswari and kamalam,1985; Biswas *et al.*, 1986; Tan *et al.*,1986*).*

The concentration of wheat bran xylan have a profound effect on the enzyme production. Among the varied concentration - 5g,10 g,15 g,20 g,15 g,30 g tested for enzyme production, *A.fumigatus* showed maximum activity than *A.terreus* in all the concentrations studied and the maximum production was recorded at 30 g (Fig. 5) similar to the results obtained by Dubey and Johri (1987).

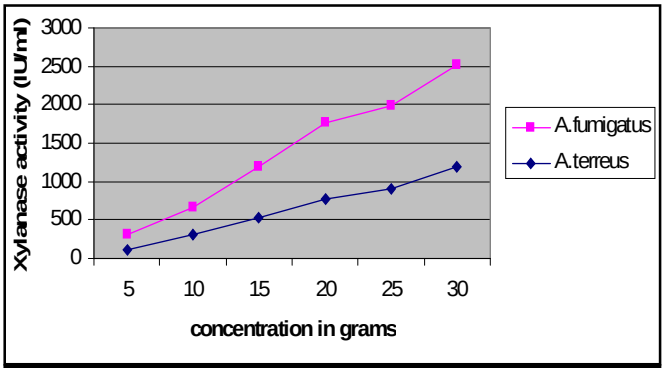

Fig. 5 : Xylanase production at different concentration of wheat bran xylan

The effect of nitrogen sources on enzyme production was studied using four different nitrogen equivalents namely $NaNO_3$, KNO_3, Glutamine, NH_4Cl. Both the species showed a good activity on $NaNo_3$ (Fig. 6) but the maximum activity was recorded by *A.fumigatus* only. KNO_3, Glutamine and NH_4Cl had only less influence on the xylanase production (Maheswari and kamalam,1985).

The Bivalent ions in the medium were replaced with other ions like $MgSO_4$, $MnSO_4$, $BaCl_2$, $CaCl_2$, and $COCl_2$ to test their effect on the enzyme production. *A.fumigatus* showed maximum activity in all the ions than *A.terreus* (Fig.7). In accordance with the result of Biswas *et al.*

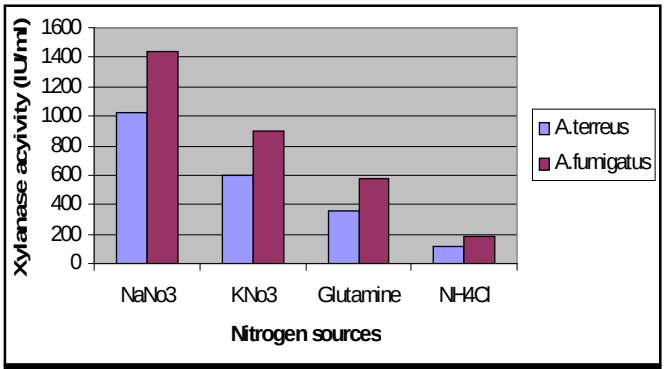

Fig. 6 : Effect of nitrogen sourceson xylanase production

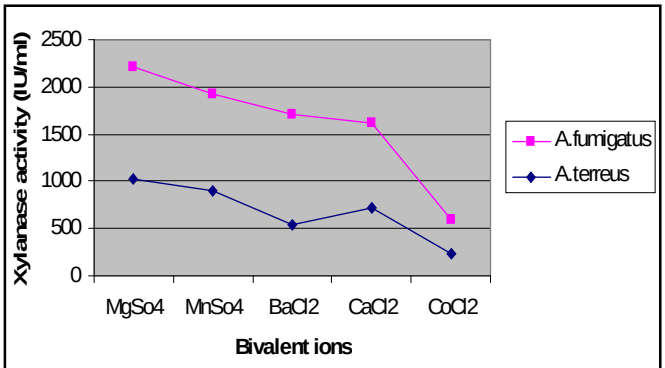

Fig. 7 : Effect of bivalen ions on xylanase production

(1989) both the fungus showed complete inhibition when grown on the medium containing inhibitors like $HgCl_2$, Sodium azide, Cadmium chloride, $MnCl_2$. With regard to stability, it was noted that the crude enzyme was found to be stable at 60 minutes and there after it was decreasing from 90 min, 120 min, and 150 min of incubation (Fig. 8).

Conclusion:

It is inferred that among all the cultural conditions studied for the maximum production of xylanase by the two fungal species, *A.fumigatus* was found to be the

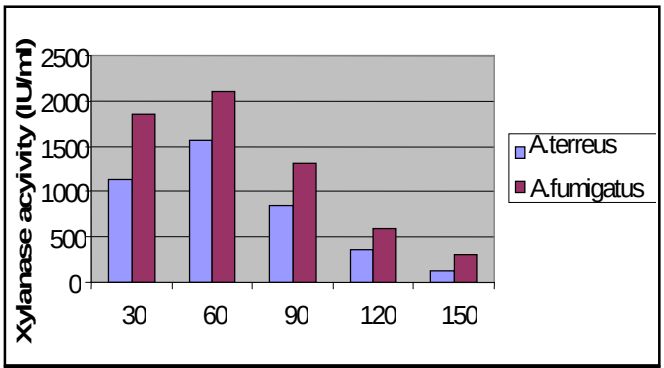

Fig.8 : Effect of time intervals on the temperature stability of the enzyme

best xylanase producer than *A.terreus.* Hence, for future studies *A.fumigatus* can be choosen as an ideal organism for maximum enzyme production.

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