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# PRODUCTION OF XYLANASE BY SOLID STATE FERMENTATION BY *ASPERGILLUS NIGER*

**WASEEM AHMAD BUTT, IKRAM-UL-HAQ, SIKANDER ALI, QADEER M. A. & *JAVED IQBAL**

*Biotechnology research laboratory*
*Botany department, government colleg,e, Lahore.*

**Abstract**

The present work describes the optimization of cultural conditions for the production of xylanase by *Aspergillus niger*. Fifty different strains of *Aspergillus niger* were isolated from different soil samples. Of all the strains tested, *Aspergillus niger* GCB-15 gave maximum production of xylanase, when wheat bran was used as substrate, urea as nitrogen source, incubation period of 72 hours and distilled water as an extractant was used.

## Introdution

Xylanase have been extensively studied and could potentially be employed for the production of hydrolysate from agro-industrial wastes, nutritional improvements of lignocelluloses feeds (Destroy 1981), processing of food and increasing animal feed digestibility (Haltrich and Steiner 1994), Agro-fibre and bio bleaching of Kraft paper pulp (Kulkarni *et al.*, 1999). Xylanase have been isolated from diversified range of micro-organism including fungi and bacteria (Monti *et al.*, 1991) but *Aspergillus* niger remains the organism of choice for the production of xylanase (Haq *et al.*, 1993). Solid-state fermentation involves growth of microorganisms on moist substrate in the absence of free water and economic recovery of required product in concentrated form (Medeiros *et al.*, 2000). Wheat bran is the best substrate among a few easily available lignocelluloses wastes. In addition to wheat straw, rice straw and bagasse etc. also produced agricultural lignocelluloses wastes estimated to be about 40 million tons per year (Archana *et al.*, 1997).The present study is concerned to develop a fermentation process by culturing the *Aspergillus niger* on various agricultural by products and wastes as substrate.

## Materials and methods

**Isolation of organism:** Different cultures of *Aspergillus niger* were isolated from soil by serial dilution method (Clark *et al.*, 1958). The cultures were maintained on Potato dextrose agar slants.

**Fermentation technique**: Ten gm wheat bran was transferred to 250ml. Conical flask and moistened by adding 10ml. of distilled water. The flasks were plugged with cotton

*Department of Botany, University of the Punjab, Quaid-e-Azam campus, Lahore.

and were sterilized in an autoclave at 121° C for fifteen minutes. The flask were then cooled at room temperature and were inoculated with 1ml. Conidial suspension prepared in 0.005% Monoxal O.T. The flasks were then incubated at 30+1° C for 72 hours. The flasks were shaken twice daily. After 72 hours, 100 ml of the distilled water was transferred to each flask. The flasks were then rotated at the rotary shaker (200 rpm) at 30° C for one hour. After one hour the fermented broth was filtered and filtrate was used for the estimation of xylanase. All the experiments were run in duplicates.

**Enzyme assay:** The estimation of xylanase was carried out according to the method of Miller (1959). One unit liberate one mole of reducing sugar measured as xylose equivalents from xylan per minute at pH 7.0 at 30° C.

**Results and discussion**

**Screening of mould culture:** The data of Table 1 shows the screening of *Aspergillus niger* strains for the production of xylanase by solid state fermentation. Of all the strains tested for xylanase production the strain GCB 15 gave maximum production of xylanase (1625 U/g). In the subsequent experiment therefore *Aspergillus niger* GCB-15 was used.

**Selection of the substrate:** The effect of different substrates for the production of xylanase by *Aspergillus niger* GCB-15 was carried out (Table 2). Maximum production of xylanase (1675 u/g) was obtained in wheat bran medium other substrate such as sunflower meal and rich husk produced the enzyme at low rate. This difference might be due to adequate amount of nutrients and porosity for oxygen supply using wheat bran as the basal substrate. Polygilenia *et al.* (1989) found that wheat bran contain 8.04% cellulose and the rest is lignin and xylan. Large quantity of xylan and increased surface area of wheat bran provided optimum support for the production of xylanase.

**Rate of xylanase synthesis:** The data of table 3 shows the effect of incubation time on the production of xylanase by *Aspergillus niger* GCB-15. The flasks were incubated at 30 °C for 24 –120 hours. The maximum production of the enzyme was obtained (1680u/g) after 72 hours of incubation. Further increase of the incubation period resulted in the decrease in the production of xylanase. The decrease in enzyme production may be lapse of time, the susceptible portion of xylan molecules rapidly digested and only crystalline portion was left behind which can be used by the organism for the conversion of the enzyme. This finding is an agreement with the work reported by Reese *et al.* (1969). This work, however, is not in good agreement with the work of Roose (1963) who reported higher values of xylanase production 96 hours, after incubation.

**Extractants of enzyme:** The data of table 4 shows the extraction of enzymes from fermented wheat bran by different extractants. The maximum extraction was obtained when distilled water was used as an extractants (1750 U/g). The extraction of microbial enzyme was greatly influenced by the pH. The extraction of enzyme with distilled water was maximum. This finding is in agreement with the work reported earlier Dubeau *et al.* (1987). In a similar study, Subramaniyan *et al.* (1997)

reported maximum xylanase activity (100.72 U/g) at pH 7.0 of the extractant used. Hence, our finding (1750 U/g) is more encouraging as compared to the work of Subramaniyan *et al.* (1997).

**Effect of different nitrogen sources:** The production of xylanase is greatly influenced by both the sources and concentration of nitrogen. In the present work the different nitrogen sources such as urea, ammonium sulphate and sodium nitrate were evaluated (Table 5). The maximum production of enzyme (1790 u/g) was obtained when urea was used as nitrogen source. It may be due to increase in the fungal growth. Montenecourt and Eveleight (1979) also selected urea as a sole nitrogen source for the maximum production of xylanase. The workers (Kulkarni *et al.*, 1999; Polygilenia *et al.*, 1989) found better yield with sodium nitrate for optimal growth of *Aspergillus niger* maximal xylanase production.

**Table 1. Screening of *Aspergillus niger* for the production of xylanase by solid state fermentation.**

| No. | Strains | Xylanase activity U/g | No | Strains | Xylanase activity(U/g) |
|---|---|---|---|---|---|
| 1. | GCB-1 | 988 | 26. | GCB-26 | 1400 |
| 2. | GCB-2 | 875 | 27. | GCB-27 | 1376 |
| 3. | GCB-3 | 75 | 28. | GCB-28 | 1265 |
| 4. | GCB-4 | 1518 | 29. | GCB-29 | 1476 |
| 5. | GCB-5 | 1150 | 30. | GCB-30 | 1515 |
| 6. | GCB-6 | 1576 | 31. | GCB-31 | 1365 |
| 7. | GCB-7 | 1596 | 32. | GCB-32 | 1456 |
| 8. | GCB-8 | 137 | 33. | GCB-33 | 1543 |
| 9. | GCB-9 | 462 | 34. | GCB-34 | 1234 |
| 10. | GCB-10 | 1443 | 35. | GCB-35 | 1445 |
| 11. | GCB-11 | 1450 | 36. | GCB-36 | 1600 |
| 12. | GCB-12 | 1584 | 37. | GCB-37 | 1575 |
| 13. | GCB-13 | 1612 | 38. | GCB-38 | 1543 |
| 14. | GCB-14 | 1468 | 39. | GCB-39 | 1234 |
| 15. | GCB-15 | 1625 | 40. | GCB-40 | 1345 |
| 16. | GCB-16 | 1443 | 41. | GCB-41 | 1245 |
| 17. | GCB-17 | 1445 | 42. | GCB-42 | 1345 |
| 18. | GCB-18 | 1342 | 43. | GCB-43 | 1435 |
| 19. | GCB-19 | 1543 | 44. | GCB-44 | 1535 |
| 20. | GCB-20 | 1515 | 45. | GCB-45 | 1234 |
| 21. | GCB-21 | 1090 | 46. | GCB-46 | 1235 |
| 22. | GCB-22 | 1276 | 47. | GCB-47 | 1543 |
| 23. | GCB-23 | 1345 | 48. | GCB-48 | 1235 |
| 24. | GCB-24 | 1265 | 49. | GCB-49 | 1455 |
| 25. | GCB-25 | 1150 | 50. | GCB-50 | 1535 |

**Table 2. Selection of substrate for the production of xylanase by *Aspergillus niger* GCB-15.**

| No. | Substrates | Xylanase activity units/g |
|---|---|---|
| 1. | Wheat bran | 1675 |
| 2. | Rice husk | 875 |
| 3. | Sunflower meal | 1225 |

**Table 3. Effect of incubation time on xylanase production of xylanase by *Aspergillus niger* GCB-15.**

| No. | Incubation time (hours) | Xylanase activity (Units/g) |
|---|---|---|
| 1. | 24 | 750 |
| 2. | 48 | 875 |
| 3. | 72 | 1680 |
| 4. | 96 | 1150 |
| 5. | 120 | 1050 |

**Table 4. Extractants of xylanase by *aspergillus niger* GCB-15**

| No. | EXTRACTANTS | pH | Xylanase activity (Units/g) |
|---|---|---|---|
| 1. | Tap water | 7.60 | 725 |
| 2. | Distilled water | 7.0 | 1750 |
| 3. | Acetate buffer | 4.5 | 650 |
| 4. | Citrate buffer | 4.8 | 750 |
| 5. | Phosphate buffer | 7.5 | 625 |

**Table 5. Effect of different nitrogen sources on the biosynthesis of Xylanase by *Aspergillus niger* GCB-15.**

| No. | Nitrogen sources | Xylanase activity units/g |
|---|---|---|
| 1. | Urea | 1790 |
| 2. | Ammonium sulphate | 700 |
| 3. | Sodium nitrate | 1025 |

Substrate=Wheat bran, pH=7.0

**References**

Archana, A., T. Satyanarayna. 1997. Xylanase production by thermophilic *Bacillus lichenifórmis* A99 in solid state fermentation. *Enzyme Microbiol. Technol.*, .21(1):12-17

Clark H. E., Bordner Geldrich, E.F., Kabler, P.W. and Huzz C. B.1958. Applied Microbiology. *International book company, New York*, pp. 27-53.

Destroy, R.W. 1981. Bioconversion of agricultural biomass to organic chemicals and inorganic chemicals from biomass (ed., I.S. Gold Stein ),pp: 1924.

Dubeau, H., D.S. Chahal and M. Ishaque. 1987. Xylanase of *Chaetomium cellulolytium*; its nature of production and hydrolysis potential. *Biotech. Lett.*, 9(4):75-287.

Gessesse, A., G. Mamo.1998. Purification and characterization of an alkaline xylanase from alkaliphilic Micrococus sp. AR-135. *J.Ind. Microbiol.*, 20(3-4):210-214.

Haltrich, D. and C. Steiner. 1994. Formation of xylanase by *Schizophyllum commune*. Effect of medium components. *J. Enzy.Microb. Technol.*, 16(3):229-235.

Haq, I., S.H. Iqbal and M.A. Qadeer. 1993. Production of xylanase and CMC cellulose by mould culture. *Pak. J. Biotechnols.*, 4(20): 403-409.

Kulkarni, M., A. Shendye, M. Rao. 1999. Molecular and biotechnological aspects of xylanase. *FEMS Microbiol. Rev.* 23(4):41-45.

Medeiros, R.G., M.L. Sozzner., J.A. Thome, A.O. Cacais, R.S. Estelles, B.C. Sales;H.M. Ferreiol, N.S.A. Lucena, J.F.G. Silva, E.X. Filho.2000. The production of hemicellulases by aerobic fungi on Medium containing residue of Banana plant as substrate. *Biotechnol. Prog.*, 16(3): 522-524.

Miller, G.L. 1959. Use of dinitrosalicyclic acid reagent for determination of reducing sugars. *J.Anal. Chem.*, 31: 426-8.

Montenecourt, B.S. and D.E. Eveleigh. 1979. Preparation of mutants of *Trichoderma reesei* with enhanced xylanase and cellulase production *Appl. Environ. Microbiol.*, 34:777-782.

Monti, R., H.F. Terenzi and J.A. Jorge. 1991. Purification and properties of an extracellular xylanase from the thermophilic fungus *Humicola grisea* var. *Thermoidea. Canadian J. Microbiol.*, 37(9): 675-681.

Polygilinia, G.V., G.G. Fain, A.P. Rukhlyadeva. 1989. Determination of cellulase in raw materials. *Dishch from st.* (7):66-68.

Reese, E., T. Lolad and J.E. Rarrish. 1969. Modified substrates and Modified products as inducers of carbohydrates. *J. Biotechnol.*, 100:1151.

Roose, F.J.1963. Advances in enzymatic hydrolysis of cellulose and related material. *Porgdaman Pres. London*.pp.50-53.

Singh, S., B. Pilly, V. Dilsook and B.A. Prior. 2000. Production and properties of hemicellulases by a *Theromyces lanuginosus* strain. *J.App. Microbio.*, 88(6):975-982.

Subramaniyan, S., P. Prema and S. V. Ramakrishna. 1997. Isolation and screening for alkaline thermostable xylanases. *J. Basic Microbiol.*, 37(6): 431-437.