ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology, Volume 2 Number 3: 185-190, 2003
ISSN 1682-2978
© 2003 Asian Network for Scientific Information

Isolation and Screening of *Aspergillus niger* Isolates for Xylanase Biosynthesis

Ayesha Khan, Ikram-ul-Haq, Waseem Ahmad Butt and Sikander Ali
Biotechnology Research Laboratory, Department of Botany,
Government College University, Lahore, Pakistan

Abstract: Present investigation deals with the isolation and screening of ***Aspergillus niger*** isolates for the biosynthesis of xylanase. About 104 different strains of ***Aspergillus niger*** were isolated from soil collected from different areas of Lahore by serial dilution method on xylene agar malt extract medium. The strains with larger whitish zones of xylene hydrolysis were picked up and transferred to the PDA slants. The cultures were incubated at 30°C for 3-5 days for maximal sporulation. The ***Aspergillus niger*** strains were screened for xylanase production by submerged fermentation. Xylanase activity was ranged from 30-225 U ml^{-1} and dry cell mass from 6.48-26.56 g L^{-1}. Maximum xylanase productivity was obtained by GCBT-35 (225 U ml^{-1}). The mycelial dry weight was 9.54 g L^{-1} while mycelia were small in size and rounded in shape.

Key words: Xylanade, biosynthesis, fermentation, ***Aspergillus niger***, xylene degradation, isolation

Introduction

Xylanases have been well characterized by their properties and mode of action towards xylene 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 naturally occurring ant nutritional effects (Chen ***et al.***, 2001). Owing to these novel applications interest has increased in microbial production of xylanase in the recent years (Kebir ***et al.***, 2000). Production of Xylanase by ***Aspergillus niger*** may be carried out by solid, surface or submerged fermentation. A successful process depends both on appropriate strain and on optimization of fermentation parameters (Haq ***et al.***, 2002a). Isolation and screening of a hyper producer strain plays a key role in the production of enzyme (Haq ***et al.***, 2002b). The strains of ***Aspergillus niger*** can be isolated from soil, water and air (Wu ***et al.***, 2000). Kutsai ***et al.*** (2001) isolated different strains of ***Aspergillus niger*** by using xylene agar malt extract medium. Clark ***et al.*** (1958) adopted the method of serial dilution for the isolation of different strains of ***Aspergillus niger***. Substantial amount of foreign exchange is being spent for the import of this enzyme in the country, so it is worthwhile to produce xylanase in laboratory and development of the potent strain of ***Aspergillus niger*** by mutation.

In this regard, different cultures of ***Aspergillus niger*** were isolated from soil collected from different areas of Lahore by serial dilution method. The cultures of ***Aspergillus niger*** were identified and screened out for the maximum production of xylanase using shake flask technique.

Materials and Methods

Isolation of xylene decomposing fungi

The different cultures of ***Aspergillus niger*** were isolated from soil collected from different areas of Lahore by serial dilution method (Clark ***et al.***, 1958). One gram of soil sample was dissolved in 100 ml of sterilized distilled water. This sample was further diluted up to 1/1000 time. 1 ml of this diluted sample was then transferred to the petri plates containing xylene agar malt extract medium, pH 5.0. The petri plates were then placed at 30°C for 3-5 days. The young colonies of ***Aspergillus niger*** were picked up, transferred to potato dextrose agar slants and stored at 4°C in a refrigerator. The sub-culturing was carried out after every 3-4 weeks.

Inoculum preparation

The conidia from 4-6 days old cultures were wetted by adding 10 ml of 0.005% Monoxal O.T (Diocetyl ester of sodium sulpho succinic acid) to each slant. The conidia were scratched with a sterilized inoculating needle and the tube was shaken gently. The supernatant containing conidia was decanted off aseptically and the suspension was used as an inoculum.

Fermentation technique

Shake flask technique in 250 ml Erlenmeyer flask was used for xylanase production. The medium containing (g l^{-1}); wheat bran 5.0, $NaNO_3$, 1.0, NH_4Cl 1.5, KH_2PO_4 1.0, $MgSO_4.7H_2O$ and Tween 80 2.0 ml at pH 4.5 was used for fermentation. 25 ml of the fermentation medium was transferred to each flask and were cotton plugged. The flasks were sterilized in the autoclave at 121°C for 15 minutes (15lbs/inch^2). After cooling the medium at room temperature, 1.0 ml of the conidial inoculum was transferred in each flask and placed at the rotary incubator shaker (rotated at 160 rpm) at 30°C for 48 h (optimized). After 48 h, the ingredients of the flasks were filtered and the filtrate was used for the estimation of xylanase.

Analysis

Mycelial dry weight was determined by filtering the culture medium through weighed Whatman filter paper No. 44 keeping at 110ºC overnight. The enzyme was assayed according to the method of Wong (1988). Sugar was estimated gravimetrically by DNS method (Miller ***et al.***, 1959). An UV/V is scanning spectrophotometer (Double beam, Cecil-CE 7200-series, UK) was used for measuring colour intensity. "One unit xylanase will liberate one μ mole of reducing sugar equivalents measured as xylose from xylene per min at pH 7.0 and 30°C. Enzyme activity was expressed as U ml^{-1}" (Wong, 1988).

Results and Discussion

Microbial production of xylanase is more preferred to plant and animal sources because of easier availability, structural stability and ease of genetic manipulations (Bilgrami and Pandey, 1992). Xylanase fermentation by locally isolated ***Aspergillus niger*** strains was carried out in 250 ml Erlenmeyer flasks using conidial inoculum.

Table 1: Screening of different strains of *Aspergillus niger*

Strains of *Aspergillus niger*	Xylanase saccha rifying activity (U ml^{-1})	Mycelial dry weight(g l^{-1})	Strains of *Aspergillus niger*	Xylanase saccha rifying activity (U ml^{-1})	Mycelial dry weight(g l^{-1})
GCBT-1	150	12.68	GCBT-46	125	24.64
GCBT-2	30	14.44	GCBT-47	45	14.25
GCBT-3	70	16.56	GCBT-48	115	26.56
GCBT-4	50	15.72	GCBT-49	45	14.55
GCBT-5	30	14.12	GCBT-50	78	22.10
GCBT-6	70	12.4	GCBT-51	97	18.90
GCBT-7	100	16.96	GCBT-52	112	17.50
GCBT-8	50	13.96	GCBT-53	145	14.55
GCBT-9	40	16.88	GCBT-54	180	6.48
GCBT-10	32	16.76	GCBT-55	190	15.80
GCBT-11	50	14.44	GCBT-56	35	16.50
GCBT-12	30	15.96	GCBT-57	90	16.92
GCBT-13	31	15.72	GCBT-58	40	13.75
GCBT-14	30	16.28	GCBT-59	55	13.95
GCBT-15	30	16.04	GCBT-60	60	12.05
GCBT-16	35	16.80	GCBT-61	155	13.64
GCBT-17	32	15.64	GCBT-62	45	18.50
GCBT-18	30	17.08	GCBT-63	155	19.52
GCBT-19	35	15.16	GCBT-64	60	15.25
GCBT-20	40	16.16	GCBT-65	35	19.00
GCBT-21	37	15.20	GCBT-66	120	12.52
GCBT-22	120	18.64	GCBT-67	105	18.44
GCBT-23	90	17.88	GCBT-68	50	14.50
GCBT-24	66	14.55	GCBT-69	55	13.25
GCBT-25	60	16.95	GCBT-70	35	18.75
GCBT-26	30	17.00	GCBT-71	95	12.54
GCBT-27	115	17.44	GCBT-72	35	17.60
GCBT-28	115	16.60	GCBT-73	40	15.25
GCBT-29	100	17.08	GCBT-74	32	18.62
GCBT-30	100	16.28	GCBT-75	55	13.50
GCBT-31	100	18.00	GCBT-76	45	15.55
GCBT-32	195	14.80	GCBT-77	65	14.76
GCBT-33	215	10.92	GCBT-78	98	21.00
GCBT-34	210	16.54	GCBT-79	110	18.06
GCBT-35	225	9.54	GCBT-80	135	17.85
GCBT-36	40	12.00	GCBT-81	60	14.05
GCBT-37	55	14.00	GCBT-82	50	12.25
GCBT-38	32	10.00	GCBT-83	50	13.50
GCBT-39	170	18.76	GCBT-84	65	12.30
GCBT-40	60	12.50	GCBT-85	48	16.65
GCBT-41	160	23.60	GCBT-86	75	11.05
GCBT-42	50	14.00	GCBT-87	100	10.35
GCBT-43	35	15.50	GCBT-88	110	15.65
GCBT-44	60	11.85	GCBT-89	105	14.35
GCBT-45	65	12.55	GCBT-90	90	13.95

Table 1: Continue

Strains of *Aspergillus niger*	Xylanase saccha rifying activity (U ml^{-1})	Mycelial dry weight(g l^{-1})	Strains of *Aspergillus niger*	Xylanase saccha rifying activity (U ml^{-1})	Mycelial dry weight(g l^{-1})
GCBT-91	85	15.45	GCBT-98	68	18.82
GCBT-92	45	14.00	GCBT-99	42	18.00
GCBT-93	65	18.05	GCBT-100	67	17.09
GCBT-94	66	15.95	GCBT-101	101	14.50
GCBT-95	121	16.00	GCBT-102	109	16.03
GCBT-96	100	19.45	GCBT-103	84	15.75
GCBT-97	105	16.43	GCBT-104	57	16.40

Temperature = 30 ± 1°C pH = 4.5

Table 1a: Sub-grouping of different strains of *Aspergillus niger* according to xylanase productivity

No. of strains	Range of xylanase productivity (U ml^{-1})
39	0 - 50
37	50 - 100
18	100 - 150
7	150 - 200
3	200 - 250

GCBT-35 gave maximum xylanase production i.e. 225 U ml^{-1}

Table 1b: Sub-grouping of different strains of *Aspergillus niger* according to mycelial dry weight

No. of strains	Range of mycelial dry weight (g l^{-1})
3	5 - 10
38	10 - 15
58	15 - 20
4	20 - 25
1	25 - 30

GCBT-48 gave maximum mycelial dry weight i.e. 26.56 g L^{-1}.

Time course study is significant because it actually determines the incubation period for any microbial culture. Rate of xylanase production by a locally isolated strain of ***Aspergillus niger*** was studied (Fig. 1). The maximum saccharifying activity (140 U ml^{-1}) was observed, 48 h after the conidial inoculation. The dry cell mass was 15.64 g L^{-1}. However, xylanase activity was not significant at time periods (24 or 72 h) other than 48 h. Time course study revealed a significant increase in enzyme production with the increase in time, which was presumed to be due to rapid hydrolysis of xylene in the medium. Further increase in incubation period (after 48 h) resulted in the decreased enzyme production. The decrease in enzyme production might be due to the rapid digestion of susceptible portion of xylene molecules and then only crystalline portion was left behind, which cannot be used by the organism for the production of the enzyme. This finding is in accordance with the work reported by Irwin and Wilron (1993). Shake flask technique was used for the screening of the culture for xylanase production in present study.

Hundred and four different strains of ***Aspergillus niger*** were isolated locally and evaluated for xylanase production (Table 1). The isolates were sub-grouped according to their enzyme productivity (Table 1a). Results shows that 39 cultures produced enzyme in the range of

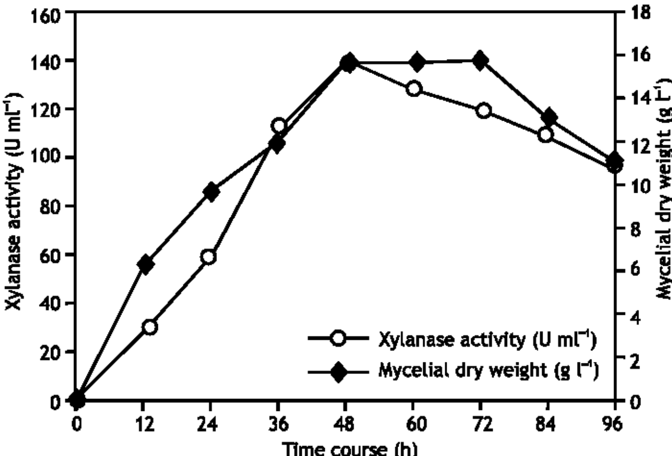

Fig. 1: Time course profile for xylanase production by locally isolated strain of ***Aspergillus niger*** GCBT-35. Temperature = 30 ± 1°C Initial pH = 4.5

0-50 U ml^{-1}; 37 cultures between 50-100 U ml^{-1}; 18 cultures between 100-150 U ml^{-1}; 7 cultures and 3 cultures were in the range of 150-200 U ml^{-1} and 200-250 U ml^{-1}, respectively. The isolates were also sub-grouped according to their mycelial dry weight (Table 1b). Results show 3 cultures produced mycelial dry weight in the range of 5.0-10.0 g L^{-1}; 38 cultures between 10.0-15.0 g L^{-1}; 58 cultures between 15.0-20.0 g L^{-1}; 4 cultures and 1 culture were in the range of 20.0-25.0 g L^{-1} and 25.0-30.0 g L^{-1}, respectively.

Of all the isolates investigated, maximum enzyme production (225 U ml^{-1}) was obtained by ***Aspergillus niger*** GCBT-35, however, maximum mycelial dry weight was obtained from GCBT-48 (26.56 g L^{-1}). Our results are more encouraging than the work reported by different authors (Uchida ***et al.***, 1992; Kebir ***et al.***, 2000 and Kutsai ***et al.***, 2001).

Acknowledgments

Authors are grateful to Pakistan Science Foundation (PSF) for all the practical and financial assistance. The research paper is related to the project no. PSF/RES/BIOTECH/PP-GC/BIO. (37) sponsored by PSF.

References

Bilgrami, K.S. and A.K. Pandey, 1992. Industry and fermentation in introduction to biotechnology (ES. K. Jain), pp: 149-165.

Chen, S.C., Y. Zhang and P.J.Gao, 2001. Studies on submerged fermentation of alkaline β-1, 4-glyconases by ***Bacillus pumilus*** A-30. Sheng Wugong Xuebao., 16: 485-489.

Clark, H.E., G.E.F. Bordner, P.W. Kabler and C.B. Huff, 1958. Applied Microbiology. International Book Company, NY, pp: 27-53.

Haq, I., A. khan, W.A. Butt, S. Ali and M.A. Qadeer, 2002. Enhanced production of xylanase by mutant strain of ***Aspergillus niger*** under solid-state fermentation conditions. Indus J. Plant Sci.,1: 22-27.

Haq, I., A. Ehsan, W.A. Butt and S. Ali, 2002. Studies on the biosynthesis of enzyme xylanase by submerged fermentation from ***Aspergillus niger*** GCBMX-45. Pak. J. Biol. Sci., 5: 1309-1310.

Irwin, D. and D.B. Wilron, 1993. Characterization and sequence of a ***Hermomonospora fusta*** xylanase. Applied and Environ. Microb., 60: 763-770.

Kebir, H., C. Dupont and R. Morasoli, 2000. Increased xylanase production in ***Streptomyces lividans*** after replacement of the signal peptide dependence on box and inverted repeat sequence. Biochem. Biophys. Acta., 149: 177-184.

Kutsai, J., A. Bata, E. Brydl, P. Rafai and V. Jurkovich, 2001. Characterization and effects of xylanase enzyme preparation extracted from ***Thermomyces lanuginosus*** cultures. Acta Vet. Hung., 49: 175-184.

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

Uchida, H. I. Kusakabe, Y. Kewabata, T. Ono and K. Morakami, 1992. Production of xylose from xylene with intracellular enzyme system of ***Aspergillus niger***. J. Ferment. and Bioeng., 7: 153-158.

Wong, K.K.Y., 1988. Production of xylanase and its unit definition. J. Microbiol., 11: 305-309.

Wu. K., C. Z. Jingmin, Jie. L. Bin, M. Tu, W.Tao and P. Renrui, 2000. Substrate specificities of Xylanases from ***Aspergillus niger*** and its relationship of xyloligosaccharide production. Quanquo Gongye Weishengwu Xinxi Zhongxin., 30: 18-20.