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Xylanase Biosynthesis by Chemically Mutated Strain of *Aspergillus niger*

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Abstract: Present investigation deals with the xylanase biosynthesis by chemically mutated strain of *Aspergillus niger*. *Aspergillus niger* strain GCBT–35 was improved by using N-methyl N-nitro N-nitroso guanidine (MNNG) as the chemical mutagenic agent. Sixty mutant strains with larger whitish zones of xylan hydrolysis were picked up and transferred to the PDA slants. The *Aspergilllus niger* strains were screened for xylanase production by submerged fermentation. Xylanase activity was ranged from 30-200 U ml^{-1}. Of all the mutants tested, *Aspergillus niger* GCBCX-20 gave maximum production (200 U ml^{-1}) of enzyme which was 1.70 folds higher than the parental strain of *Aspergillus niger* (115 U ml^{-1}). Time course study was also carried out by the potent mutant GCBCX-20 for production of Xylanase. Maximum enzyme production (215U ml^{-1}) was observed 48 hours after inoculation.

Key words: Xylanase, biosynthesis, MNNG, submerged fermentation, mutation, *Aspergillus niger* and xylan degradation

Introduction

Xylanase is an extracellular enzyme which hydrolysis the ß1- 4, D- xylosidic linkage of highly polymerized and substituted ß1- 4 linked-D-xylobiose, xylotriose glucuronosyl residues (Kulkarni *et al.*, 1999). Xylanase shows a wide variety of applications including bio pulping, nutritional improvement of lignocellulosic feed stock, production of ethanol, methane, other products and in the processing of food (Nunez *et al.*, 2001, Haq *et al.*, 2002 and Bocchini *et al.*, 2003). These uses have placed greater stress on increasing xylanase production by fermentation. There is more availability of nutrient to the organism used for fermentation. The time required for submerged fermentation is less and there is sufficient supply of oxygen (Bim and Franco, 2000). Microbial production of xylanases is preferred to plant and animal sources due to easier availability, structural stability and ease of genetic manipulations (Bilgrami and Pandy, 1992). Mutation can enhance enzyme productivity that may be several folds higher than the parental strain (Chadha *et al.*, 1999). It was proved that unlike conventional physical and chemical mutagens, polynucleotides selectively induce visible and lethal mutations preferentially in certain genes, different in case of treatment with different polynucleotides and these mutations may induce certain genes to be more efficient (Gershenson. 1996). In the present study, strain of *Aspergillus niger* was chemically improved by using MNNG and screening of best chemically mutated strain of *Aspergillus niger* for xylanase biosynthesis was carried out. For this purpose, shake flask technique was employed as the method of choice.

Materials and Methods

Organism and Culture Maintenance: *Aspergillus niger* strain GCBT-35 was maintained on potato dextrose agar slants (Merck, Germany), pH 4.5 and stored at 4°C in refrigerator.

Strain Improvement: *Aspergillus niger* strain GCBT-35 was improved by using N-methyl N-nitro N-nitroso guanidine (MNNG) as the chemical mutagenic agent. (Pontecarvo *et al.*, 1953).

Preparation of Conidial Suspension: The conidia from 4-6 days old cultures were wetted by adding 10 ml of 0.9% sterilized saline water. The conidia were scratched with a sterilized inoculating needle and the tube was shaken gently. This suspension was then diluted up to 10^{-2} to 10^{-4} times.

Mutation Technique: Five ml of prepared (50-300 μg ml^{-1}) N-methyl N-nitro N-nitroso guanidine (MNNG or NG) was transferred to each sterilized centrifuge tube containing 5 ml of conidial suspension. The tubes were placed at room temperature for different time intervals such as 5-60 minutes. After the time interval, these tubes were centrifuged at 6,000 rpm for 20 minutes. The supernatant was discarded to remove the MNNG from the cells. Five ml of saline water was added to each centrifuge tube. The tubes were re-centrifuged for the removal of traces of chemical reagent from the cells. This process was repeated three times. After washing the cells, 10 ml of sterilized saline water was added to each tube to form conidial suspension. The tubes were shaken vigorously and approximately 0.5 ml of this suspension was transferred to each petriplate containing potato dextrose xylan agar medium and incubated at 30°C for about 48 hours. The mutant strains with larger white zones of xylan hydrolysis were

picked up and transferred to PDA slants. The cultures were incubated at 30°C for 3-5 days for maximum sporulation.

Fermentation Technique: Shake flask technique in 250 ml Erlenmeyer flask was used for xylanase production. The medium containing (g l^{-1}); wheat bran 20, $NaNO_3$, 1.0, NH_4Cl 1.5, KH_2PO_4 1.0, $MgSO_4.7H_2O$ 0.3 and Tween 80 2.0 ml at pH 4.5 was used for fermentation. Twenty-five 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 15minutes (15lbs $inch^{-2}$). After cooling the medium at room temperature, 1.0 ml of the conidial suspension was transferred in each flask and placed at the rotary incubator shaker (rotated at 200 rpm) at 30°C for 48 hours (optimized). After 48 hours, the ingredients of the flasks were filtered and the filtrate was used for the estimation of xylanase.

Analysis: Xylanase activity was determined by the method of Wong (1988). An UV/V is scanning spectrophotometer (Double beam, Cecil-CE 7200-series, UK) was used for measuring colour intensity at 546nm. "One unit xylanase will liberate one μ mole of reducing sugar equivalents measured as xylose from xylan per minute at pH 7.0 and 30°C. Enzyme activity was expressed as U ml^{-1}" (Wong, 1988).

Results and Discussion

Mutation enhances the efficiency of some genes through duplication and deletion mechanism (Stadler, 1997). For the improvement of fungus for Xylanase production, the conidial suspension of parental *Aspergillus niger* GCBT-35 was subjected to different doses of MNNG for 5-60 minutes (Table 1).

Table 1: Screening of MNNG treated *Aspergillus* mutants for Xylanase biosynthesis after different time intervals

No.	Treatment time (min)	No. of survivals
1	5	4
2	10	8
3	15	10
4	20	14
5	25	8
6	30	6
7	35	4
8	40	3
9	45	2
10	50	1
11	55	Nil
12	60	Nil

Table 2: Screening of different mutant strains of *Aspergillus niger* for Xylanase biosynthesis

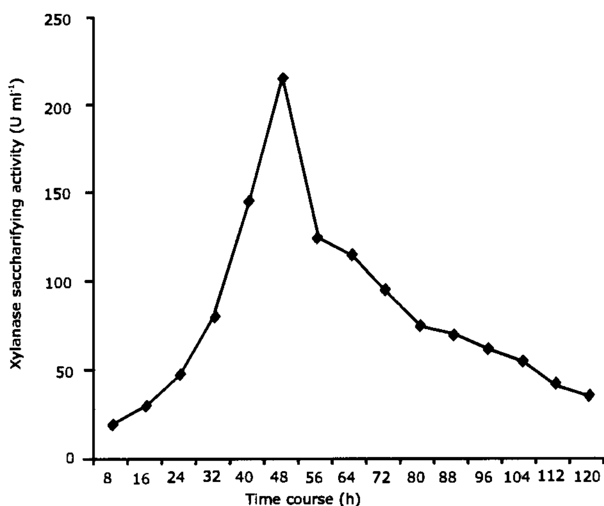
Mutant Strains of *Aspergillus niger*	Xylanase saccharifying activity (U ml^{-1})
GCBCX-1	85
GCBCX-2	90
GCBCX-3	75
GCBCX-4	100
GCBCX-5	75
GCBCX-6	95
GCBCX-7	79
GCBCX-8	125
GCBCX-9	130
GCBCX-10	75
GCBCX-11	65
GCBCX-12	85
GCBCX-13	70
GCBCX-14	90
GCBCX-15	165
GCBCX-16	125
GCBCX-17	100
GCBCX-18	125
GCBCX-19	95
GCBCX-20	200
GCBCX-21	135
GCBCX-22	110
GCBCX-23	110
GCBCX-24	60
GCBCX-25	75
GCBCX-26	85
GCBCX-27	75
GCBCX-28	90
GCBCX-29	60
GCBCX-30	75
GCBCX-31	70
GCBCX-32	100
GCBCX-33	75
GCBCX-34	65
GCBCX-35	110
GCBCX-36	100
GCBCX-37	95
GCBCX-38	70
GCBCX-39	70
GCBCX-40	65
GCBCX-41	40
GCBCX-42	50
GCBCX-43	90
GCBCX-44	65
GCBCX-45	65
GCBCX-46	80
GCBCX-47	65
GCBCX-48	55
GCBCX-49	95
GCBCX-50	35
GCBCX-51	75
GCBCX-52	60
GCBCX-53	55
GCBCX-54	65
GCBCX-55	55
GCBCX-56	70
GCBCX-57	40
GCBCX-58	30
GCBCX-59	35
GCBCX-60	30

Table 2a: Sub grouping of mutant strains of *Aspergillus niger* for Xylanase biosynthesis

No. of mutant strains	Range of xylanase productivity (U ml^{-1})
7	30 - 50
43	50 - 100
8	100 - 150
2	150 - 200

So GCBCX-20 is used in further experiments for xylanase production

Temperature = 30 ± 1°C pH = 4.5



Temperature = 30 ± 1°C Initial pH = 4.5

Fig. 1: Time course profile for xylanase production by hyper-secretive mutant strain of *Aspergillus niger* GCBCX-20

Sixty mutant strains of *Aspergillus niger* were isolated by observing bigger zones of hydrolysis of xylan in the petriplates. These mutants were than evaluated for Xylanase production (Table 2) Xylanase activity was ranged from 30-200 Uml^{-1}. The isolates were sub grouped according to their enzyme productivity (Table 2a). Results showed that 7 cultures produced enzyme in the range of 30-50 U ml^{-1}; 43 cultures between 50-100 U ml^{-1}; 8 cultures and 2 cultures in the range of 100–150 Uml^{-1} and 150–200 Uml^{-1}, respectively. Of all the mutants investigated, maximum enzyme production (200 U ml^{-1}) was obtained by mutant strain GCBCX-20, isolated after 20 minutes of chemical treatment. The enzyme production from this mutant was found to be 1.70 folds higher than the parental strain of *Aspergillus niger* (115 Uml^{-1}). Couri and Defarias, 1995 studied that MNNG produces O-6 alkylation of guanine residues and its mutagenic action may be due to misreplications. It has preferential effect at DNA replication point and therefore tends to produce clusters of closely linked mutations (Rowland *et al.*, 1995). The complete death of fungus was observed when the treatment time was increased from 50 minutes or with higher dose of MNNG. In a similar study, Chen *et al.* (2001) also reported maximum xylanase production by a mutant strain of *Aspergillus niger*.

Time course study is significant because it actually determines the incubation period for any microbial culture. Rate of xylanase production by mutated strain of *Aspergillus niger* was studied (Fig. 1). The maximum saccharifying activity (215 U ml^{-1}) was observed, 48 h after the conidial inoculation. 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 xylan 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 xylan 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).

References

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

Bim, M.A. and T.T. Franco, 2000. Extraction inaqueous two- phase systems of alkaline xylanase production by *Bacillus pumilus* and its applications in Kraft pulp bleaching. J. chromatogr; B: Bio med-Sci. Appl; 743: 349-356.

Bocchini, D., V. Damiano, E. Gomes and R. Da Silva, 2003. Effect of *Bacillus circulans* D1 Themostable Xylanase on Bio bleaching of Eucalyptus Kraft Pulp. Appl Biochem Biotechnol., 106: 393-402

Chadha, B.S., K. Jaswinder, K. Rubinder, H.S. Saini and S. Singh, 1999. Xylanase production by *Thermomyces lanuginosus* wild and mutant strains. World J. Microbiol. Biotechno., 15: 195-198.

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

Couri, S. and A.X. Defarias, 1995. Genetic manipulation of *Aspergillus niger* for increased synthesis of pectinolytic enzymes. Revista de Microbiologia 26: 314-317.

Gershenson, S.M.,1996. Selectivity of the mutagenic action of DNA and other polynucleotides. Zhurnal Obshchei Biologii. 57:661-683.

Haq, I. A. khan, W.A. Butt, S.Ali and M.A. Qadeer, 2002. Enhanced production of xylanase by mutant strain of *Aspergillus niger* GCBMX-45 under solid state fermentation conditions. Indus J. Plant Sciences., 1: 22-27.

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

Kulkarni, M., A. Shendye and M. Rao, 1999. Molecular and biotechnological aspects of xylanase. FEMS Microbiol. Rev. 23: 41-456.

Maximo, C., M. Costa-Ferreira and J. Duarte, 1998.Some properties of eucalyptus kraft pulp treated with xylanase from *Aspergillus niger* World J. Microbiol. Biotechnol. 14:365-367.

Monfort, A., A.Blasco, J.A. Prieto and P. Sanz, 1997. Construction of bakers yeast strains that secrete different xylanolytic enzymes and their use in bread making. J. Cereal Sci., 26:195-199.

Nunez, L.Z., R. Rodrignez and M. A. Baez, 2001. Gas retention mechanism in wheat bread doughs. The role of xylanase and arabionoxylain fractions. J. Biochem., 56:31-33.

Pontecarvo, J., J. A. Roper, L. M. Hemmous, K.P. Macdonald and A. W. J. Buftan, 1953. Strain improvement through various mutational techniques. Adv. Genet., 5: 141-283.

Rowland, G.G., A. Mchughen, R. S. Bhatty, S.L.Mackenzie and D.C. Taylor, 1995. The application of chemical mutagenesis and biotechnology to modification of Linseed (Linum usitatissimuml) Euphytica. 85: 317-321.

Stadler, D., 1997. Ultraviolet-induced mutation and the chemical nature of the gene. Genetics. 145: 863-865.

Williamson, G., 1995. Influence of different xylanases on the activity of ferulic acid esterase on wheat bran. Biotechnology and Applied Biochemistry. 22: 65-73.

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