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ENHANCED BETA-GLUCOSIDASE PRODUCTION BY MUTAGENESIS IN *Penicillium purpurogenum*

A. B. Dhake and M. B. Patil*

University Department of Biochemistry, L.I.T. Premises, Nagpur University, Nagpur - 440 033 (India)

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ABSTRACT

P. purpurogenum isolated from decaying wood sample in our laboratory produces comparable amount of beta-glucosidase. The intracellular and extracellular enzymes reached to the peak after 4 and 8 days respectively. Germinating spores of this strain were subjected to mutagenesis with U.V and NTG (3-nitro, 5 methyl guanidine). After mutagenesis, both the treatments have shown induction in beta-glucosidase production to significant level.

Keywords: Beta-glucosidase, mutagenesis and *Penicillium purpurogenum.*

INTRUDOCTION

The use of microbial enzymes is of increasing importance in industries now a days. β-glucosidase or β-glucoside glucohydrolase [EC 3.2.1.21] is one of the enzymes amongst them. It catalyzes the hydrolysis of alkyl and aryl-β-glucosides. The enzyme is widely used for ethanol production from cellulosic waste and in the synthesis of useful β-glucosides[1-4]. In flavor industries, the enzyme is used to release the aromatic compounds from glucosidic precursors present in fruit and fermenting products[5,6].

β-glucosidase production was studied most amongst the organisms like *Bretlanomyces bruxellensis*[6], *Aspergillus oryzae*[7], *Fusarium oxysporum*[8], *Trichoderma reesei*[9] and *C. peltata*[10]. The mutations, which are intentionally produced with the help of mutagen are the induced mutations. Both the chemical and physical mutations fall under the category of induced mutation.

The chemical mutagen incorporates mutation by modifying purine and pyrimidine or by distortion of DNA. In case of physical mutation mutation is due to formation of pyrimidine dimmer. Mutations are reported to enhance cellulase production in *Aspergillus* sps[11], xylanase production in *A. niger*[12], *Thermomyces lanuginosus*[13] inulinase production in *Aspergillus fumigatus*[8], *A. niger*[14] and *P. purpurogenum*[15]. In the present investigation, attempts have been made to enhance beta-glucosidase production by chemical and physical mutagenic methods.

MATERIAL AND METHODS

Chemical

Sucrose; p-nitrophenyl-ß-D-glucopyranoside (β-PNPG) and NTG (3-nitro, 5 methyl guanidine) were the products of SRL, Mumbai, India. All other chemicals were of analytical grade.

Microorganism

The organism P. purpurogenum was isolated in our laboratory and identified by IMT, MTCC, Chandigarh, India, was used.

Culture condition and Cultivation medium

The microorganism prefers subculturing on potato dextrose agar (PDA) slants after every 2 months. The cultures were stored at a temperature of 4°C. Czapek Dox (CD) medium used by Patil and Shastri (1981) was employed for the cultivation of *P. purpurogenum* having composition [0.6% NaCl, 1% Sucrose, 0.05% $MgSO_4 \cdot 7H_2O$, 0.15% KH_2PO_4, 2.5% $NaNO_3$, pH 5.5][16]. Sucrose was autoclaved separately and mixed with sterilized CD medium to get 1% concentration. Before autoclaving the pH of CD medium was adjusted to 5.5. Autoclaving was done at 15 lbs steam pressure for 15 min. Erlenmeyer flasks of 250 ml containing 50 ml medium were inoculated with 0.1 ml spore inoculum of organism (10^4 Spores/ml) and the incubation was carried out at 30°C under static condition.

Inoculum preparation

A spore suspension was prepared by adding sterile distilled water containing 1% (V/V) Tween 80 to 4-day-old slant culture.

Extraction of Enzyme

After 4 days of incubation the mycelial mass was collected by filtration, washed with distilled water and crushed in cold distilled water. The mycelial extract was centrifuged at 5,000g for 20 min at 4°C. The supernatant was used as the source of intracellular enzyme, where after 8 days mycelial mass was removed by filtration and the filtrate was centrifuged at 5,000g for 20 min at 4°C. The supernatant was used as extracellular enzyme.

Enzyme Assay

The enzyme assay was based on the procedure described by Riou *et al*. (1998). ß-glucosidase activity was assayed by mixing 0.1 ml 5 mM P-nitrophenyl ß-D-glucopyranoside (pNPG) in 0.4 ml 0.1 M Sodium acetate buffer at pH 5.5 + 0.1 ml enzyme. After incubation for 10 min at 50°C, the reaction was stopped by addition of 2 ml of 1 M sodium carbonate and the liberated P-nitrophenol was monitored at 420 nm, running suitable control simultaneously. One unit of β-glucosidase activity corresponded to the release of 1 mmol of *p*- nitrophenol min^{-1} under these conditions. Activity has been reported to be units/g/min and units/ml/min for intracellular and extracellular β-glucosidase respectively.

Physical Mutagenesis

The physical mutagenesis performed as reported by Sharma *et al.* (2002). 0.1 ml of the inoculum (10^4 Spores/ml) was poured centrally to the agar plates and spread with the help of sterile glass spreader. The plates were exposed to U.V light (60 W) for 0 to 10 min at a distance of 15 cm from UV source and incubated at 30°C for 4 days. After the incubation period the plates with 1 % survival rate were selected. The well-separated colonies were picked up with the help of sterile loop and transferred to PDA slants and incubated at 30°C. The spore suspension was prepared from culture slants as described above.

Chemical Mutagenesis

The chemical mutagenesis was performed as suggested by Sharma *et al.* (2002). Different amounts of NTG (0-2 mg) were added to spore suspension. The mixture was incubated for 30 and 60 min, respectively. Further, dilution was made to hundred fold with 0.1 M phosphate buffer (pH 5.5) and 0.1 ml of the spore suspension was spread on PDA plates with the help of glass spreader. Plates were incubated at 30°C and treatments giving lowest number of colonies were further studied for β-glucosidase production.

RESULTS AND DISCUSSION

All the experiments were done in triplicate and the results are the mean of three findings. The results are critically analyzed by single linear regression analysis. The percent survival rate was lowest for the U.V treatment of 10 min, while the percent survival declines with rise in U.V exposure time (Fig. 1). Total 20 UV mutants were screened after 10 min UV treatment. These mutated strains were then tested for intracellular and extracellular β-glucosidase production. Amongst them 5 strains (UVm3, UVm7, UVm12, UVm15, UVm17) show higher production upto 2 fold as compared to control (Parental) strain of *P. purpurogenum*. The UVm12 shows maximum 2-fold induction for intracellular and extracellular β-glucosidase production Table - 1. Similar induction in production was also obtained for xylanase by Butt *et al.* (2002) and Chadha *et al.* (1999). After UV treatment Vishwanthan and Kulkarni 1995 also reported 2-fold increase in inulinase production[14]. The UV

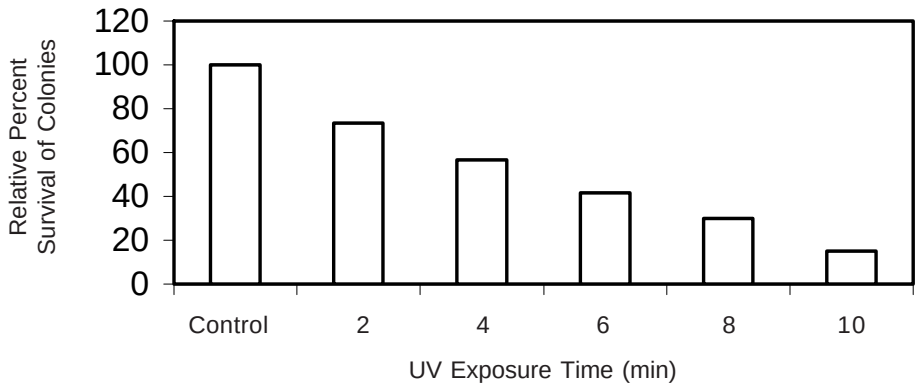

Fig. - 1 : Percent survival of colonies after UV treatment for different time interval

Table-1: Extracellular and intracellular β-glucosidase production by control (Parental) and UV mutated *P. purpurogenum* strains

Mutated Colony No	Extracellular β-glucosidase (U/g/min)	Intracellular β-glucosidase (U/g/min)
Control	3742	7386
UVm3	5709	11123
UVm7	6332	11198
UVm12	7484	13694
UVm15	6764	11738
UVm17	6716	11563

treatment for 30 to 60 min was used by Chand *et al.* (2005) to induce the cellulase production in 7 different fungi[12]. The NTG mutagenesis shows 2-fold increase in invertase production for *A. fumigatus* by Fiedurek *et al.* (2000).

Further, chemical mutagenesis by using NTG has been carried out in order to increase β-glucosidase production. When the concentration of NTG mutagen was increased from 0 to 2 mg/ml simultaneous decrease in the percent survival was reported showing the least survival at concentration of 2 mg/ml for both 30 and 60 min treatment (Fig. -2). In case of NTG treatment for 30 and 60 min to the fungal spores total 32 mutants were screened. NTG is a chemical mutagen. It mainly causes mutagenesis by causing transitions and transvertions (Bajon *et al.,* 1984). After 30 min treatment with NTG 5 mutants namely (NTGm2, NTGm7, NTGm8, NTGm11, NTGm13) and after 60 min treatment with NTG 6 mutants (NTGm17, NTGm19, NTGm25, NTGm27, NTGm29, NTGm30) were selected for further β-glucosidase production respectively. Amongst these 11 mutants NTGm27 shows 3 fold increase in both extracellular and intracellur β-glucosidase production as compared to parental (unmutated) *P. purpurogenum* strain Table - 2.

Chemical mutagenesis by Haq *et al.* 2002 also shows induction in alpha amylase production for *B. substilis* using NTG as chemical mutagens[19]. Chemical treated mutated strain of *Thermomyces lanuginosus* also showed 2.5 fold rise in xylanase production as reported by Chadha *et al.*1999[13]. The chemical mutagenesis by MNNG (1-methyl-3-nitro-1-nitrosoguanidine) and ethidium bromide at 0.1 and 0.2 mg ml^{-1} showed the increased cellulase production for 7 different fungi by Chand *et al.* (2005). Fiedurek *et al.* (2000) for *A. fumigatus* reported 2-fold increase in invertase production after NTG treatment[17].

Amongst the physical and chemical mutagenesis, chemical mutagenesis gives better results as compared to physical mutagenesis showing 3-fold increase in β-glucosidase production by *P. purpurogenum*.

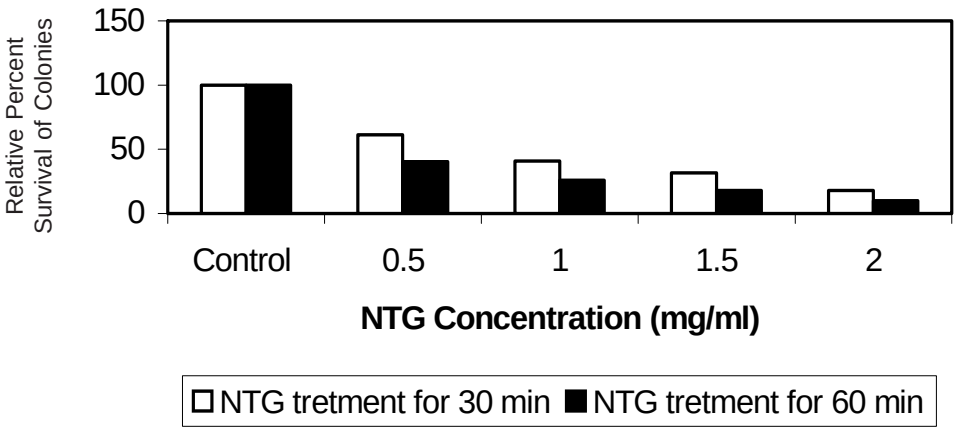

Fig-2: Percent survival of colonies after NTG treatment for different concentrations

Table-2: Extracellular and intracellular β-glucosidase production by control (Parental) and NTG mutated *P. purpurogenum* strains for 30 and 60 min

Mutated Colony No	Treatment time (min)	Extracellular β – glucosidase (U/g/min)	Intracellular β – glucosidase (U/g/min)
Control		3742	7386
NTGm2	30	9211	16056
NTGm7	30	10074	17618
NTGm8	30	8155	17698
NTGm11	30	5661	10490
NTGm13	30	8779	10238
NTGm17	60	6284	17040
NTGm19	60	8155	17240
NTGm25	60	9307	17332
NTGm27	60	11178	21650
NTGm29	60	8923	17698
NTGm30	60	8155	15145

The hyper enzyme productive mutants were tested for stability and enzyme production. All the mutants were found to be stable and did not loose the capacity of enzyme production when checked for a period of 3 months after every 15 days. Further characterization of mutants (UVm12 and NTGm27) is in progress to get enhanced β-glucosidase production. Work on this line is in progress.

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