

Effect of UV Induced Mutation on Production of Xylanase Enzyme from *Bacillus subtilis*

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ABSTRACT

Research on Xylanase enzyme has markedly increased due to its potential applications in several industries include pulping and bleaching processes, where it is using cellulose free preparations, textile processes, the enzymatic saccharification of lignocellulosic materials and waste treatment. The impact of ultra violet radiations on xylanase producing ability of *Bacillus subtilis* was studied. Maximum enzymatic activity (crude) upto 17 folds were obtained from mutant strain exposed to 60 minutes ultra violet irradiation in comparison to parent strain. Purified enzyme showed molecular weight of 38 KDa and 44 KDa by SDS-PAGE in both parent and mutant strains. The SDS-PAGE of mutant strain also showed the presence of other bands of higher molecular weight indicating the presence of additional proteins resulting due to UV exposure, leading to the higher production of xylanase. Result of the Agarose gel electrophoresis of genomic DNA of parent and mutant strain showed that changes in the gene expression due to impact of UV does not change the genome of mutant strain of *Bacillus subtilis*.

Keywords: *Bacillus subtilis*, xylanase, enzymatic activity, SDS-PAGE.

INTRODUCTION

Biodegradation of xylan, a component of the plant cell wall, is a complex process that requires the combined action of several enzymes, among which xylanase (1,4- β -D-xylan xylanohydrolase; EC 3.2.1.8) which cleaves internal linkages on the β -1,4-xylose backbone, play a key role¹. It has been shown that many kinds of bacteria and fungi hydrolyze β -1,4 xylan by the use of xylanolytic enzymes, such a β -1,4 xylanases, β - xylosidases, and esterases^{2,3}. β -1,4 xylanases are the key enzymes that hydrolyze the backbone structure of β -1,4 xylans to initiate degradation of the complex polysaccharides by microorganisms. A number of β -1,4 xylanases have been purified from fungi and bacteria, and the genes encoding β - 1,4 xylanases have been cloned and characterized. Several microorganisms produce multiple xylanases, implying a strategy for effective hydrolysis of β -1,4 xylan^{3,4}. Recently the interest in xylanases has markedly increased due to the potential applications in pulping and bleaching processes using cellulase free preparations, textile processes, the enzymatic saccharification of lignocellulosic materials and waste treatment⁵.

The present investigation deals with the enhancement of the production of industrially important xylanase, by subjecting *B. Subtilis* strain to mutagenesis by UV radiation. The enzymatic activities were then compared between the wild and mutant strains. The enzymes were then precipitated with ammonium sulphate and the collected purified protein were detected by SDS-PAGE technique.

MATERIALS AND METHODS

Bacterium and growth conditions: The bacterial culture *Bacillus subtilis* was obtained from GKVK Bangaluru, Karnataka, India. It was maintained on nutrient agar medium (g/l: nutrient broth 13.0g, agar 15.0g, pH 7.0). The growth was determined by taking optical density of *B. subtilis* using UV-VIS spectrophotometer at 660 nm at every 15 min⁶.

Mutagenesis: UV irradiations (253.7nm) were used to obtain mutants. Bacterial suspension (0.5 ml, 1 week old) was transferred to sterile petriplates and exposed to UV irradiation (253.7 nm) for 5min, 10 min, 15 min, 20 min, 25 min, 30 min, 35 min, 40 min, 45 min, 50 min, 55 min and 60 min. The distance between lamp and petriplates was adjusted to 55 cm and incubated for 24 h. UV plates were placed in the incubator at 37°C for 24 h⁷. Colonies after UV treatment were recorded to determine survival of the target strain. Sub culturing of resistant colonies was done and then reexposed to UV irradiation to obtain mutant colonies. Thus reversal was also examined. Then screened mutant derivatives were assayed quantitatively for enzymatic activity by shake flask method^{8,9}.

Fermentation media: Media supplemented with components as peptone 0.6%, KCl 0.05% (w/v), MgSO₄.7H₂O 0.05% (w/v) and starch (1%) was used for xylanase production for target bacterial strain along with screened mutants^{7,10}.

Protein estimation and xylanase assay: The protein was determined by the Lowry's method using bovine serum albumin (BSA) as standard¹⁴. Xylanase activity was measured according to Bailey *et al.*¹¹. A 900 µL of 1% solubilised birchwood xylan solution was added with 100 µL enzyme solution in a test tube. 1.5mL DNS reagent was added and incubated at 50°C for 5min in water bath^{10,12}. The absorbance was measured at 540 nm. The reaction was terminated at zero time in the control tubes. The standard graph was prepared using 0–500 µg xylose. An autozero was set in UV-VIS spectrophotometer (Hitachi, Japan) using buffer solution. One unit of xylanase activity was defined as the amount of enzyme that liberates 1micromole of reducing sugars equivalent to xylose per minute under the assay conditions described. Solubilised xylan was prepared by stirring birchwood xylan with 1M NaOH for six hours at room temperature followed by centrifugation and freeze drying the supernatant after neutralising the alkali with 1M HCl.

Protein purification and molecular weight determination: Xylanase produced was partially purified by precipitation with ammonium sulphate (60%) at 4°C and followed by dialysis with phosphate buffer (0.001M) for 24 h. The molecular weight of the purified xylanase was estimated by SDS PAGE performed by Laemmli's method¹³ using 12% acrylamide gel. Molecular mass for SDS-PAGE were calculated by using Diastase and Bovine serum albumin protein as standard.

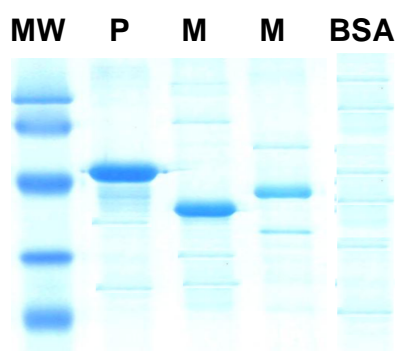
Agarose gel electrophoresis: The most efficient xylanase producing strain from UV mutants was selected and compared with parent strain. Genomic DNA was extracted and studied with the help of agarose gel electrophoresis using Bangalore Genei kit. Visualization of bands was done under transilluminator. Bands were studied by using control DNA (marker) as standard.

RESULTS AND DISCUSSION

Effect of UV exposure of *B. subtilis* for different time intervals (5 min to 60 min) was evaluated by constructing a survival curve itself. The results revealed a gradual decline in percentage of survivors with increase in UV exposure time (Graph 1). Exposure for 20min was assumed sublethal resulting 50% mortality. Exposure for 60 min was lethal resulting in 90% mortality. Xylanase activity of mutants was more¹⁴ as compared to the parent. The study showed that the enzymatic activity of mutant strains irradiated with UV radiation was different at different duration of radiation exposure. There was a maximum enhancement in xylanase activity at 60min exposure. It has also been observed that the maximum xylanase production occurred when cell growth reached the peak at their late exponential and early stationary phase of growth. This fact is also supported by N Tahar *et al.*¹⁵ who reported that the effective production of xylanase may not occur until the stationary phase has been reached. Total protein in the crude enzyme of the parent strain was found to be 292 mgL⁻¹ whereas in the mutant strain it was 618 mgL⁻¹ which was two times higher than parent strain.

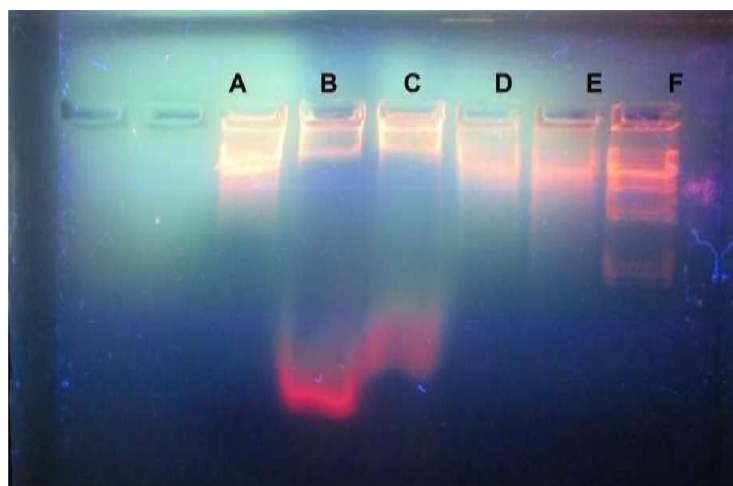
Total enzymatic activity of the crude enzyme of parent strain was found to be 17.5 Uml⁻¹ whereas in the mutant strain the enzymatic activity of the crude enzyme was 303 Uml⁻¹ which was 17 times higher than parent strain. (Table 1). Purified enzyme showed molecular weight of 38 KDa and 44 KDa by SDS-PAGE in both parent and mutant strains (Fig1). The SDS-PAGE of mutant strain also showed the presence of other bands of higher molecular weight indicating the presence of additional proteins resulting due to UV exposure, leading to the higher production of xylanase. Result of the Agarose gel electrophoresis showed that there are no changes in the genome of mutant strain due to uv irradiation (Fig.2). The increase in xylanase activity in mutant strains attributed to possible changes in the promoter zones of the genes coding for these enzymes due to the ultraviolet exposure. The radiation might have deregulated the transcription of the mRNA corresponding to enzyme, leading to an increased production¹⁶. It is assumed that xylanase production is under the control of such regulation. There are evidences that indicate the implementation of mutagenesis through UV for strain improvement¹⁷.

Fig. 1: Results of the SDS-PAGE of the parent and the mutant strain



(MW: Molecular wt marker, M: Mutant strain, P: Parent strain, BSA: Bovine Serum Albumin,)

Fig. 2: Results of the Agarose Gel Electrophoresis of the parent and the mutant strain



(Crude DNA, B: Crude DNA (mutant), C: Crude DNA (Control), D: Purified DNA (mutant), E: Purified DNA (Control), F: DNA Marker

Table 1: Purification of xylanase from *Bacillus subtilis* (parent and mutant strain)

Stages	Total protein (mgL ⁻¹)		Enzyme activity (Uml ⁻¹)		Specific activity		Purification fold	
	Mutant	parent	Mutant	Parent	Mutant	parent	Mutant	Parent
Crude	618	292	303	17.5	24.50	3.03	1.0	1.0
Precipitate	250	85	400	7.50	80.00	4.41	3.26	1.45
Dialysed enzyme	262	70	56.2	8.20	97.00	5.80	4.04	1.91

CONCLUSION

Hyper xylanase producing mutant strain of *Bacillus subtilis* was obtained by subjecting it to different periods of UV irradiations. Xylanase activity of mutants was more as compared to parent. *Bacillus subtilis* subjected to 60 minutes of UV irradiation showed the highest amylase activity (303U/ml crude activity). SDS-PAGE results showed different protein patterns for both parent and mutant strain (60 min exposure) of *Bacillus subtilis*. The protein pattern of mutant strain showed the presence of other proteins of higher molecular weight. Thus the results indicated that xylanase activity was affected and improved due to stress proteins produced by UV irradiation. There was increase in the xylanase activity with increase in UV irradiation, highest (about 17 times increase in the activity of crude enzyme) being produced by 60 min exposure of ultra violet radiation. Result of the Agarose gel electrophoresis showed that changes in the gene expression due to impact of UV do not change the genome of *B. subtilis* i.e. there is no changes in the genome of mutant strain of *B. subtilis*.

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