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EFFECT OF UV INDUCED MUTATION ON AMYLASE PRODUCING POTENTIAL OF *BACILLUS SUBTILIS* (2620)

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ABSTRACT

The impact of ultra violet radiations on alpha amylase producing ability of *Bacillus subtilis* (2620) 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 68 KDa and 54 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 amylase. 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*, amylase, enzymatic activity, SDS-PAGE**

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INTRODUCTION

α-Amylase [E.C 3.2.1.1] is an enzyme that catalyzes the hydrolysis of α-D-(1, 4) glycosidic linkage in starch components and related carbohydrates. It is a key enzyme in the production of starch derivatives and thus is of great industrial importance [1]. Amylase constitute a class of industrial enzymes, which alone contributes approximately 25% of enzyme market covering many industrial processes such as sugar, textile, paper[2], tanning, liquefaction[3] as well as in food processed industry such as baking, high fructose corn syrup and also in distillation. Amylase can be obtained from several sources such as plant, animal, and microbes [4]. The microbial sources of amylase are preferred to other sources because of its plasticity and vast availability[5]. Highly active enzyme is generally required for the conversion of starch into oligosaccharides. So it is worthwhile to select potent strain of micro-organism for enzyme production. *Bacillus* species such as *B. amyloliquefacien*, *B. subtilis*, and *B. licheniformis* are known as potent producers of amylase [6]. UV- light has also been reported to be mutagenic in variety of organisms[7]. The correlation between the quantity of energy absorbed by DNA and the observed biological effects are illustrated in the wavelength region between 254 and 320 nm[8]. In recent years, attempts have been made for the overproduction of microbial enzyme by induced mutagenesis[7]. Improvement in *Rhizopus oryzae* strain by UV, resulted in the over production glucoamylase as compared to parent strain[9]. The present investigation deals with the enhancement of the production of industrially important α-amylase, 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

1. Bacterium and growth conditions: The bacterial culture *Bacillus subtilis* 2620 was obtained from IMTECH, Chandigarh. 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 min.

2. 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 petri plates was adjusted to 55 cm and incubated for 24 h. UV plates were placed in the incubator at 37°C for 24 h[10]. 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[10, 11].

3. Fermentation media: Media supplemented with components as peptone 0.6%, KCl 0.05% (w/v), $MgSO_4.7H_2O$ 0.05% (w/v) and starch (1%) was used for amylase production for target bacterial strain along with screened mutants.

4. Iodine test: Fermentation media with 2% agar was used. Colonies of the isolated strain were transferred by replica plating on to starch agar plates and incubated at 37°C for 24h. Plates with bacterial colonies were then

flooded with Gram's iodine reagent (0.01M I_2-KI solution) [12] [13].

5. Protein estimation and amylase assay The protein was determined by the Lowry's method using bovine serum albumin (BSA) as standard [14].Amylase activity was assayed by using a reaction mixture comprising crude enzyme 0.5ml, 0.5ml of 1% (w/v) soluble starch solution in 0.1M phosphate buffer (pH 6.0) by the Dinitrosalicylic acid method (Miller, 1959)[11] and absorbance was read at 540 nm using UV-VIS Spectrophotometer. Amylase activity was determined using formula:

$$\text{Amylase Activity} = \frac{\text{Standard factor} \times \text{absorbance}}{\text{Time of incubation} \times \text{dilution}}$$

$$\text{Standard Factor} = \frac{\text{Concentration (µmol/ml) of standard solution}}{\text{Absorbance}}$$

6. Protein purification and molecular weight determination: Amylase 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 α-amylase was estimated by SDS PAGE performed by Laemmli's method[15] using 12% acrylamide gel. Molecular mass for SDS-PAGE were calculated by using Diastase and Bovine serum albumin protein as standard.

7. Agarose gel electrophoresis: The most efficient amylolytic 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* (2620) 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. Alpha amylase activity of mutants was more [16] 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 amylase activity at 60min exposure (Graph 2). It has also been observed that the maximum α-amylase production occurred when cell growth reached the peak at their late exponential and early stationary phase of growth. This fact is also supported by Asgher et al.[17] who reported that the effective production of α-amylase 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). Results of the SDS-PAGE (Fig. 2) showed the presence of alpha and beta amylase in both parent and mutant strain. Mutant strain showed the presence of other proteins of higher molecular. Result of the Agarose gel electrophoresis showed that there are no changes in the genome of mutant strain due to uv irradiation (Fig. 3). The increase in amylase activity in mutant strain

is 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 production[18]. It is assumed that amylase production is under the control of such regulation. There are evidences that indicate the implementation of mutagenesis through UV for strain improvement [19] [20].

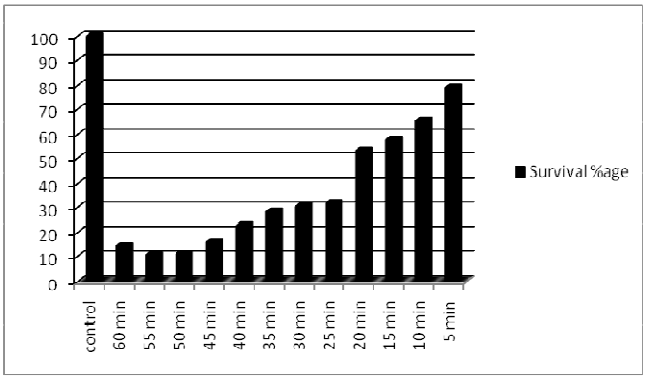
Table 1

Purification of amylase from Bacillus subtilis (2620) (parent and mutant strain)

Stages	Total protein (mgL^{-1})		Enzymatic activity (Uml^{-1})		Specific activity		Purification fold	
	Mutant	Parent	Mutant	Parent	Mutant	Parent	Mutant	Parent
Crude enzyme	618	292	303	17.5	24.51	3.03	1	1
Precipitated Enzyme	250	85	400	7.50	80.00	4.41	3.26	1.45
Dialysed enzyme	262	70	56	8.20	97.00	5.80	4.04	1.91

Graph 1

Survival Graph of Bacillus subtilis after UV irradiation



Graph2

Amylase activity and protein content of mutant strains of Bacillus subtilis after different time intervals of UV exposure

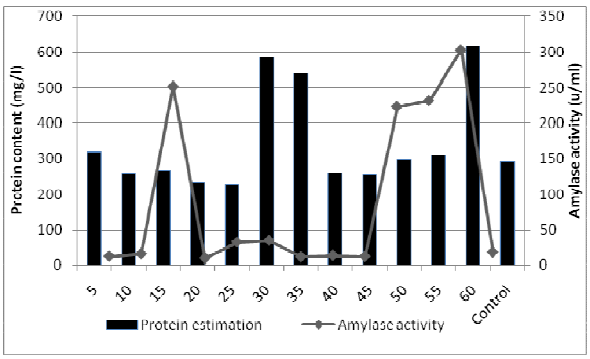


Figure 1
Iodine test of the amylase activity of the mutant strain

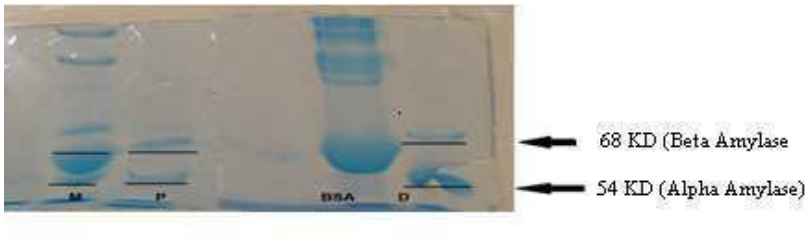


Figure 2
Results of the SDS-PAGE of the parent and the mutant strain (M: Mutant strain, P: Parent strain, BSA: Bovine Serum Albumin, D: Diastase).

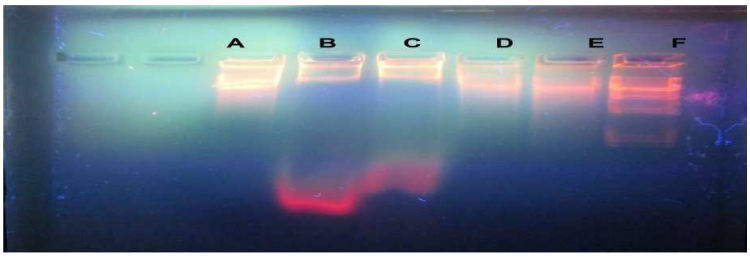


Figure 3
Results of the Agarose Gel Electrophoresis of the parent and the mutant strain (Crude DNA, B: Crude DNA (mutant), C: Crude DNA (Control), D: Purified DNA (mutant), E: Purified DNA (Control), F: DNA Marker

CONCLUSION

Hyper amylase producing mutant strain of *Bacillus subtilis* was obtained by subjecting it to different periods of UV irradiations. Alpha amylase 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 α-amylase activity was affected and improved due to stress proteins produced by UV irradiation. There was increase in the amylase 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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