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Production of Amyloglucosidase by UV Irradiated Strain of *Aspergillus niger*

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Abstract: The parental strain of *Aspergillus niger* was subjected to UV irradiations for 5-30 minutes. One hundred and ten mutants of *Aspergillus niger* were isolated by observing the hydrolysis of starch in the petri plates. Of all the mutants tested, the mutant strain of *Aspergillus niger* GCBU-25, isolated after 25 minutes of UV irradiation gave the maximum production of AMG (136.1 IU/ml/min), which gave two fold increase in the production of the enzyme than the parental strain. The production of amyloglucosidase reached its maximum (183 IU/ml/min) when starch at 1% level and $(NH_4)_2SO_4$ at 0.2% level of nitrogen was used as carbon and nitrogen source respectively.

Key words: Amyloglucosidase, UV, mutation, *Aspergillus niger*, carbon, nitrogen

Introduction

The availability of highly active amyloglucosidase is very essential for the conversion of oligosaccharides into glucose (Similey *et al*., 1964; Mackenzi *et al*., 2000). This enzyme hydrolyze 1-4 linkage of starch of oligosaccharides into glucose. So, this enzyme is extensively used in glucose production, textile industries etc (Nigam and Singh, 1995). These uses have placed greater stress on increasing amyloglucosidase production and search for more efficient processes. Recently some attempts have been made in this laboratory for the optimization of the cultural conditions for the production of amyloglucosidase by *Aspergillus niger* (Haq *et al*., 1997). The UV irradiations were found to be best for the improvement of the strain of *Aspergillus niger* for maximum production of amyloglucosidase (Ghosh *et al*., 1991; Navalaina *et al*., 1980; Kang *et al*., 1999). The selection of suitable carbon and nitrogen sources are also very essential for the better production of amyloglucosidase (Lineback *et al*., 1966).

The present study is concerned with the improvement of *Aspergillus niger* for the production of amyloglucosidase after exposing to UV irradiations. The different carbon and nitrogen sources were also evaluated for the production of amyloglucosidase.

Materials and Methods

The strain of *Aspergillus niger* GCBA-20 was used for the production of the production of amyloglucosidase. The strain was obtained from Biotechnology Laboratory, Department of Botany, Govt. College, Lahore. The strain was maintained on potato dextrose agar medium. The

fermentation was carried out in 250ml Erlenmeyer flask. Fifty ml of the fermentation medium containing (g/l) Starch 20.0, lactose 10.0, nutrient broth 10.0, $(NH_4)_2SO_4$ 5.0, $CaCl_2$ 2.0, NaCl 2.0 in 1000 ml of Phosphate buffer (pH 7.5) was transferred to 250 ml cotton plugged Erlenmeyer flask. The flasks were sterilized in the autoclave and cooled at room temperature. One ml of conidial suspension was aseptically transferred to each flask. The flasks were then placed in the rotary incubator shaker (200 rpm) at 30°C for 72h. After 72 h, the fermented broth was centrifuged at 5000 rpm for 15 min. The solids free supernatant was used for the estimation of amyloglucosidase. All the experiments were run in triplicates. The statistical analysis were compared by the method of Snedecor and Cochrane (1980). Significance has been presented as Duncan's Multiple Range test in the form of probability (*P*) values.

Enzyme assay: The amyloglucosidase estimation was carried out according to DNS method of Caldwell *et al.* (1976). One unit of activity is that amount of enzyme, which liberates 1mg glucose per hour from 5% soluble starch. The enzyme solution at pH 5.0 was incubated at 40°C using 5% soluble starch solution. The reducing sugars were measured by adding 3,5-dinitro salicylic acid reagent, boiling for 5 min, cooling and measuring the OD at 540 nm in the spectrophotometer (Model CECIL CE7200) against maltose as standard.
The amyloglucosidase activity was determined in IU/ml/min by applying the following formula:

$$\text{IU/ml/min} = \frac{\text{Activity of enzyme}}{\text{Molecular wt. of glucose x time of incubation}} \times 100$$

Mutagenic treatment: One ml of the conidial suspension was diluted up to 10^{-3}-10^{-5} times. This conidial suspension was used for mutagenic treatment. Ten ml of the diluted suspension was transferred to the sterilized petri plates. The petri plates were then placed under the UV lamp, (emitting the energy of 1.6 x 10 $J/m^2/s$) for 5-40 minutes. After different time intervals, 0.5 ml of the conidial suspension was transferred to the petri plates containing potato dextrose starch agar medium. The plates were then placed in the incubator at 30°C for 3-5 days. After 3-5 days the *Aspergillus niger* colonies showing bigger zones of starch hydrolysis as compared to parental strain (also run in parallel) were picked up and then transferred to the potato dextrose starch agar slants. These mutant strains were screened for the production of amyloglucosidase in 250 ml Erlenmeyer flask (as described above).

Results

Screening of UV irradiated mutants: The data in Table 1 shows the screening of the UV irradiated mutants for the production of alpha AMG. The conidia of *Aspergillus niger* were exposed to UV irradiation for 5-40 minutes. One hundred and sixteen mutants were isolated in the petri plates. Of

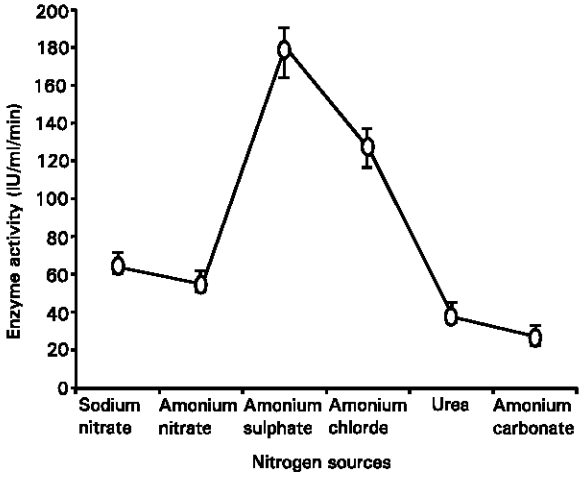

Fig 1: Effect of different concentrations of starch on the production of amyloglucosidase by *Aspergillus niger* GCU-25. Each value is an average of three replicates. Y error bars indicate the standard error. The values differ significantly at P = 0.05

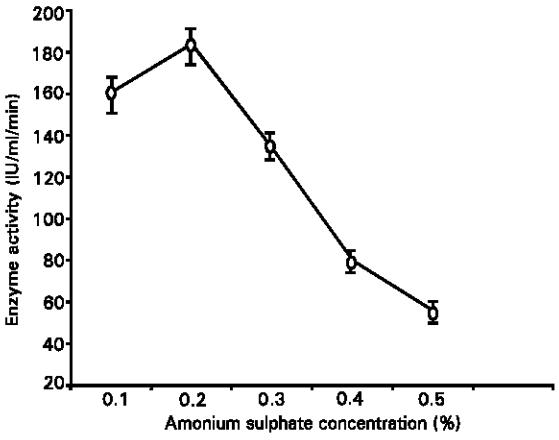

Fig 2: Effect of different nitrogen sources on *Aspergillus niger* GCU-25.Each value is an average of three replicates. Y error bars indicate the standard error. The values differ significantly at P = 0.05

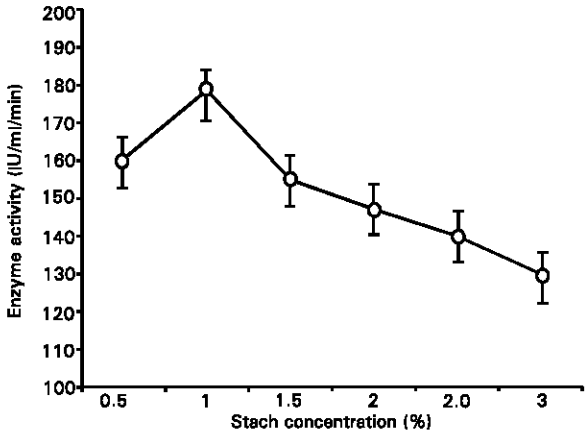

Fig. 3: Effect of different concentrations of $(NH_4)_2SO_4$ on the production of *Aspergillus niger* GCU- 25. Each value is an average of three replicates Yerror bars indicated the standared after time intervals of UV irradiation.

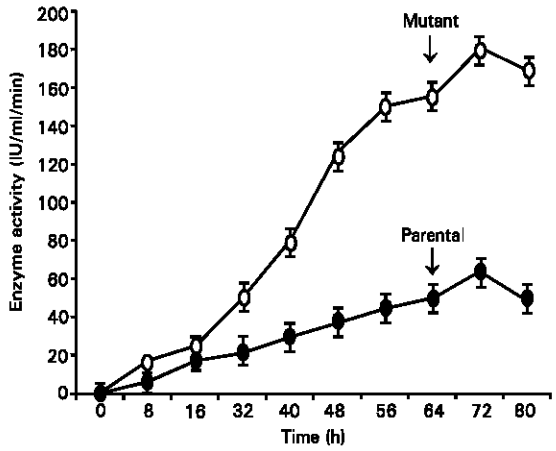

Fig. 4: Comparison between parental and mutant strain of *Aspergillus niger* for the production of amyloglucosidase Each value is an average of three replicates. Yerror bars indicated thestandard error. The values differ significantly at P = 0.05.

Table 1: Screening of *Aspergillus niger* mutants for the production of amyloglucosidase isolated after different time intervals of UV irradiations

No.	Exposure time (min)	No. of survivers	Range of AMG (IU/ml/min)
1	5	36	430-1260
2	10	30	75-1321
3	15	26	750-1390
4	20	16	340-921
5	25	04	526-1470
6	30	04	810-975
7	35	Nil	Nil
8	40	Nil	Nil

Temperature = 30 °C pH =6.0 Incubation period = 72h

all the mutants tested, the *Aspergillus niger* GCBU-25, isolate after 25 min of UV irradiations, gave maximum production of amyloglucosidase (136.1 IU/ml/min). This strain was selected for further studies.

Effect of different concentrations of starch: The effect of different concentrations of starch was investigated for the production of AMG by *Aspergillus niger* GCBU-25 (Fig 1). The starch was added of three replicates. Y error bars indicated the standard error. The values differ significantly at P = 0.05 in the medium at 0.5-3.0% levels. The production of the enzyme was obtained maximum (179. IU/ml/min) when 1.0 % starch was added to the fermentation medium. Further increase in the amount of starch, resulted in the decrease production of enzyme. However 1% starch was found optimum for the production of amyloglucosidase.

Effect of different nitrogen sources: The different nitrogen sources such as $(NH_4)_2SO_4$, NH_4NO_3, NH_4Cl, $NaNO_3$, $(NH_4)_2CO_3$ or urea were evaluated for the production of AMG by *Aspergillus niger* GCBU-25 (Fig 2). Nitrogen sources on the basis of 0.2% nitrogen were added to the fermentation medium. The maximum production of enzyme (178 IU/ml/min) was achieved when $(NH_4)_2SO_4$ was added to the medium.

The effect of different concentrations of $(NH_4)_2SO_4$ was also studied for the production of enzyme (Fig. 3). The maximum production of AMG (183 IU/ml/min) was obtained when 0.2% nitrogen was added to the medium. Further increase in the concentration of nitrogen however, reduces the production of enzyme. Hence 0.2% nitrogen in the form of $(NH_4)_2SO_4$ was selected for further studies.

Comparison between parental and mutant strains of *Aspergillus niger:* The mutant strain was compared with the parental strain for the production of enzyme, cell mass formation and substrate consumption (Fig. 4). The cell mass formation and substrate consumption were almost same for both the parent and mutant strain. But the production of the enzyme was significantly increased with mutant strain of *Aspergillus niger* than the parental strain which was 183 IU/ml/min and 65 IU/ml/min respectively.

Discussion

The improved production of amyloglucosidase can be achieved by using hypersecretive mutant of *Aspergillus niger*. The parental strain of *Aspergillus niger* GCBA-20 was treated with UV irradiation for 5-40 minutes. One hundred and twenty six mutants showing bigger zone of starch hydrolysis as compared to parental strain were picked up. Among all the mutant strains tested, the mutant isolated after 25 minutes of UV irradiation gave maximum production of AMG. The complete death of the fungi was observed after 40 minutes of UV exposure. Thus 25 minutes was selected because it gave an enhancement of AMG formation. It may be due to the relationship between mutation rate and the amount of dose to the fungi (Gardner *et al.*, 1991). UV induced mutation has given a stable and viable mutant for hyper-production of AMG. The productivity was two fold increased than the parental strain. This enhancement may have occurred either due to increase in gene copy number or either improvement in the gene expression or both.

The different concentrations of starch were evaluated for the production of AMG. The maximum production of enzyme was achieved when only 1% starch was added to the fermentation medium. When the amount of starch was increased the production of the enzyme was reduced. It may be due to, with the increase in the amount of carbon source than the optimum level, leads towards the reduction of enzyme formation (Ariff and Webb, 1998).

The inorganic nitrogen sources have inducing effect on the production of AMG. Among different nitrogen sources evaluated, the ammonium sulphate was found to be the best inducer of AMG. Lineback *et al.* (1966) have reported the regulation of AMG formation by nitrogen source; easily metabolizable nitrogen source like ammonium sulphate was better than the other nitrogen sources. It was observed that urea although released ammonium ion slowly was not a good nitrogen source. This was attributed to low urease activity of the organism. Pandey *et al.* (1994) reported that *Aspergillus niger* following growth on rice bran show that addition of ammonium ion increase AMG production. But in present study, the production of enzyme was increased when wheat bran along with ammonium sulphate was used in the fermentation medium.

The comparative study between mutant and parental strains show that although the substrate consumption and cell mass formation was same but the enzyme production was significantly increased by the mutant strain. It may be due to the increase in the gene on the DNA, which may occur due to the mutation (Gardner *et al.*, 1991). This increase in gene number may cause the hyper production of the amyloglucosidase by *Aspergillus niger*, which was more significant for the yield of the enzyme.

The Ultraviolet radiations were found to be the best for the production of best mutants of *Aspergillus niger* for amyloglucosidase fermentation. The high dose of UV resulted complete death of the fungi. The selected mutant strain required fewer amounts of carbon and nitrogen sources as compared to parental strain for the production of amyloglucosidase, which makes the study more economical.

Acknowledgments

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