Journal of Food Technology 3 (1): 68-71, 2005
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Production of Amyloglucosidase by UV Irradiated Strain of Aspergillus Niger Using Solid State Fermentation

Koukab Rana, Ikram-ul-Haq and Hamad Ashraf
Biotechnology Research, Centre Department of Botany, Govt.College. University Lahore, Pakistan

Abstract: The present study is concerned with the production of amyloglucosidase by UV irradiated strain of Aspergillus niger. The parental strain of Aspergillus niger was subjected to UV irriaditions for 5-30 minutes. One hundred and ten mutants of Aspergillus niger were isolated by observing the hydrolysis of starch in the petriplates. 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 was two fold increase in the production of the enzyme than the parental strain. The production of AMG reached 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, Aspergillus, Ammonium sulphate, Solid state UV, Wheat bran

Introduction

The availability of highly active amyloglucosidase is very essential for the conversion of oligosaccharides into glucose (Similey *et al.*, 1964; Nigam and Sing,1995; Mackenzi *et al.*, 2000). Recently some attempts have been made in this laboratory for the optimisation of the culturals conditions for the production of amyloglucosidase in submerged fermentation conditions by Aspergillus niger (Haq *et al.*, 1997). Solid state fermentation holds tremendous potential for the biosynthesis of amyloglucosidase. 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). Easily available carbon and nitrogen sources may improved the production of biomass while the induction of the enzyme may be reduced due to catabolite repression (Akpan, and Adelaja, 2004).

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.

Material and Methods

Organism: 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.

Fermentation Technique: Solid state fermentation technique was employed for the production of amyloglucosidase. Ten gram of wheat bran was transferred to 250 ml cotton plugged conical flask. The wheat bran was moistened with 0.01N HCl in the ratio of 1:1. The flasks were sterilized in an autoclave at 15lb pressure (121°C) for 15 min and then cooled at room temperature. Each flask was inoculated with 1.0 ml of inoculum. The flasks were placed in an incubator at 30"1EC for 48 hrs.

Extraction of Enzyme: Hundred ml of 0.01N HCl was added to each flask, 48 hours after incubation. The flasks were rotated at rotary shaker (200 rpm) for one hour. Then the fermented fungal bran was filtered and filtrate was used for estimation of amyloglucosidase.

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.

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 petriplates. The petriplates were then placed under the UV lamp, (emitting the energy of 1.6 x 10 J/m^2/s) for 5-30 minutes. After different time intervals, 0.5 ml of the conidial suspension was transferred to the petriplates containing potato dextrose starch agar medium. The plates were than placed in the incubator at 30EC 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.

Results

Screening of UV Irriadated Mutants: The data of 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 petriplates. Of 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.

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

No.	Exposure	No. of	Range of
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

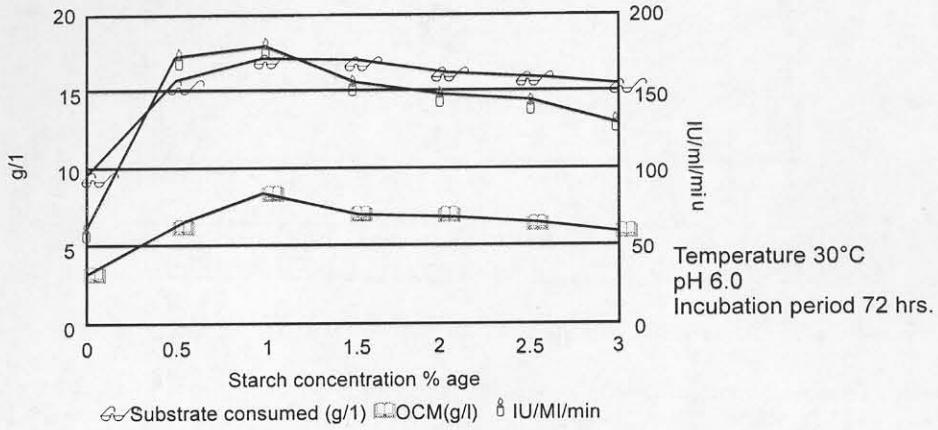

Fig. 1: Effect of different concentration of starch on the production of amloyghucosidase by Aspergllus Niger GCU-25

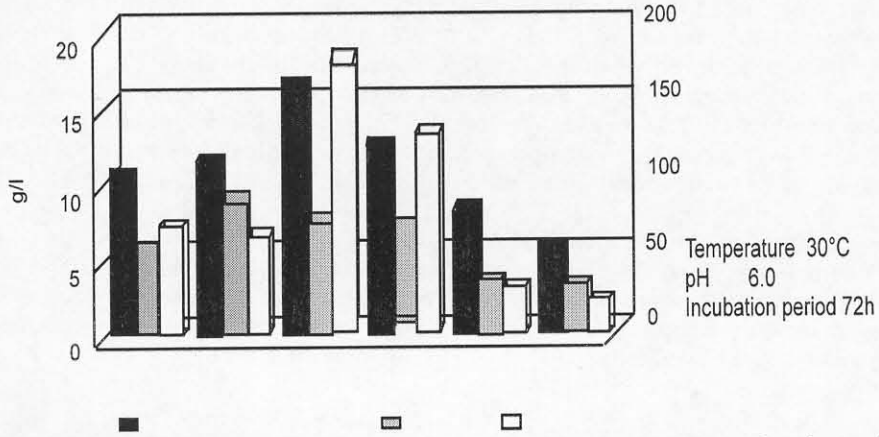

Fig. 2: Effect of different nitrogen sources on the production of amloyghucosidase by Aspergllus Niger GCU-25

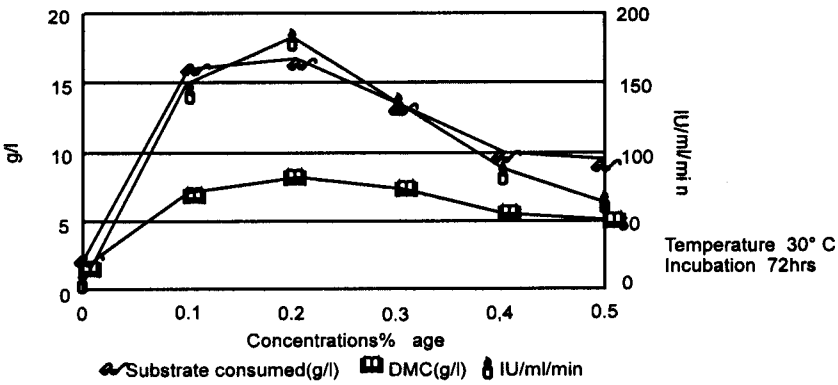

Fig. 3: Effect of different concentration of $(NH_4)_2SO_4$ on the production of amyghucosidase by Aspergllus Niger GCU-25

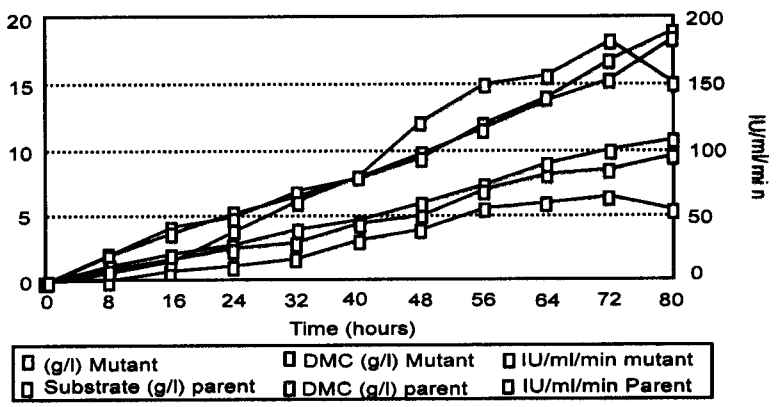

Fig. 4: Comparison between parental and mutual strain of Aspergllus Niger for the production of amyghucosidase

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 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. by

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 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 Strain 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 was 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 hyperscreative 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 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 increase than the parental strain. This enhancement may have occurred either due to increase in gene copy no. or either improvement in the gene expression or both. The mutant of Aspergillus niger has advantages of hyper-production of AMG and may serve as a starting strain for further genetic improvement. (Ghosh *et al.* 1991).

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 inducery effect on the production of AMG. Among the 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 the 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. It may be due to the increase in the gene on the DNA, which may occure due to the mutation. This increase in the gene No. may cause the hyper production of the amyloglucosidase by the Aspergillus niger, which was more significant for the yield of the enzyme

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