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Biosynthesis of Amyloglucosidase by *Aspergillus niger* Using Wheat Bran as Substrate

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Abstract: The present study is concerned with the production of amyloglucosidase by *Aspergillus niger* GCUCM-36. Effect of addition of different carbon sources and nitrogen sources on the production of enzyme was investigated. The enzyme formation was maximum (1180 IU/g/min) in the presence of glucose (1.0%) and NH_4Cl (1.5% nitrogen). The production of enzyme reached maximum, (1180 IU/g/min) at 48 hours after incubation.

Key words: Amyloglucosidase, *Aspergillus niger*, acetate buffer, glucose, ammonium sulphate

Introduction

Amyloglucosidase, an extracellular enzyme, degraded α,1-4 and α,1-6 glucosidic linkages of starch into short chain saccharides (Pazur and Ando, 1959; Dubey *et al*., 2000). The enzyme is extensively used in glucose production, brewing, textile, food, paper and pharmaceutical industries (Nigam and Singh, 1995; Mamo and Gessesse, 1999). Wheat bran was found to be an excellent substrate for the production of amyloglucosidase by *Aspergillus niger*. The production of amyloglucosidase by *Aspergillus niger* is greatly affected with various carbon and nitrogen sources (Sinker and Lewis, 1980). Pandey and Radhakrishanan (1993) has studied the supplementation of wheat bran with other starches. The maximum production of amyloglucosidase was obtained by mixing corn starch with wheat bran. Qadeer *et al*. (1985) have reported that the addition of glucose or ammonium sulphate to the fermentation medium enhanced the enzyme production. The addition of small amount of yeast extract into a medium containing $(NH_4)_2SO_4$ and starch, at a balanced carbon to nitrogen ratio increased amyloglucosidase production (Ariff and Web, 1998). Pakistan is mainly an agricultural country. Many agricultural by-products such as wheat bran, rice bran, rice husk, sunflower meal, cotton seed meal and oat bran are abundantly available for their conversion to products of economic importance. Bioconversion of these agricultural by-products to enzyme like amyloglucosidase offers an alternative for their utilization. Pakistan imports a lot of amyloglucosidase enzyme spending a huge amount of foreign exchange. The present study is concerned with the optimization of the cultural conditions for the production of amyloglucosidase by mutant strain of *Aspergillus niger* GCUCM-36 in solid state fermentation. The substrate wheat bran, a by-product of wheat flour industry was used for the production of enzyme.

Materials and Methods

Organism: The mutant strain of *Aspergillus niger* GCUCM-36 was used in present study (one year). The strain was obtained from Biotechnology Research Laboratory, Department of Botany, Government College, Lahore. The culture was maintained on potato dextrose agar medium.

Inoculum preparation: The conidial suspension was used as inoculum in present study. The conidial suspension was prepared in sterilized 0.005% Monoxal O.T. (Di-octyl ester of sodium sulpho succinic acid). Ten ml of sterilized Monoxal O.T. was transferred to each slant having profuse conidial growth on its surface. The test tube was shaken vigorously for breaking the clumps of conidia. Each ml of conidial suspension contained about 2.6×10^6 conidia.

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 acetate buffer in the ratio of 1:1. The flasks were sterilized in an autoclave 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± 1°C for 48 hours. One hundred ml of acetate buffer 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 assay of amyloglucosidase was carried out according to the method of Caldwell *et al*. (1968). The enzyme solution at pH 4.5 was incubated at 40°C using 5% soluble starch solution. The reducing sugars were measured by adding 3, 5-dinitro salicylic acid reagent, boiled for 5 min, cooled and measured the O.D at 540 nm in the spectrophotometer (Model CECIL CE7200) against glucose as standard. "One unit of activity is that amount of enzyme, which liberates one mg of glucose per hour from 5% soluble starch". The enzyme activity was then converted into IU/g/min by applying the following formula:

$$\text{IU/g/min} = \frac{\text{Sugar released}}{100 \times 60} \times 1000$$

Carbon sources: The effect of carbon sources such as glucose, sucrose, xylose, lactose, maltose or starch were tested for the production of amyloglucosidase.

Nitrogen sources: The effect of nitrogen sources such as NH_4NO_3, $(NH_4)_2HPO_4$, NH_4Cl, $NaNO_3$, $(NH_4)_2SO_4$ or urea)were tested for the production of amyloglucosidase.
All the contents of culture media unless other wise stated were of analytic grade.

Statistical Analysis: Post Hoc Multiple Comparisons were applied for different tests (ANOVA I design). Significance has been presented in the form of probability (p) values (Snedecor and Cochrane, 1998).

Results and Discussion

The optimization of the cultural conditions is very essential for the enhanced production of amyloglucosidase. In present study, the effect of different carbon sources was evaluated for the production of amyloglucosidase by *Aspergillus niger* GCUCM-36 (Fig. 1). The carbon sources at 1% (w/w) level were added to the fermentation medium. The maximum production of amyloglucosidase was found in the medium containing glucose (1010 IU/g/min). It might be due to that, the fungus required simplest carbon source for its initial growth. The fungus after initial growth can attack the complex carbohydrates of wheat bran for its further growth. Ariff and Web (1998) have reported that starch was a better carbon source for fermentation of amyloglucosidase. But in present study, the production of enzyme

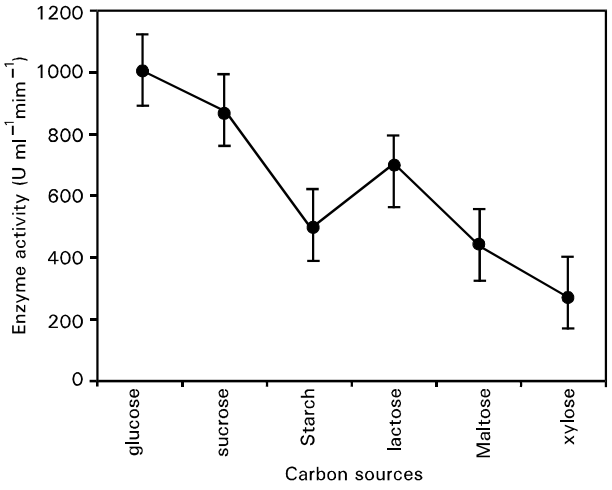

Fig. 1: Effect of different carbon sources on the production of amyloglucosidase by *Aspergillus niger* GCUCM-36

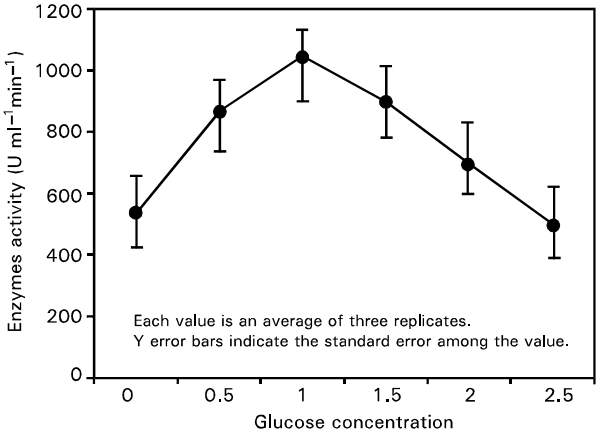

Fig. 2: Effect of different concentrations of glucose on the production of amyloglucosidase by *Aspergillus niger* GCUCM-36

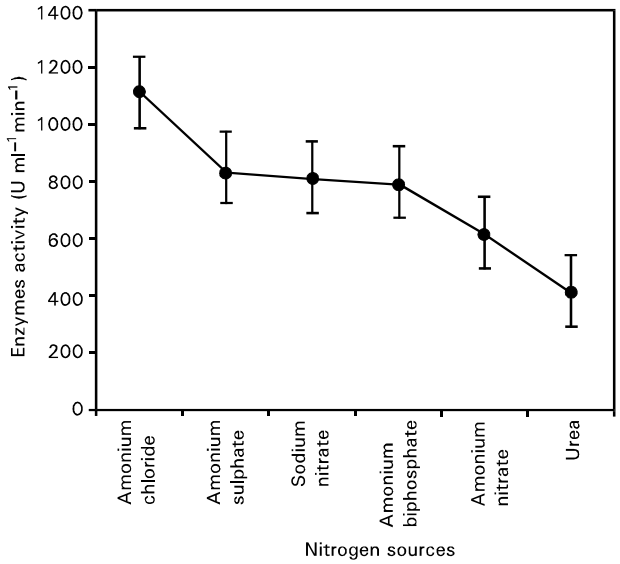

Fig. 3: Effect of different nitrogen sources on the production of amyloglucosidase by *Aspergillus niger* GCUCM-36

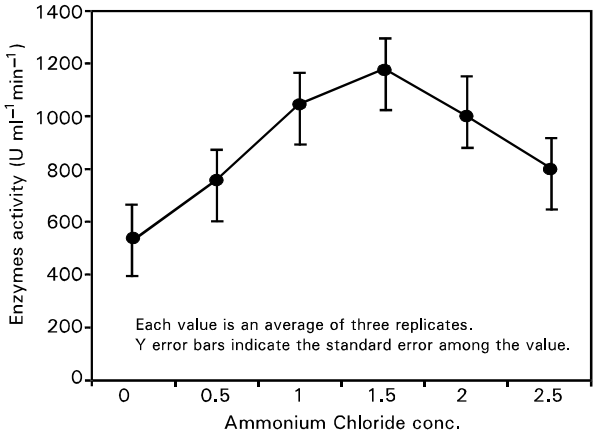

Fig. 4: Effect of different concentration of ammonium chloride on the production of amyloglucosidase by *Aspergillus niger* GCUCM-36

was significantly decreased with the supplementation of starch. It might be due to the complex carbon source such as starch, which acted as inhibitor for the initial growth of *Aspergillus niger*. However, glucose, which gave the best results, was selected as carbon source and its different concentrations (0-2.5 %) were also evaluated for the production of amyloglucosidase (Fig. 2). Among all the concentrations tested, 1% glucose was found to be optimum for better production of amyloglucosidase. As the amount of glucose was increased, production of the enzyme was decreased. It might be due to catabolite repression of enzyme at higher concentrations of glucose. This finding is an agreement with the work of Nandakumar *et al.*, 1999 and Pedersen *et al.* (2000). Since the addition of glucose to the wheat bran substrate enhanced the enzyme formation, therefore, it was added to the medium in further experiments.

Nitrogen sources have inducer effect on the growth of fungi and production of amyloglucosidase (Mamo and Gessesse, 1999). In present study, the effect of different nitrogen sources such as NH_4Cl, $(NH_4)_2SO_4$, $NaNO_3$, $(NH_4)_2HPO_4$, NH_4NO_3 or urea on the production of amyloglucosidase from *Aspergillus niger* GCUCM-36 was tested for the production of enzyme (Fig. 3). The maximum production of amyloglucosidase (1115 IU/g/min) was obtained when NH_4Cl was added to the fermentation medium. Thus NH_4Cl as nitrogen source was selected for its addition to the basal medium in further experiments. The effect of different concentrations of NH_4Cl (0-2.5%w/w) was also investigated on the production of amyloglucosidase by *Aspergillus niger* GCUCM-36 (Fig. 4). The production of enzyme was found maximum (1180 IU/g/min) when 1.5% nitrogen in the form of NH_4Cl was added to the medium. The synthesis of amyloglucosidase was affected by increasing the level of NH_4Cl in wheat bran substrate. The enzymic activity was quite low (460 IU/g/min) when NH_4Cl was not added to the medium. Therefore, 1.5% nitrogen in the form of NH_4Cl was selected for the biosynthesis of amyloglucosidase. Lineback *et al.* (1966) have reported the regulation of amyloglucosidase formation by nitrogen source. Easily metabolizable nitrogen source like $(NH_4)_2SO_4$ was better than other nitrogen sources. But in present work, NH_4Cl was found to be the best nitrogen source. It might be due to the fact that NH_4^+ ions were easily available, with supplementation of NH_4Cl in wheat bran medium. The production of enzyme was greatly inhibited with the addition of urea in the fermentation medium. It might be due to the fact that urea released NH_4^+ ions a bit slowly as compared with NH_4Cl. This was attributed to low urease activity

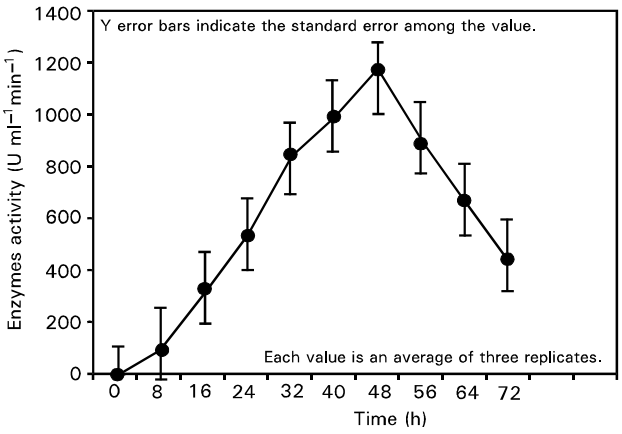

Fig. 5: Rate of amyloglucosidase fermentation by *Aspergillus niger* GCUCM-36

of the organism, which is in accord with the work of Qadeer *et al.* (1985). Thus, it was concluded that the addition of NH_4Cl as nitrogen source in the fermentation medium was necessary for enhanced amyloglucosidase production.

The optimization of the time course of fermentation is very necessary for the production of amyloglucosidase. The fermentation medium was incubated at 30°C for 0-72 hours. The production of enzyme was determined at different time intervals. The production of amyloglucosidase was reached maximum 48 hours after conidial inoculation (1180 IU/g/min). Further increase in the incubation period did not show any significant increase in the enzyme production rather it was decreased (Fig. 5). It might be due to the depletion of available nutrients for the growth of *Aspergillus niger*. Ramadas *et al.* (1996) have reported that maximum production of enzyme was obtained, 96 hours after inoculation. But in present study, the optimum production was achieved after 48 hours of incubation. So, our finding is more significant than the work of Ramadas *et al.* (1996).

In conclusion the glucose and ammonium chloride were the best source of carbon and nitrogen respectively for the production of alpha amylase. The C:N ratio 1:1.5 was found to be the best for optimum production of alpha amylase. Our findings are more encouraging and lead towards the production of alpha amylase on industrial scale.

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