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Extracellular Glucoamylase from the Isolate *Aspergillus fumigatus*

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Abstract: *Aspergillus fumigatus* Fresenius was isolated from goat's rumen and exhibited highest amylase production at temperature 37°C, medium pH 7.0 and 3 days of incubation period. Four percent starch as carbon source and 0.25% $(NH_4)_2HPO_4$ as nitrogen source was found to induce amylase production by the isolate *Aspergillus fumigatus*. Maximum glucoamylase activity was achieved at 35°C temperature and pH 7.0 along with 4% starch as substrate concentration during enzyme-substrate reaction.

Key words: *Aspergillus fumigatus*, amylase

INTRODUCTION

Human beings have utilized enzymes since ancient times, well before their nature was understood. With the understanding of nature of enzymes and their catalytic potentiality, the use of enzymes has gradually been extended to variety of fields such as food production, brewing, pharmaceuticals, textiles and detergent[1].

Up to the early 1970's it was considered that plant and animal materials were the best sources of enzymes but now a days, microbial enzymes are becoming increasingly important for its technical and economical advantages[2]

Amylases have important application in diverse industries such as backing, brewing, detergent, medicine, textile, paper and pharmaceuticals. Extracellular amylase, have been found in various species of fungi[3-6] and bacteria[7-9].

Different culture conditions greatly affect on the production of amylase. So it becomes necessary to investigate different factors involved in maximum production of amylase. Fungal amylase production reported at different pH[6,10], temperature[10-12] incubation time[10,12,18] and also in presence of various carbon and nitrogen sources[6,13-15].

Fungal amylase activity also influenced to a great extends by pH[15-18], temperature[15,19,20], various metal ions[19,20] and also substrate concentration.

In the present study, screening was carried out with a fungal isolate, which was isolated from goat's rumen. Studies were also conducted to determine the optimum culture conditions and factors involved in maximum production of amylase for the selected isolate.

MATERIALS AND METHODS

Microorganism: The isolate *Aspergillus fumigatus* Fresenius (R_1) was isolated from goat's rumen.

Isolation and screening of the isolates: For the isolation of amylolytic microbes enrichment media technique was followed. Primary screening was done by starch agar plate method. During secondary screening three different broth media such as defined salt medium {containing KH_2P0_4 0.5%, K_2HP0_4. 0.67%, $(NH_4)S0_4$ 0.1%, $MgS0_4 7H_20$ 0.01% and soluble starch 4%[21] }, Starch yeast extract medium {containing soluble starch 0.5%, KH_2P0_4 0.1%, yeast extract 0.2%, $MgS0_4 7H_20$ 0.05%[22]} and standard production medium {containing soluble starch 4%, $(NH_4)_2HP0_4$ 0.5%, yeast extract 0.5%, sodium citrate 0.2%, $MgS0_4 7H_20$ 0.05%, $CaCl_2 2H_20$ 0.008%[23] }were used.

Measurement of enzyme activity: Amylase activity was determined by measuring the increase in reducing sugar formed by the enzymatic hydrolysis of starch. Five mL 1% soluble starch, 1mL 0.2 M acetate buffer, 1mL deionized water and 1mL enzyme solution were mixed and incubated at 40°C in water bath for 1h[18]. The amount of reducing sugar liberated was quantified by Nelson's modification of Somogyi method[24]. Enzyme activity expressed in Unit, which was defined as the amount of enzyme that released 1 μg of glucose mL^{-1} of enzyme h^{-1}.

Biomass yield: Biomass was determined by dry weight method.

Optimization of culture conditions

Effect of incubation time, temperature and medium pH: To ascertain the effect of culture conditions the present study was carried out at different incubation time (2, 3, 4, 5 and 6 days), temperature (10, 27, 37 and 45°C), medium pH (5.0, 6.0, 7.0 and 8.0). Their effect on biomass characteristics, biomass yield, reducing sugar and amylase production were also recorded.

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Effect of carbon and nitrogen sources: The production of extracellular amylases under different carbon and nitrogen availability were studied in liquid medium. Four carbon sources such as starch, wheat powder, rice powder, potato and five nitrogen source such as di-ammonium hydrogen phosphate, ammonium sulphate, potassium nitrate, malt and yeast extract were added to the basal medium and their effect on production of amylase, extracellular protein, biomass yield was recorded.

To ascertain optimum percent of carbon and nitrogen sources, the study was carried out with 0.25 to 2% nitrogen source and 0.5 to 4.5% carbon sources keeping other experiment conditions at optimum level.

Factors involved on enzyme activity

Enzyme substrate reaction time: To ascertain the effect of incubation time on enzyme activity the enzyme substrate reaction mixture were incubated for different incubation time i.e. 15, 30, 45, 60 and 75 min and its effect on enzyme activity were studied.

Substance concentration: The effect of substrate concentration of the reaction mixture on enzyme activity was measured at different concentration of starch solution (i.e. 1.0, 2.0, 3.0, 4.0 and 5.0%).

Temperature and pH: The effect of pH on amylase activity was determined by incubating the reaction mixture at pH values ranging from 5.0 to 8.0 using citrate phosphate buffer and optimum temperature for enzyme activity was determined by conducting the assay at various temperature range from 30 to 50°C during enzyme substrate reaction.

RESULTS AND DISCUSSION

Twenty-three microbial isolates were isolated from rumen of goat. The isolates were then purified and screened for amylolytic activity.

Screening of isolates: The primary screening was carried out by starch hydrolysis method. Three different liquid medium were used during final selection of the isolate. Among the isolates the fungal isolate R_1 exhibited higher amylolytic activity in broth medium and was selected for further studies.

Identification of selected isolate: On the basis of cultural and morphological characteristics the isolate R_1 was found to belong to the genus *Aspergillus* and closely related to the species *Aspergillus fumigatus* Fresenius, while compared with the standard description of 'A Manual of soil fungi'[25].

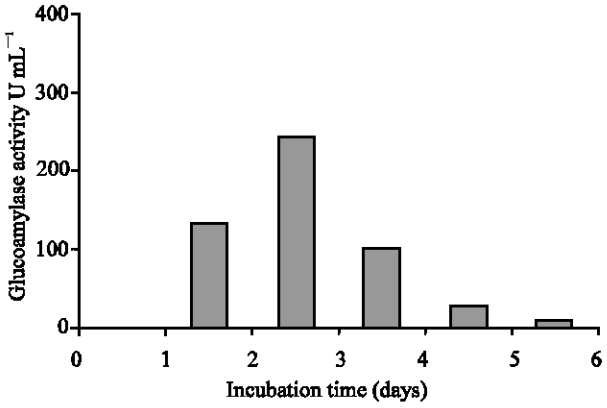

Fig. 1: Effect of incubation time on production of amylase by the isolate *Aspergillus fumigatus*

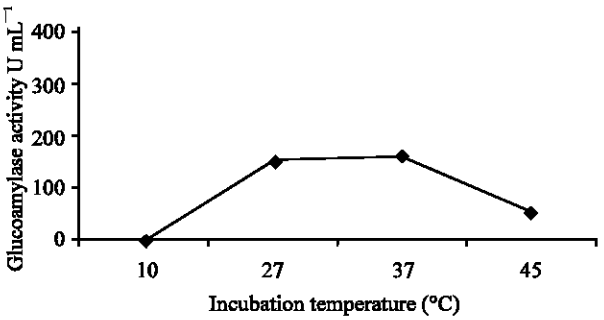

Fig. 2: Effect of temperature on production of amylase by the isolate *Aspergillus fumigatus*

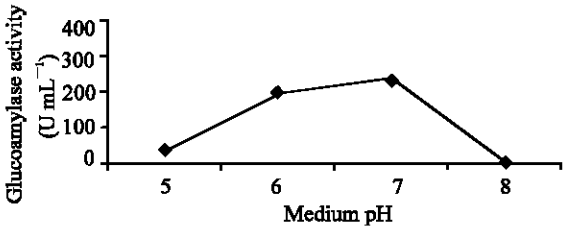

Fig. 3: Effect of medium pH on production of amylase by the isolate *Aspergillus fumigatus*

Optimization of culture conditions

Effect of incubation time: The fungal isolate showed different amylase activities at different incubation period and it was found that the isolate showed maximum activity after 3 days of incubation (Fig. 1) but highest biomass yield was recorded after 4 days of incubation time. The pH of the supernatant was found to range from 5.92 to 6.20. Production of amylase after 3 days of incubation time by *Aspergillus* sp. was also reported by Rahman *et al.*[18]

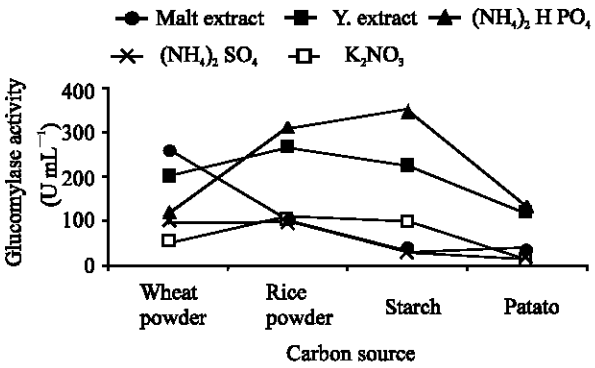

Fig. 4: Effect of carbon and nitrogen sources on production of amylase by the isolate *Aspergillus fumigatus*

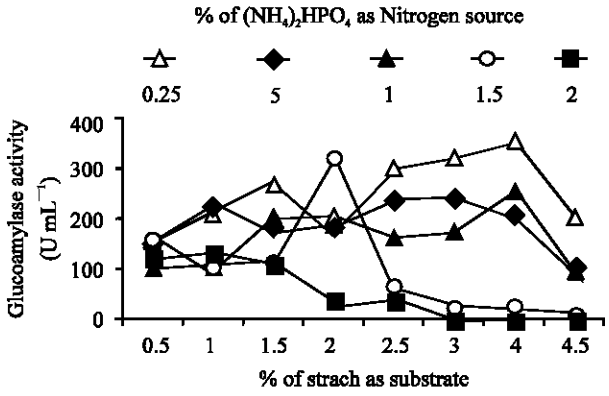

Fig. 5: Effect of concentration of starch and $(NH_4)_2HP0_4$ on production of amylase by the isolate *Aspergillus fumigatus*

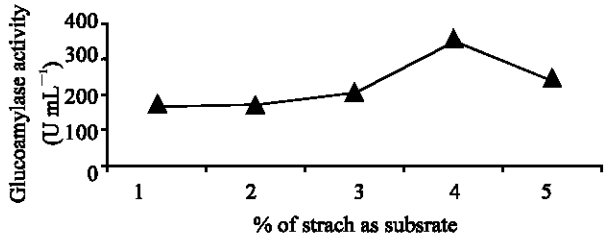

Fig. 6: Effect of substrate concentration on activity of glucoamylase of the isolate *Aspergillus fumigatus* during enzyme-substrate reaction

Effect of temperature: The isolate R_1 showed maximum enzyme activity and highest biomass yield at 37°C (Fig. 2). Similar observation was also made by Shah *et al.*[26] and Shahida *et al.*[27]

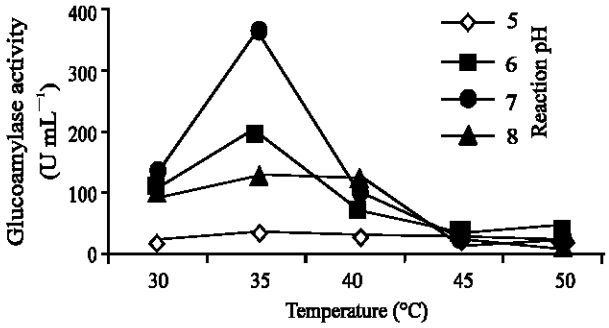

Fig. 7: Effect of pH and temperature on activity of glucoamylase of the isolate *Aspergillus fumigatus* during enzyme-substrate reaction

Effect of medium pH: Medium pH also plays an important role on production of microbial enzyme. In the present study, maximum production was achieved at medium pH 7.0 (Fig. 3). The color of the supernatant was recorded as golden yellow and the pH of the culture filtrates were ranged from 4.90 to 6.93. Similarly Mahmoud[6] isolated *Aspergillus fumigatus* from poultry feeds and maximum production was found at medium pH 7.0.

Effect of carbon and nitrogen sources: Figure 4 shows different enzymes activity on the production of amylase with the presence of various carbon and nitrogen sources by the isolate *Aspergillus fumigatus*. Maximum production of amylase was achieved when starch as carbon and $(NH_4)_2HPO_4$ as nitrogen source were used with the basal medium. The pH of the culture filtrates varied with different carbon and nitrogen sources. Maximum biomass yield was recorded when starch as carbon and $(NH_4)_2SO_4$ as nitrogen source were used. Amylase production induced by starch also reported by Lachmund and Ruttkowski[14], Lin[28], Frost and Moss[29].

After determination of suitable nitrogen and carbon source, it is also necessary to find out the concentration of suitable carbon and nitrogen source in the medium for maximum enzyme production. Figure 5 shows that the fungal isolate *Aspergillus fumigatus*, released maximum amylase when 0.25% $(NH_4)_2HPO_4$ and 4% starch were added in the growth medium. In our study, the concentration of carbon and nitrogen in the medium was found to have influence on the production of amylase by the isolate *Aspergillus fumigatus*.

Factors involved on enzyme activity: Enzyme activity depends upon enzyme-substrate reaction pH,

temperature, times, substrate concentration and many other factors. So it becomes necessary to find out different limiting factors for maximum activity of amylase.

Enzyme-substrate reaction time: The culture filtrates of the fungal isolate R_1 exhibited highest glucoamylase activity when enzyme substrate reaction mixtures were incubated at 35°C for 60 min.

Substrate concentration: The crude enzyme extract of isolate R_1, were allowed to react with different substrate concentrations (1 to 5%) and maximum activity was found with 4% of starch as substrate (Fig. 6).

Temperature and pH: Figure 7 shows that temperature and pH are also most important factors, which markedly influenced on enzyme activity. Maximum glucoamylase activity of the crude enzyme extract of fungal isolate R_1 was recorded at reaction mixture temperature 35°C and pH 7.0. Similar results also reported by Abou-Zeid and Alaa[24] while working on *Aspergillus flavus*.

REFERENCES

1. Kennedy, F., 1987. Enzyme Technology. In Biotechnol Vol. 7a. Ed. Rehm, H.J. and G. Reed, V.C.H. Weinheim.
2. Kelly, C.T. and W.M. Fogarty, 1976. Microbial alkaline enzymes. Process Biochem, 11: 3-9.
3. Tani, Y., V. Voungsuvaneert and J. Kumunantu, 1986. Raw cassava starch digestive glucoamylase of *Aspergillus* sp. N-2 isolated from cassava starch. J. Ferment. Technol., 64: 405-412.
4. Kanlayakrit, W., K. Ishiatsu, M. Nakao and S. Hayshida, 1987. Characteristics of raw starch degrading glucoamylase from thermophilic *Rhizomucor pusilus*. J. Ferment Technol., 65: 379-385.
5. Steverson, E.M., R.A. Korun, W. Admassu and R.C. Heimasab, 1984. Kinetics of the amylase system of *Saccharomyces fibulegera*. Enzyme and Microbiol., Technol., 6: 549-554.
6. Mahmoud, A.L.E., 1993. Different factors affecting growth and amylase production by fungi inhabiting poultry feeds. J. Basic Microbiol., 33: 187-192.
7. Srivastava, R.A.K. and J.N. Baruah, 1986. Culture condition for production of thermostable amylase by *Bacillus stearothermophilus* Appl. Environ. Microbiol, 51: 179-184.
8. Syu, M.J. and Y.H. Chen, 1997. A study on the α-amylase fermentation performed by *Bacillus amyloliquefaciens*. Chem. Eng. J., 65: 237-247.
9. Tanaka, T., E. Ishimoto, Y. Shimomura, M. Tariguchi and S. Oi, 1987. Purification and some properties of raw starch binding amylase of *Clostridium butyricum* T-7 isolated from mesophilic methane sludge. Agri. Biol. Chem., 51: 399-405.
10. Bormiss, R., Schmidt, Dieter, Gorges, Eberhardt, Ruftoff, Heinz, Taeufel, Alfred, Lietz, Peter, Steffen, Heinig, Wolfgany and Gergs, Ursula, 1981. Enzyme mixture for saccharification of grain mash. Ger (East) D.D. 152: 142.
11. Hizukuri and Susumu, 1983. *Aspergillus* strains and their use to produce D-glucose from starch. Eur. Pat. Appl. Ep.,134: 546.
12. Marzan, L.W., M.A. Manchur, M.T. Hossain and M.N. Anwar, 2001. Production of protease and α-amylase by *Fusarium poae*. Bangladesh J. Microbiol., 18:127-134.
13. Guo, Y., L. Haung, M. Zhao, H. Chen, X. Peng, G. Luo and L. Li, 1988. Production of glucoamylase by immobilized cells. 11. Conditions for batch fermentation and semicontineuous fermentation, Shipin Yu Fajiao Gongye, 2: 28-31.
14. Lachmund, A. and E. Ruttkowski, 1990. Investigations on the expression and regulation of the α-amylase gene from *Aspergillus oryzae*. DECHEMA Biotechnol. Conf., 4: 399-402.
15. Okolo, B.N., Ezeogu, Lewis, I. Mba and N. Charles, 1995. Production of raw starch digesting amylase by *Aspergillus niger* grown on native starch sources. J. Sci. Food Agric., 69: 109-115.
16. Filipov, S.A., S.P. Avakyants and S.P. Bartenev, 1982. Conditions for the immobilization of cells of microscopic fungi and their use for enzymatic hydrolysis of starch. Ferment. Spirt. Prom-st., 2: 30-31.
17. Kachelkina, A.I. and L.I. Oreshchenko, 1980. Separating α-amylase from a culture medium. U.S.S.R., 668: 348.
18. Rahman, A.K.K.M.S., M.F. Rahman, P.K. Nath, M.M. Hoq and M. Hossain, 1993. Glucoamylase activity of some selected strains of *Aspergillus niger* and *A. oryzae*. Bangladesh J. Microbiol., 10: 107-110.
19. Olasupo, N.A., O.D. Teniola, R. Okasun, A. Omawaye, S.O. Olatrope and E.M.B. Scott, 1996. Studies on an amylolytic strain of *Saccharomyces cerevisiae* isolated from yam tuber. J. Basic Microbiol., 36: 293-288.
20. Abou-Zeid Alaa, M., 1997. Production, purification and characterization of an extracellular α-amylase enzyme isolated from *Aspergillus flavus*. Microbios, 89: 55-66.

21. Almeida, E.M. D.S., K. Mizuta and J.R. Gigilo, 1997. *Pycnoporus sanguineus*: A novel source of α-amylase. Mycol. Res., 101: 188-190.
22. Adams, P.R., 1997. Growth and amylase production in *Sporotrichum thermophile* Apinis. Biotechnol. Appl. Biochem., 26: 169-170.
23. Kwan, H.S., K.H. So, K.Y. Chan and S.C. Cheng, 1993. Production of thermotolerant α-amylase by *Bacillus circulans*. W.J. Microbiol and Biotechnol., 9: 50-52.
24. Nelson, N., 1944. A photometric adaptation of the Somogy method for the determination of glucose. J. Biol. Chem., 153: 375-380.
25. Gilman, J.C., 1975. A Manual of Soil Fungi. Second Indian Reprint. Oxford and IBH Publication Co. New Delhi.
26. Shah, N.K., V. Ramamurti and R.M. Kothari, 1991. Comparative profiles of fungus α-amylase production by submerged and surface fermentation. Biotechnol. Lett., 13: 361-364.
27. Shahida, A., M.A. Manchur, M.T. Hossain and M.N. Anwar, 1999. Isolation of amylase producing fungi and their amylase activity. The Chittagong Univ. J. Sci., 23: 107-113.
28. Lin, L.L., M.R. Tsau and W.S. Chu, 1994. General characteristics of thermostable amylopullulanases and amylases from the alkalophilic *Bacillus* sp.TS-23. Appl. Microbiol. Biotechnol., 42: 51-56.
29. Frost, G.M. and D.A. Moss, 1997. Production of Enzyme by Fermentation. In: Biotechnology. Kennedy, J.F., Ed., pp: 65-211.