HYPER PRODUCTION OF $\beta$-GLUCOSIDASE BY AN ASPERGILLUS sp.¹

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SUMMARY
An Aspergillus sp. was isolated which secreted high levels of $\beta$-glucosidase in growth medium. The maximum activity (10 IU/ml of $\beta$-glucosidase and 22.6 IU/ml of cellobiase) was obtained in cellulose medium supplemented with wheat bran. The pH and temperature optima for this enzyme were 4.5 and 65°C respectively.

INTRODUCTION
During cellulose degradation cellobiose is a potent inhibitor of endo- and exo-glucanases and this effect is more pronounced when the organism is deficient or inefficient in $\beta$-glucosidase production as in the case of Trichoderma reesei (Bisset and Sternberg, 1976). In order to eliminate the inhibition of endo- and exo-glucanases by cellobiose and thereby to improve the rate of saccharification of cellulose, Sternberg et al. (1977) have recommended the supplementation of T. reesei cellulases with $\beta$-glucosidases from other sources. In this paper we report the isolation of a fungus, identified as Aspergillus sp., which secretes unusually high amounts of $\beta$-glucosidase in the culture filtrate.

METHODS AND MATERIALS
The following reagents were purchased from the suppliers indicated: cellulose 100 powder (Cellulose product of India Ltd, Ahmedabad), xylan (Fluka AG, Switzerland), Solka Floc SW40 (Brown Co., Berlin NH), Avicel P.H.101 (Honeywill and Stein Ltd., U.K.), sodium salt of carboxymethylcellulose and cellobiose (Sigma Chemical Co., U.S.A.) and p-nitrophenyl-$\beta$-D-glucoside (Koch-Light, U.K.). All the other chemicals used were of analytical grade.

Enzyme production: Growth medium described for T. viride by Reese and Mandels (1963) containing specified quality of cellulose was used in enzyme production. The pH of the medium was adjusted to 5.5 prior to autoclaving at 121°C for 20 min. Spores of the Aspergillus culture grown on potato dextrose agar were inoculated in 500 ml Erlenmeyer flask containing 100 ml of the growth medium. The flasks were incubated at 30°C on rotary shaker for 12-14 days and then harvested by centrifugation at 5000 rpm in Sorvall RC 5B centrifuge. The clear supernatant was used to estimate various enzyme activities.

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Enzyme assays: γ-Glucosidase activity was determined by a modified method of Eberhart (1961) using p-nitrophenyl-β-D-glucoside (pNPG) as a substrate. Cellobiase activity was measured by the modified method of Umezurike (1971) using cellobiose as substrate. The glucose produced was estimated by glucose peroxidase test (Bergmeyer et al., 1974; Wood and McCrae, 1972).

One unit of activity is expressed as the number of moles of glucose or p-nitrophenol produced per min per millilitre of the culture filtrate.

RESULTS AND DISCUSSION

Enzyme production in presence of various cellulosic and other carbon sources: The effect of different cellulosic and other carbon sources on β-glucosidase production by Aspergillus sp. is shown in Table 1. The enzyme was produced in presence of all the substrates used. Wheat bran was found to be the best substrate for enzyme production. Production was further enhanced when 3% wheat bran and 2% cellulose powder were used together as substrate. Among the other carbon sources studied, lactose and starch induced more enzyme activity than cellulose at the same concentration. The highest enzyme activity was detected in the culture filtrate when 3% xylan was used as a source of carbon. Very low levels of enzyme activity was observed when the culture was grown in presence of glucose, maltose, glycerol or cellobiose as carbon sources. The culture also exhibited low levels of carboxymethylcellulase (CMCase) and filter paper activities (data not shown).

Comparison of β-glucosidase by Aspergillus sp. and other fungal cultures: The amount of enzyme secreted by the present isolate when compared with the values reported so far for other Aspergillus cultures and basidiomycete CPC-142, was higher (Table 2). The highest cellobiase activity reported for T. reesei cultures is 0.5-1.3 IU/ml (Montenecourt and Eveleigh, 1977) which is suboptimal for conversion of cellulose to glucose (Sternberg et al., 1977). With pNPG or cellobiose as the substrate the optimum pH and temperature for the enzyme activity were 4.5 and 65°C respectively. The yield on the other hand, was the highest at pH 5.5 and 30°C. Isolate of Aspergillus sp. reported here is apparently the most suitable candidate as the source of extracellular β-glucosidase for supplementation of T. reesei cellulases to enhance the rate of saccharification of cellulose.

REFERENCES


