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NUTRITIONAL STUDIES OF ASPERGILLUS AWAMORI FOR THE PRODUCTION OF AMYLOGLUCOSIDASE

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Of all the strains of *Aspergillus* species *A. awamori* gave the best results of amyloglucosidase. The enzyme formation in general was sensitive towards medium composition. Penicillin waste mycelium greatly improved the amyloglucosidase formation than the other sources of nitrogen. The amyloglucosidase formation was decreased in the order of Penicillin waste mycelium, cornsteep liquor, $NaNO_3$, NH_4Cl, $(NH_4)_2SO_4$ and urea. Glucose was equally good source of carbon in the culture medium like yellow corn-flour.

Amyloglucosidase, the enzyme which converts starch specifically and completely to glucose, capable of hydrolysing both α-1,4- and α-1,6-glucosidic linkages,[1–3] finds large applications in the preparation of syrups of special properties.[4–7] Extensive research is being carried out in various laboratories to determine optimal conditions for the production of amyloglucosidase by mould fermentations.[1,6,8–14] Strains of *Rhizopus*[15–16] and *Aspergillus* [17–20] species are generally used for the production of the enzyme on commercial scale.

Important factors for the synthesis of amyloglucosidase are medium composition such as carbon and nitrogen sources, trace metals and magnesium ions.

The present study describes the selection of mould strains capable of producing amyloglucosidase and their nutritional requirements for optimum yield in shake flasks.

Materials and Methods

Microorganisms.—The mould strains of *Aspergillus niger* NRRL-337, *Aspergillus awamori* NRRL-3112 and *Aspergillus phoenicis* IFO-6649 were used in the present study.

The cultures were maintained on the agar medium consisting of (g/l): sucrose, 30.0; $NaNO_3$, 3.0; K_2HPO_4, 1.0; $MgSO_4.7H_2O$, 0.5; $FeSO_4.7H_2O$, 0.01 and agar, 15.0. The cultures were incubated at 30°C for 5–7 days for maximum sporulation and then slants were kept in the refrigerator.

Inoculum Preparation.—The spores from 5–7 days old cultures were wet with 5 ml of 0.05% Monoxal O.T. (diacetyl ester of sodium sulphosuccinic acid). The supernatant containing spores suspension was decanted off asceptically. The agar surface was washed twice with sterile distilled water. The combined washings were made up to 25 ml and shaken with glass beads to break the clumps of spores. The spore concentration in the inoculum was 1.50×10^6/ml. The counting was carried out by using Thomas counting chamber.

Fermentation Medium.—All media, given in Table 1, unless otherwise stated, were autoclaved at 121°C for 15 min. The fermentation medium (25 ml) including 1 ml spore suspension was held in 250 ml conical flask. The flasks were shaken on a rotary shaker, fabricated in the Workshop of PCSIR Labs, Lahore (throw 1½"). The shaker was rotated at 150 rev/min. The shaker was placed in an air-conditioned room and the temperature was kept at 30°C.

Analytical Method.—Extracellular enzyme activity was determined in the culture filtrate obtained after the removal of mycelium by filtration or centrifuging at 3000 rev/min for 15 min. The enzyme activity was determined by measuring the glucose liberated from starch.

One unit of the amyloglucosidase enzyme was taken as that amount of enzyme required to digest one tenth of starch essentially to dextrose in 1 hr at pH 4.0 and at a temperature of 55°C.

The enzyme liquids were assayed using 4% starch solution thinned with 0.05 g α-amylase per 80 g starch. Glucose was estimated by ferricyanide reduction method, a modification of Fujita and Iwatake.[21]

Results

Selection of Strain.—The three strains of *Aspergillus* species, i.e. *A. phoenicis* IFO-6649, *A. niger* NRRL-

Table 1.—Percentage Composition of Fermentation Media.

Constituents	M1	M2	M3	M4
Corn-flour	7.0	—	—	—
Yellow corn-flour	—	1.00	5.0	5.0
$(NH_4)_2SO_4$	0.7	0.70	—	—
Corn-steep liquor	—	—	5.0	5.0
$K_2HPO_4.3H_2O$	0.10	0.10	—	—
$MgSO_4.7H_2O$	0.05	0.05	—	—
KCl	0.05	—	—	—
$FeSO_4$	0.001	—	—	—
Acetic acid	0.90	—	—	—
NaOH	0.54	—	—	—
KOH	—	—	0.05	0.2
α-Amylase	—	—	0.00225	0.0025

TABLE 2

Strains used	Days	Units/ml
A. phoenicis IFO-6649	5	3.0
A. niger NRRL-337	5	2.5
A. awamori NRRL-3112	5	8.0

TABLE 3.—PRODUCTION OF AMYLOGLUCOSIDASE BY *A. awamori* NRRL-3112 ON DIFFERENT FERMENTATION MEDIA.

Medium	pH	Days	Units/ml
M1	5.6	6	11.5
M2	5.8	7	13.0
M3	4.3	4	18.0
M4	4.3	4	20.0

337 and *A. awamori* NRRL-3112 were examined for their amyloglucosidase producing abilities in shake flasks (Table 2). The cultures were grown in the fermentation medium M2 for 5 days. The strain of *A. awamori* gave the highest yield of amyloglucosidase, i.e. 8 units/ml. In subsequent experiments, therefore, the strain of *A. awamori* was used for the production of enzyme.

The data in Table 3 shows the amyloglucosidase formation by *A. awamori* in different media. The cultures were analysed at different periods after inoculation. Amyloglucosidase yield was maximum in media M3 and M4, i.e. 18 and 20 units/ml respectively. The enzyme synthesis, however, was lower in synthetic media M1, i.e. 11.5 units/ml. It follows that medium composition has great influence on the production of amyloglucosidase. The maximum yield in the natural media may be due to the availability of certain minerals and amino acids present in the ingredients. The synthetic media, however, was selected for investigating the effect of various nitrogen and carbohydrate sources on the production of amyloglucosidase.

Effect of Carbon Sources.—The data in Table 4 shows the effect of different carbon sources on the production of amyloglucosidase by *A. awamori*. The amount of each carbon source was kept at 5% in the medium M1. The yield of amyloglucosdiase was lower in the presence of lactose and sucrose, that is, 0.7 and 0.5 units/ml respectively. Glucose and yellow corn-flour gave maximum production of amyloglucosidase than that obtained in the presence of fructose, maltose or potato-starch, i.e. 1.5 units/ml.

To determine the optimum concentration of yellow corn-flour in the culture medium, effect of different levels of yellow corn–flour (2–10%) was studied on the synthesis of amyloglucosidase in medium M1. The production of enzyme was increased with the increasing concentration of

TABLE 4.—EFFECT OF DIFFERENT CARBON SOURCES ON THE PRODUCTION OF AMYLOGLUCOSIDASE BY *A. awamori* NRRL-3112 IN MEDIUM M1

Carbon source	Units/ml
Lactose	0.7
Fructose	1.5
Glucose	2.0
Maltose	1.5
Sucrose	0.5
Corn-flour	1.0
Potato starch	1.6
Yellow corn-flour	2.1

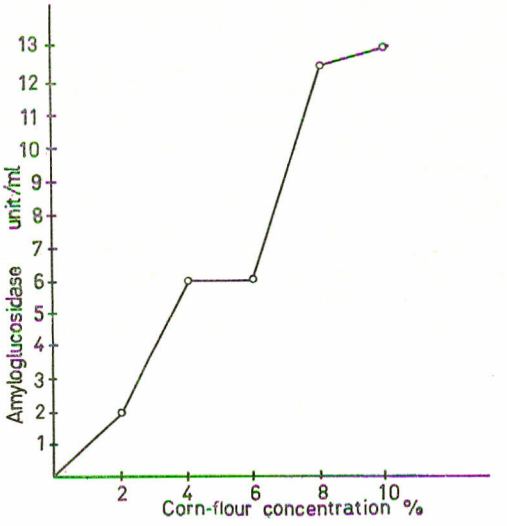

Fig. 1.—Effect of corn-flour on the production of amyloglucosidase by *A. awamori* NRRL 3112.

corn-flour. In the presence of 4–6% corn-flour, the amount of enzyme produced was 6.0 units/ml. Further increase in the amount of yellow corn-flour i.e. 8 and 10%, greatly improved the yield of amyloglucosidase i.e. 12.5 and 13.0 units/ml, respectively.

The culture medium was highly viscous when concentrations above 6% were used with the result that both the agitation and aeration of the shake flask cultures was greatly affected. The corn–flour concentration of 5%, was therefore employed in further study.

Effect of Nitrogen Sources.—Effect of different sources of nitrogen such as NH_4Cl, $NaNO_3$, $(NH_4)_2SO_4$, urea, corn-steep liquor and penicillin-waste mycelium was studied on amyloglucosidase formation by *A. awamori* (Fig. 2–7). The nitrogen level in the culture medium was maintained at 0.85 g/l throughout in the present investigation. $NaNO_3$ and penicillin waste mycelium gave the maximum yield of amyloglucosidase among the inorganic and organic nitrogen sources respectively.

The results of enzyme synthesis, however, were in the decreasing order of $NaNO_3$, NH_4Cl, $(NH_4)_2SO_4$ among the inorganic nitrogen sources and penicillin-waste mycelium, corn-steep liquor and urea were out of organic nitrogen sources. The yield of amyloglucosidase, 6–7 days after spore inoculation was 5.0, 3.6, 3.0, 12.0, 5.5 and 3.0 units/ml, respectively. The enzyme synthesis, in general, was at peak at 48 or 72 and 144 hr after spore inoculation.

Discussion

The strain of *Aspergillus awamori* produced maximum amount of amyloglucosidase than other strains of *Aspergillus* species. The factors which control the synthesis of enzyme are: the adequate levels and type of both the carbon and nitrogen sources. The organic nitrogen source such as penicillin-waste mycelium results in maximum enzyme formation. This may be attributed to the availability of vitamins, minerals, carbohydrates and amino acids. The use of corn-steep liquor had less stimulatory effect on the amyloglucosidase formation. The amount of enzyme produced was about the same as when inorganic nitrogen sources such as $NaNO_3$ were used (5.00 units/ml); other inorganic nitrogen sources like NH_4Cl, $(NH_4)_2SO_4$ and urea, an organic nitrogen source in the culture medium, produced about 3.00 units/ml of amyloglucosidase. Of all the carbohydrates, yellow corn-flour and glucose, gave better

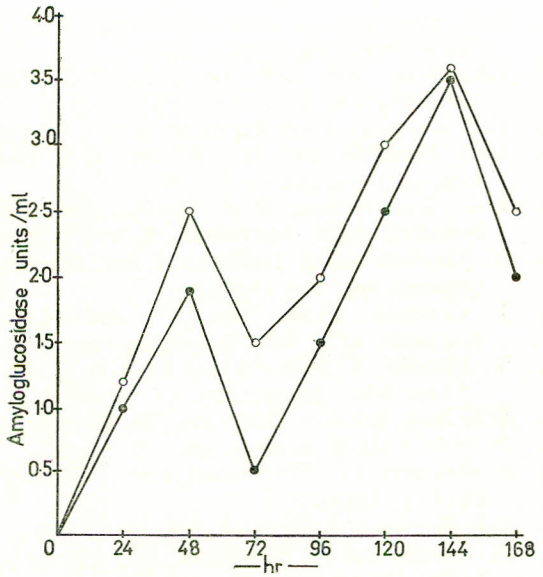

Fig. 2.—Production of amyloglucosidase by *A. awamori* NRRL 3112 with ammonium chloride as nitrogen source, 5% corn-flour (—O—) or 5% glucose (—●—) as carbon source.

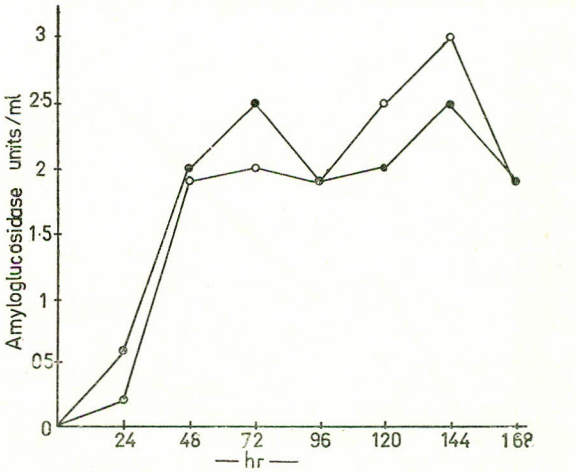

Fig. 4.—Production of amyloglucosidase by *A. awamori* NRRL 3112 with ammonium sulphate as nitrogen source, 5% corn-flour (—●—) or 5% glucose (—O—) as carbon source.

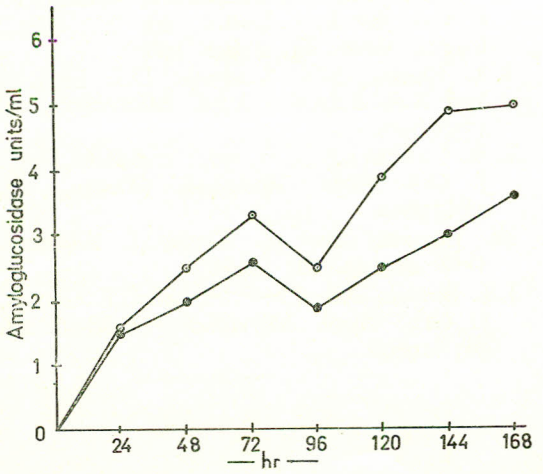

Fig. 3.—Production of amyloglucosidase by *A. awamori* NRRL 3112 sodium nitrate as nitrogen source, 5% corn-flour (—⊙—) or 5% glucose (—●—) as carbon source.

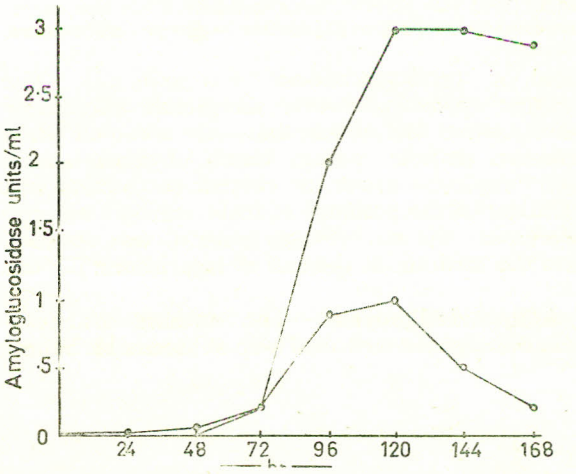

Fig. 5.—Production of amyloglucosidase by *A. awamori* NRRL 3112 with urea as nitrogen source, 5% corn-flour (—O—) or 5% glucose (—●—) as carbon source.

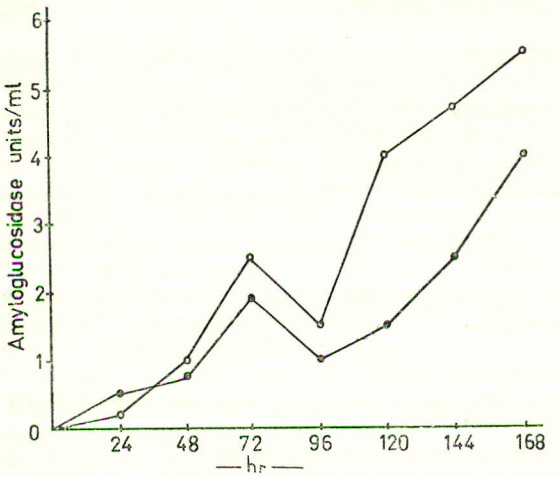

Fig. 6.—Production of amyloglucosidase by *A. awamori* NRRL 3112 with corn-steep liquor as nitrogen source, 5% corn-flour (—O—) or 5% glucose (—●—) as carbon source.

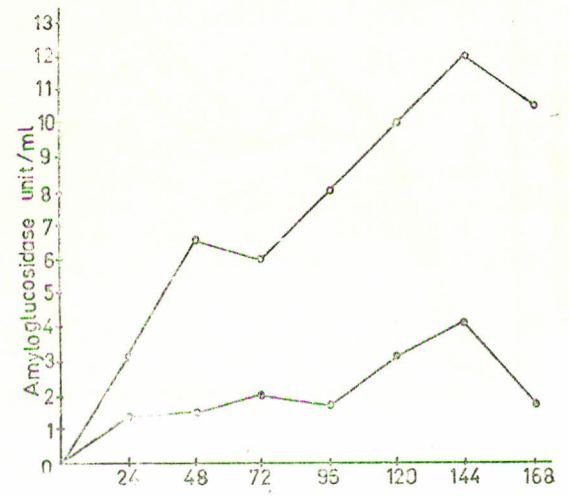

Fig. 7.—Production of amyloglucosidase by *A. awamori* NRRL 3112 with pencillin waste mycelium as nitrogen source, corn-flour (—O—) 5% or 5% glucose (—●—)as carbon source.

yield of amyloglucosidase (2.0 units/ml). The enzyme synthesis, however, was greatly affected by using lactose and sucrose than that obtained when fructose, maltose, potato starch or maize-starch were employed where the enzyme production was identical in the presence of these carbon sources. Moreover, the enzyme, in general, was secreted into the medium at the end of logarithmic period.

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