Madras Agric. J., 87(7-9): 367 - 369 July - September 2000
https://doi.org/10.29321/MAJ.10.A00472

# Microbial production of glucoamylase from agro wastes

C. THAMARAI CHELVI AND D. PURSHOTHAMAN
*Department of Agric. Microbiology, Agricultural College and Research Institute, Madurai - 625 104.*

**Abstract** : Fungal glucoamylases were among the first microbial enzymes shown to have a strong affinity for starch granules and to hydrolyse them effectively. While many microorganisms produce glucoamylase, the thermophilic ones are preferred for industrial fermentations, for, they have higher enzyme production potential and the contamination hazards can be avoided. A study was conducted at Agricultural College and Research Institute, Madurai during 1994 to produce glucoamylase from pearlmillet waste by a themophilic fungus, *Aspergillus niger*. ***(Key words** : Glucoamylase, Thermophilic fungus, Apergillus niger, Pearlmillet).*

Pearlmillet waste was collected from the biocontrol agent production unit of the Department of Agricultural Entomology, Agricultural College and Research Institute, Madurai. Pearlmillet grains were used as a feed for rearing *Corcyra cephalonica* (Kulasekaran *et al.*, 1997) an insect host for the biocontrol agent. After the pearlmillet waste was utilized by the caterpillars, it is thrown as a waste. Thermophilic fungus *Aspergillus niger* F.ST. SW.1 was obtained from pearlmillet waste by enrichment technique which exhibited higher glucoamylase activity.

## Materials and Methods

A study was conducted at Agricultural College and Research Institute, Madurai during 1994 to produce glucoamylase by themophilic *Aspergillus niger* from pearlmillet waste. Fifteen fungal isolates were isolated by enrichment technique (Subba Rao, 1997).

*Hydrolysis of starch waste*

Composition of glucoamylase enzyme production medium (EPM)

| | | |
|---|---|---|
| Pearlmillet waste | : | 5.0 g |
| Peptone | : | 1.0 g |
| Ammonium Sulphate | : | 0.5 g |
| Glucose | : | 5.0 g |
| pH | : | 5.0 |
| Tap water | : | 1000 ml |

*Assay of glucoamylase activity (E. C. 3.2.1.3):*

The assay system consisted of 2 ml of 0.5 per cent soluble starch (Merck - Germany) in distilled water, 2 ml of 0.1 M phosphate buffer (pH 6.5) and 1 ml of the enzyme source. The enzyme reaction was allowed to proceed for 60 min, both at room temperature (28°C-30°C) and 50°C in a thermostatically controlled agitating water bath after which the activity was terminated by the addition of 0.5 ml of 10 per cent trichloroacetic acid (TCA). Appropriate enzyme control and substrate control were also maintained. After termination of enzyme activity, 1 ml quantity was withdrawn from the reaction mixture transferred to a clean tube. The quantity of reducing sugars liberated was determined following Nelson's method, (Nelson, 1944).

Starch content of pearlmillet was estimated using Anthrone reagent (Jeyaraman, 1972).

The presence of glucose, the end product of the reaction mixture was confirmed by a thin layer chromatography (TLC).

*Purification of glucoamylase : Partial purification of glucoamylase*

A quantity of 200 ml of the enzyme preparation was dialysed for 24 hr against distilled water. Dialysis was carried out at 4°C with changes of distilled waster for every 4 hr. The dialysate was used for further purification (Jeyaraman, 1972).

*Purification of glucoamylase by ammonium sulphate percipitation*

A quantity of 200 ml of the clear dialysate was taken in a 500 ml Erlenmeyer flask cooled to 4°C and added slowly with pre-cooled, saturated ammonium sulphate (pH 8.0) solution. The contents were held at 4°C for 24 hr and centrifuged at 2,100 x G for 30 min. The precipitate was dissolved in 10 ml of 0.2 M sodium acetate buffer (pH 5.2). The enzyme activity was assayed.

*Production of glucoamylase through solid state fermentation technique (SSF)*

A quantity of 100 g of pearlmillet waste was

taken in a 500 ml of Erlenmeyer flask. Tap water was used as the moistening agent and the ratio of the solids to the moistening agent was 2:1 (v/v). The flask was autoclaved at 121°C for 15 min. allowed to cool to 35°C and inoculated with 4 ml. of fungal inoculum. The ratio of the inoculum used was 2.0 ml per 50 g of moist medium. The flask was incubated in a slanting position in an incubator with 65-70 per cent relative humidity (Ramesh and Lonsane, 1990).

*Extraction of glucoamylase enzyme from solid sate fermentation*

The enzyme from the fermented substrate was extracted with five volumes of 10 mM phospate buffer (pH 7.0) at 28°C for 60 mm. The slurry was squeezed through a moist cheese cloth. The left over solids were again subjected to extraction using the same procedure and the extracts were pooled. The extract was centrifuged at 3,200 x G. The clear supernatant was used as the enzyme. Tap water was also used as extratant. (Ramesh and Lonsane, 1990).

*Enzyme production*

The enzyme production broth was prepared with 15 g $L^{-1}$ pearlmillet waste. The broth was dispensed in 200 ml in 500 ml Erlenmeyer flasks. Sterilized in an autoclave for 15 min cooled and inoculated with one ml inoculum of selected isolate of *Aspergillus niger*. The flasks were incubated at 50°C for 7 days under static conditions. After the incubation period, the contents were filtered and the filterate centrifuged at 3100 x G for 15 min. The clear supernatant served as the source of enzyme.

*Characterization of glucoamylase*

*Effect of pH on the activity of glucoamylase*

Two of the enzyme source was added to 4 ml of reaction mixture containing 2 ml of 0.5 per cent soluble starch, 2 ml of buffer in 50 ml flask. The pH values of the reaction mixture were varied by using different buffers. The reaction mixture was incubated at 60°C for one hour and the reaction was stopped by adding 0.5 ml of 10 per cent trichloroacetic acid. Glucoamylase activity was determined by Nelson's method.

*Effect of temperature of glucoamylase activity*

The assay system consisted of 2 ml of 0.5 ml per cent soluble starch, 2 ml of 0.1 M phosphate buffer pH (6.5) and 1 ml of enzyme source. The enzyme reaction was allowed to proceed for 60 min at varying temperature of 10, 20, 30, 40 50, 60, 70 and 80°C in a thermostatically controlled water bath shaker.

The reaction was terminated by adding 0.5 ml of 10 per cent trichloroacetic acid, glucoamylase activity was determined as detailed earlier.

*Effect of substrate concentration on glucoamylase activity*

The assay system consisted of 2 ml of 0.1 M phosphate buffer (pH 6.5), 1 ml of the enzyme source and 2 ml of soluble starch at different concentrations, i.e. 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.2 and 2.4 per cent. The reaction was allowed to take place for 60 min at 60°C and the reaction was terminated by adding 0.5 ml of 10 per cent TCA and glucoamylase activity was determined by Nelson's method.

*Production of glucose syrup using glucoamylase*

A quantity of 500 g of pearlmillet waste was taken in 2L flat bottomed flask, added with 250 ml of 0.1M phosphate buffer (pH 6.5) and sterillised in an autoclave at 121°C for 30 min, cooled and 150 ml of enzyme source was added. The flask was incubated at 30°C for 7 days. The contents of the flask was filtered off throught layers of cheese cloth. One ml of the filterate was taken for estimation of reducing sugars following Nelson's method (Lonsane, 1987). Glucose syrup was also produced using starch wastes like sorghum, maize and thippi as described earlier.

**Results and Discussion**

From pearlmillet waste by enrichment technique, fifteen fungal isolates were obtained. All the fungal isolates exhibited starch hydrolysis. Glucoamlyase activity and the biomass of the thermophilic fungal isolates are presented in Table 1. A higher gluocamylase activity was observed at 50°C than at 28°C. The isolate, F.ST. SW.1 (*Aspergillus niger*) isolated from pearlmillet waste exhibited higher glucoamylase activity at 50°C after 10 days incubation period. Solid state fermentation was done by using the pearlmillet waste and enzyme was extracted after the fermentation process with phosphate buffer where the recovery of enzyme was very high (Table 2).

In the present study, an attempt has been made to purify the enzyme partially. The dialysis of culture

filterate and ammonium sulphate precipitation resulted in higher enzyme activities (Yu and Hang, 1991).

Glucoamylase activity was assayed at different pH levels, 60°C temperature and substrate concentration. The results have indicated that pH 5.0 appears to be optimum for enzyme activity. The optimum temperature for the enzyme activity was 60°C and 2 per cent starch was optimum substrate.

Glucose syrup was produced using glucoamylase with different sources of starch wastes like pearlmillet, sorghum, maize, and thippi. Among the four sources of starch wastes hydrolysis of pearlmillet waste was more effective and 81.42 per cent conversion to glucose was achieved in 7 days.

The efficiency of conversion appears to be reasonably high also. It becomes clear that the starch rich pearlmillet waste and similar agricultural wastes can be used as a substrate for glucoamylase production. By using thermophilic fungi and through solid state fermentation technique glucoamylase for commercial use can be prepared.

**References**


Jeyaraman, J. (1972). Techniques in Biology, pp.67-72. Higginbothams Ltd., Madras.

Kulasekaran, K., Velayutham, B. and Banjervi, P.S. (1977). Studies on the suitability of economic medium for the mass multiplication of *Corcyra cephalonicas*. *The Farm Sci.* **4**: 8-11.


**Table 1.** Glucoamylase activity of the culture filtrate of the selected thermophillic fungal isolate F.ST.SW.1 *Aspergillus niger* on pearlmillet waste

| Incubation period (days) | Glucoamylase activity (E.U $ml^{-1}$ $hr^{-1}$) | | Specific activity (E.U $mg^{-1}$ of protein) | | Bio mass $g^{-1}$ dry weight |
|---|---|---|---|---|---|
| | 28°C | 50°C | 28°C | 50°C | |
| 5 | 0.34 | 0.48 | 30.00 | 42.35 | 3.40 |
| 7 | 0.49 | 0.54 | 47.05 | 62.17 | 3.60 |
| 10 | 1.95 | 3.85 | 80.30 | 158.54 | 3.80 |

EU : Enzyme Unit

**Table 2.** Production of glucoamylase enzyme through solid state fermentation

| Fungal Isolate | Enzyme extraction | Glucoamylase activity (E.U $ml^{-1}$ $hr^{-1}$) | |
|---|---|---|---|
| | | 28°C | 50°C |
| F.ST.SW.1 | Tap water | 14.46 | 18.38 |
| | Phosphate buffer | 21.18 | 31.50 |
| F.ST.SW.1 | Tap water | 7.65 | 12.75 |
| | Phosphate buffer | 19.90 | 21.00 |

E.U. - Enzyme unit

Data (Mean of three determinations)

| | S.E. | C.D.(P=0.05) |
|---|---|---|
| Isolates | 0.082 | 0.190 |
| Enzyme extraction | 0.082 | 0.190 |
| Temperature | 0.082 | 0.190 |
| I x E x T | 0.164 | 0.380 |


Lonsane, B.K. (1987). Studies on production of glucose syrup from starchy materials. pp. 116-118. *Annual Report,* 1985-86. CFTRI, Mysore.

Nelson, N. (1944). A photometric adoption of the Sumogyi's method for the determination of glucose. *J. Biol. Chem.* **153**: 315-380.

Ramesh, M.V. and Lonsane, B.K. (1990). Critical importance of moisture content of the medium in alpha - amylase production by *Bacillus licheniformis* 1727 in a solid state fermentation system. *Appl. Microbiol Biotechnol.* **33**: 501-505.

Subba Rao, N.S. (1977). Soil microorganisms and plant growth. Oxford and IBH Publishing Co., New Delhi. p. 253.

Yu, R.C. and Hang. Y.D. (1991). Purification and characterization of a glucoamylase from *Rhizopus oryzae*. *Food Chem.* **40**: 301 - 308.


(Received : April 1999 ; Revised : March 2001).