Amylase Production by Rhizopus nigricans Using Mashed Maize

T AYOGU, E AMADI

Citation

T AYOGU, E AMADI. *Amylase Production by Rhizopus nigricans Using Mashed Maize*. The Internet Journal of Microbiology. 2009 Volume 8 Number 1.

Abstract

Rhizopus nigricans isolated from stagnant water was studied as a source of amylase using boiled mashed maize (yellow variety) as sole source of carbon, while ammonium sulphate was used as nitrogen source. Concentrations of mashed maize and ammonium sulphate were 50g/l and 20g/l respectively. These levels were optimal for growth and amylase production at 30°C and pH 6.0. Partial purification of the enzyme by salting, desalting and elution using DEAE-Sephadex A 50 were carried out to increase enzyme activity. Amylase activities using some native starches as test substrates showed a maximum of 7.0U/L for rice starch, followed by potato starch 6.5U/L. Mashed maize, therefore, could serve as sole source of carbon for amylase production by Rhizopus
nigricans.

INTRODUCTION

Microbial amylases are distributed among bacteria, protozoa, algae and fungi(1). The fungal amylases occur mainly in the species of Aspergillus and Rhizopus (2). The use of these microorganisms in the production of enzymes on an industrial scale has obvious advantages, which include the fast rate of multiplication, diversity of enzymes present and the possibility of genetic manipulation(3) . Microbial amylases are used extensively in industrial practice. The alpha-amylases from Bacillus species are used routinely in the brewing, textile and paper industries(4) . In the brewing industries, amylase facilitates the use of high levels of adjuncts such as barley, maize and sorghum(4). It hydrolyses starch to a product of suitable viscosity for coating the surface of paper and in the textile industry it is used for designing purposes(5).

Usually amylase production from fungi has been carried out using well defined chemical media by submerged fermentation (SMF) and solid state fermentation (SSF) in recent times(6). The economics of enzyme production using inexpensive raw materials can make an industrial enzyme process competitive(7) . Maize grains are produced abundantly in the Eastern part of Nigeria especially during the cropping season between April and September. Their high content of starch has prompted the present attempt to use grains as a medium for amylase production from Rhizopus nigricans. The aim of this study therefore, was to explore the use of local and readily available material (mashed maize) to produce amylase from Rhizopus nigricans.

MATERIALS AND METHODS

COLLECTION OF SAMPLES

Stagnant water found around the environment of the Institute of Management and Technology was chosen as the source of the isolation of Rhizopus nigricans. 10ml of the stagnant water samples were collected in sterile bottles and taken to the laboratory for analysis.

ISOLATION OF

The starch medium was produced by the method described by Higashiara and Okada(8) with slight modification. An increased quantity of ammonium sulphate was added to the medium and subsequently used for the isolation of Rhizopus. Pure culture of the isolate was obtained after several cycles of growth. Identification and characterization of the Rhizopus species was based on morphological characteristics of plate and slide cultures. Prepared samples were observed under the light microscope and identified. Positive cultures were screened for amylase production using various concentrations of the mashed maize and ammonium sulphate, as sole sources of carbon and nitrogen

respectively and sugar production was tested by the method of Somogyi(9).

PREPARATION OF CRUDE ENZYME

The medium composition for amylase production contained 50g/L boiled mashed maize (yellow variety) and 2.0g/L ammonium sulphate $(NH_4)_2 SO_4$ as nitrogen source, adjusted to pH 6.0 and sterilized by autoclaving. Fermentation was carried out in 500ml flasks with a working volume of 250ml at 30°C in a rotary shaker at 100rpm for 72h. The fermentation was started with pure culture 10% (v/v) inoculum (10^7 spores/ml) of 24h growth suspension of the organism, obtained from the starch medium.

Periodical analysis of samples taken was carried out for amylase activity. Enzyme recovery from the fermentation broth was by filtration and the filtrate served as the crude enzyme.

AMMONIUM SULPHATE PRECIPITATION

150ml of the crude amylase sample was brought to 70% ammonium sulphate saturation by adding 22.18g of crystalline ammonium sulphate. This was carefully stirred and left in the refrigerator overnight.

DESALTING ON SEPHADEX G- 25

A few grams (15g) of sephadex G – 25 were swollen in sufficient amount of 10mM sodium acetate buffer pH 5.0 containing 1mM EDTA and packed into a column (1.20 x 14cm). The samples were loaded onto different columns and eluted with the same buffer.

BATCH ELUTION WITH DEAE – SEPHADEX A50

The desalted sample was mixed separately with DEAE-Sephadex 50 resin in 50mM tris buffer pH 7.0. The mixture was stirred and centrifuged at 1500 rpm for 30 mins. The supernatant collected was used for enzyme assay.

ENZYME ASSAY

The method described by Somogyi (9) was used to determine amylase activity. In this method, one unit of amylase activity was defined as one micromole of reducing sugar (as glucose) under the assay condition while protein concentration was determined by the method of Lowry et al.,(10) using albumin as standard. The above tests were repeated twice and mean values recorded.

EFFECT OF SUBSTRATE CONCENTRATION ON GROWTH AND AMYLASE PRODUCTION

The effect of substrate concentration (mashed maize) on the growth and amylase production was determined.

RESULTS AND DISCUSSION

Rhizopus nigricans produced a significant quantity of amylase in a very simple medium of boiled mashed maize, as sole source of carbon and ammonium sulphate as the only nitrogen source. Typical growth and enzyme production profiles in the medium showed that amylase production was highest in the mashed maize concentration of 50g/L, while the biomass and protein concentration was 70g/L and 45g/L respectively (Table 1).

Although the higher the mashed maize concentration, the higher the starch concentration, amylase production did not follow that pattern. It can be seen from table 1, also, that beyond 50g/L concentration, amylase production declined. However, protein levels increased slightly with increased mashed maize concentration, up to a maximum value of 70g/L concentration of mashed maize. Similar patterns have been reported(4) during amylase production using Bacillus subtilis. In this study, amylase production occurred at the end of the exponential phase and entering stationary phase. Protein concentration increased in the medium with the secretion of amylase into the medium.

The effect of different concentrations of the ammonium sulphate in the fermentation medium on the growth and enzyme production and hence protein concentration is shown in table 2, while table 3 shows the maximum activities of the amylase, on starches from different cereals grains. The result of this work shows that large scale production of microbial enzyme (amylase) especially from Rhizopus nigricans could be possible using a very cheap local raw material like mashed maize as carbon source. This growth medium if developed, could replace more expensive chemical media.

Figure 1

Table 1: Effect of Substrate concentration on growth and amylase production

Conc. of Mashed Maize (g/L) Dry wt	Maximum amylase Activity (U/ml)	Biomass (g/L) Dry wt	Protein (mg/ml) Dry wt
30.0	4.0 ± 0.15	0.50	0.024
35.0	4.2 ± 0.24	0.75	0.028
40.0	5.5 ± 0.45	0.80	0.030
45.0	6.4 ± 0.52	0.98	0.035
50.0	7.5 ± 0.69	1.70	0.032
60.0	7.1 ± 0.66	1.76	0.026
65.0	6.2 ± 0.48	1.82	0.031
70.0	5.8 ± 0.51	2.1	0.022
75.0	4.5 ± 0.36	1.8	0.024

Values for the maximum amylase activity are the means of duplicate determination, ± SD

Figure 2

Table 2 : Effect of ammonium sulphate concentration on growth of and amylase production at mashed maize concentration of 50g/L

Conc. Of $(NH_4)_2SO_4$ (g/L)	Maximum activity (U/ml)	Biomass g/l (Dry wt)	Protein mg/ml (Dry wt)
1.5	3.5 ± 0.14	0.74	0.023
1.8	4.2 ± 0.18	0.96	0.028
2.2	7.5 ± 0.66	1.65	0.030
2.3	6.4 ± 0.50	1.45	0.022
2.5	3.8 ± 0.15	1.08	0.016
2.8	2.0 ± 0.11	0.98	0.024
Control	0.4 ± 0.20	0.42	0.025

Values for the maximum amylase activity are the means of duplicate determination, ± SD

Figure 3

Table 3 : Maximum activity of amylase produced by (growth on mashed maize) on some starch sources

Sources of Starch	Maximum amylase activity (U/mL)
Rice	7.0 ± 0.65
Potato	6.5 ± 0.55
Tapioca	5.5 ± 0.50
Millet	3.5 ± 0.23
Sorghum	4.0 ± 0.18

Values are the means of duplicate determination, ± SD

References

1. Fisher, E.W.; Stein, E.A.(1960). Alpha-amylase. In: The enzyme. Boyer PD, Lardy, H (Ed). New York, Academic Press.
2. Asia, T.; Sata, S.T.; Mujasaka, S.; Izumida, M.(1952). The amylase production by submerged culture of Aspergillus. J Agric Chem Soc. 25, 352-360.
3. Okafor, N.(1987). Industrial Microbiology. University Ife Press, Ile Ife Nigeria, first edition.
4. Oguntimein, G.B.(1993).Growth and amylase production by Bacillus licheniformis isolated from cassava processing waste. Nig. Food J. 2: 58-69.
5. Lonsane, B.K.; Ramesh, M.V.(1990). Production of Bacterial thermostable alpha-amylase by solid-state fermentation: a potential tool for achieving economy in enzyme production and starch hydrolysis. Adv. Appl. Microb. 35 : 1-56.
6. Miranda, O.A.; Salgueiro, A.A.; Pimental, N.C.B.; Limafilho, J.J.; Melo, E.H.M.; Duran, N.(1999). Lipase production by a Brazilian strain of Penicillium citrinium using industrial residue. Bioresources Tech. 69 : 145-149.
7. Couto, S.R.; Sanroman, M.A.(2006). Application of solid-state fermentation to food industry. J Food Engr. 76: 291-302.
8. Higashihara, M.; Okada, S.(1974) Studies on Beta-amylase of Bacillus megaterium . Agric Biol Chem. 38: 1023-1029.
9. Somogyi, N.(1952). Notes on sugar determination. J Biol Chem. 195 : 10-23.
10. Lowry, O.N.; Rosenbrough, N.J.; Farr, A.C.; Randall, R.T.(1951). Protein measurement with the folin phenol reagent. J Biol Chem. 103: 265-275.

Author Information

T.E AYOGU, PhD
DEPARTMENT OF FOOD TECHNOLOGY, INSTITUTE OF MANAGEMENT AND TECHNOLOGY

E.S AMADI, PhD
DEPARTMENT OF APPLIED MICROBIOLOGY, FACULTY OF BIOLOGICAL SCIENCES, EBONYI STATE UNIVERSITY