EVALUATION OF VEGETABLE WASTES FOR ENHANCED PRODUCTION OF AMYLASE BY ASPERGILLUS NIGER ISOLATED FROM MARINE WATER ADOPTING SOLID STATE FERMENTATION

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Abstract

The present study undertaken to evaluate vegetables wastes as a substrate for amylase production by solid state fermentation by *Aspergillus niger* originally isolated from marine water. Among 123 different fungal isolates reported,35 isolates belong to *Aspergillus niger* showed strong amylase activity. The wastes supported growth and enzyme activity at all parameters tested.Maximum activity was recorded at 0% moisture content,70 C and pH 9 at all incubation time

Key words: Amylase, Aspergillus niger,enzyme activity

I. INTRODUCTION

Amylases are one of the most important industrial enzymes that have a wide variety of applications ranging from conversion of starch to sugar syrups, to the production of cyclodextrins for the pharmaceutical industry. These enzymes account for about 30 % of the world's enzyme production. The a-amylase family can roughly be divided into two groups: the starch hydrolyzing enzymes and the starch modifying, or transglycosylating enzymes (1)

Solid-state fermentation has emerged as a potential technology for the production of microbial products such as feed, fuel, food, industrial chemicals and pharmaceutical roducts. Its application in bioprocesses such as bioleaching, biobeneficiation, bioremediation, biopulping, etc. has offered several advantages. Utilisation of agro-industrial residues as substrates in SSF processes provides an alternative avenue and value-addition to these otherwise under- or non-utilised residues Enzymes are among the most important products obtained for human needs through microbial sources. A large number of industrial processes in the areas of industrial, environmental and food biotechnology utilize enzymes at some stage or the other. Current developments in biotechnology are yielding new applications for enzymes. Solid state fermentation (SSF) holds tremendous potential for the production of enzymes. It can be of special interest in those processes where the crude fermented products may be used directly as enzyme sources (2)

In the present study vegetable wastes were evaluated for amylase production and effect of parameters by *Aspergillus niger* isolated from marine water

II. MATERIALS AND METHODS

A. Sample collection

The sea water was collected in different sites of Marina Beach Chennai (Asia's 2nd largest beach) By using the sterile autoclaved 2 liter water bottle and brought to the Laboratory immediately. And the samples were kept in the refrigeration at 4°C till samples were processed.

B. Isolation of fungi

The SDA media was used for isolation. The media was prepared in sea water, sterilized by autoclaving and poured in to sterile Petri plate and allowed to solidify. 25 ml of the collected sea water was aseptically transferred to 250 ml of sterile distilled water blank and mixed thoroughly. 10 ml of the sample was transferred to 90 ml of distilled water, shook vigorously and diluted. 0.1 ml of the aliquot was spread plated on SDA media prepared with sea water, incubated at 30°C for 3-4 days.

The fungal colonies appeared on respective dilution was countered and streaked on SDA media separately to get pure culture. A single monosporic colony was selected and streaked on SDA slant as monosporic culture for further studies. The slants were stored at 4°C in refrigeration. Glycerol storage was also maintained.

C. Identification

The respective fugal pure cultures was identified based on their morphological and cultural characteristics, microscopic examination of fungal spores with lactophenol cotton blue as per the criteria suggested by Humber (3).

D. Screening for amylase production

Primary screening was carried out to evaluate amylase producing fungi using Starch Agar media. 100 ml of the Starch Agar media was prepared in 250 ml of conical flask, sterilized by autoclave and poured into the sterile Petri plate, allowed for solidification. A fully grown mycelial plug (4 mm) was cut 4 days old fungal culture on SDA plate, transferred to Starch agar media and incubated at 32°C for 3-4 days. The plates were flooded with iodine solution. After the incubation period a clear zone was observed around the fungal colonies indicating amylase production.

The amylase producing fungi were selected and used for further study.

E. Inoculum preparation

The spores were obtained from 10 days old SDA slant culture of the fungi by scraping of the slant surface with sterile distilled water containing few drops of tween-20. The slurry obtained was filtered through masculine cloth to remove the mycelial debris and it was used as a source of inoculum. The spore count was done by the Heamocytometer.

100 ml of inoculum media was sterilized and inoculated with 0.1 ml of spore suspension. (10^8 spores/ml) of respective amylase producing fungi are incubated at 32°C for 4 days in a shaker at 250 rpm. After the incubation period the media filtered through filter paper, centrifuged at 10,000 rpm for 10 min; the collected supernant was used as source of enzyme.

F. Amylase assay

1 ml of the supernant were added in the sterile test tube and it is diluted with 1 ml of distilled water in addition to this 1 ml of starch, 1 ml of NaOH was added and kept in water bath at 40°C for 10 mins after the incubation period 1 ml of DNS (Di nitro salicylic acid) is added in the test tube and OD was measured using colorimeter at 540 nm.

G. Amylase Production Using Vegetable Wastes

Peel, pulp or non used portion of (Tomato, Potato, beans, onion, carrot, brinjal, and cauliflower) were collected from university mess in sterile polythene bags and brought to the laboratory. The wastes were allowed to shade dry for few hours to remove moisture content.

25 gms of the mixed waste vegetable transferred to 100 ml of conical flask, the moisture content for each flask was adjusted to 0, 10, 20, 40, 60% with distilled water. The flask was sterilized by autoclave and it is inoculated with 0.1 ml of fungal spore from inoculums media all the flask were kept at 30°C for 4-days and each day the enzyme assay was preformed.

H. Enzyme extraction

After the incubation period 5 gms of mixed waste vegetables from the respective flasks were transferred to beakers and flooded with distilled water and mixed thoroughly with sterile glass rod, shook vigorously, filtered the suspension with filter paper and collected filtrates were used as crude enzyme source for further studies.

I. Effect of pH

1 ml of crude enzyme was incubated with phosphate buffer of different pH ranged from 5, 6, 7, 8, 9 and 10 respectively at 32°C for 10 min. The enzyme assay was carried out as described earlier.

J. Effect of Temperature

1 ml of crude enzyme was incubated with different temperature ranged from 30°C, 40°C, 50°C, 60°C, 70°C respectively for 10 minutes. The enzyme assay was carried out as described earlier.

III. RESULTS AND DISCUSSION

A. Generic composition of fungi isolates

A total of 155 isolates belong to five different species were isolated and their generic composition was presented in Table 1. The isolated fungi were Aspergillus niger, Penicillium expansum, Gliocladium sp, Aspergillus sp and Penicilliium sp. Penicillium sp and Penicillium expansum were dominant followed by Aspergillus niger (Table 1) Burteseva *et al.,* (2003) explained on filamentus marine fungi as producers of O-glycosyl hydrolase: β-1, 3-glucanase from Chaetomium indicum and suggested that 90 fungal strains (42 species) isolated from marine habits were studied for their ability to produce extra cellular enzyme.

Cultural filtrates of these strains were shown to contain a series of glycosidases which varied with habitat. The level of activity depended on the species of fungi. Optimal condition for growth of Chaetomium indicum and for biosynthesis of β-1,3-glucanase were determined β-1,3-glucanase was isolated using ion exchange chromatography, ultra filtration and gel filtration. The presence of two enzyme forms were shown: both forms were exo β-1, 3-glucanase.

Table 1. Generic composition of fungi isolated from marine water.

S.No	Fungi	Composition
1	*Aspergillus niger*	50%
2	*Gliocladium sp*	25%
3	*Aspergillus sp*	15%
4	*Penicillium expansum*	6%
5	*Penicillium sp*	4%

B. Screening of Amylase Producing Fungi In Solid Plate Assay

The different fungal species were tested for screening of amylase production on solid plate assay using starch agar media. Among the five species *Aspergillus niger* and *Gliocladium sp* showed strong amylase activity as shown in Table 2.

Table 2. Screening of amylase production

S.No	Fungi	Productivity of Amylase
1	*Aspergillus niger*	+++
2	*Gliocladium sp*	++
3	*Aspergillus sp*	+
4	*Penicillium expansum*	+
5	*Penicillium sp*	+

+++- high activity, ++ - moderate activity, + - less activity

C. Amylase Production Son Vegetable Waste

Effect of moisture content

Among the different moisture content, enzyme activity was found to be maximum at 0% level followed by 10% and enzyme production was high at 96 hours of incubation (Table 3). The enzyme activity at 24, 48, 72 and 96 hours was 50, 54, 62 and 74 Units/ml. (Table 3) similarly 43, 45, 55, 62 Units/ml at 10%, 41, 44, 47, 61 units/ml at 20%, 36, 43, 46, 56 units/ml at 40%, 30, 41, 44, 56 Units/ml at 60% was observed at 24, 48, 72, 96 hours of incubation respectively. Anil.Patel (1) have reported on β-amylase from fungal culture grown on oil cakes and its properties, the solid state fermentation was carried out for the production of β-amylase using *Aspergillus oryzae.* The oils such as sesame oil cake, groundnut oil cake, palm kernel cake and olive oil cake were screened to be used as substrate for the enzyme production and also compared with wheat bran (WB).

Table 3. Effect of moisture content (%) on amylase activity

S.No	Incubation time (Hours)	Enzyme activity (u/ml)				
	0	10	20	40	50	
1	24	50.0	43	41	36	30
2	48	54.0	45	44	43	41
3	72	62.0	55	47	46	44
4	96	74.0	62	61	56	56

Effect of pH and temperature on amylase activity

Maximum enzyme activity was recorded at 9.0 followed by 10.0 (62.1 and 66.6 units/ml respectively) 58.5, 60.7 and 61.2 units/ml activity was observed in the range of 5,6 and 7 respectively (Table 4)

Table 4. Effect of pH on amylase activity

S.No	pH	Enzyme activity (Units/ml)
1	5.0	117.7
2	6.0	118.2
3	7.0	118.5
4	8.0	120.2
5	9.0	121.6

Among the different temperature tested Maximum enzyme activity was recorded at 70°C followed by 60°C (167 and 196 units/ml) and 59.2°C units/ml) respectively (Table 5)

Table 5. Effect of Temperature on enzyme activity

S.No	Temperature (C)	Enzyme activity (Units/ml)
1	30	121
2	40	127
3	50	141
4	60	167
5	70	196

Mohapatra *et al.* (4) showed that associated with marine sponge *spirastrella sp*., grown at 30°C. the enzyme has an optimal pH of 5.0 and an optimum temperature of 60°C. the half lives of the partially purified enzyme at 55 and 60°C were 120 and 50 minutes.Frolova *et al.* (5) introduced the activity of the isolated amylase from *Aspergillus flavipes* forms decreased in the presence of proteolytic enzyme. New, highly stable forms of amylase (with pH optimum of 5.5 and 7.5 and the maximum activity at 60 - 80°*C*) were synthesized in the presence of Diisopropyl flourophosphate, an inhibitor of Protease. Poornima *et al* (6) proposed α-amylase activity of *s.aureofasciculus* was studied and the maximum enzyme activity is found at pH 9, temperature 45°C, 0.05% Nacl concentration, carbon compound mannose and nitrogen compound L-histidine.

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