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EFFECTS OF CULTURAL CONDITIONS ON α-AMYLASE PRODUCTION BY *PENICILLIUM EXPANSUM*

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Experiments were conducted to investigate the conditions of α-amylase (E.C. 3.2.1.1) production by *Penicillium expansum* strain CMI 39761. The optimal conditions of α-amylase production in submerged culture with culture time 48 hr, temperature 37° and initial pH 6.5. Millet husk finely powdered was used as a carbon source. The addition of starch, maltose as C sources and $NaNO_3$, peptone as N sources to millet husk fine powder medium were effective for α-amylase production.

Key words: Penicillium expansum, Millet husk, α-Amylase.

INTRODUCTION

α-Amylase have a wide use in industrial productions, mostly in glucose and high maltose syrups [1]. Several raw starch digesting amylases are produced from *Aspergillus niger* [2], *Aspergillus oryzae* [3], *Rhizopus sp.* [4], *Bacillus subtilis* [5,6] *Bacillus licheniformis* [7], and *Penicillium expansum* [8].

However promising studies reported regarding the fermentation of agricultural waste (soyabean meal, wheat straw, wheat bran, rice bran, rice husk and peanut meal) to α-amylase production by *Bacillus subtilis* under submerged or surface culture conditions of growth [1,6].

Above communications inspired to develop a fermentation process for hydrolysates of agricultural waste for amylase production by *Penicillium expansum* link CMI 39761. The organism *Penicillium expansum* link grow well on a variety of cellulosic materials include textiles and paper but is best known as the cause of distinct brown rot of apples in storage and also to a lesser extent in a number of soft fruits [9]. In the present work this versatile substrate accepting characteristic of *Penicillium expansum* was therefore exploited for the production of α-amylase using millet husk as a carbon and energy source. In present work therefore an attempt has been made to use millet husk to study the possibility of its use as carbon source and effects of cultural conditions on the production of amylase. However literature survey indicates that such work on α-amylase production by *Penicillium expansum* has not been done.

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MATERIAL AND METHODS

Strain. Penicillium expansum strain CMI 39761 obtained from Department of Microbiology, University of Glasgow was used. The stock culture was maintained on agar slants, containing (gl^{-1}) dextrose 20.0, peptone 10.0, agar 20.0 and distilled water. The ingredients were thoroughly mixed and kept in culture tubes sterilized at 1.5kg/cm^2 for 20 minutes. The sterilized slants were inoculated with *Penicillium expansum* and incubated at 27° to obtained luxuriant growth.

Inoculum. A spore suspension was prepared by adding sterile water to stock culture to get $50x10^6$ spores/ml.

Basal medium. Basal medium was used for the growth of *Penicillium expansum* as reported by Burrel *et al.* [10] containing following reagents (per litre of solution): glucose, 10.0 g; $(NH_4)_2SO_4$, 2.5 g; fumaric acid, 2.0 g; $KH_2PO_4.2H_2O$, 1.0 g; $MgSO_4.7H_2O$, 0.5 g; $(NH_4)Fe(SO_4)_2.12H_2O$, 0.2 mg; $ZnSO_4.7H_2O$, 0.2 mg; $MnSO_4.H_2O$, 0.1 mg and thiamine hydrochloride, 0.1 mg. The pH of the medium was adjusted to 6.0.

Fermentation medium. Fifty ml of basal medium with and without glucose supplemented with 0.5 g of millet husk fine powder (40 mesh) was taken in a 250 ml flask. The pH of the medium was adjusted at 6.0. The flasks were plugged with cotton wool and sterilized at 120° for 20 min, and cooled at room temperature, were inoculated with 0.5 ml of the inoculum containing $50x10^6$ spores/ml. The flasks were incubated at 27°. The flasks were shaken manually twice a day. The culture broth was filtered from mycelium after an interval of 24 hours incubation period, through Whatman No. 1 filter paper. The recovered mycelium were dried at 110° and weighed.

Determination of protein. Protein content of the broth was determined by Lowry *et al.* [11] method with bovine serum albumin as standard.

Determination of α-amylase activity. α-Amylase activity was determined by Fischer and Stein [12]. method 1.0 ml of culture broth was added to 1.0 ml of 1 % soluble starch solution adjusted at pH 6.3 with phosphate buffer, and incubated at 25° for 3 minutes. The reaction was terminated by the addition of 2.0 ml DNS (3.5-dinitro salicylic acid) reagent. Colour developed due to the reducing sugar by heating the reactant in a boiling water bath for 5 minutes and then rapidly cooling to room temperature. The extinction value determined at 550 mμ.

One unit of activity was the amount of enzyme which liberated reducing group from 1 % soluble starch in 3 minutes corresponding to 1.0 mg maltose hydrate.

Analysis of millet husk. The ash was determined by incineration of known weight of the sample at 750°. Protein (Nx6.25) was determined by the macro kjeldal method, and starch by McCready *et al.* method [13]. Hemicellulose, cellulose and lignin was determined by Anderson and clydesdale [14] procedure.

RESULTS AND DISCUSSION

Chemical composition of millet husk. Millet husk is a agricultural waste and contains variable ingredients (Table-1). Millet husk contains mineral matter (ash), carbohydrate (cellulosic and noncellulosic), starch, nitrogen and lipid, which may be used as a medium for the growth of *Penicillium expansum* and formation of α-amylase.

Fermentation period. The results obtained (Fig. 1) show that the fermentation period influenced the bio-

Table 1. Chemical constituents present in millet husk.

Components	Percentage g/100 g of millet husk
Ash	7.66
Total lipids	4.00
Cellulose	5.10
Hemicellulose	33.50
Non-cellulosic carbohydrate	19.54
Starch	0.123
Lignin	5.28
Nitrogen	1.06
Protein (Nx6.25)	6.625

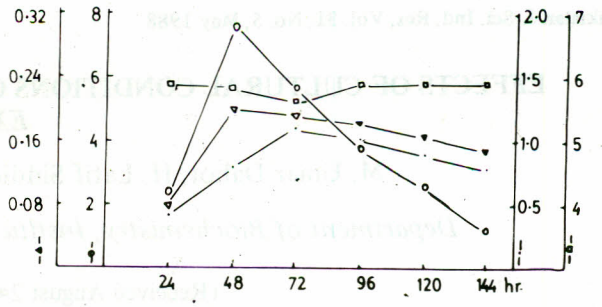

Fig. 1. X-axes: The effect of time period on enzyme formation by Penicillium expansum.

Y-axes: – △ – Soluble protein mg ml^{-1}; – ○ – α-amylase activity units ml^{-1}; – . – Biomass weight g L^{-1}; – □ – Final pH.

synthesis of α-amylase by *Penicillium expansum* (CMI 39761) when fermentation medium contained only millet husk as a carbon source. The maximum yield of α-amylase was 7.5 units/ml at 48 hrs, and then declined. The pH of the medium decreased with the increase of incubation period reaching lowest pH in 72 hrs and then rose become constant at 5.92. Thus for enzyme production the cultures were incubated for 48 hrs.

Takasaki [15] is of the opinion that there is no definite relationship between time of growth and extracellular enzyme synthesis, varying with the conditions of growth, in terms of pH, temperature, medium compositions and the organism employed.

Effect of incubation temperature on enzyme formation. Inoculated flasks were incubated at 25 to 60° as shown in (Fig. 2). Maximum α-amylase production was obtained at 37°

Effect of initial pH on enzyme formation. The effect of initial pH values (4-8) indicates that maximum produc-

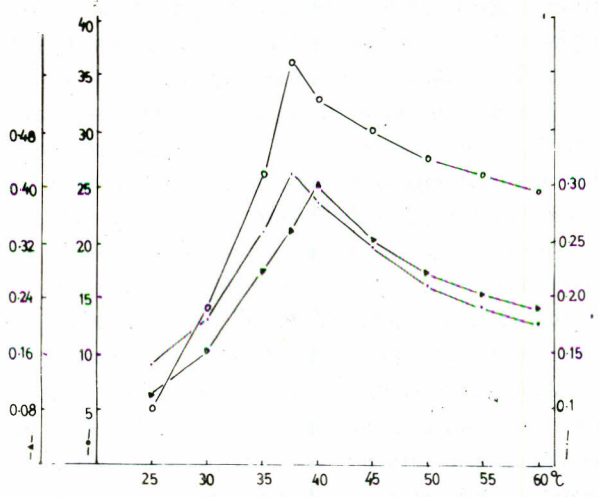

Fig. 2. X=axes: The effect of incubation temperature on enzyme formation by Penicillium expansum.

Y=axes: – ○ – α-amylase activity units ml^{-1}; – △ – Soluble protein mg ml^{-1}; – . – Biomass weight g L^{-1}.

tion of α-amylase was found at pH 6.5 and then declines abruptly (Fig. 3). At the end of cultivation on millet husk medium a slight decrease of final pH values were recorded (Fig. 3). This declination of pH may have been caused by

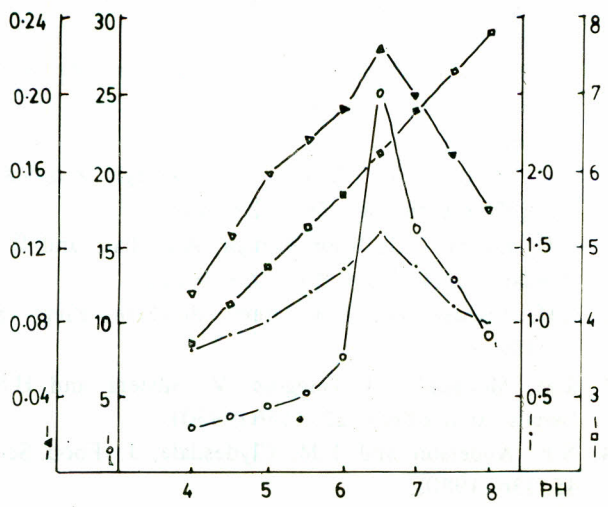

Fig. 3. X-axes. The effect of initial pH of the fermentation medium on enzyme formation by Penicillium expansum.

Y-axes; – △ – Soluble protein mg ml^{-1}; – ○ – α-amylase activity units ml^{-1}; – . – Biomass weight g L^{-1}; – □ – Final pH.

extracellular acid proteases [16,17]. At initial fermentation pH 5 and 8 only the maximum α-amylase production decreased upto 20 % and 36 %. Similar optimum conditions of initial pH and temperature were studied for Bacillus amyloliquefaciens with soluble starch as a carbon source [18].

Effect of carbon sources on enzyme formation. The effect of carbon sources such as glucose, maltose and starch alongwith millet husk on α-amylase formation has been investigated. The results (Table 2) indicate that *Penicillium expansum* is able to produce and excrete α-amylase when grown on most varied carbon sources, although the extent of formation varies. The carbon sources apparently poorly utilized as energy source for growth, synthesized larger amount of enzyme, than the carbon sources used for energy.. In present study it was observed that starch and maltose alongwith millet husk stimulated the maximum production of α-amylase 20 % & 12.5 % respectively whereas glucose reduced 30 % α-amylase production. During the course of growth on glucose the pH shifted towards acidic side, but with other carbon sources, the pH remained at pH 6.2 & 6.3. It is reported that α-amylase is apparently inducible and its synthesis depends on the presence of starch or maltose in the fermentation medium [18,19].

Effect of nitrogen sources on enzyme formation. Fermentation without $(NH_4)_2SO_4$ was supplemented with

Table 2. The effect of carbon and nitrogen sources on α-amylase formation by *Penicillium expansum* strain CMI 39761.

Nutrient 1 % wt/vol	Final pH*	Dry biomass $mgml^{-1}$	α-amylase activity units ml^{-1}
Carbon sources			
Control**	6.40	2.6	40
Glucose	4.64	3.10	21
Glucose + Millet husk	5.56	2.85	28
Maltose + Millet husk	6.30	2.08	45
Starch + Millet husk	6.21	2.40	48
Nitrogen sources			
Control***	6.40	2.6	40
$NaNo_3$+Millet husk	6.64	2.45	45
Peptone+Millet husk	6.72	2.51	44
Urea + Millet husk	6.90	2.78	30

*The initial pH value of the fermentation medium adjusted at 6.5 .

**The basal fermentation medium contained millet husk fine powder

***The basal fermentation medium contained millet husk fine powder 10 mg ml^{-1} and $(NH_4)SO_4$ 0.25 mg ml^{-1}.

different nitrogen sources with finely powdered millet husk as carbon source. The inoculum was incubated for 48 hrs at 37°, with the initial pH 6.5. The production of α-amylase was stimulated 10 % & 12.5 % with peptone and $NaNO_3$ respectively. The incorporation of urea in fermentation medium as nitrogen source lowered enzyme production to about 25 % (Table 2). During the course of growth on nitrogen sources the final pH was increased from pH 6.5 The stimulation effect of peptone on α-amylase production by microorganisms is reported in literature [20]. The inhibitory effect of urea is also reported by other workers [1,3].

CONCLUSION

Among enzymes produced by microorganisms, fungal α-amylase was marketed first. The commercial production of fungal α-amylase in synthetic, semisynthetic and natural media is done by *Aspergillus oryzae*. The search for new cheaper raw material has increased prevailing higher due to the sugar prices. Developing of an economically

feasible biotechnical process for fermentable sugars from cellulose is not easy. It involves break-up of cellulosic material by *Penicillium expansum* used as carbon and energy source [9]. In the present work expensive carbohydrates were replaced by millet husk cellulosic material, containing hemicellulose, cellulose, lignin, starch alongwith organic nitrogen, mineral matter (ash) and lipid. While *Aspergillus oryzae* and Bacillus subtilis do not degrade cellulosic material easily but grow well on starches from wheat bran, rice bran & maize bran [1,6,21].

In present study *Penicillium expansum* link CMI 39761 produces α-amylase 40 units/ml broth/4000 units/g of millet husk. Results indicate that no significant difference in enzyme formation was noted when compared with *Bacillus subtilis* [6] which produces 3800 units/g of wheat bran and 2500 units/g of maize bran while Aspergillus foetidus [22] produces α-amylase activity 43 units/ml broth when rice flour was used as a carbon source. The induced 20 % and 12.5 % when millet husk was supplemented with starch and maltose.

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