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PRODUCTION OF AMYLASE FROM *CUCUMIS MELO* USING *ASPERGILLUS NIGER* BY LIQUID FERMENTATION

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Abstract:

Submerged fermentation was carried out using muskmelon shell as a substrate for the production of amylase using Aspergillus niger. It was observed that the activity started to peak at 60 hrs as 102.6 µg/ml/min, reached maximum at 118.56µg/ml/min at the 84th hrs and then went on decreasing at 108 hrs to111.72 µg/ml/min, respectively. The results show that the amylase activity was decreasing after the 3rd day of incubation in the same optimal conditions. The optimum temperature maintained for amylase activity, was 30°C at pH 8.The process parameters influencing the production of α-amylase were optimized.

Key words: *Cucumis melo, Aspergillus niger, α-Amylase, Submerged Fermentation*

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INTRODUCTION:

Amylase is one of the important and well-known industrial enzymes that cause the breakdown of starch or glycogen. Use of microorganism for the production of amylase is economical since microbes are easy to manipulate for obtaining enzymes of desired characteristics. Amylases stand out as a class of enzymes useful in food, brewing, textile, detergent and pharmaceutical industries. They are mainly employed for starch liquefaction, production of maltose, oligosaccharide mixtures, high fructose syrup and malto-tetrose syrup. They are applied during detergent production to improve cleaning effect and are also used for starch de-sizing in textile industry. Amylase can be produced either by submerged fermentation (SMF) or by solid state fermentation (SSF) procedures [1, 2, 3].

Musk melons (*Cucumis melo*) are extensively grown in almost all the countries of the world. At the global level the musk melon productions are very high. Melon husks are shells that are discarded after processing or shelling of melon seeds. Large quantities of the melon husks are discarded and burnt, which pollute the environment. It was reported by Ogbe and George, 2012, that carbohydrate content of musk melon husk was as much as 61.01 ± 0.35 [4].

A wide range of microbes such as bacteria and fungi are used for the production of industrial enzymes. However, fungi are preferred to bacteria for enzyme production because of their filamentous nature, which helps in its penetration through the submerged substrate. In the present study *Aspergillus niger* was used which has the additional advantage of controlling bacterial contamination due to its capacity of surviving at high degree of acidity (pH<3).

White biotechnology poses a challenge to economically well-established chemical processes that have been optimized for years [5]. This is a welcome sign due to the obvious reasons like, easy processing, cheap raw materials and it being eco-friendly [6]. Over a period of time we can do away with the chemical processing thus saving the environment. The aim of this study therefore was to determine the production of α amylase from the muskmelon shells using *Aspergillus niger.*

Amylase produced from fungal cultures was found to be more stable than the bacterial produced amylases on a commercial scale. A lot of research has been made to optimize culture conditions and suitable strains of fungi [7, 8]. Molds are capable of producing high amounts of amylase and *Aspergillus niger* is one such commonly fungus which is used for commercial production of amylase. Studies on fungal amylases especially in developing countries have concentrated mainly on *Aspergillus niger*, probably because of their ubiquitous nature and non-fastidious nutritional requirements. Solid State Fermentation holds tremendous potentials for the production of enzymes. It can be of special interest in those procsses where the crude fermented product may be used directly as the enzyme source [9, 10, 11].

The free water is indispensable to the microorganism's growth and is adsorbed on a solid support or complexed into the interior of a solid matrix [12]. This method has economic value for countries with abundance of biomass and agro industrial residues, as these can be used as cheap raw materials. Solid state fermentation which has been reported to be cheaper because of the enzyme extraction procedures (Kirankumar *et al*., 2011, Kumar and Duhan, 2011) and this method is a ray of hope for cheaper alternative for enzyme production [13, 14]. In case of SSF the cost of the substrate also plays a key role in deciding the cost of production. Agro industrial wastes have been reported to be good substrate for the cost effective production of alpha amylases and are thus attracting researchers for using agro industrial waste as a substrate for alpha amylase production [15, 16, 17, 18, 19]. Fungal species have been studied extensively for the production of alpha amylase because of the low cost of substrates used for the production [20, 21, 22].

METHODS:

Source of Microorganism

The standard strain of *Aspergillus niger* was obtained from the Jayagen Biologics Analytical Laboratory, Chennai.

The culture of *A. niger* is shown in Figure 1.

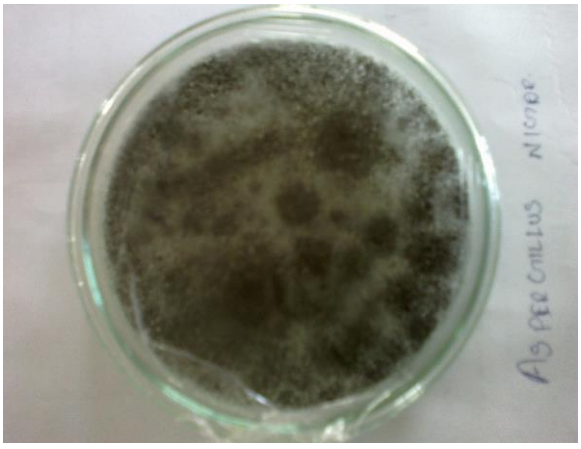

Fig.1: The culture of *A. niger.*

Subculturing
Frozen stocks on agar slants were activated periodically (fortnightly) and maintained on PDA-agar slants.

Collection of Samples
Cucumis melo fruits were collected from local market at Chennai. The shells were separated from the fruits and were sun-dried for over 12 days. The dried shells were powdered to be used for the fermentation process.

Sample Preparations
The dried shells were powdered for the fermentation process using mixer grinder.

Preparation of Culture Medium
The juice is produced by heating the powdered fruit shell in water at 85°C for 45 min with continuous stirring. The extract is filtered, decanted, further clarified through and sterilized at 120°C during 20 min.

Fermentation Process
The strains of *A. niger* were inoculated in sterile 250 ml Erlenmeyer flasks containing 20 ml of culture medium composed of (g/L): glucose, 20.0, yeast extract 5.0 and KH_2PO_4, 5.0 at pH 7.0 The cultures were developed in 100 ml Erlenmeyer flasks containing 10ml of fruit syrup inoculated with 2% (w/v) inocula levels and incubated at 30°C for shaker incubator.

Collection of Crude Enzyme
After 60 hour of incubation the samples were collected at every 24 hours interval from the fermented flask. [23] Each collected sample was homogenised gently with mortar and pestle and then centrifuged at 10,000rpm for 30mins at 4°C and analysed the activity of enzyme calorimetrically. The enzyme sample was stored at 4°C until the assay. All the processes were carried out under appropriate conditions. [24]

Enzyme Assay in Crude Enzyme Sample (A-AMYLASE)
Enzyme assay was carried out by DNS method of (Miller, 1954) in which 0.5ml enzyme was treated with 0.5 ml of substrate (0.5 % starch in 100 mM Tris buffer) under standard reaction conditions and the reaction was stopped by adding DNS reagent. [25] The amount of maltose released was determined by comparing the absorbance reading of the test enzyme at 540 nm with the standard graph plotted by treating the known concentration of maltose ranging from 0.05mg/ml to 0.5mg/ml. One unit amylase activity was defined as amount of enzyme that releases one micromoles of maltose per minute under standard reaction conditions.

Amylase was assayed by adding 1 ml of enzyme fermented broth supernatant to 1 ml of 0.5% soluble starch and incubated for 30 min at 37°C. The reaction was stopped by adding 3 ml of 1-dinitro-salicylic acid, followed by boiling for 10 min. The final volume was made to 10 ml with distilled water and the absorbance was measured at 540 nm calorimetrically. A calibration curve of absorbance and concentration of D-glucose was established with known amount of glucose. [26] One unit of amylase activity was defined as the amount of enzyme that releases 1 µ mol of reducing sugar as D-glucose per min under the assay conditions. The results are presented as specific activity (µ mol/L/min) (Table 1, 2, 3 and Figure 1).

RESULTS AND DISCUSSION:

Table 1: Amylase enzyme OD values (Incubated at 60 hours)

S.No	Enzyme solution(ml)	0.5% Starch solution (ml)	Incubation for 15 minutes	DNS solution(ml)	Boiling for 5 minutes	Rochelle salt(ml)	OD value at 540 nm
1.	1	1		3		1	0.44
2.	1	1.5		3		1	0.45
3.	1	2		3		1	0.45
4.	1	2.5		3		1	0.50
5.	1	3		3		1	0.52

Table 2: Amylase enzyme OD values (Incubated at 84 hours)

S.No	Enzyme solution(ml)	0.5% Starch solution (ml)	Incubation for 15 minutes	DNS solution(ml)	Boiling for 5 minutes	Rochelle salt(ml)	OD value at 540 nm
1.	1	1		3		1	0.32
2.	1	1.5		3		1	0.42
3.	1	2		3		1	0.46
4.	1	2.5		3		1	0.51
5.	1	3		3		1	0.53

Table 3: Amylase enzyme OD values (Incubated at 108 hours)

S.No	Enzyme solution(ml)	0.5% Starch solution (ml)	Incubation for 15 minutes	DNS solution(ml)	Boiling for 5 minutes	Rochelle salt(ml)	OD value at 540 nm
1.	1	1		3		1	0.34
2.	1	1.5		3		1	0.36
3.	1	2.0		3		1	0.41
4.	1	2.5		3		1	0.45
5.	1	3		3		1	0.46

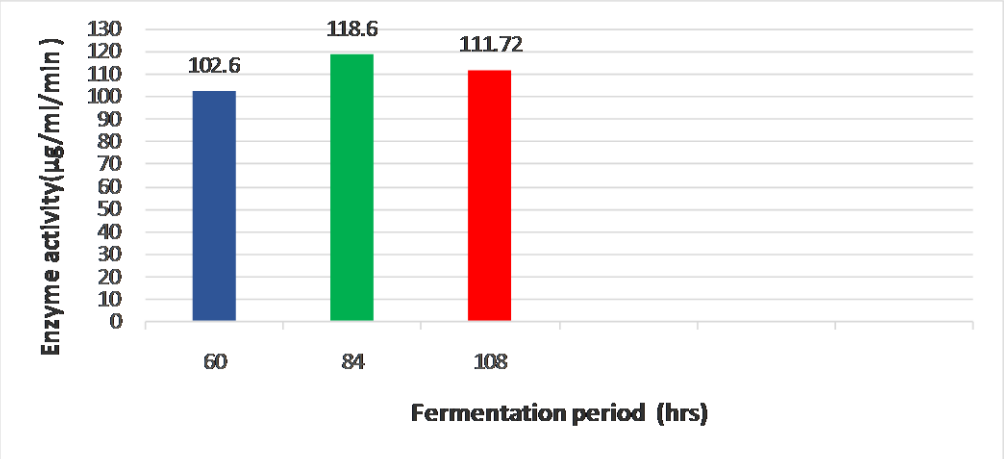

Fig. 2: Graphical representation of enzyme activities at different fermentation periods

DISCUSSION

The present experimental strain of *A. niger* chosen was able to grow on and was capable of producing α-amylase. It is found that the enzyme produced by Musk melon shell produced encouraging results at 102.6 units at 60th hr, a maximum of 118.6 units at 84th hr and 111.72 units at 104th hr, respectively, as shown in Figure 2. Beyond this period the enzyme production reduced considerably. Khan and Yadav, 2011, have used *A. niger* to obtain Amylase from various vegetable wastes by SSF at 28^0 C and Ph 6.2 [10]. Mrudula and Anitharaj, 2011 have reported pectinase production using *A. niger* with orange peel as substrate by SSF [11]. Radha Krishna *et al*, 2012, have compared the production of amylase by SSF and SMF with banana peel as substrate and using *A. niger* as the fungal strain [3]. Vijayaraghavan *et al*, 2011 have used *Penicillium* strain obtaining maximum production of Amylase on the 96 hrs at 37^o C, pH 7 using banana peel as substrate. [23]

CONCLUSION:

Thus it is evident that *A. niger* is a good, cheap and easily available source for production of Amylase

enzyme and so also musk melon shells. This strategy will help in industrial exploitation of these two naturally available resources thus helping green technology. Further work is warranted before this report is industrially commercialised.

COMPETING INTERESTS

This is to inform that no conflict of interest exist among the authors.

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