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Production of Lipase enzyme by *Aspergillus flavus* using Groundnut waste

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Abstract: The present study was carried out to determine the production of the lipase enzyme from *Aspergillus flavus* under submerged fermentation. Lipase containing protein content was estimated. Effect of bio process variables such as p^H, temperature and substrate concentration of Lipase activity were also monitored during lipase production. The enzyme was active at p^H 5, 32°c and 7gm substrate concentration in submerged fermentation. The amount of enzyme produced at p^H 5 was 2.501IU/ml, temperature 32°c was 2.380 IU/ml, 7gm substrate concentration was 2.358 IU/ml.
Key words: *Aspergillus flavus*, Ground nut shell, temperature, p^H, Substrate concentration.

INTRODUCTION

Lipases are enzymes belonging to the group of serine hydrolases (E.C.3.1.1.3). Their natural substrates are triglycerides and their mode of action is similar to that of the esterases . The lipases are able to catalyze hydrolysis, Esterification, transesterification and lactonization [1].

In 1856, Claude Bernard first discovered a lipase in pancreatic juice as an enzyme that hydrolysed insoluble oil droplets and converted them to soluble products.

Lipases have traditionally been obtained from animal pancreas and are used as a digestive aid for human consumption either in crude mixture with other hydrolases (pancreatin) or as a purified grade.

Lipases can be found in animal and vegetable cells. Certain micro organisms are the source of choice for lipase productions[2].Lipolytic enzymes are currently attracting an enormous attention because of their biotechnological potential [3]. They constitute the most important group of biocatalysts for biotechnological application. Further more, novel biotechnological application have been successfully established using lipases for the synthesis of biopolymers and biodiesel, the production of enantiopure pharmaceuticals, agrochemicals and flavour compounds [1].

Lipases from a large number of bacterial, fungal and plant and animal sources have been purified to homogeneity [4]. Lipases isolated from different sources have a wide range of properties depending on their sources

with respect to positional specificity, fatty acid specificity, thermostability, p^H optimum, etc[5].

Microbial lipases constitute and important group of biotechnologically valuable enzymes, mainly because of the versatility of their applied property and ease of mass production. Microbial lipases are widely diversified in their enzymatic properties and substrate specificity, which make them very attractive for industrial application. The lipase catalyst transesterification in organic solvents is an emerging industrial applications such as production of cocoa butter equivalent, human milk fat substitute "Betapol", pharmaceutically important polyunsaturated fatty acid (PUFA) rich / low calorie lipids, "designers fats or structured" and production of biodiesel from vegetable oils [6,7].

Lipases may be used as digestive aids [8]. Lipases are the activators of Tumor Necrosis factor and therefore can be used in the treatment of malignant tumors [9].

Lipases have earlier been used as therapeutics in the treatment of gastrointestinal disturbances, dyspepsia's, cutaneous manifestations of digestive allergies, etc[10].

MATERIALS AND METHODS

SAMPLE COLLECTION

The groundnut shell was collected from Eadayarnattham, Thanjavur District. The ground nut shell was dried for 10days and grind well to make powder. The ground samples were used as powder for the production of lipase using *Aspergillus flavus* then lipase activity was analyzed.

MICROORGANISMS USED

Lipase producing strain of *Aspergillus flavus* was obtained from Amphigene Research Centre, Thanjavur. Waste ground nut shell used as a substrate. The organisms were subculture on Rose Bengal agar medium incubated for 72 hours at 28 ^{0} C. The spores of each slant was scraped into 5 ml sterile water and shaken well with sterile beads on a rotary shaker for 30 minutes to break the spore chains and to make uniform suspension. This suspension was filtered through sterile cotton to remove the hyphal filaments.

FERMENTATION PROCESS (SHAKE FLASK CULTURE METHOD)

After preparation of production (Ground nut shell powder-5 gm Peptone-2.5 gm Distilled water-250 ml) media 10 % of inoculums was transfer to the production medium and incubated at 28 ^{o} C on a rotary shaker at 150 rpm for 72 hours. The mycelium was separated by filtration was used for the enzyme assay.

ENZYME EXTRACTION

After 7 days incubation, 1% (W/V) Triton X100 with Phosphate buffer 20 mM (pH8) was added to each flask. The flasks were kept in a rotary shaker at200 rpm for one hour at 250C. The ingredients of the flask were subsequently filtered and the culture filtrate was used as the enzyme source. There lative humidity and dry matter were determined by drying 5g of fermented solid matter at 1000C for 24h.

ULTRAFILTRATION

Cell-free culture supernatant (2 liters) was concentrated to 200 ml with an Amicon ultra filtration unit and YM 30 membranes (exclusion limit, 30K) at 5°C under a pressure of 2.5 bar of N2.Then 200 ml of the above Tris hydrochloride buffer (pH 8.0) was added and again concentrated to 200 ml, and this step was repeated 10 times. Finally, the samples were concentrated to 25 ml, and the same volume of 5 mM EDTA in Tris hydrochloride buffer (pH 8.0) was added. The sample was further concentrated to 6 ml, and1.5-ml portions were stored at -70°C.

ASSAY METHOD
PROCEDURE

Determination of lipase activity was done with the help of cup-plate method. The medium(X) contains Difco peptone-10g, NaCl-

5g, CaC12.2H2O-1.0g, Agar 20g and 10ml lipid substrate Serbitan mono laurate (Tween-20) (Pre-sterilized), distilled water- 1000ml was added to it. The pH of the medium was adjusted to 6.0. The medium was poured in each Petri plate. On solidifying the medium with the help of a cork borer (No.4) of 8mm diameter well was made in the centre of the plate and was filled with 0.1ml culture filtrate. The plates were incubated at 28°C. After 24 hours, a clear circular zone was measured (mm) as lipase activity.

PRODUCTION AT DIFFERENT TEMPERATURES, P^H ,SUBSTRATE CONCENTRATION

Aspergillus flavus were inoculated onto the organic waste medium and were then incubated at 32 °C to 50 °C, P^H 5-6, 5gm and 7gm respectively. The growth was checked for 4-5 days and protein content were analyzed [11].

RESULTS AND DISCUSSION

In our present investigation the lipase production in submerged fermentation of ground shell medium and ground nut shell with peptone medium was carried out.

A.flavus produces green colour colonies on the Rose Bengal Agar plate. *A.flavus* is mostly or entirely uniseriate aspergilla, strongly roughened conidia, and a more somber color described as “dark yellow green”.

The *A.flavus* produced significant quantities of enzyme when grown in synthetic oil based medium under submerged fermentation[12].

The lipase enzyme production was carried out in ground nut shell medium in submerged fermentation. The lipase production was monitored at 32°c, 40°c, 50°c and p^H 5, 5.6, 5.8, 6 is 2.380 Iu/ml, 0.760 Iu/ml, 0.560 Iu/ml, 2.501 Iu/ml, 2.197 Iu/ml, 2.068 Iu/ml, 0.881 Iu/ml. The lipase production was increased at p^H 5, 32°c. The enzymes were used for removal of lipid strains.

The maximum lipase production for ground nut shell medium was maintained at temperature 32°c. Lipase production by *A.flavus* was in correlation with the growth temperature, whereby 32°c was the optimum temperature for incubation time. Low lipase production and growth rate were observed at 40°c and 50°c. Being a thermostable enzyme, lipase was optimally active at its optimum growth temperature to fulfill nutritional requirements [13]. Maximum lipase production by *A.flavus* was obtained at the ground nut shell medium of p^H 5.0. At p^H 6.0 lipase productions decreased up to 80%. Extra cellular lipase production was tested over abroad p^H range (p^H 5.0 to p^H 6.0). The result showed that p^H 5.0 supported both good growth and high lipase production. However, at p^H 6.0 both the growth and lipase production were totally decreased growth was only observed at narrow pH range of 5.0 to 6.0 which indicated that the fungal physiology depended on environmental p^H.

The maximum lipase production was observed at 7 gm ground nut shell medium. Minimum lipase production was observed in ground nut shell with peptone medium.

Extra cellular lipase production was tested over abroad various nutrient compositions. The result showed that substrates such as Ground nut shell medium supported the good growth and high lipase production.

The total protein content was monitored on ground nut shell medium was 85.47 and ground nut shell with peptone medium was 60.0. This result concludes that the protein content was increased in ground nut shell medium.

The *A.flavus* produce maximum amount of enzyme lipase at p^H 5, 32°c and 7 gm substrate concentration. The enzyme was used for removal of lipid strains.

PRODUCTION OF LIPASE BY *A.flavus* AT DIFFERENT TEMPERATURE, P^H AND SUBSTRATE CONCENTRATION

	TEMPERATURE			
Strain Name	32°C	40°C	50°C	-
A.flavus	2.380	0.760	0.560	-
	P^H			
	5	5.6	5.8	6
	2.501	2.197	2.068	0.881
	SUBSTRATE CONCENTRATION			
	5gm	7gm	-	-
	2.300	2.358	-	-

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