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STUDIORUM PROGRESSUS

Studies on Mold Lipase

Comparative Study of Lipases Obtained from Molds Grown on Cocoanut

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Lipase had been observed to be present in *Aspergillus niger* by CAMUS[2]. HAEHN[3] had explored the possibility of fat synthesis by fungal enzymes. ARCHIBALD RAYNER[4] observed that certain molds decompose the edible oils into fatty acids and finally into CO_2 and CH_4. FODOR and CHARI[5] found that mycelial lipase of *Aspergillus niger and penicillium roqueforti* showed the maximum lipolytic activityatan optimum pH of 6·5. RAMAKRISHNAN and NEVGI[6] investigated the different oil seeds and RAMAKRISHNAN and BANERJEE[7] the different oil seed cakes for their lipolytic activity. In continuation of their search for a cheap and active lipase, the authors are investigating the molds grown on different oil seeds for their lipolytic activity. The present paper deals with the results of their investigations on mold lipases from cocoanut source.

Experimental

Cocoanut kernel in different forms—kernel in milk, slightly matured kernel, matured kernel and dried kernel (Kopra) were kept in desiccators over water at 28°C and exposed to air. The molds began to grow on them after four days.

It was found that the mold growth was maximum in the case of kernel in milk and decreased in the order—kernel in milk (moisture 72·2%; free fatty acid —1·4%), slightly matured kernel (moisture 65·1%; free fatty acid 2·1%), matured kernel (moisture 53·2%; free fatty acid —2·2%) and dried kernel (moisture 2·8%; free fatty acid —2·4%).

The molds grown were subcultured in Czapek agar medium in Petri dishes and pure strains were prepared. Staining methods used by MISWARD BAYLISS, DAVID GLICK, and ROBERT SIEM[8] as well as the biological methods used by GRABILL and REED[9] were used to detect the lipolytic molds from the pure strains obtained.

The pure strains of the lipolytic molds were grown in liquid Czapek medium at 37°C, and after a week the mats formed were removed, washed well with sterilised water, and extracted with low-boiling petroleum ether to remove the fat. They were completely dried at room temperature to remove the solvent, powdered well, sieved through a 60 mesh sieve, and the powder was used.

The activity of these mold lipases was studied by investigating the hydrolysis of freshly prepared groundnut oil using these lipases and disodium phosphate—citric acid buffer of varying pH.

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Each set of the experiments consisted of 1 cm³ of oil, 5 cm³ of water, 0·1 g of mold lipase, 2 cm³ of buffer mixture of varying pH, and a few drops of toluene in a flask incubated at 37°C for 24 hours after a thorough shaking. Always a blank accompanied each sample. After the period of incubation, the contents were removed and titrated against 0·1 N NaOH after the addition of 25 cm³ of neutral alcohol and warming for some time. Necessary precautions were taken to obtain readings under sterile conditions. The difference between the sample and the blank in terms of cm³ of 0·1 N sodium hydroxide will give the activity of the lipase. The results are given in Table I.

From the above table, it can be seen that, in general, the strains isolated from molds grown on kernel in milk show high lipolytic activity. The optimum pH for all the lipolytic molds is 6·2. *Aspergillus flavus, Aspergillus oryzae* and *Aspergillus niger* A_2 strains are highly lipolytic in nature.

These three strains were grown on cocoanut cake medium. In different culture flasks, 200 cm³ of cake solution containing 15% of oil-free cake was added and different amounts of cocoanut oil (f. f. a. = 0·05%) were added. They were sterilised for 10 minutes under 15 lbs. pressure, inoculated with the strains and incubated at 37°C. After four days, the mats were removed and the lipase powder was prepared. The activity of these mold lipases was determined by studying the hydrolysis of freshly prepared grand nut by oil using these lipases and disodium phosphate-citric acid buffer of 6·2 pH. The results are given in Table II.

Table II

Medium	Growth	Lipase activity in terms of difference in cm³ of 0·1 N NaOH between the sample and the blank for		
		A. flavus	*A. oryzae*	*A. niger* A_2
Czapek	Appreciable growth	6·8	6·1	5·8
Cocoanut cake alone	Not satisfactory	5·2	4·8	4·3
Cocoanut cake + 2% cocoanut oil	Slight growth	5·8	5·3	4·9
Cocoanut cake + 5% cocoanut oil	Good growth	6·9	6·5	6·1
Cocoanut cake + 10% cocoanut oil	Very good growth	*12·9*	*10·8*	*9·5*
Cocoanut cake + 15% cocoanut oil	Slight growth	7·1	6·8	6·2
Cocoanut cake + 20% cocoanut oil	Slight growth	5·2	4·7	4·4

From the above table, it can be seen that the strains grown on cocoanut cake medium containing 10% oil show high lipolytic activity. This gives an indication that the cake medium, on further investigation, may prove to be a good source for the growth of lipolytic molds on a large scale.

Synthetic activity of the lipolytic molds

Acetone-dried lipase was prepared from the molds according to RAMAKRISHNAN and NEVGI's[1] method. The

[1] C. V. RAMAKRISHNAN and G. V. NEVGI, J. Univ. Bombay *19*, Part 3, p. 36 (1950).

Table I

Name of the mold	Activity of the mold lipase in terms of difference in cm³ of 0,1 N NaOH between the sample and the blank when the pH of the mixture is							
	3·1	3·6	4·2	4·8	5·4	6·2	6·8	7·2
Aspergillus niger A_1								
From kernel in milk	2·9	3·2	3·5	4·2	4·9	*5·2*	4·6	4·1
From slightly matured kernel	2·7	2·9	3·2	4·0	4·5	*5·0*	4·2	3·8
From matured kernel	2·2	2·6	2·9	3·6	4·1	*4·7*	4·0	3·5
From dried kernel	1·9	2·2	2·6	3·0	3·9	*4·2*	3·8	3·0
Aspergillus niger A_2								
From kernel in milk	3·1	3·3	3·8	4·9	5·1	*5·8*	5·2	4·7
From slightly matured kernel	2·5	2·8	3·0	3·8	4·5	*5·2*	4·8	4·2
From matured kernel	2·8	3·0	3·5	4·5	4·9	*5·5*	5·0	4·5
From dried kernel	2·2	2·5	2·8	3·2	3·8	*4·5*	4·2	4·0
Aspergillus flavus								
From kernel in milk	3·8	4·2	4·8	5·2	5·6	*6·8*	5·9	4·3
From slightly matured kernel	2·9	3·9	4·5	4·7	5·2	*6·2*	5·2	4·1
From matured kernel	2·5	3·4	3·9	4·1	4·9	*5·2*	4·8	3·7
From dried kernel	2·3	2·8	3·1	3·8	4·1	*4·8*	4·1	3·2
Aspergillus oryzae								
From kernel in milk	3·0	3·7	4·5	5·0	5·2	*6·1*	5·1	4·9
From slightly matured kernel	2·8	3·4	3·8	4·8	5·0	*5·5*	5·0	4·5
From matured kernel	2·5	2·9	3·2	4·2	4·8	*5·1*	4·7	4·1
From dried kernel	2·4	2·6	2·9	3·9	4·2	*4·7*	4·2	3·8
Penicillium sp.								
From kernel in milk	2·5	2·8	3·1	3·5	3·6	*4·2*	4·0	3·5
From slightly matured kernel	2·0	2·6	2·9	3·2	3·4	*4·1*	3·8	3·2
From matured kernel	1·9	2·4	2·6	2·8	3·0	*3·7*	3·5	3·0
From dried kernel	1·5	2·0	2·2	2·5	2·9	*3·2*	2·9	2·5
Yellow mold								
From kernel in milk	2·7	2·9	3·1	3·3	3·5	*4·0*	3·4	2·9
From slightly matured kernel	2·5	2·5	2·8	2·9	3·2	*3·5*	3·2	2·7
From matured kernel	2·2	2·1	2·5	2·7	3·0	*3·2*	2·9	2·2
From dried kernel	1·8	1·8	2·2	2·4	2·8	*3·0*	2·5	1·8
Aspergillus fumigatus								
From kernel in milk	1·2	1·8	2·1	2·4	2·8	*3·2*	2·5	1·8
From slightly matured kernel	1·0	1·5	1·8	2·0	2·5	*3·0*	2·2	1·9
From matured kernel	0·8	1·2	1·5	1·5	2·2	*2·8*	2·0	1·4
From dried kernel	0·5	0·9	1·1	1·5	1·8	*2·6*	2·0	1·1
Rhizopus sp.								
From kernel in milk	1·0	1·2	1·5	2·0	2·2	*2·5*	2·1	1·5
From slightly matured kernel	0·7	0·8	1·2	1·8	1·9	*2·2*	1·8	1·0
From matured kernel	0·4	0·6	0·9	1·4	1·5	*1·8*	1·5	0·8
From dried kernel	0·2	0·4	0·6	1·0	1·4	*1·6*	0·9	0·7

synthesis of butyl oleate was carried out using these lipases.

In different conical flasks, equimolecular quantities (0·054 g molecule) of butyl alcohol [$CH_3(CH_2)_2\,CH_2OH$— B. P. 117°C; density = 0·809 g cm³ molecular weight 74·1] and oleic acid [$C_8H_{17}CH = CH(CH_2)_7$—COOH B. P. 286°C 100 mm density = 0·895 g/cm³; molecular weight 282·4) were added. The lipase (1 g) and ether solvent (10 cm³) were added to each flask, shaken well, and kept in the incubator at 37°C after corking well. At different intervals of time 1 cm³ from each flask was taken, 25 cm³ of neutral alcohol added, warmed for some time and titrated against 0·1 N sodium hydroxide. Always a blank accompanied each sample. The difference between the blank and the sample was calculated in terms of cm³ of 0,1 N sodium hydroxide. From this the percentage synthesis was calculated. The results are given in table III.

Table III

Solvent: Ether Synthesis of butyl oleate

Set No.	Name of the lipase	Percentage synthesis on day						
		1	2	3	4	5	6	7
1	*Aspergillus flavus*	7·9	14·8	16·5	20·1	29·7	*40·2*	35·6
2	*Aspergillus oryzae*	6·9	12·7	15·1	18·2	20·6	*32·8*	29·8
3	*Aspergillus niger* A_2	5·8	11·1	13·8	15·6	19·8	*30·7*	27·6

From Table III, it can be seen that the mold lipases can also synthesise esters which suggests that these might be utilized for the preparation of synthetic fat.

The above observations prove that these mold lipases can be used for fat hydrolysis if a pilot plant can be erected to grow the lipolytic molds on a large scale. The strains obtained from the molds grown on other oil seeds will also be analysed for their lipolytic activity, and if no other strain with better activity is found, these will be investigated as to whether lipase can be extracted from them on a large scale.

Our thanks are due to the Indian Council of Medical Research for having awarded a Fellowship to one of us to carry out this investigation.

Zusammenfassung

Stämme von *Aspergillus flavus*, *Aspergillus oryzae* und *Aspergillus niger*, die aus auf Kokosnuß gezüchteten Kolonien isoliert waren, erwiesen sich als lipolytisch aktiv. Das Medium wird gegenwärtig dahin entwickelt, daß die lipolytischen Schimmelpilze in großem Maßstab gezüchtet werden können.