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SCREENING OF FUNGAL STRAINS FOR THE ISOLATION OF LIPASE ENZYME

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ABSTRACT

Twenty-five fungal strains of Aspergillus, Mucor, Penicillium and Rhizopus species isolated from soil and other food products have been tested for their lipolytic activity. Under specified assay conditions it was found that strains of Aspergillus species exhibited maximum lipase activity and protein contents showed a good correlation with their lipolytic activity pattern. It was also noted that *Aspergillus niger*, *Aspergillus oryzae*, and *Aspergillus flavus* isolated from soil contained more enzymatic activity than those isolated from other sources. It was evident that thermotolerant strains were more potent in lipase activity than the non-thermotolerant ones. The details concerning optimum lipase production from the strains of *Aspergillus niger*, *Aspergillus oryzae* and *Aspergillus flavus* under similar assay conditions have been discussed.

INTRODUCTION

The wide use of lipase preparations in cosmetics, pharmaceutical preparations, food processing, elimination of fat from bones and chemical detergent industries have prompted the manufacture of this enzyme from cheap sources on commercial scale (Somkuti and Babel, 1968; Robert *et al.*, 1968; Laboureur and Villalon, 1969; Patrick and Lake, 1969; Fukomoto and Mieko, 1970 and Kobayashi, 1971). It may be assumed that microbial sources may be cheaper for the isolation of this enzyme in comparison to animal sources. Moreover, more information about the isolation and characterization of this enzyme will be very helpful to inactivate this enzyme in rice bran, a by product of rice crop, containing a good amount of edible oils. The objective of the present

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investigation was to make a comparative study to screen the twenty-five fungal strains of Aspergillus, Mucor, Penicillium and Rhizopus species, isolated from various sources for their lipase activity.

MATERIALS AND METHODS

i) *Sample Collection*

Twenty-five pure and indentified fungal-strains of Aspergillus, Mucor, Rhizopus and penicillium species were obtained from PCSIR, Labs., Lahore, Plant Pathology Department, University of Agriculture, Faisalabad and Food Science Division, Nuclear Institute for Agriculture and Biology, Faisalabad. These strains were propagated and maintained by following standard microbial methods (Duggar, 1909).

ii) *Enzyme Preparation*

The collected strains of Aspergillus, Mucor, Rhizopus, and Penicillium species were subjected to Kundu and Pal (1970) method for the isolation of crude lipase enzyme.

Ten grams of fresh commercial wheat bran were added separately into three 250 ml conical flasks and were moistened with 40 per cent sterilized water. The flasks were plugged with sterilized cotton and were autoclaved at 15 lb PSI at 120°C for twenty minutes. The isolated strains of different species were inoculated on this sterilized bran medium in such a way that equal amount of inoculum was introduced into each flask. Thus each strain got duplicate samples and one was kept as control (non-inoculated bran sample) flask. The inoculated flasks alongwith their control were incubated at 30 ± 1°C for 24, 48, 72 and 96 hours.

After the completion of the required incubation time period, the grown cultures (mouldy bran) in each flask were extracted by adding sterilized water (1 : 4) to each flask. These flasks were vigorously shaken for 30 minutes at 5°C. The aqueous extract was centrifuged at 650 x g at 5°C for 20 minutes using Centrifuge Refrigerator Chrison 11KS (German).

The clear supernatant fraction was collected and 20 ml of 70 per cent

ethanol were added to it. The mixture (containing freshly formed precipitate was centrifuged at (650 xg) for 20 minutes at 5 °C and after centrifugation, the residual pellet was dissolved in 100 ml of 0.2 M phosphate buffer of pH 6.5. This fraction was used as crude lipase enzyme.

iii) *Incubation studies*

The optimal growth period and temperature were determined, studying the lipolytic activity of the strains of *Aspergillus flavus, A. niger and A. oryzae,* following prescribed assay conditions from 24 hours to 144 hours and temperature studies at 20, 25, 30, 35 and 40 °C, respectively.

iv) *Hydrogen-ion concentration*

A. flavus, A. niger and *A. oryzae* were assayed for lipolytic activity using the prescribed assay conditions except the variation in pH, ranging from pH 4-9 with interval of pH 0.5.

v) *Substrate-specificity*

A. oryzae A. niger and *A. flavus,* were assayed for their lipolytic activity by using 5 gm, of commercial grade olive oil, coconut oil, cotton seed oil and butterfat in the substrate mixture. Freshly prepared homogenized substrate emulsion was used each time.

vi) *Optimal temperature*

Strains of *A. oryzae, A. niger* and *A. flavus* were assayed at variable temperatures, i. e., 5, 20, 25, 30, 35, 37, 40, 45, 50, 55, 60 °C and the incubation time period (60 minutes) was kept constant for all variable temperatures and the prescribed procedure for lipolytic activity determination was followed.

vii) *Estimation of total protein*

Total protein concentration in the cell free broth samples was estimated by the method of Lowry *et al.* (1951).

viii) *Statistical methods employed*

The completely randomized design with three replications was used for this experiment. The data collected were subjected to the analysis of variance.

Fisher's L.S.D. test was used for planned comparison of paired means.

RESULTS AND DISCUSSION

The details of twenty-five strains of Aspergillus, Mucor, Rhizopus and Penicillium species have been recorded in Table 1. Lipolytic activity of twenty-five locally isolated strains assayed by the method of Kundu and Pal (1970) have been reported in Table 2.

The variations in the enzymatic activity of the twenty-five fungal strains can be explained from their protein contents determined by the method of Lowry *et al.* (1951). It was seen that all the fungal strains differed significantly among themselves in their protein contents except two strains (*A. flavus* and *P. nigricans*) with the same protein contents. The protein contens were higher in the strains which exhibited maximum lipase activity in the prescribed assay conditions. Similarly, those strains which exhibited weaker lipase producing capacity, contained less amount of protein.

Since *A. niger*, *A. oryzae* and *A. flavus* showed maximum enzymatic activity in the above prescribed assay system, it, therefore, seemed quite logical that detailed studies were required in order to characterize the optimal parameters, for the maximum lipase production of these three strains under uniform assay conditions. The optimal hydrogen ion concentration for *A. niger*, *A. oryzae* and *A. flavus* were found to be 5.5, 6.0 and 5.0, respectively. These findings differed from the observations of Kundu and Pal (1970) who reported that pH 6.5 was optimum for the lipase activity in the assay system. Although the source of the fungal strain was soil in both the cases, but the difference in the enzymatic activity at different pH's in similar assay conditions could only be explained on the basis of difference in the nature of the soil.

The substrate specificity of these three strains indicated that the substrates differed significantly among themselves.

It was observed that emulsified coconut oil and butterfat were excellent substrates for these strains. Olive oil and cotton seed oil were also better substrates but corn oil was less attacked by these strains. Olive oil, cotton

Table 1. *Details of twenty-five fungal strains*

Name of fungal strain	Name of institution from whore strains collected	Source of isolation	Medium used
A. oryzae	NIAB*	Soil	Czapek's dox
A. flavus	NIAB	Soil	Czapek's dox
A. niger	,,	,,	,,
A. candidus	,,	,,	,,
A. versicolor	,,	,,	,,
A. sydowi	,,	,,	,,
A. fumigatus	UA**	Food grains	,,
A. terreus	,,	Soil	,,
A. sydowi	,,	,,	,,
A. ochraceus	,,	,,	,,
A. unilateralis	,,	Food	,,
A. violaceous	,,	,,	,,
A. nidulans	,,	Straw	,,
A. niger	PCSIR***	Food	Malt extract
A. flavus	,,	,,	,,
P. duponti	,,	Animal dung	,,
P. nigricans	,,	Soil	,,
P. purpurogenum	,,	Animal dung	,,
P. requeforti	,,	Wheat grain	,,
P. cephalophora	,,	Animal dung	,,
M. pusillus	UA	Wheat grain	YPSS
M. miehei	,,	,,	,,
M. indicus	PCSIR	Animal dung	Czapek's dox
M. variens	,,	,,	,,
R. arrhizus	,,	Food grain	,,

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Table 2. *Lipase activity of fungal strains*

Name of fungal strain	Volume of 0.05N alcoholic KOH consumed for unboiled enzyme (ml)	Volume of 0.05N alcoholic KOH consumed for boiled enzyme (ml)	Lipase activity units/5 ml
A. flavus*	28.9	11.2	17.7***
A. niger*	35.8	11.3	24.5
A. oryzae*	32.9	11.0	21.9
A. candidus	27.1	11.2	15.9
A. flavus**	25.6	11.3	14.3
A. sydowi	24.7	11.2	13.5
A. terreus	25.4	11.3	14.1
A. violaceous	13.4	11.0	2.4
A. fumigates	23.5	11.0	12.5
A. ochraceus	22.5	11.3	11.2
A. unilateralis	14.3	11.1	3.2
A. versicolor	22.4	11 0	11.5
A. nidulans	13.5	11.0	2.5
A. paraciticus	12.9	11.2	1.7
A. niger**	31.3	11.1	20.2
M. miehei	26 6	11.2	15.4
M. variens	13.5	11.2	2.3
M. pusillus	28.0	11.3	16.7
M. indicus	13.9	11.1	2.8
P. nigricans	28.0	11.0	17.0
P. duponti	14.3	11.2	3.1
P. cephalophora	13 6	11.3	2.3
P. purpurogenum	14.7	11.2	3.5
P. requeforti	23.9	11.0	12.9
R. arrhizus	25 0	11.2	13.8

* Isolated from soil. ** Isolated from food.

*** Each reading is the mean of triplicate readings.

seed oil and corn oil which mainly contained, unsaturated fatty acids of 16 and 18 carbon atoms, were less hydrolyzed, while coconut oil which contained saturated fatty acids of 6-10 carbon atoms was very well attacked by the fungal lipases from three fungal strains. Similarly, emulsified butterfat which contained predominantly lower saturated fatty acids of 4 and 6 carbon atoms were also well hydrolyzed. From these results, it may be concluded that emulsified coconut oil and butterfat are excellent substrates for *A. niger* and *A. flavus* and their specificity to triglycerides of lower molecular weight fatty acids and a variety of higher molecular weight of oils in the emulsified state has been confirmed. These results are in agreement with the findings of Somkuti and Babel (1968) regarding the emulsified butterfat as substrate, but these results have shown that emulsified coconut oil is an excellent substrate in comparison to olive oil. This difference can be explained on the basis that fungal strain tested by Somkuti and Babel 1968) belonged to non-thermotolerant class, whereas strains tested in the present studies belonged to thermotolerant class. The maximum activity exhibited by these three strains have been three days for *A. niger* and *A flavus* and two days for *A. oryzae*. These observations are contrary to those made by Kundu and Pal (1970) except for *A. oryzae*.

The growth pattern of these strains in terms of enzymatic activity showed a gradual increase and decrease with time. The optimum temperatures noted for initial incubation are 30°Cand 25°C for *A. oryzae A. niger* and respectively. Previous studies also reported that fungal strains of Aspergillus species normally showed maximum lipase activity at 25°C to 30°C (Kundu and Pal, 1970; Fukomoto *et al.*, 1964). Similarly, optimum temperature for these strains for the hydrolysis reaction was found to be 37°C. The maximum enzymatic activity ranged between 37-40°C and complete inactivation was observed from 60°C onward. Similar observations have been reported by other workers while assaying plant lipases or microbial lipases (Akhtar *et al.*, 1975).

The reported work provides a general survey of screening the local fungal strains for their lipase activity. All the isolates subjected for lipase activity

were without any purification and therefore more detailed work for the isolation and purification of this enzyme is suggested.

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