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Isolation and Screening of Lipase Producing Organisms Using De-oiled Seed Cake as a Substrate

Asmita R Shringeri and G. R. Naik
Department of Post Graduate & Research in Biotechnology, Gulbarga University, Kalaburagi - Karnataka, India.

Abstract: *Microbial enzymes are often more useful than enzymes derived from plants or animals because of the great variety of catalytic activity. Lipases are the acyl hydrolases and water soluble enzymes that play a role in fat digestion by cleaving long chain triglycerides into polar lipids. Because of an opposite polarity between the enzyme (hydrophilic) and their substrate (lipophilic), lipase reaction occurs at the interface between the aqueous and oil phase. The main purpose of the study is to utilize Seed cake for the production of industrially important enzyme. In this study the soil sample was collected from garage and petrol bunks, screened for lipase producing organisms grown on tributyrin agar media. The isolates showing maximum lipase activity were studied using de-oiled seed cake as substrate for biochemical parameters.*
Keywords: *Lipase, Tributyrin, De-oiled seed cake.*

I. Introduction

Lipases are ubiquitous water soluble enzymes which have the ability to hydrolyse triacylglycerols to release free fatty acids and glycerol. Lipases constitute a major group of biocatalysts that have immense biotechnological applications. Lipases have been isolated and purified from fungi, yeast, bacteria, plant and animal sources [7]. Of all these, bacterial lipases are most economical and stable [12]. Microbial lipases are of special interest because of their stability in organic solvent, do not require any cofactor for their activity, and has a broad substrate specification and high enantio selectivity [13]. Oils cakes are the important and quick acting organic nitrogenous manure. Non edible oil cakes are not suitable for feeding to animals. Therefore they are mainly used as manures. 35-60% of oil cake is obtained. It is a rich source of carbohydrate and other nutrients is an ideal candidate for the production of liquid bio-ethanol [3]. The seed cakes have been exploited in the field of fermentation technique which has resulted in the production of bulk chemicals and value added products such as amino acids and enzymes

At present bacterial lipases are of great demand for potential industrial applications. The present paper is mainly focused on the isolation and screening of microorganisms producing lipase and enzyme activity using *pongamia pinnata* and *jatropha curcus* oil cake.

II. Materials and Methods

(i) Sample Collection:

Soil samples were collected from oil contaminated soils from oil mills, garage and petrol bunks situated in and around Kalaburagi city. The de-oiled seed cakes were obtained from Biofuel Information and Demonstration Centre, Gulbarga University, Kalaburagi, and were used as substrate for the lipase production.

(ii) Isolation of Lipolytic microorganisms:

Soil samples have been serially diluted and plated on to tributyrin agar base containing 0.5% (w/v) peptone, 0.3% (w/v) yeast extract, 1% (v/v) Tributyrin and 2% agar, pH 7.0 by spread plate method [3]. Plates were incubated at 37^{o}C for two to three days. Axenic cultures of isolates were maintained on minimal media agar slants (3% yeast extract, 3% NaCl, 5% Peptone and 20% agar, pH 7.0) and were subcultured every 15 days.

(iii) Screening of the isolates for Lipase Activity:

(a) Primary Screening

Screening of the isolates using Tributyrin (Qualitative assay):
Lipolytic organisms were screened by qualitative plate assay, using tributyrin agar base and incubated at 37^{o}C for 48hrs. Potential isolates were selected by measuring the diameter of zone of hydrolysis exhibited by the isolates.

(b) Secondary Screening

The isolates showing potentiality in primary screening were further screened for the ability to utilize the de-oiled seed cake as substrate. This was done in three different steps, firstly the isolates were cultured

containing 1% tributyrin as substrate, and in second step the isolates were cultured containing 1gm *pongamia pinnata* seed cake as substrate, in third step the isolates were cultured containing 1gm *jatropha curcus* seed cake as substrate, for 18hrs at 37°C in orbital shaker at 160rpm.

(iv) Enzyme Assay

(a) Lipase Activity Assay by Titrimetric Method:

Lipase was assayed by a standard titrimetric method.

Sample preparation: An aliquot of enzyme extract was dispensed to a tributyrin – potassium phosphate buffered solution emulsified with gum Arabica, incubated for 15 mins at room temperature.

After incubation the reaction mixture was cooled quickly in ice and the reaction was terminated with addition of 1ml of acetone: ethanol solution. Enzyme activity was calculated.

(b) Specific Activity:

Specific activity of enzyme was determined by estimation of protein content of enzyme fraction using de-oiled seed cake as substrate which was determined by comparing with the standard BSA curve and the specific activity of lipase was calculated.

(v) Results

(a) Isolation and screening of isolates for Lipase activity:

Lipase producing microbial culture were isolated from different sites of Kalaburagi by serially diluting the samples were plated in Tributyrin agar plate. Lipolytic bacteria were isolated from the plate and enriched in Nutrient broth by periodic sub culturing. Forty lipase producers gave positive results in plates showing lipolytic zone. Twenty one different bacterial strains (KAR1-KAR21) were screened which are capable of producing lipolytic zone and were used for further studies.

(b) Assay for lipase activity

After 64 hrs of incubation, lipase activity was calculated by using standard titrimetric method. Activity of enzyme was observed to be 14400U/ml of *pongamia pinnata* and 14200U/ml of *jatropha curcus*.

Table 1:- Enzyme activity using *Pongamia pinnata* as seed cake.

Isolates	Crude extract (U/ml)	Partially purified (U/ml)
KAR 15	14000	13600
KAR 21	14400	14000

Table 2:- Enzyme activity using *Jatropha curcas* seed cake as substrate.

Isolates	Crude Extract (U/ml)	Partially Purified (U/ml)
KAR 15	13800	13800
KAR 21	14200	14200

Protein Estimation by Lowry's Method:

The amount of protein was 760µg/ml for KAR15 strain and 850µg/ml for KAR21 strain.

Fig No-1:- Total Protein content of enzyme fraction extracted using *Pongamia pinnata* seed cake as substrate.

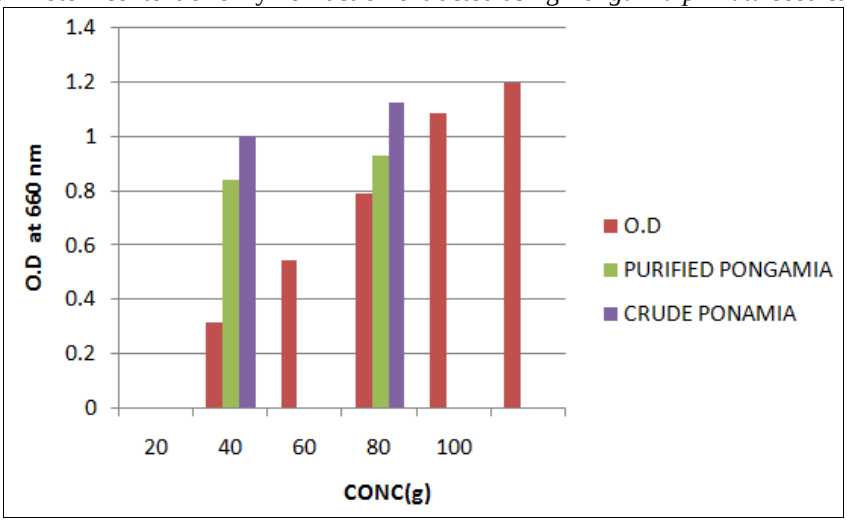
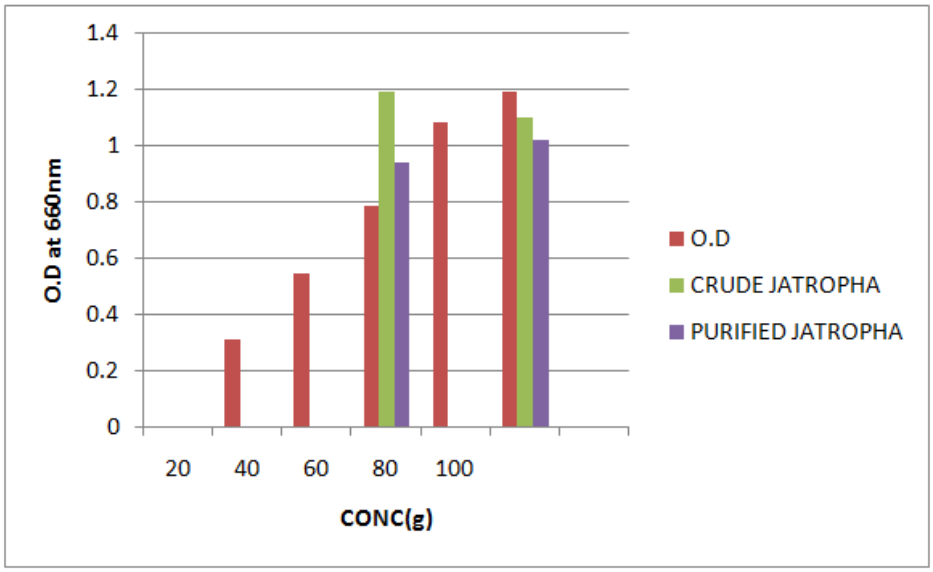


Fig No-2:- Total Protein content of enzyme fraction extracted using *jatropha curcus* seed cake as substrate.



Specific Activity:

Specific activity of enzyme was estimated using crude and partially purified samples of *Jatropha curcus* and *Pongami pinnata* seed cake.

Table 3:- Specific activity of isolates on both seed cake *Pongamia pinnata* and *Jatropha curcus* seed cake as substrate

Isolate	**Crude Extract (µ/mg)**		**Partially Purified (µ/mg)**	
	Pongamia pinnata	***Jatropha curcus***	***Pongamia pinnata***	***Jatropha curcus***
KAR 15	18.42	15.33	21.25	19.43
KAR 21	16.94	16.90	19.71	18.20

III. Conclusion

In this study, soil sample was collected from oil mills, garage and petrol bunks for screening of lipase producing microorganism using tributyrin agar plate method. The isolated microorganisms were screened using qualitative assay. The isolated bacteria were grown on three different substrates. Among which *pongamia pinnata* showed maximum enzyme activity (0.4675 U/ml) as compared to *jatropha curcus.*

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