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Isolation and Optimization of Lipase Producing Bacteria from Oil Contaminated Soils

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Abstract: Lipolytic bacteria were isolated from oil contaminated soils and grown on tributyrin media containing 1% (w/v) olive oil. The isolate showing maximum activity was identified by following Berger's manual. Different media parameters were optimized for maximal enzyme production. Peak lipase activity was observed for palm oil as carbon source, peptone as nitrogen source, at pH 7.0 and temperature at 37°C.

Key words: Lipases · Tributyrin · Extracellular · Oil

INTRODUCTION

Lipases (Triacylglycerol lipases, EC 3.1.1.3) are 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 biotechnology applications. Lipases have been isolated and purified from fungi, yeast, bacteria, plant and animal sources [1]. Of all these, bacterial lipases are more economical and stable [2].

Bacterial lipases are used extensively in food and dairy industry for the hydrolysis of milk fat, cheese ripening, flavour enhancement and lipolysis of butter fat and cream [3]. Lipases are also used in detergent industry as additive in washing powder [4], textile industry to increase fabric absorbency [5], for synthesis of biodegradable polymers or compounds [6] and different transesterification reactions [7]. In addition, the enzyme is used as a catalyst for production of different products used in cosmetic industry [8], in pulp and paper industry [9], in synthesis of biodiesel [10], degreasing of leather [11] and in pharmaceutical industry [12].

Currently bacterial lipases are of great demand because of potential industrial applications. The present paper focused on screening and isolation of microorganisms and optimisation of different parameters for maximal enzyme activity.

MATERIALS AND METHODS

Sample Collection: Samples were collected from oil and fat contaminated soils of diary and oil refinery industries situated in and around Hyderabad andhra Pradesh, India.

Isolation of Lipolytic Bacteria: 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 [13]. Plates were incubated at 37°C for two days. Pure cultures of the isolates were maintained on minimal media agar slants (yeast extract, NaCl, Peptone and 2% agar, pH 7.0) and were subcultured every 15 days.

Screening of the Isolates for Lipase Activity: Lipolytic organisms were screened by qualitative plate assay. Isolates were grown on Tributyrin agar base plates and incubated at 36°C for 2 days. Zone of clearance was observed due to hydrolysis of tributyrin.

Culturing and Characterization of the Isolates: The isolate showing maximum zone of clearance hereby referred as L2 is selected for further analysis. Morphological and biochemical characteristics of the isolate have been studied for the identification of the isolate.

Lipase Assay: Lipase activity was measured by titrimetric method using olive oil as a substrate. Olive oil (10% v/v) was emulsified with gum Arabic (5% w/v) in 100mM potassium phosphate buffer pH 7.0. 100 µl of enzyme was added to the emulsion and incubated for 15 minutes at 37°C. The reaction was stopped and fatty acids were extracted by addition of 1.0ml of acetone: ethanol solution (1:1). The amount of fatty acids liberated were estimated by titrating with 0.05M NaOH until pH 10.5 using a phenophathelin indicator [14].

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One unit of enzyme is defined as the amount of enzyme required to hydrolyse µmol of fatty acids from triglycerides.

Optimization of Media Parameters for Profound Enzyme Activity

Effect of Incubation Period on Lipase Activity: L2 was cultured in Tribuytrin broth containing yeast extract, NaCl, peptone and 1% (w/v) olive oil at 36°C in an inorbital shaker at agitation speed of 150rpm. The culture broth was harvested at 8h intervals by centrifugation at 10,000 g, 30 min, 4°C. The supernatant collected was used as crude enzyme solution and was assayed for enzyme activity.

Effect of Different Oils as Carbon Source on Lipase Activity: Olive oil present in the growth media was replaced with different oils like palm oil, ghee, coconut oil, ground nut oil, sunflower oil and mustard oil at a final concentration of 1% (w/v) by keeping remaining parameters same.

Effect of Different Nitrogen Sources on Lipase Activity: Different nitrogen sources like yeast extract, soya bean meal, $NaNO_3$, tryptone and peptone were added to the broth at a final concentration of 1% (w/v). Remaining parameters were unaltered.

Effect of Temperature on Lipase Activity: For selection of optimum temperature for the production of lipases, the temperatures varying from 21to 42°C were selected by keeping the remaining parameters same.

Effect of pH on Lipase Activity: The optimum pH for enzyme production was selected by varying the pH of the tributyrin broth from 5 to 9 whereas the other parameters were unaltered.

Effect of Agitation Speed on Lipase Activity: To determine the optimal agitation speed for peak enzyme activity, the L2 was cultured in an orbital shaking incubator at 36°C at varying agitation speed from 120-200 rpm.

RESULTS AND DISCUSSION

Screening, Isolation and Identification of Lipolytic Bacteria: 10 different soil samples collected from different areas show high bacterial count. The colony labelled as L2 showed maximum zone of clearance when plated on tributyrin agar base. Morphological and biochemical studies were done on L2 isolate. By following the bergey's manual, the isolate was identified likely to be belonging to genus *Staphylococcus*.

Effect of Incubation Period on Lipase Activity: The L2 isolate was inoculated in Tributyrin broth and was harvested at 8 hours interval. Maximum enzyme activity was observed at 48 hours at the early stationary phase. The activity of the enzyme gradually decreased after 48 hours (Figure 1).

Effect of Carbon and Nitrogen Sources on Lipase Activity: For selection of optimum carbon and nitrogen source, different carbon and nitrogen sources were used for the media. Peak enzyme activity was obtained when palm oil and peptone were used as carbon and nitrogen source (Figures 2, 3).

Effect of Temperature, pH and Agitation Speed on Lipase Activity: Lipase activity was observed at broad range of temperature, pH and agitation speed. Maximum activity was observed at 36°C temperature, pH 7.0 and at agitation speed of 160rpm (Figures 4,5,6).

In conclusion, bacterial lipases are one of the enzymes having huge market demand. The isolated *Staphylococus sps* isolate have shown the production of extracellular lipases. Optimization studies on media parameters for maximum lipase activity were done on isolated lipolytic bacteria. The isolate has shown a broad range of pH and temperature. The extracellular lipase enzyme can be further purified and used in different industrial applications.

Table 1: Morphological and biochemical characterisation of L2 isolate

Substrate, Test		Result
Gram Staining		Positive
Morphology		Cocci
Citrate		Negative
Catalase		Positive
Gelatin liquefaction		Negative
Nitrate reduction		Weakly positive
Oxidase		Negative
Fermentation of gas and acid		
	Glucose	Positive
	Sucrose	Positive
	Lactose	Positive
Indole		Negative
Methyl red		Negative
Voges-Proskauer		Negative
Urease		Negative

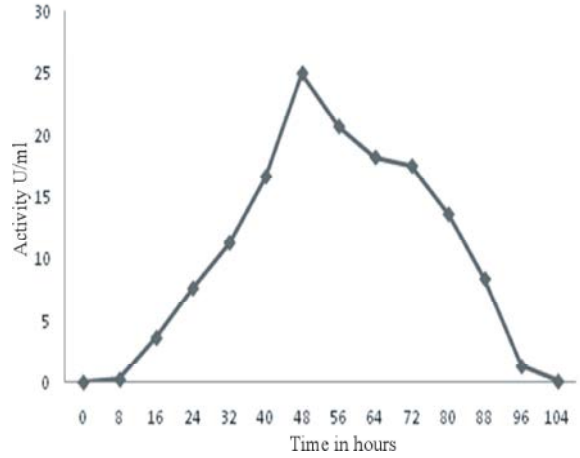

Fig. 1: Effect of incubation period on lipase activity

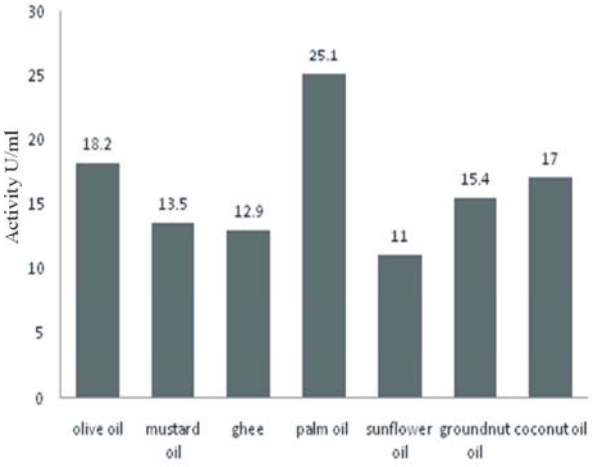

Fig. 2: Effect of different carbon sources on lipase activity

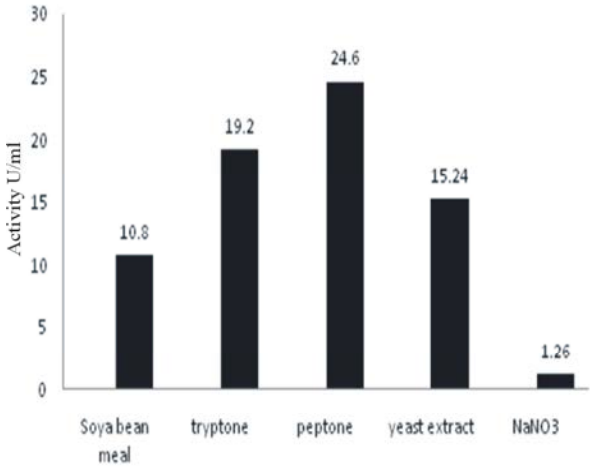

Fig. 3: Effect of different nitrogen sources on lipase activity

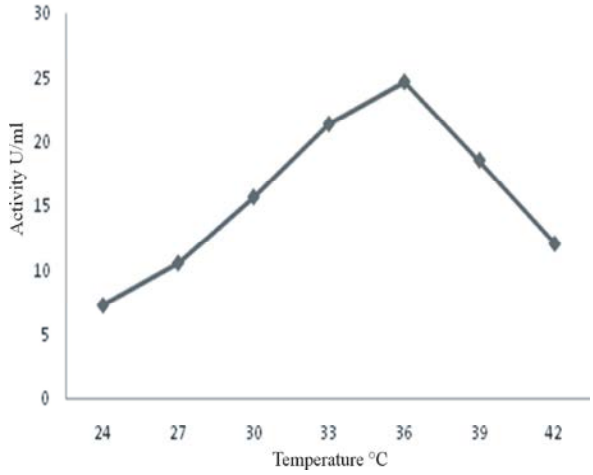

Fig. 4: Effect of temperature on lipase activity

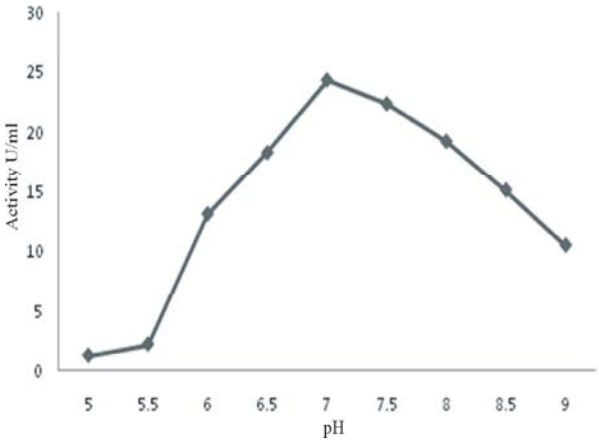

Fig. 5: Effect of pH on lipase activity

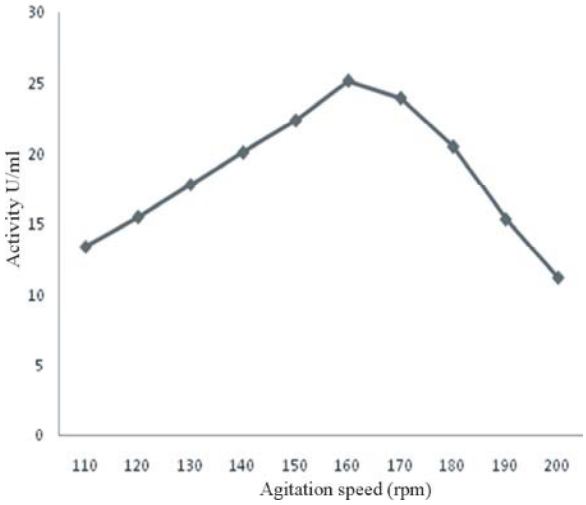

Fig. 6: Effect of agitation speed (rpm) on lipase activity

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