ISOLATION AND CHARACTERIZATION OF LIPASE PRODUCING BACTERIA FROM VELLORE SOIL

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Summary

The present study was focus on isolation of lipase producing bacteria from the soil sample of Vellore, Tamil Nadu, India. Lipase has wide variety of applications in many industries like pharmaceuticals, food and chemical. Bacterial strains (*Bacillus* sp.) having high lipase productivity are isolated by enrichment culture method using Tributyrin agar and their characterization was also done followed by the partial purification involving Ammonium sulphate precipitation and dialysis. Growth parameters are analyzed against varying temperature and pH, with olive oil substrate. This study suggests that optimum temperature and pH for the lipase producing strains is 37°C, and pH 7.0 respectively.

Keywords: Lipase, Soil, *Bacillus* sp., Tributyrin agar, Ammonium Sulphate.

Introduction

Lipase is a water soluble enzyme that acts on the hydrolysis of carboxylic ester bonds in water insoluble, lipid substrates. Lipases thus comprise a subclass of the esterase. They catalyze the hydrolysis of triacylglycerols to glycerol and free fatty acids. In contrast to esterase, lipases are activated only when adsorbed to an oil–water interface and do not hydrolyze dissolved substrates in the bulk fluid. They constitute a large part of earth's biomass and lipolytic enzymes play an important role. They catalyze at lipid-water interfaces involving interfacial adsorption and subsequent catalysis[1].

Lipases are widely found throughout animal and plant kingdoms as well as moulds and bacteria. There are many bacterial, fungal, plant and animal species from which lipases have been isolated. Apart from their natural functions they can also catalyze esterification, interesterification and transesterification reactions in non-aqueous media. This versatile nature helps lipases to become a potential choice in various applications.

Industrial scale extraction of lipases is carried out in bacteria, fungi, actinomycetes and cultures of plant and animal cells. Lipases are also used in fat and oleo chemical industry, production of biodegradable polymers, textile industry, detergent industry, food processing, diagnostic tools, tea processing, medical applications, biosensors, degreasing of leathers, waste, sewage, effluent treatment, oil biodegradation and pulp and paper industry production of biodiesel. In addition, lipases have been used for development of flavors in cheese ripening, bakery products, and beverages and are used to aid removal of fat from meat and fish products [2].

High production cost have become a limitation for the industrial use of lipases and this limitation has been overcome by using molecular technologies which enabled its production in high levels in purified form. With the rapid development in enzyme technology microbial lipases are receiving much attention[3]. The present study focus on isolation and partial purification of lipolytic bacteria from Vellore soil and optimization of lipase with olive oil substrate was determined.

Materials and Methods

Sample Collection

Soil sample was collected aseptically from the different part of Vellore district, Tamilnadu. India and transferred into a sterile container for the isolation of lipase producing organisms under laboratory conditions.

Isolation of lipase producing bacteria

Bacteria were isolated from the collected soil sample for which 10 gm of soil was dissolved in 100 ml of distilled water. Seven test tubes were taken and it was serially diluted from 10^{-1} to 10^{-6} dilutions. The diluted samples were plated on Nutrient agar in aseptic conditions. The plates were incubated at 37°C for 24hrs

Screening and identification of lipolytic strains

Lipase production of bacteria was determined using Tributyrin Agar plates[4]. In Tributyrin agar base, tributyrin (1%) was added and the pH of the medium was adjusted to 7.0.The bacterial cultures were inoculated on to the tributyrin agar plates and incubated at 37°C for 24 hrs. Formation of the halo zones around the colonies was considered to be positive.

Lipase production

The lipolytic bacteria were inoculated to salt basal media (consisting of 12.8 g $Na_2HPO_4.7H_2O$,3 g KH_2PO_4, 0.5 g NaCl, 2 Mm $MgSO_4.7H_2O$, 1 g NH_4Cl, 2 g Glucose and 10 ml) with additional Olive oil substrate (2.5 %) i.e. 2.5 ml in 100 ml of broth[5]. The media pH was adjusted to 7.0 and 8.0 subsequently for each strain and incubated for 24-48 hours at 37°C and 42°C.

Lipase activity

Lipases basically work by breaking the triacylglycerides into free fatty acids and glycerol. This concept is utilized for assaying the specific activity of the lipase by titrimetric method[6]. Olive oil substrate emulsion contained 10% (w/v) olive oil, 10% (w/v) Arabic gum, 0.5 M sodium chloride and 20 mM Calcium chloride was blended for 2 min at the maximum speed in a blender. 2 ml of lipase was incubated with 20 ml of substrate solution at a shaker incubator for 125 rpm for 30 minutes. The reaction was then terminated my adding 10ml of ethanol: acetone (1:1). This was then titrated against 0.02 N NaOH by adding phenolphthalein indicator until the end point was reached.

One unit of lipase activity (µg/ml/min) is defined as the amount of enzyme releasing one mole of free fatty acids in one minute under fixed parameters.

$$\text{Lipase Activity} = \frac{\text{Volume of alkali consumed} * \text{Normality of NaOH}}{\text{Time of Incubation} * \text{Volume of Enzyme solution}}$$

Partial purification of extracellular lipase

Ammonium sulfate precipitation and dialysis

An enzyme preparation was obtained by precipitation with 30-90% ammonium sulfate fractionation. The crude extract of pH 7.0 was saturated with 60% ammonium sulfate and of pH 8.0 was saturated with 40 % ammonium sulfate. Further this was refrigerated at 4°C for 24 hours. The precipitate was collected by centrifugation at 10000 rpm for 20 minutes at 4°C .The precipitate was dissolved in 5ml Tris-Hcl buffer, pH 6.8. The fractions were dialyzed in the Tris-Hcl buffer. This process was called as desalting and this was continued till last trace of ammonium sulphate is removed.

Identification of Lipolytic bacteria

The isolated lipolytic bacteria were identified based on morphological, biochemical and physiological characters according to Bergey's manual of determinative bacteriology[7]

Statistical Analysis

All tests were conducted in triplicate. The zone of inhibition reported as means ± standard deviation (SD).

Results and Discussions

Enrichment culture technique enabled the isolation of lipolytic bacteria in Tributyrin media plates. Out of 25, seven showed lipase activity. From the seven isolates collected from soil sample two (D1 and D2) showed high lipolytic activity (Fig.1) and they were further screened. The strains had varying clear zone size around them which resembled their differential lipase activity. Madhusudan et al reported bacterial count varied from 1.0×10^8 CFU/g to 5.2×10^8 CFU/g of the Vellore soil sample[8].

Table 1. Variation in the clear zone size of the isolates

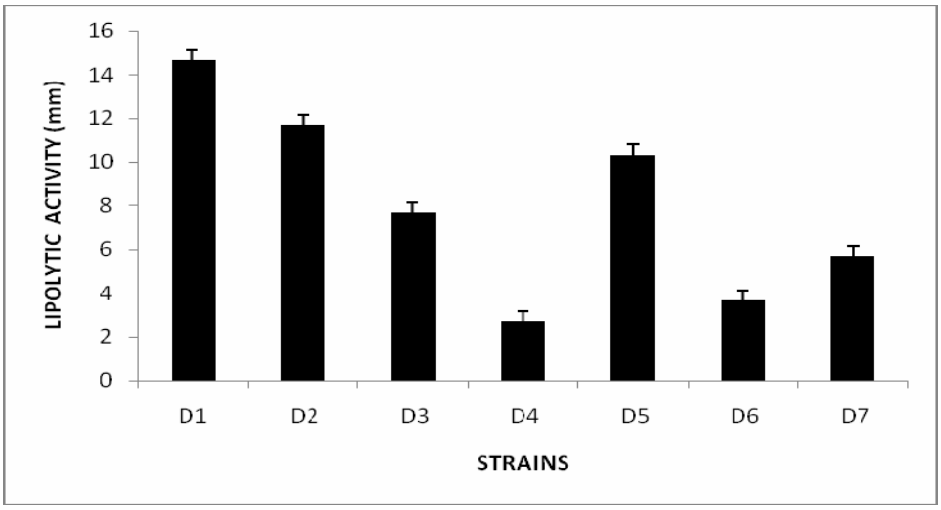
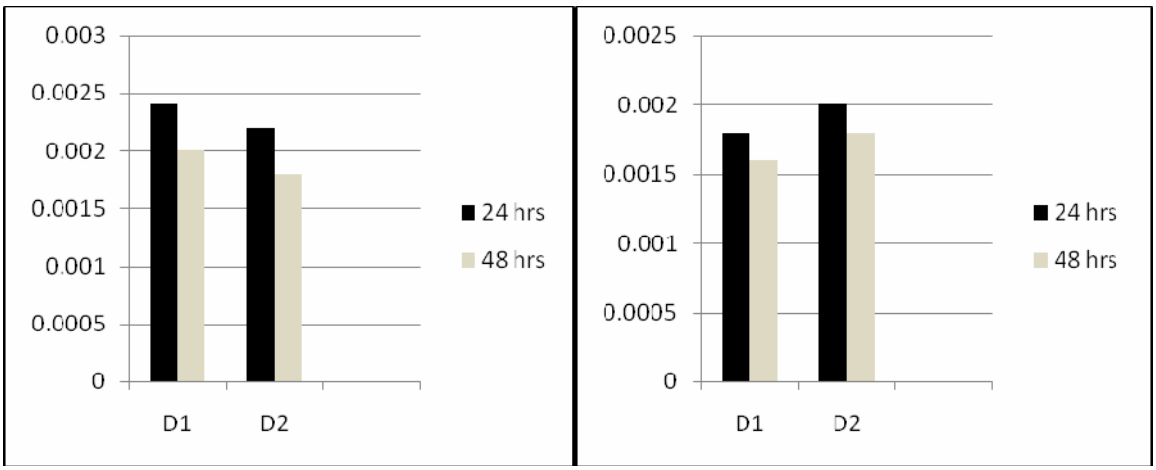


Figure 2: Lipase activity with olive oil substrate at varying medium pH.

pH 7.0 **pH 8.0**



From the study it has been found that lipase production is higher at pH 7.0 when compared to pH 8.0 for the tested stains D1 and D2 (Figure.2). The specific activity of D1 strain was (0.0024µg/ml/min). In literature, maximum activity at higher pH values was also reported. Sevgi et al reported bacteria prefer pH around 7 for best lipase production[9]. Lipase activity was also compared for different temperatures and it was found that activity was higher at 37°C over a 24 hour incubation period (Figure.3). The maximum activity was noted for D2 strain (0.0022µg/ml/min). Production of a thermostable lipase from thermophilic *Bacillus sp*. strain Wai 28A 45, in the presence of tripalmitin at 70°C, was described by Janssen et al[10].

Figure 3: Lipase activity at different temperatures (37°C and 47°C) at different incubation period (24 and 48 hrs)

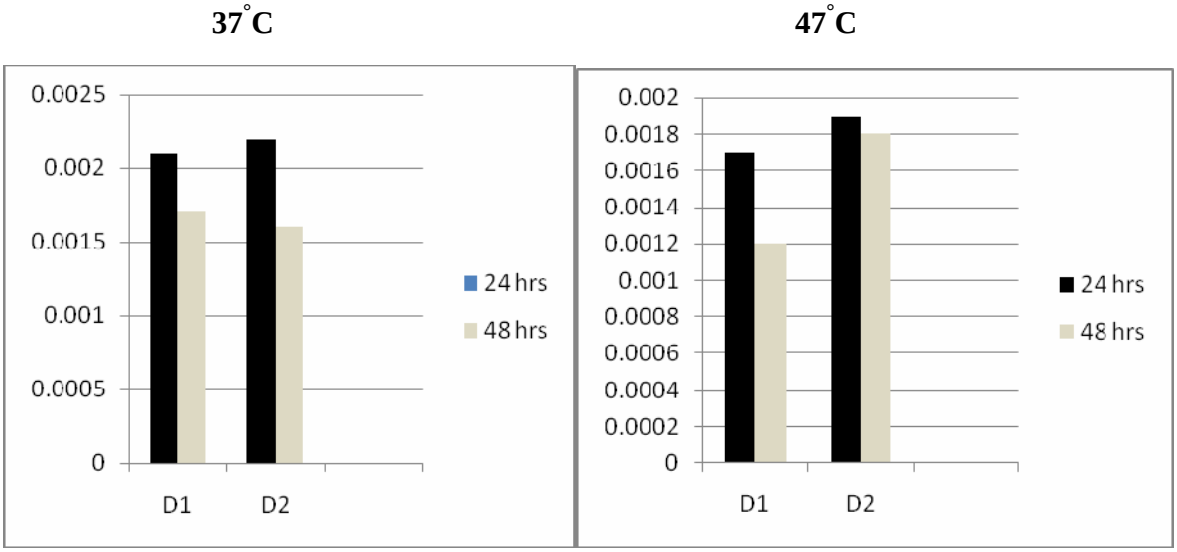


Table 1.

Characterization	Test	*Bacillus* sp (D1)	*Bacillus* sp (D2)
Cultural characterization	Colony morphology	Large, Irregular, Flat, Opaque, Smooth	Small, Undulate, Flat, Opaque, Smooth
Microscopic characterization	Spore staining	Spore forming	Spore forming
	Gram staining	Gram positive rods	Gram positive rods
	Motility	Non motile	Non motile
Biochemical characterization	Indole	positive	positive
	Methyl Red	Positive	negative
	Voges Proskauer	Negative	negative
	Citrate utilization	Positive	positive
	Catalase	Positive	positive
	Oxidase	Negative	negative
	Urease	Positive	positive
	Nitrate reduction	Positive	positive
	Gelatin liquefaction	Positive	positive
	Starch hydrolysis	Positive	positive

Lipase activity was found to be maximum in at pH7.0 over an incubation period of 24 hours and it gradually decreases with greater incubation time and temperature as well as pH. Wang et al reports thermophilic *Bacillus* strain A30-1 (ATCC 53841) produced maximal levels of thermostable alkaline lipase when corn oil and olive oil (1%) were used as carbon sources[11]. The present study is in consistence with the earlier work.

The cell free filtrate was extracted and it was further partially purified using Ammonium Sulphate precipitation. Precipitation at pH 7.0 was obtained at 60% of ammonium sulphate and for pH 8.0 at 40%. This was dialyzed against buffer (Tris-Hcl, pH 6.8) and partially purified lipase extract was obtained. Most commercial applications of lipases do not require highly pure enzyme. Excessive purification is expensive and reduces overall recovery of the enzyme[12]. Finally the lipolytic organisms were identified as *Bacillus* sps based on the colony morphology and biochemical studies (Table. 1). *Bacillus* sps are good lipase producers and are preferred in the food industry for the enzyme production due to their non-pathogenecity. So far 15 different *Bacillus* sp reported for lipase production but no one commercialized.

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