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Optimization of Lipase by Bacillus Cereus Isolated From Fish Gut

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Abstract. Lipase production by fish gut gut isolate *Bacillus cereus* was investigated and optimized. The lipidic substrate tested were: groundnut oil cake, sesame oil cake, coconut oil cake, fish bone and coconut oil sediment waste. Among these, coconut oil sediment waste supported maximum lipase production (0.0624 / ml / min.). Effect of pH and temperature indicated that, the lipase production was maximum at pH 7.0 (0.035 U/ml/min) and at 37^0C (0.041 U/ml/min). In metal ions added medium calcium chloride and magnesium sulphate were positively influenced the lipase production by *B. cereus.*

Keywords: Lipase, Lipidic substrates, *B. cereus*

1. Introduction

Lipases are considered to be the largest group of industrial enzyme, subsequent to proteases and amylases. Lipases are glycerol ester hydrolases which hydrolyze ester linkages of glycerides at water–oil interface. During hydrolysis reaction they take acyl group from glycerides and form lipase–acyl composite, and then transfer the acyl group to OH group of water (Martinelle and Hult, 1995). Lipase occurs widely throughout the world's flora and fauna. In eukaryotes, lipases are involved in various stages of lipid metabolism including fat digestion, absorption, reconstitution, and lipoprotein metabolism. In plants, lipases are found in energy reserve tissues. Numerous species of bacteria, yeasts and moulds produce lipases with different enzymological properties and specificities (Akram Kashmiri *et al.,* 2006).

Lipases mainly applied on manufacturing of soaps and detergent and they are used in the hydrolysis of tallow (hard fat made from the body parts of animals, such as horses and cattle), which is necessary for soap production. Lipases are also used as stain-digesters in detergents. Also lipases have potential applications in oleo chemical, paper manufacturing, cosmetics, pharmaceuticals and agrochemical industries. They are also employed in organic chemical processing, biosurfactant synthesis, nutrition and biomedical sciences (Pandey *et al.,* 1999).

In fermentation process, lipase production is mainly influenced by carbon, nitrogen sources, agitation, and dissolved oxygen concentration etc. Lipases are mostly inducible enzymes and inducers such as oils are necessary for lipase production (Gerhatz, 1990). Considering the information given above the present study was undertaken to optimize the lipase production by fish intestinal isolate *Bacillus cereus* using different substrates, pH, temperature and metal ions.

2. Materials and Methods

2.1. Microorganism

The bacterial strain used in this study was isolated from the intestine of the fish collected from the local market. The lipase production by this strain was observed by using tributyrin agar medium. The strain was identified as *Bacillus cereus* by using biochemical characteristics and 16s rRNA gene sequencing.

2.2. Lipase Production

The bacterium was initially cultured using medium containing (w/v): yeast extract (0.15%), peptone (0.5%), sodium chloride (1.0%) and olive oil (0.5%), at pH 7, and 32°C for 24 h. Then, 5% of enriched seed culture was inoculated into a 50 ml medium (w/v) containing potassium peptone 0.5%, dihydrogen orthophosphate 0.1%; sodium chloride 1% and magnesium sulphate 0.01%. Then it was incubated at 32°C at 150 rpm. After incubation it was centrifuged at 10000 rpm and the supernatant was used for lipase activity determination.

2.3. Lipase Assay

Lipase activity was assayed through spectrophotometric method by using p-nitrophenol palmitate as substrate. The reaction mixture containing 100 l of 50 mM Tris buffer (pH-7.0), 50 l of substrate solution (1mM p-NPP containing 1% Triton X-100), 350 l of H_2O and the reaction was initiated by adding 100µl of enzyme solution. After incubation of 10 min, the reaction was stopped by adding 1 ml of 2% sodium dodecyl sulphate (SDS) solution. The absorbance was red at 420 nm using UV–vis–spectorophotometer. One unit of lipase activity was defined as the amount of enzyme releasing 1 µmol of p-nitrophenol per minute.

2.4. Optimization of Lipase Production

2.4.1. Effect of Different Lipid Substrates on Lipase Production

Effect of different lipid substrates on lipase production was determined by using coconut oil cake, groundnut oil cake, sesame oil cake, coconut oil sediment and fish bone. Their influence on lipase production was determined at different concentrations (0.5, 1.0, 1.5 and 2.0%) and also at different time intervals (24, 48 and 72h).

2.4.2. Effect of pH on Lipase Production

The effect of pH on lipase production was determined at different pH such as 5, 7 and 9. Their influence on different time interval (24, 48, 72 h) was also determined.

2.4.3. Effect of Temperature on Lipase Production

Effect of temperature was determined by inoculating the organism on lipase production media at different temperatures such as 27^{o}C, 37^{o}C and 47^{o}C with various time intervals (24, 48 and 72h).

2.4.4. Effect of Metal ions on Lipase Production.

The effect of different metal ions on lipase production was determined by using metals such as manganese sulphate, ferrous sulphate, copper sulphate, magnesium sulphate, and calcium chloride at 0.1% concentration.

3. Results and Discussion

The effect of different concentrations of lipid substrates on lipase production at various time intervals is shown in Figs 1, 2 and 3. Irrespective of the substrate tested, the lipase production was high at 1.0%concentration.At the tested time intervals the lipase production was maximum at 48h of incubation when compared to 24 h and 72 h (0.062 U/ml/min). Among the tested substrates coconut oil sediment was emerged as the best substrate for maximum lipase production(0.062 U/ml/min)The triglycerdies present in the coconut oil sediment may be responsible for the enhanced production of lipase by *Bacillus cereus* . Falony et.al (2006) reported that Triglycerides like coconut oil was found to be the inducer of lipase production by *Aspergillus niger*

The effect of different medium pH at various incubation periods on lipase production resulted that pH 7 was the optimum for maximum lipase production(0.049 U/ml/min). In this study pH7.0 positively supported lipase production in all the tested incubation time (24, 48 and 72 h) (Fig 4). This inferred that the bacterial strain does not prepare acidic or alkaline pH for lipase production. This was supported by the study of Sekhon et al. (2005) *on Bacillus megaterium* AKG-1, which has optimum activity at pH 7. Similarly Esakkiraj et al., (2010) reported that lipase production by *Staphylococcus epidermidis* CMST-Pi 1 isolated from the gut of shrimp was maximum at pH 7.

The effect of different incubation temperatures on lipase production at various time intervals revealed that 37ºC was optimum for maximum lipase production(0.047 U/ml/min) in all the incubation period. This indicated that *B.cereus* was mesophilic organisms and that couldn't tolerate higher or lower temperature (Fig 5). Optimization of temperature is vital for cell growth and enzyme production. The present result was supported by the report of Shariff *et al.,* 2007 for optimum lipase production by *Bacillus* sp. strain L2 at 37-40^0C. Also Baharum *et al.,* (2003) found that lipase production by *Pseudomonas* sp. strain S5 was maximum at 37^oC

Metal ions are the important nutrients that decide the optimum lipase production by microbial strains. In this study five metal ions were screened and the results showed that calcium chloride supported higher lipase production (0.056 U/ml/min) (Fig.6). Other best metal ions observed for maximum lipase production was magnesium sulphate. Maximum lipase production by *Bacillus coagulans* was also an evidence for Ca^{2+} ions mediated lipase production (Alkan *et al.,* 2007). This was also in consistence with the Ca^{2+} increased lipase production by *Pseudomonas fluorescens* 2D (Makhzoum *et al.,* 1995) and *Antrodia cinnamomea* (Lin *et al.* (2006). Zhen-qian and Chun-yun (2009) have reported that lipase production by *Enterobacter agglomerans* increased very much by Mg^{2+} and Zn^{2+}. This study clearly indicated that, the lipidic substrate coconut oil sediment waste could be effectively used as the low cost substrate for the lipase production by *B. cereus*.

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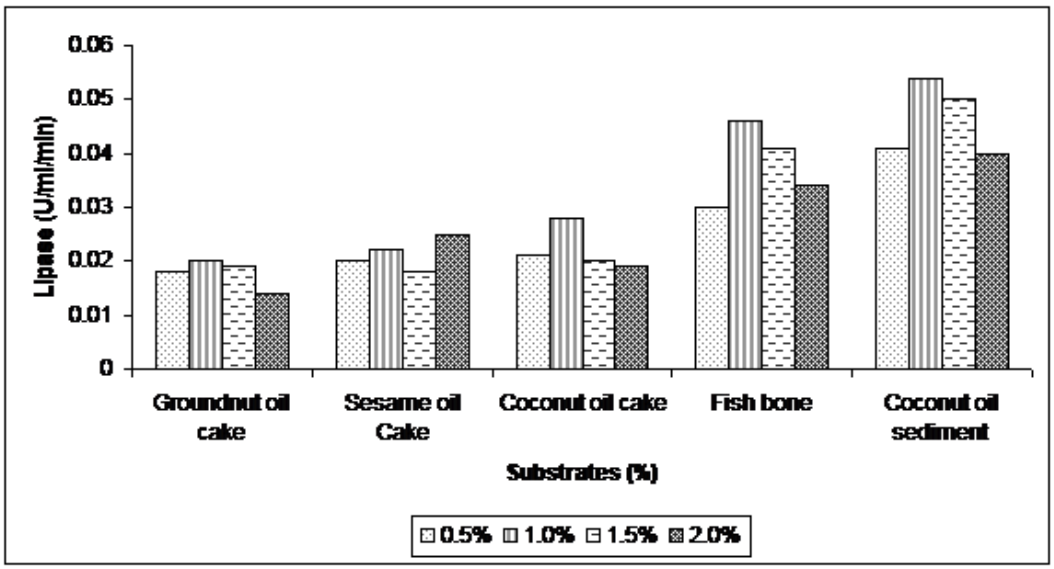

Fig. 1: Effect of different concentrations of lipid substrates on lipase production by *Bacillus cereus* after 24 h incubation

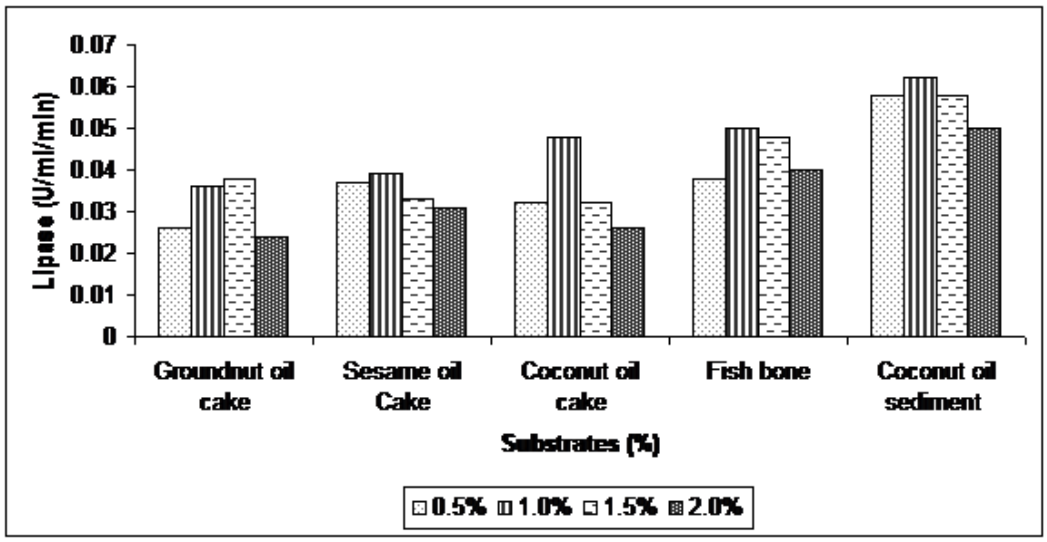

Fig. 2: Effect of different concentrations of lipid substrates on lipase production by *Bacillus cereus* after 48 h incubation

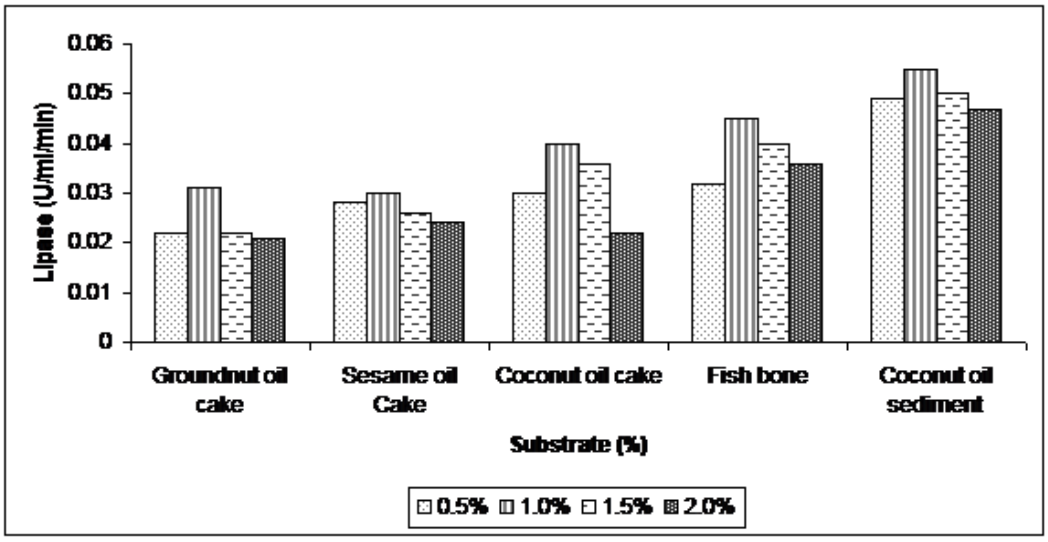

Fig. 3: Effect of different concentrations of lipid substrates on lipase production by *Bacillus cereus* after 72 h incubation

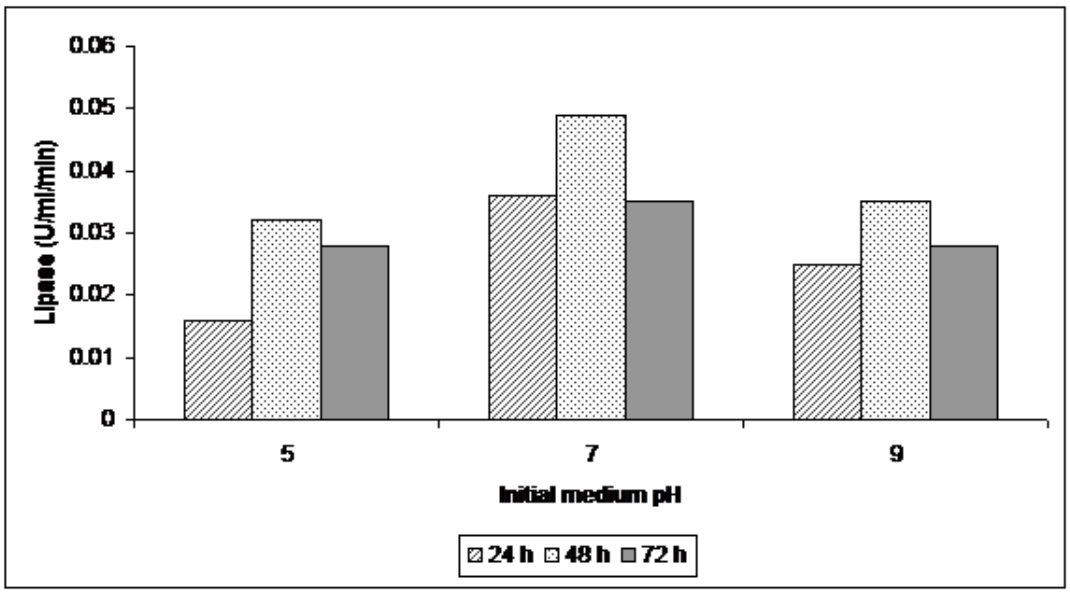

Fig. 4: Effect of different pH levels on lipase production by *Bacillus cereus* at different time intervals

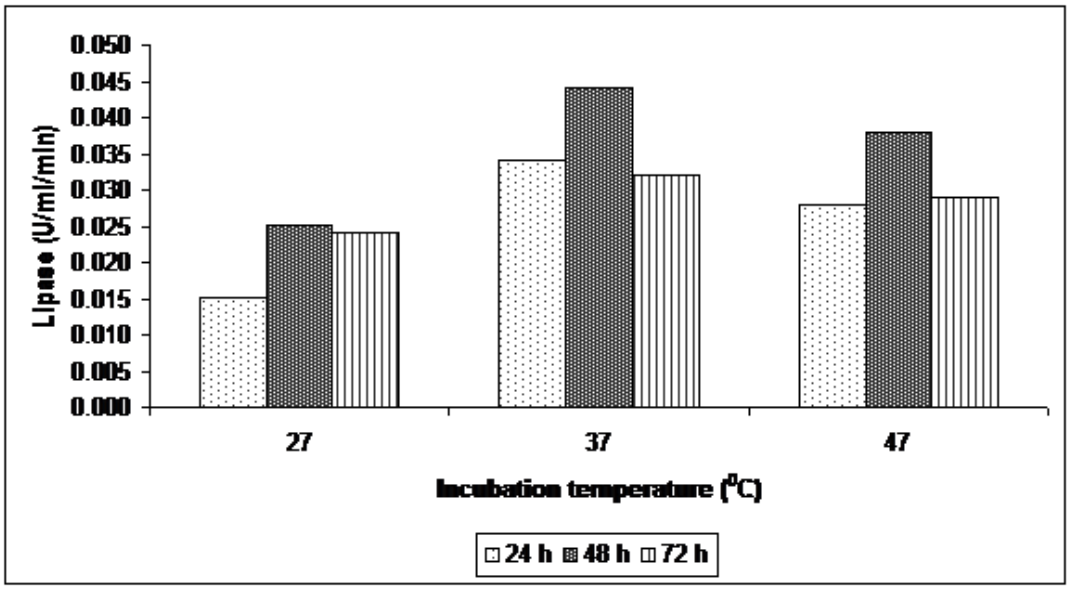

Fig. 5: Effect of different incubation temperatures on lipase production by *Bacillus cereus* at different time intervals.

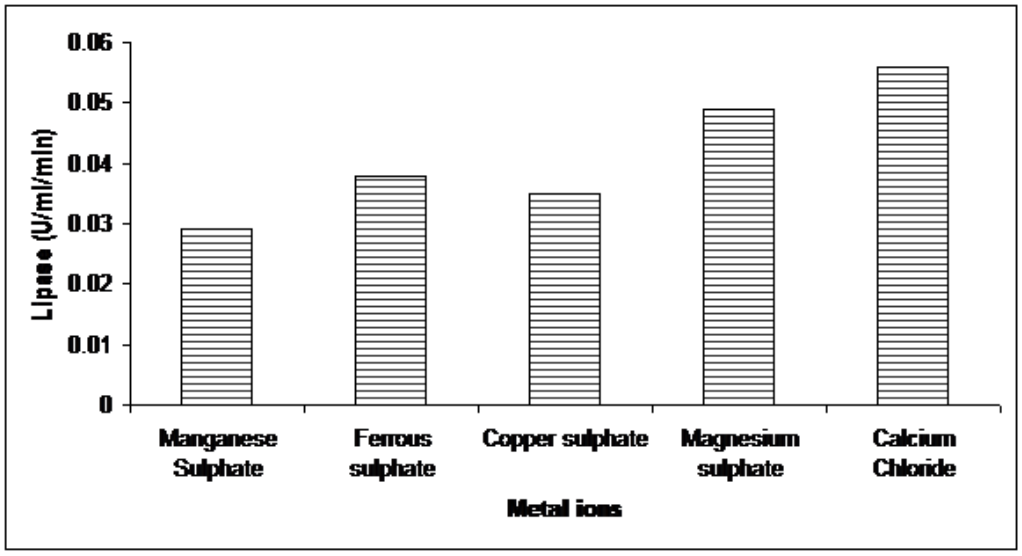

Fig. 6: Effect of different metal ions on lipase production by *Bacillus cereus*