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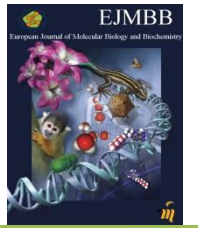

OPTIMIZATION OF LIPASE ENZYME ACTIVITY PRODUCED USING *BACILLUS MEGATERIUM* ISOLATED FROM GARDEN SOIL

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ABSTRACT

Lipase enzymes are monomeric proteins which are highly exploited due to its vast applications in food, dairy pharmaceutical. The present investigation describes the aim of producing lipase enzyme from olive oil using *Bacillus megaterium* which is isolated from garden soil. The strain, *Bacillus megaterium* was identified through biochemical characterization and subjected into lipase production by shake flask fermentations. The enhanced production of lipase enzyme by *B. megaterium* was achieved by optimized conditions such as carbon source, nitrogen source, substrate, pH, and temperature and incubation time. Result shows that highest lipase activity for *Bacillus megaterium* was achieved at pH 7 at 30°C for incubation period of 45 hour in the media containing soyameal as carbon source, casein as nitrogen source and gingly oil as substrate source. This optimized condition highly releases the lipase enzymes.

INTRODUCTION

Enzymes are proteins which are biological catalysts that they convert substrates into products. Microbial enzymes were established their application in different processes in food, pharmaceutical, textile, paper, leather and other industries [1, 2]. Microorganisms were present in everywhere like soil, water, ocean sediments and polluted area. Microorganisms have the ability to break down a variety of carbon and energy sources and convert into useful products like aminoacids, nucleotides, vitamins, carbohydrates and fatty acids by producing specific enzymes. Due to this ability microorganisms have been considered as little bio-factories [3].

Microbiallipase enzymes hydrolases that they involved in the hydrolysis of natural organic compounds [4] and have different enzymological properties and substrate specificities. Many species of bacteria, yeast and molds are found to produce lipases [5]. Bacterial lipases were highly stable and versatile.

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Production of lipases using bacteria is low cost and easy to manipulation. Lipases are triacylglycerol acylhydrolasesand water-soluble enzymes which have the ability to hydrolyze triacylglycerol into fatty acids and glycerol at an oil-water interface [6]. Lipases are monomeric proteins having high molecular weights in the range of 16,000-670,000 Daltons and mostly stable in organic solvents [7]. Production of enzymes was influenced by physical and chemical factors, such as temperature, pH, nitrogen and carbon sources, inorganic salts, agitation and dissolved oxygen concentration [8, 9]. The source for lipase production is usage of cheaper substrates like oil seed cakes, coconut cake [10], agricultural residues [11], cotton seed cake, soybean bran and sludge [12], groundnut oil cake [13] and oil mill effluent etc., [14]. Lipases play an important role in agriculture, food, dairy, oil, detergent, leather [15], cosmetics [16] and perfume [17], textile, paper and pharmaceutical applications. In this study, isolation of lipase producing bacteria *Bacillus megaterium* from garden soil aiming at the attainment of a lipase formulation for industrial applications.

The better enzyme producing bacterial isolate was enhanced under physical and chemical conditions.

MATERIALS AND METHODS

Sample Collection and Isolation of Bacteria

Garden Soil from Around Sri Akilandeswari women college, Wandiwash was collected in a sterile container and it was brought to the laboratory for further processing. The collected sample was serially diluted up to 10^{-7} dilution using sterile saline as a blank and the diluted samples were plated into the sterile Nutrient agar (NA) using spread plate method. The NA plates were incubated at 37°C for 24 hours.

The isolated colonies were further purified by streak plate method using sterile media plates. The pure cultures were inoculated into sterile nutrient agar slants and nutrient broth for further identification and characterization process. The morphological and physiological characterization of the isolate was performed according to the methods described in Bergey's Manual of determinative bacteriology [18].

Isolation of Lipolytic Microbes

For the isolation of lipolytic microbes, 1.0 gm of sample was mixed in 100 ml of double distilled water. It was then serially diluted (10^{-1} to 10^{-7}) and the diluted samples were spread on tributyrin agar plates. The formation of clear zone around the colony on the plate was considered as lipolytic microbes.

Lipase enzyme production and optimization

Preparation of Inoculum for bacteria

The inoculum for further production of enzyme and other studies was prepared using Luria broth (LB). The pure culture was inoculated into sterile inoculum broth and was incubated at 37°C in a rotary shaker for overnight. The fresh overnight culture was used as an inoculum for production of Enzyme.

Enzyme Production

The enzyme production was carried out by shake flask fermentation using production mediumwhich comprising of Glucose as a carbon source and amended with olive oil as a lipase substrate with pH 7. The enzyme production medium (100 ml) containing Olive oil (5%),Yeast extract(0.2g), Peptone (0.5g), MgSO (0.05g), NaCl (0.05g), Agar (2g), pH -7.

Five hundred ml of sterile Production broth was prepared in one-liter conical flask and 5% inoculum was transferred aseptically in to the production medium. The inoculated medium was incubated at 37°C for 48 hours. The medium was agitated at 200 rpm for better aeration and growth of the organism.

Optimization of pH

The standard production medium was adjusted to different pH ranges from 4 to 10 using 0.1 N HCl and 0.1N NaOH, respective organisms was inoculated to check the optimum pH and its effect on lipase production.

Optimization of Incubation Temperature

The standard production medium was inoculated and incubated at temperatures ranging from 4 to 60°C to test for their effect on lipase production and the optimum temperature for maximum lipase production.

Optimization of Incubation Period

The production medium was incubated under standard conditions for a time period of 15 to 70 hrs individually on the organisms to test the effect of time in the production of lipase.

Optimization of Carbon Source

The effect of carbon source on lipase production was studied using fructose, lactose, sucrose, glucose, starch, mannitol, glycerol, groundnut meal and soyameal which were substituted in standard production media.

Optimization of Nitrogen Source

For the increased production of lipase enzyme by *Bacillus megaterium*various nitrogen sources were typically supplemented in standard production medium by replacing with organic and inorganic nitrogen sources like peptone, soyatone, yeast extract, tryptone, beef extract, casein, ammonium chloride, ammonium nitrate, ammonium sulphate and sodium nitrate.

Optimization of Substrate Source

By using different substrates sources such as neem oil, palm oil, pongemia oil, ground nut oil, soyabean oil, sun flower oil, olive oil, seasame oil, castor oil, hippe oil, mustard oil, coconut oil, gingly oil and cod liver oil, their effect on lipase production was assessed at optimum pH, incubation temperature and time.

Enzyme Assay

Lipase assay was carried out using tributyrin agar plate assay as qualitative test to detect lipase activity [19].

Lipase Activity

Lipase activity was determined by NPP (-nitrophenyl palmitate) method [20]. The coefficient of extinction of -nitrophenol (NP), 1.5. 104 L/mol/cm, was determined from the absorbance measured at 410 nm of standard solution NP. One unit was defined as the amount of enzyme liberating 1µmol of - nitrophenol per minute at 37°C.

Table 1. Biochemical characters of *Bacillus megaterium*

S.No	Biochemical Tests	*Bacillus megaterium*
1	Gram staining	Gram +ve, Rod
2	Agar slant	Abundant, white, waxy
3	Motility	Motile
4	Spore staining	Positive
5	Indole	Negative
6	Methyl red	positive
7	VogesProskauer	Negattive
8	Citrate Utilization	Positive
9	Starch hydrolysis	Positive
10	Oxidase	Positive
11	Catalase	Positive
12	Urease	Negative
13	H_2S Production	Positive
14	Mannitol salt agar	Positive
15	Triple sugar iron agar test	Acid and alkaline

Figure 1. Effect of pH on Lipase Activity

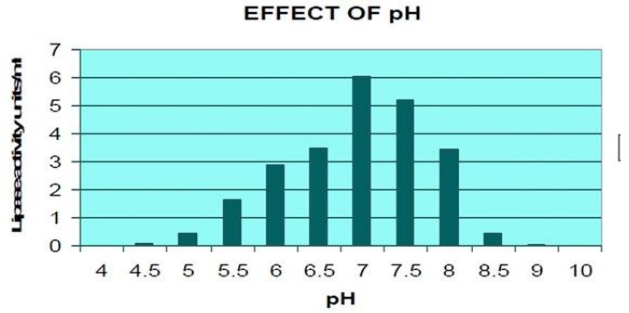


Figure 2. Effect of Temperature on Lipase Activity

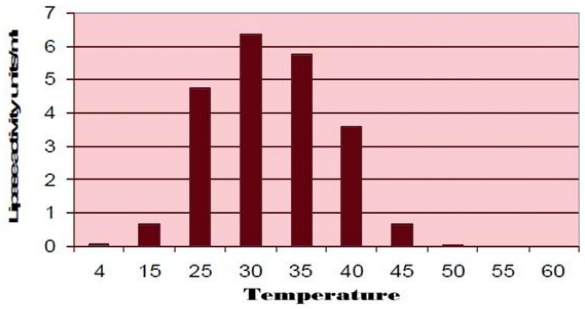


Figure 3. Effect of Incubation Time on Lipase Activity

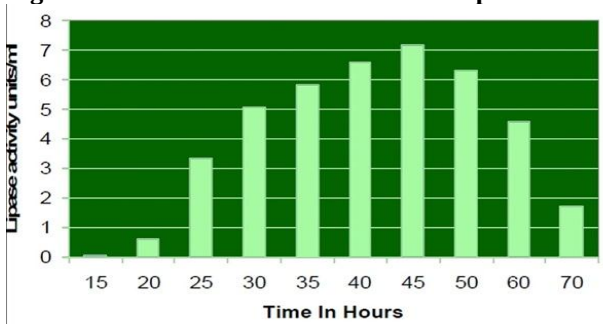


Figure 4. Effect of Carbon Sources on Lipase Activity

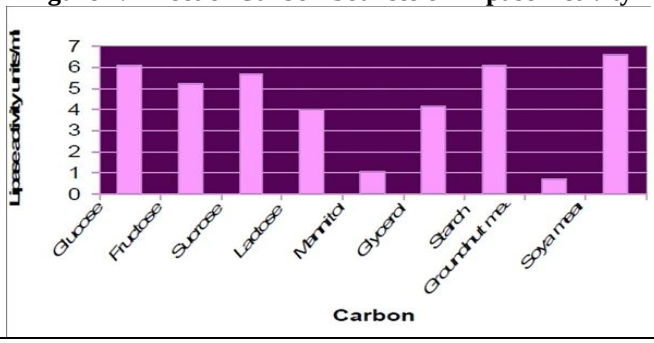


Figure 5. Effect of Nitrogen Source on Lipase Activity

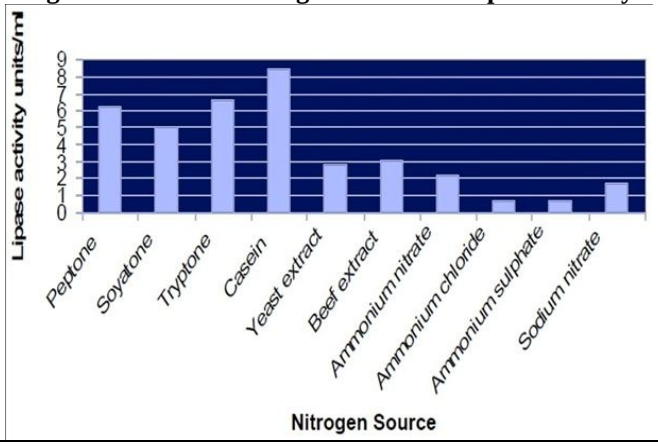
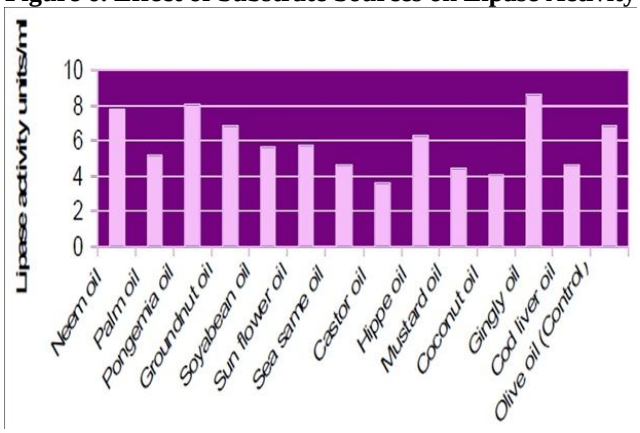


Figure 6. Effect of Substrate Sources on Lipase Activity



RESULTS AND DISCUSSION

Identification of Bacterial culture

The isolated microorganisms were examined using various cultural and biochemical test for identification (Figure1-4). The cultural characteristics of microorganisms as a help in identifying and classifying organisms into taxonomic groups. From this biochemical studies the bacteria identified from the soil source is *Bacillus megaterium* (Table 1).

Optimization of culture conditions for lipase production

The efficiency of lipase activity was analyzed NPP assay for different pH, temperature, incubation time, carbon, and nitrogen and substrate sources as shown in (Figure 1-6).

Result shows that highest lipase activity for *Bacillus megaterium was* achieved at pH 7 at 30°C for incubation period of 45 hour in the media containing soyameal and glucose as carbon source, casein as nitrogen source and gingly oil as substrate source. The production of extracellular lipase in submerged culture of *Bacillus megaterium*has been investigated. The lipase production was optimized in shake flask experiments.

The observed pH and temperature range optimum for maximum lipase production were 7-8 and 30-40°C [21]. The lipase production by *Bacillus megaterium* prefers neutral pH (6.5-7) and *Pseudomonas aeruginosa* MB prefers neutral pH [22, 23]. The present study revealed that lipase production by *Bacillus megaterium was* high in pH 7. The lipase activity by *Bacillus megaterium was* found to be maximum at pH 8 [24]*B. thermocatenulatus* and *B. stearothermophilus* produce lipase with similar properties. Their molecular mass is approx. 45 KDa and they display maximal activity at pH 9 and 65°C [25, 26], but in the present study highest lipase activity for *Bacillus megaterium was* achieved at 30°C for incubation period of 45 hour. In the present study different carbon sources were screened for their efficiency to support lipase production.

Among the tested, *Bacillus megaterium*results maximum lipase activity in the media containing soyamealas carbon source. Takahiro et al., [27] achieved lipase production by a fedbatch culture of *Pseudomonas fluorescens*. During the cultivation, temperature, pH and dissolved oxygen concentration. Olive oil was used as a carbon source for microbial growth.

A number of factors affecting the production of extracellular lipase by C*ryptococcus sp.* S-2 were investigated. Consecutive optimization of nitrogen, carbon sources and inducers enhanced lipase activity and under optimum conditions the lipase activity was 65.7 U/ml of the culture medium in 120 h at 25°C and at pH 5.6. Sardine oil, soy bean oil and triolein were effective inducers for lipase production [28]. Among the nitrogen sources, casein produced maximum lipase compared to others. This is because casein is a simple milk protein and can be easily utilized by a candidate species.

In the present study gingly oil show maximum lipase activity as substrate source. Supachoket al. [29] found highest activity with -nitrophenylestercaprate as the synthetic substrate and tricaprylin as the triacylglycerol.

CONCLUSION

In this study, bacterial lipase enzyme was produced using *B. megaterium* by shake flask fermentation process. Production of lipase is easy to manipulation, low cost and cheap materials used as source of substrate and eco-friendly. The present study concluded that olive oil is potential substrate for lipase production. The fermentation time is 45 hours, pH 7, temperature 30°C for incubation period of 45 hour in the media containing soyamealas carbon source, casein as nitrogen source and gingly oil as substrate source.

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CONFLICT OF INTEREST: NIL

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