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Research Article

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Production of Lipase enzyme from *Pseudomonas aeruginosa* isolated from lipid rich soil

Prasad M. P.*
Sangenomics Research Labs, Domlur, Bangalore-560071, India
*Corresponding Author E-mail: prasad_m_p@hotmail.com

ABSTRACT

Lipases are enzymes which have numerous applications in many industries like leather, soaps and detergents, pharmaceuticals, biofuel, food, textile etc. Many organisms like plants, fungi, bacteria are known to produce lipase. The present study was aimed at isolating lipase producing microorganisms from different soil samples which are rich in lipid content like oil mills. As oil mill samples are rich in lipid and fatty acid content it makes a very good source to find microorganisms capable of degrading lipids by producing lipase enzymes. In the present study the isolates were identified by morphological, biochemical and molecular characterization. Production media was optimized by varying both physical and chemical parameters such as pH, temperature, incubation period, carbon, nitrogen and substrate sources. The maximum lipase activity by the isolate Pseudomonas aeruginosa was at pH 7 at 35° C for 45 hours. Lipase activity was high in media containing glycerol, casein and pongemia oil. Statistical analysis revealed that variation in lipase production is highly influenced by changes in physical and chemical parameter.

Key words : *Pseudomonas aeruginosa, Lipase, Lipids, Enzyme, Effluents, Pongemia oil.*

INTRODUCTION

Although the existence of lipolytic bacteria has been recognized for many years, our understanding of bacterial lipolysis stems for the most part from studies with crude enzyme systems[1]. Lipases are serine hydrolases of considerable physiological significance and industrial potential that can catalyze numerous reactions such as hydrolysis, interesterification, esterification, alcoholysis and aminolysis[2,3]. *Pseudomonas* species, ubiquitous in soil and water, are of considerable scientific and technological importance and comprise a taxon of metabolically versatile organism capable of utilizing a wide range of simple and complex organic compounds[4]. *Pseudomonas* lipases are very interesting that are not common among lipases produced by other microorganisms, such as their thermoresistance and activity at alkaline pH [5]. The lipases from *Pseudomonas* are ordered into three groups according to their molecular mass and biochemical properties with the following representative enzymes[6].

P.aeruginosa lipase for group I, *P.cepacia* and *P.glumae* which have been renamed *Burkholderia cepacia* and *B.glumae* for group II and *P.fluorescens* lipase for group III. *Pseudomonas aeruginosa* secretes an extracellular lipase, which has been isolated from culture media of either industrial fermentation of wild type *Pseudomonas aeruginosa* PAC1R or an overexpressing *P. aeruginosa* strain carrying a plasmid with the cloned lipase gene[7]. The objective of the current work was therefore to optimize lipase production by a locally isolated strain of *P. aeruginosa* from oil mill effluent.

MATERIAL AND METHODS

Sample Collection

For the present study, effluent and soil samples were collected from different oil Refinery mills like groundnut oil, palm oil and coconut oil in a sterile container for the isolation of lipase producing organisms under laboratory condition. The samples were stored under refrigeration conditions until further use.

Isolation of Lipolytic Microbes and Identification

For the isolation of lipolytic microbes, 1.0 gm of sample was dissolved in 100 ml of double distilled water. It was then serially diluted (10^{-1} to 10^{-6}) and the diluted samples were plated on tributyrin agar plates. The formation of clear zone around the colony on the plate was considered as positive for lipolytic microorganisms.

Spot inoculation of the positive organisms was done on tributyrin agar and the zone of hydrolysis was measured for a period of 7 days. The bacteria which formed the largest clear zone around the colony was finalized as the test organism and was identified based on morphological, biochemical and molecular characterization according to Bergey's manual of determinative bacteriology[8]. The test organism was maintained on nutrient agar slant supplemented with 5% olive oil.

Production Media Composition

The standard liquid medium contained (per liter) Olive oil 5%, peptone 5gm, yeast extract 5gm, glucose 5gm, NaCl 0.25 gm and $MgSO_4$. $7H_2O$ 0.5 gm. Different chemical and physical parameters were optimized using the standard production media keeping the other variables constant.

Optimization of Lipase production

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

Temperatures ranging from 4° C to 60° C were used to test for their effect on lipase production and the optimum temperature for maximum lipase production.

The production medium was incubated under standard conditions for a time period of 15 to 72 hrs to test the amount of lipase production over a given period of time.

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

To check the effect of nitrogen on the production of lipase enzyme by the test organism various nitrogen sources were typically supplemented in standard production medium by replacing it with organic and inorganic nitrogen sources like peptone, soyatone, yeast extract, tryptone, beef extract, casein, ammonium chloride, ammonium nitrate, ammonium sulphate and sodium nitrate.

Substrates which acted as inducers in the production of lipase, 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 and their effect on lipase production was assessed at optimum pH, incubation temperature and time.

Enzyme Assay and Activity

Lipase assay was carried out using tributyrin agar plate assay as qualitative test to detect lipase activity[9]. Lipase activity was determined by ρNPP (ρ-nitrophenyl palmitate) method [10]. The coefficient of extinction of ρ-nitrophenol (ρNP) 1.5 10^4 L/mol/cm, was determined from the absorbance measured at 410 nm of standard solution ρNP. One unit was defined as the amount of enzyme liberating 1μmol of ρ-nitrophenol per minute at 37 °C.

RESULT

Enrichment culture technique enabled the isolation of strains from oil mill effluent with lipolytic activity in tributyrin agar plate. The lipolytic microbes were further screened and characterized by their features and reactions and then identified as Gram negative, rod shaped motile organisms (Table-1). Finally morphological, biochemical and molecular characterization using 16sRNA studies indicated that the suspected organism was *Pseudomonas aeruginosa*.

The efficiency of lipase activity was analyzed by standard ρNPP method, and was assayed for different physical and chemical parameters like pH, temperature, incubation time, carbon, nitrogen and substrate sources as shown in (Fig-1a, b, c, d, e and f).

Fig 1a: Effect of pH on Lipase Activity

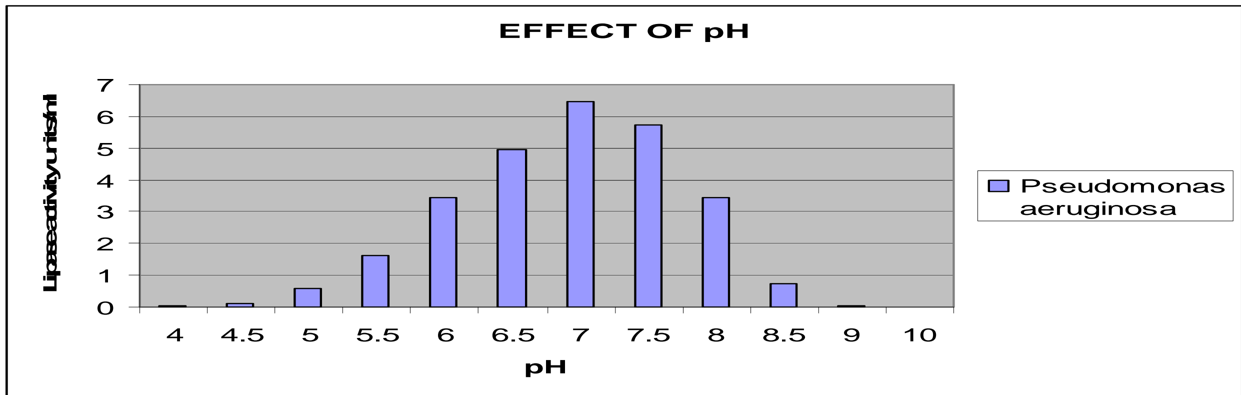


Fig 1b: Effect of Temperature on Lipase Activity

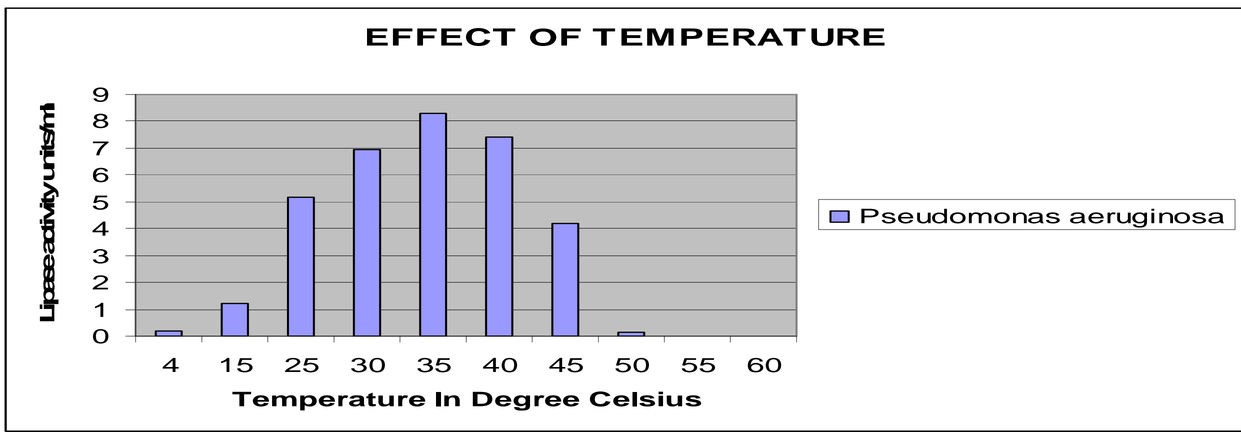


Fig 1c: Effect of Incubation Time on Lipase Activity

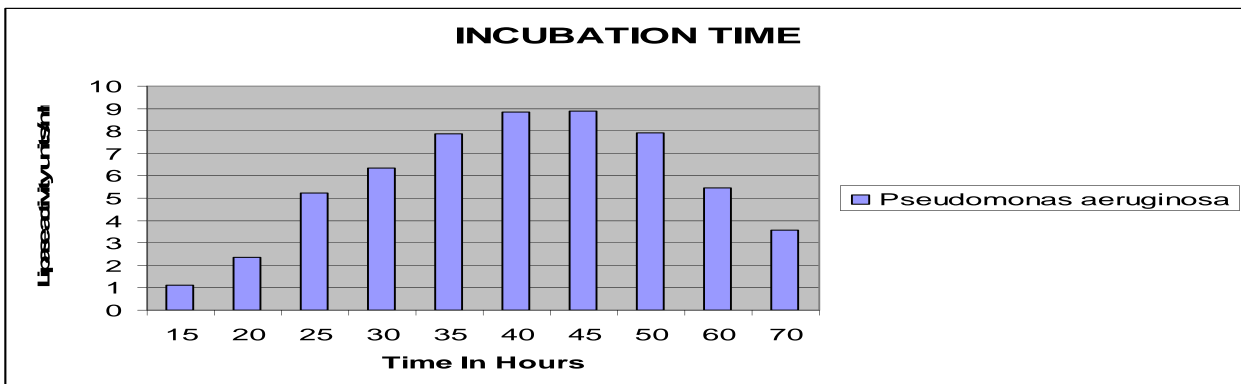


Fig 1d: Effect of Carbon Source on Lipase Activity

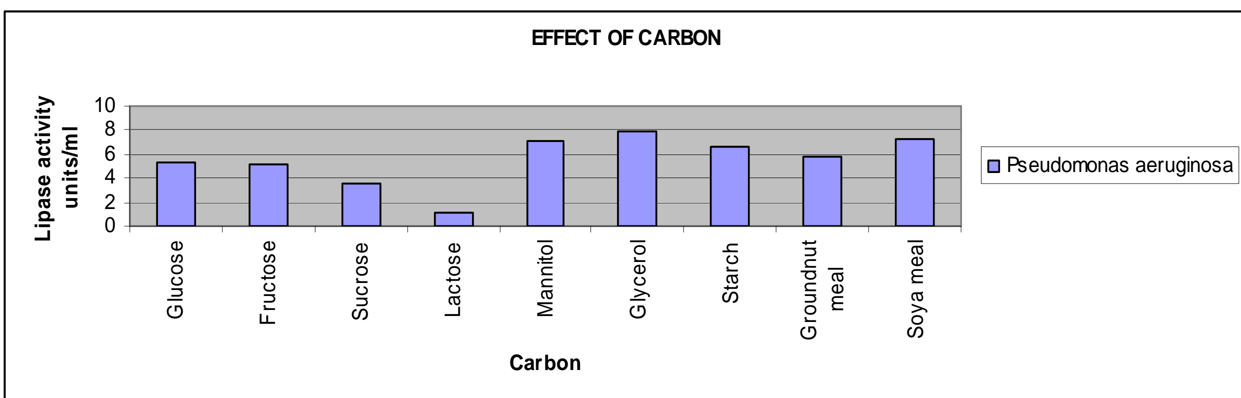


Fig 1e: Effect of Nitrogen Source on Lipase Activity

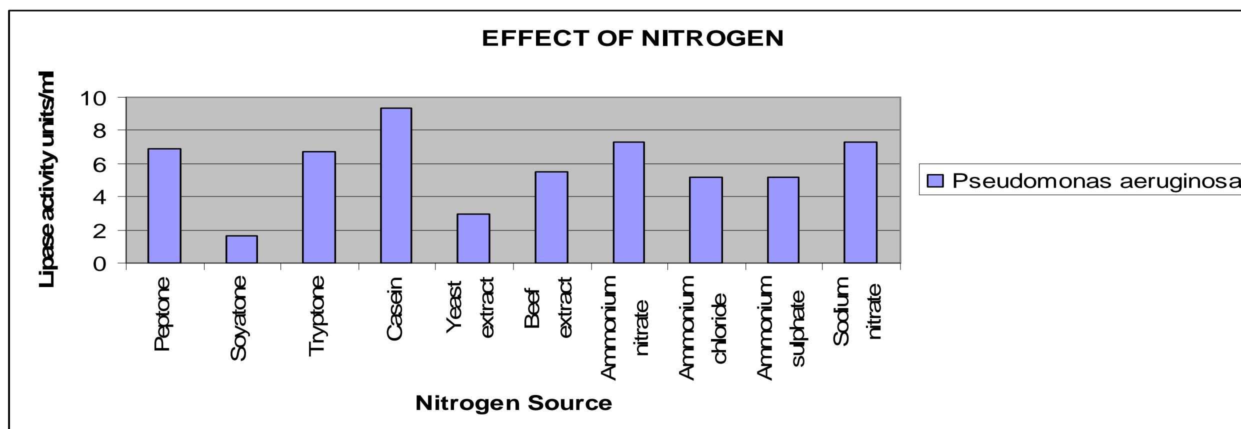
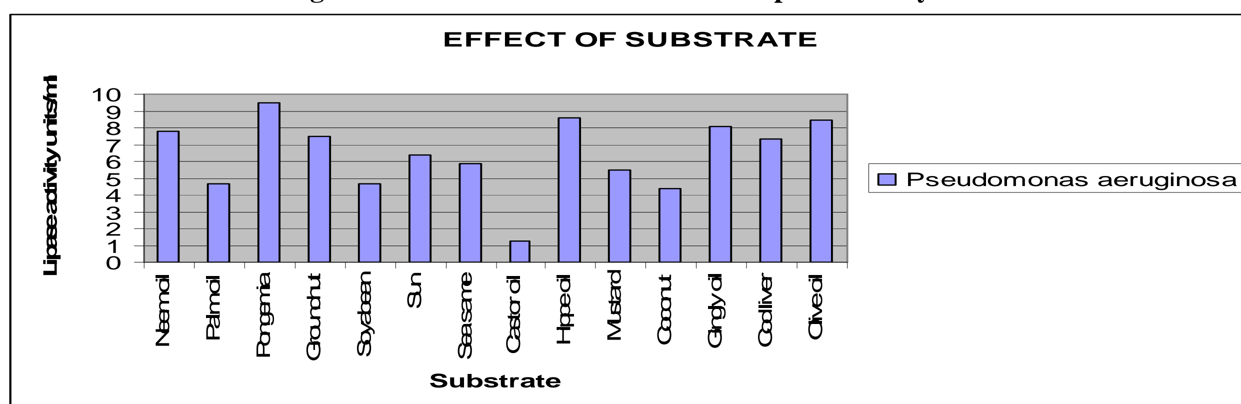


Fig 1f: Effect of Substrate Sources on Lipase Activity



Result showed that highest lipase activity for *Pseudomonas aeruginosa* was achieved at pH 7.5 at 35 °C for incubation period of 45 hour in the media containing glycerol as carbon source, casein as nitrogen source and pongemia oil as substrate source.

DISCUSSION

Bacterial true lipases were formerly ordered in the so-called *Pseudomonas* groups 1, 2 and 3 because *Pseudomonas* lipases were probably the first to be studied and have a preponderant role in industry. [11]. *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[12,13]. In the present study maximal activity was seen at pH 7.5 for 45 hours of incubation at 35 to 40°C. High concentration production of an extracellular enzyme lipase was achieved by a fed-batch of *Pseudomonas fluorescens*. During the cultivation, temperature, pH and dissolved oxygen concentration were maintained at 23 ° C, 6.5 and 2-5 ppm, respectively. Olive oil was used as a carbon source for microbial growth[14].

The enzyme production level can be markedly increased by several different fatty acid esters (eg, triglycerides and Tweens) and represents the key mechanism for making fatty acid carbon source available to the cell during growth on such compounds [15,16]. *Pseudomonas aeruginosa* resulted in maximum lipase activity in the medium containing glycerol as carbon source in the present study. The effect of lauric acid or Tween 20 extended also to the disassociation of the enzymes from the cell surfaces, leading to an almost complete release of the enzyme into the supernatant of the culture[17].

Consecutive optimization of nitrogen, carbon sources and inducers enhanced lipase activity under optimum conditions. Sardine oil, soy bean oil and triolein were effective inducers for lipase production[18]. Lipase activity was high in media containing glycerol as carbon source, casein as nitrogen source. Compared to olive oil which was used as standard substrate, pongamia oil as an inducer showed high lipase activity in the present study.

Pseudomonas aeruginosa lipase isolated showed broad substrate specificity, the highest specific activity towards the substrates ρ-nitrophenyl palmitate and 1, 2-dilaurylrac-glycerol-3-glutaric acid resorufin ester as compared to bacterial lipases isolated from *B.subtilis*, *P.alcaligenes*, *B.cepacia* and *P.fluorescens* [19]. *Pseudomonas aeruginosa* in the present study isolated from the oil mill effluents showed a broad lipase activity towards different substrates (neem oil, groundnut oil, hippe oil, pongamia oil, gingely oil, olive oil and cod liver oil) and also to a wide variety of carbon sources (mannitol, glycerol, starch and soyameal).

Although lipases have been found in many species of animal, plant and microorganism, microbial lipases are the most promising for the industrial applications of lipase-catalyzed reactions.

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