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# **Isolation Of Pseudomonas Aeruginosa from Soil and Production** of Lipase Enzyme

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#### Abstract

The isolates of *Pseudomonas aeruginosa* bacteria were uncovered in the soil surrounding the roots of palms and public gardens in Baghdad for the production of lipase enzyme. The lipase enzyme has many applications that are included in the textile and food industry, and the manufacture of detergents and medical preparations. Several tests such as temperature change, incubation period, change of lipid sources, nitrogen sources such as peptone and tryptone, and carbon sources such as glucose and lactose were carried out to choose suitable conditions for bacterial growth. The results indicated studying the conditions affecting production, it was noted that the best production was when using the culture medium to which 1% of corn oil was added, pH 7, at a temperature of 37 °C and an incubation period of 24 hours in vibrating incubator at 151 rpm, The soil surrounding the roots of the plant is a good reservoir for the presence of Pseudomonas aeruginosa bacteria

Keywords: Bacteria, pseudomonas aeruginosa, Lipase assay, Enzyme, Lipase purification

#### **1. Introduction**

Lipases are becoming more and more popular in the field of biotechnology, as they have received great interest in industrial applications because of their properties [1]. The uniqueness and its ability to benefit from a wide range of reaction materials and its high stability towards temperature and acidity function and organic solvents, as most industrial processes are carried out at a high temperature and therefore enzymes with high stability [2]. The temperature has attracted the interest of researchers and industry workers, as many lipases have been produced from animals and plants and microorganisms. Plant lipases are candidate alternative to microbial lipases, as 29 plant lipases have been mentioned until now in the database. Interest in lipase produced from grains is due to its availability and ease of isolation and purification from sources [3], the plant and the possibility of using it as a substitute for commercial enzymes and for use in the food and detergent industries. Bacterial lipases have been of great importance in the industrial field, especially because of its direct relationship to the appearance of the rancid flavour of the milk that benefited from it in the ripening of cheese [4]. This enzyme was also used in the manufacture of detergents and in the pharmaceutical industries, such as manufacture of alkaloids. As one of the catalytic enzymes, bacterial lipase enzyme is impacted by physical and chemical circumstances. In the presence of fats such as oil or fatty acids, these enzymes are usually generated [5]. Lipase is an enzyme capable of hydrolyzing esters and is insoluble in water, having a different distribution of hydrophobic substances [6]. Natural oils like

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olive oil, corn oil, and coconut oil improve the lipase enzyme's activity **[7]**. Due to the importance of studies on lipase enzymes produced from aeruginosa bacteria strains; this study came in order to obtain a productive isolate. For the enzyme, selecting the optimal conditions affecting its production, and knowing the effect of some source of carbon and nitrogen and lipids on the activity of the enzyme

# 2. Material and Method

# 2.1. Sample collection and isolation of Pseudomonas aeruginosa

Twenty soil samples were obtained in Baghdad from various locations, including the soil surrounding the roots of plants and public gardens in Baghdad. It was placed in sealed plastic bags and kept at a temperature of 6 degrees Celsius until use. To isolate the bacterial strains, five grams of soil samples were dissolved in 50 ml phosphate buffered saline solution and left in a shaker for one hour. Then,1 mL of the sample was cultivated for 24 hours at 37 °C in 100 mL of nutrient broth. They were then cultivated for 48 hours at 37 °C on nutrient agar. *Pseudomonas aeruginosa* isolates were diagnosed using biological and chemical testing [8].

# 2.2 Screening of lipase producing

The Tributyrin agar plate test method was used to screen isolated bacterial strains for lipolytic activity (TBA). The tributyrin agar media was made with 1.0 percent (v/v) olive oil and sterilized for 15 minutes at 121 °C before being placed in a Petri plate. To observe the zone, isolated bacteria were streaked on tributyrin agar plate and incubated at 37 °C for 24 hours [9].

# 2.3. Lipase assay

To produce enzymes, bacteria were cultured in a lipase-producing medium. 2 % yeast extract, 2 gram NaNO<sub>3</sub>, 0.5 gram KCl, 0.14 g KH<sub>2</sub>PO<sub>4</sub>, 1.2 g K<sub>2</sub>HPO<sub>4</sub>, 0.1 g CaSO<sub>4</sub>,1 percent olive oil, and 100 mL distilled water in a 250 mL conical flask as submerged fermentation method, inoculated flask was incubated at 37 °C for 24 hours[**10**]. After 24 hours of incubation, the fermentation broth was collected in falcon tubes. The supernatant from these falcon tubes was centrifuged for 10 minutes at 6000 rpm and used as the crude enzyme. This crude enzyme was mixed with 2 mL olive oil, 8 mL polyphenol alcohol as an emulsifier, and 0.3 M ammonium chloride, which was then titrated against 0.05 M NaOH to determine its enzyme activity. The amount of NaOH required to match the amount of acid in the solution, which was proportional to the amount of lipase generated, was calculated **[11]**. The amount of acid was determined using the following formula:

 $\mu$ mol fatty acid (U) = (mL NaOH for sample – mL NaOH for blank) × N ×1000 /M

# Where;

U: Denotes the amount of fatty acid (µmol) of fatty acid released/mL

N: Stands for the normality of the NaOH titrant used (0.05 in this case)

M: The total volume of the reaction mixture that was employed

One lipase unit is defined as the amount of enzyme that releases one mol of fatty acid per mL under standard assay conditions (U=mol of fatty acid released/mL) [12].

# 2.4 Determination of optimal culture media for Pseudomonas aeruginosa

2.4.1. Optimum pH and temperature

The organism was cultivated in mineral salt media containing 1% oil (olive oil); media were adjusted to different pH (4, 6, 7, and 9) and incubated at 30°C for 12 hours; once the best pH was established, the production medium was evaluated in a temperature range of  $25-40^{\circ}$ C [13].

#### 2.4.2. Optimum lipid source

Replacement of olive oil with sunflower oil and addition of corn oil (1 percent v/v) to the production medium in 400 ml Erlenmeyer flasks holding 100 ml of the liquid medium on a rotary shaker (151 rpm) and incubation at optimal temperature were used to test enzyme production. The enzyme was examined after 24 hours.

#### 2.4.3. Optimum carbon source

The enzyme's production was investigated using several carbon sources. Glucose, Starch, and Lactose were introduced to the production medium at a concentration of 3% (w/v) in 400 mL Erlenmeyer flasks containing 100 mL liquid media on a shaker incubator (151 rpm) and kept at temperature for 24 hours before the enzyme was tested **[14]**.

#### 2.4.4. Optimum nitrogen source

Peptone, yeast extract, and tryptone were added to the media at a final concentration of 3% (w/v) and incubated at optimum temperature for 24 hours in a shaker incubator at 151 rpm to study the effect of nitrogen sources on lipase synthesis [15].

#### 2.4.5. Optimum time incubation

To choose the optimal incubation periods for bacteria multiplication and lipase production, the culture medium was applied at different incubation times (24, 48, 72) h, the medium was inoculated with a volume of 100  $\mu$ l of *P. aeruginosa*, and incubated at optimum temperature and pH , activity of enzyme was determined **[16]**.

#### 2.5 lipase purification

The bacterial culture that has grown in mineral salt medium (MSM) were centrifuged at 8000 rpm for 20 minutes at 4 °C in a refrigerated centrifuge. The cell free supernatant was saturated with (0-70 percent) ammonium sulfate after 20 minutes of continuous stirring at 4 °C and centrifugation at 14,000 rpm. The ammonium sulphate fraction was dialyzed for 6 hours at 4 °C against 50 mM Tris-Chloride buffer in a Dialysis tube (pH 8.0). The concentrated enzyme was injected onto the Sephadex G-100 column after dialysis. The enzyme was eluted from the column at a rate of 1 ml/min. At 280 nm, the protein concentration of enzyme fractions (5 mL each) was measured using spectrophotometry. Lipase assay was performed using fractions containing highest protein content [17].

#### 3. Results and discussion

#### 3.1 Isolation and screening of lipase production from P.aeruginosa

Ten environmental isolates of *Pseudomonas aeruginosa* were obtained from soil (obtained from public gardens). Morphological and biological studies were carried out on the resulting isolates, which were phenotypically diagnosed as shown in Figure (1) by producing the green pigment (pyocyanin) when grown in a different medium. This is consistent with a previous study that demonstrated the production of Pseudomonas aeruginosa green pigment [18].



Figure 1: Growth of bacterial isolates in the culture media

# 3.2 Screening lipase producing

Based on clear zone generation on tributyrin agar plate, seven bacterial strains were identified as lipase producers (Figure 2). Only P1, P2, P4, P5, P6, P7, and P8 bacterial strains had a distinct zone around the colonies, but P3, P9, and P10 did not. The screening medium for lipase-producing bacterial strains was olive oil. Among natural oils, olive oil has been praised as one of the greatest inductors and substrates for lipase production. Bharathi and Rajalakshmi founds the areas appeared stained with bacterial strains green spots appeared when bacteria were cultured on Tributyrin agar plates at 36 °C **[19].** 



Figure 2: Lipase activity assay on Tributyrin agar plates.

**Figure (2)** shows the shape of the productive isolates as it showed a clear area after incubated for 24 h at 37 °C.

#### 3.3. Lipase assay

After the examination, the activity of seven isolates that had the ability to produce lipase enzyme was measured after culturing them in (MSM) to choose the isolate with the highest activity as shown in Table (1). This method is consistent with Malunavicius where a liquid medium was used and incubated at 37 °C for 24 hours in a shaker incubator at 150 rpm **[20]**.

Isolate	Enzyme activity
P1	21.9
P2	23.7
P4	24.2
P5	28.4
P6	19.6
P7	12
P8	20.9

Table (1): P.aeruginosa activity to produce lipase enzyme

### 3.4. Determination of optimal culture media for Pseudomonas aeruginosa

#### 3.4.1 Effect of pH and temperature

The initial pH of the growing media influences the rate of lipase production. The results indicated that the bacteria can produce lipase at pH 4.0 to 9.0 depending on the original pH of the medium. The amount of enzyme produced ranged from 5 to 29 Uml<sup>-1</sup>. *Pseudomonas aeruginosa* produces the most lipase, with a value of 29 Uml<sup>-1</sup> at pH 7 as shown in figure (3). However, it was discovered that increasing the pH from pH 7.0 to pH 9 decreased lipase synthesis. The maximum activity of lipase is seen at pH 7, at a temperature of 30 ° C. Temperature is a vital parameter that must be kept under control, and it differs from organism to organism. The physical features of the cell membrane are altered by temperature, which impacts extracellular enzyme secretion. In previously published research, it was found that the enzymatic activity was 30 U/ml at pH 7.5 and temperature of 35 °C [21].



Figure 3: Medium pH efficiency of lipase production

The bacteria generate lipase in a wide range of temperatures, from 25 °C to 40 °C, according to studies undertaken for temperature optimization. Figure (4) shows the effect of temperature on the lipase activity. The lipase enzyme activity produced at various temperatures ranged from 4.6 to 30 Uml<sup>-1</sup>. At a temperature of 37°C, the maximal lipase synthesis was 30 Uml<sup>-1</sup>. When the temperature was raised from 37°C to 40°C, the enzyme production dropped. It was also discovered that at a temperature of 40°C, the manufacturing of the lipase enzyme ended. *Pseudomonas aeruginosa* produced the most lipase at 37°C, according to another study [22]. It was also discovered that Pseudomonas fluorescens grew best and produced the most lipase enzyme when grown at 36°C.



Figure 4: The effect of incubation temperature on lipase production by *P.aeruginosa* at pH 7 and incubation time 24 h

#### 3.4.2. Optimum lipid source

Lipase synthesis is influenced by the type and concentration of lipid sources. In comparison to a control media, studies were undertaken on the effect of lipids offered as a substrate source for lipase synthesis. The use of corn oil increased the enzyme activity compared to the medium containing olive oil and sunflower as shown in figure (5). It has been reported in previous research that corn oil was the best fat source for lipase production.





#### 3.4.3. Optimum carbon source

The kind and concentration of nitrogen and carbon sources, the culture pH, growth, temperature, and dissolved oxygen level all affect lipase production **[23]**. Studies on the effect of sugars supplied as supplementary carbon sources have not boosted lipase production when compared to the control environment. Other sugars investigated reduced lipase enzyme synthesis when compared to control medium, as seen in figure 6. Lactose was shown to be the optimum carbon source for lipase synthesis **[24]**.





#### 3.4.4. Optimum nitrogen source

Lipase synthesis has been affected by the addition of amino acids as a nitrogen source. When organic nitrogen sources are employed, such as peptone and yeast extract, which have been used for lipase production by different thermophilic Pseudomonas, bacteria produce significant yields of lipase. For the three different organic nitrogen sources the activity of enzyme reached to  $32 \text{ U ml}^{-1}$ ,  $31.5 \text{ U ml}^{-1}$ , and  $28 \text{ U ml}^{-1}$  with peptone, yeast and tryptone respectively as shown in figure 7, that means the peptone enhanced lipase production. In previous research published, it was found that the addition of tryptone reduces the activity of the enzyme [25]. In a previous paper published by Mobarak- Qamsari was agreed with present results that enzymatic activity increases when peptone is added [26].



**Figure 7:** Effect of Nitrogen source on lipase activity at Temperature 37 °C, pH 7 and incubation time 24 h.

3.4.5. Optimum time incubation

The incubation period affects the growth rate of bacteria responsible for lipase production. The results indicated that bacteria can generate lipase during incubation periods ranging from 24 to 72 hours, with the enzyme production ranging from 20 to 60 Uml<sup>-1</sup>. *Pseudomonas aeruginosa* has an optimal lipase production at a 48 hour incubation period (60 Uml<sup>-1</sup>). However, it was discovered that as the incubation time was increased to 57 hours, lipase synthesis reduced. In previous research published, it was shown that the best time for bacterial growth and enzyme activity was 48 hours [**27**].



Figure 8: Effect of time incubator on lipase production by P.aeruginosa at temperature 37 °C and pH 7

#### 3.5. Lipase purification

Purification of lipase was carried out in order to isolate a protein of interest and eliminate nonessential proteins. The purification of lipase takes place in a progressive order. The enzyme was refined by salting out the proteins with ammonium sulphate precipitation after 48 hours of culture. Desalting was done to boost enzymatic activity by removing traces of salt. With a total yield of 47.7% and purification of 75.50 fold, Sephadex G-150 column chromatography was used to purify an extracellular lipase from *P.aeruginosa*. The enzyme's low yield could be attributed to difficulties in removing the significant amount of lipopolysaccharide found in *P.aeruginosa*, along with lipid hydrolysis **[28]**.

#### 4. Conclusions

To maximize lipase synthesis by Pseudomonas species, this study can be used to improve culture parameters such as pH, temperature, carbon sources, and nitrogen sources. Lipase-producing bacteria are abundant in soil, according to these studies. The ideal growing conditions discovered in this study can be used on a large scale in industrial applications.

The results that were reached on the properties of lipase in the soil surrounding the roots of plants in Baghdad is not very different from the properties of lipase found in other parts of the world, according to previous studies, despite the different soil, climate and environment. The variation in lipase activity in the conditions of different experiments was clear from the results of the research, the optimum pH of the enzyme was at pH7, and the optimum temperature was 37 °C, where the lipase activity is proportional to the increase in the concentration of enzyme activity but in a non-linear way, the enzyme reaches its best state at using corn oil as a substrate compared to other seed oils used in the research. Carbon and nitrogen sources differ in their activating and inhibitory effects on enzyme activity.

#### References

- Almeida JM., Martini V, Iulek J, Alnoch R, Moure V, Müller-Santos M and Krieger N 2019. Biochemical characterization and application of a new lipase and its cognate foldase obtained from a metagenomic library derived from fat-contaminated soil. International journal of biological macromolecules, 137, 442-454.
- [2] Sahu G and Martin M 2011. Optimization of growth conditions for the production of extracellular lipase by bacterial strains from dairy industry effluents. Biotechnol Bioinf Bioeng, 1(3), 305-311.
- [3] Rajan L, Palaniswamy D and Mohankumar S 2020. Targeting obesity with plant-derived pancreatic lipase inhibitors: a comprehensive review, Pharmacological research, 155, 104681.
- [4] Razzaq A, Shamsi S, Ali A, Ali Q, Sajjad M, Malik A and Ashraf M 2019. Microbial proteases applications. Frontiers in bioengineering and biotechnology, 7, 110.
- [5] Veerapagu M, Sankara A, Jeya K and Alagendran S 2014. Isolation and identification of a novel lipase producing bacteria from oil spilled soil. Int J Innov Res Sci Eng Technol, 3(5), 111-9.
- [6] Wu Q and Reetz, M 2020. Are Cysteine-lipases Involved in the Immune System?. Journal of Cellular Immunology, 2(4).
- [7] Seth S, Chakravorty D, Dubey V and Patra S 2014. An insight into plant lipase researchchallenges encountered. Protein Expression and Purification, 95, 13-21.
- [8] Duza M and Mastan, S 2014. Optimization of lipase production from Bacillus thuringiensis (TS11BP), Achromobacter xylosoxidans J2 (TS2MCN)-isolated from soil sediments near oilseed farm. IOSR J. Pharm. Biol. Sci, 9, 66-76.
- [9] Veerapagu M, Narayanan A, Ponmurugan K and Jeya K 2013. Screening selection identification production and optimization of bacterial lipase from oil spilled soil. Asian J. Pharm. Clin. Res, 6(3), 62-67.
- [10] Sharma M, Gat Y, Arya S, Kumar V, Panghal A and Kumar A 2019. A review on microbial alkaline protease: an essential tool for various industrial approaches. Industrial Biotechnology, 15(2), 69-78.
- [11] Bacha A, Moubayed and Abid I 2015. Thermostable, alkaline and detergent-tolerant lipase from a newly isolated thermophilic Bacillus stearothermophilus
- [12] Zouaoui B and Bouziane A 2011. Isolation, optimisation and purification of lipase production by Pseudomonas aeruginosa. J. Biotechnol. Biomater, 1(7), 1-4.
- [13] Abdullah R, Qaiser H, Iftikhar T, Kaleem A, Iqtedar M, Saleem F and Haq I 2018. Application of response surface methodology for statistical optimization of lipase production by Penicillium sp. employing solid state fermentation. Revista Mexicana de Ingeniería Química, 17(3), 863-875.
- [14] Amin M and Bhatti H 2014. Effect of physicochemical parameters on lipase production by Penicillium fellutanum using canola seed oil cake as substrate. International Journal of Agriculture and Biology, 16(1).
- [15] Phukon L, Chourasia R, Kumari M, Godan T, Sahoo D, Parameswaran B and Rai A 2020. Production and characterisation of lipase for application in detergent industry from a novel Pseudomonas helmanticensis HS6. Bioresource technology, 309, 123352.
- [16] Ilesanmi O, Adekunle A, Omolaiye J, Olorode E and Ogunkanmi A 2020. Isolation, optimization and molecular characterization of lipase producing bacteria from contaminated soil. Scientific African, 8, e00279.
- [17] Tripathi R, Singh J, kumar Bharti R and Thakur I 2014. Isolation, purification and characterization of lipase from Microbacterium sp. and its application in biodiesel production. Energy Procedia, 54, 518-529.
- [18] Jebur S, Al-Hamadani AH, Sh J 2015. Evaluation of genetic study and bacterial culture for diagnosis of Pseudomonal eye infections. Int J Curr Microbiol AppSci. 3:348–356
- [19] Bharathi D and Rajalakshmi G 2019. Microbial lipases: An overview of screening, production and purification. Biocatalysis and Agricultural Biotechnology, 22, 101368.

- [20] Malunavicius V, Druteika G, Sadauskas M, Veteikyte A, Matijosyte I, Lastauskiene E and Gudiukaite R 2018. Usage of GD-95 and GD-66 lipases as fusion partners leading to improved chimeric enzyme LipGD95-GD66. International journal of biological macromolecules, 118, 1594-1603.
- [21] Gurkok S and Ozdal M 2021. Purification and characterization of a novel extracellular, alkaline, thermoactive, and detergent-compatible lipase from Aeromonas caviae LipT51 for application in detergent industry. Protein Expression and Purification, 180, 105819.
- [22] Baloch K, Upaichit A, Cheirsilp B and Fibriana F 2021. The Occurrence of Triple Catalytic Characteristics of Yeast Lipases and Their Application Prospects in Biodiesel Production from Non-Edible Jatropha curcas Oil in a Solvent-Free System. Current Microbiology, 78(5), 1914-1925.
- [23] Jaiganesh R and Jaganathan M 2018. Isolation, purification, and characterization of lipase from bacillus sp. From kitchen grease. Asian J Pharm Clin Res, 11(6), 224-227.
- [24] Kathiravan T, Marykala J, Sundaramanickam A, Kumaresan S and Balasubramanian T 2012. Studies on nutritional requirements of Pseudomonas aeruginosa for lipase production. Adv Appl Sci Res, 3(1), 591-8.
- [25] Ginting E, Kemer K, Wullur S and Uria A 2020. Identification of proteolytic thermophiles from Moinit Coastal hot-spring, North Sulawesi, Indonesia. Geomicrobiology Journal, 37(1), 50-58.
- [26] Mobarak-Qamsari E, Kasra-Kermanshahi R and Moosavi-Nejad Z 2011. Isolation and identification of a novel, lipase-producing bacterium, Pseudomnas aeruginosa KM110. Iranian journal of microbiology, 3(2), 92.
- [27] Silva C, Delatorre A and Martins M 2007. Effect of the culture conditions on the production of an extracellular protease by thermophilic Bacillus sp and some properties of the enzymatic activity. Brazilian Journal of Microbiology, 38, 253-258.
- [28] Raza F, Sabri A, Rehman A and Hasnain S 2017. Characterization of thermophilic alkaline lipase produced by Staphylococcus aureus suitable for leather and detergent industries. Iranian Journal of Science and Technology, Transactions A: Science, 41(2), 287-294.