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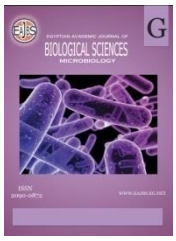

Screening For the Production of Lipase and Its Optimum Conditions from Bacterial Isolates

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ABSTRACT

Lipases, also known as triacylglycerol hydrolases, are a class of biotechnologically essential enzymes that have several uses in the dairy, detergent, culinary, and pharmaceutical sectors. Microbes are primarily manufactured of lipases, and bacterial lipases in particular play a major role in commercial operations. The goal of this study is to screen and design optimal conditions for lipase synthesis in bacterial isolates. 25 bacterial isolates were collected at random from various habitats, including soil and contaminated soil, for this study. The isolates that could produce lipase were chosen qualitatively using a rapid plate test method. The following conditions were found to be ideal for lipase production: pH 7.0, incubation temperature of 40°C for 4 days, and carbon and nitrogen supplies of olive oil and peptone, respectively. The highest amounts of lipase synthesis were achieved under these conditions.

INTRODUCTION

Lipases, classified as EC 3.1.1.3, are enzymes that are soluble in water and facilitate the breakdown of long-chain triglycerides by hydrolyzing them, typically acting on substrates that are insoluble. Lipases catalase acidolysis, alcoholysis, and aminolysis on triglycerides in addition to hydrolysis. They have a wide range of substrate specificity. and excellent activity across a wide temperature range. Lipases are thus the most versatile biocatalysts. (Singh *et al.*, 2019) [23]. Lipases are extremely important in the food, detergent, chemical, and pharmaceutical sectors. The industry has paid a lot of attention to fungus lipases. because of their substrate selectivity and stability under a variety of chemical and physical conditions. Lipases are secreted by many microorganisms, including bacteria, yeast, and fungi. Numerous environments have been found to contain lipase-producing microbes, such as industrial waste, plant oil processing facilities, dairies, and soil contaminated with oil., etc. (Sztajer *et al.*, 1998) Fungal enzymes are extracellular in nature and may be easily isolated, lowering the cost and making this source superior to bacteria (Mehta *et al.*, 2017).

Penicillium, Rhizopus, Aspergillus, and Mucor species are among the genera that contain lipase-producing fungi that are significant for commerce. All four Rhizopus species Rhizopus arrhizus, R. delemar, *R. oryzae*, and *R. niveus* produce lipase effectively. (Ayinla *et al.,* 2017). Extracellular bacterial lipases are very important commercially since bulk production is significantly easier. Even though there are many sources of lipase-producing bacteria, only a small number of these strains either wild or recombinant are used commercially. (Palekar *et al.,* 2000). Achromobacter, Alcaligenes, Arthrobacter, Bacillus, Burkholderia, and Pseudomonas are the most important. Lipases from Pseudomonas are commonly used in a variety of biotechnological applications. (Beisson *et al.,* 2000) [20] . The stability of the temperature is an essential need for industrial lipases because it allows enzyme reactions to be carried out Under higher temperature conditions, which aids in increasing currency exchange rates, increasing the solubility of substrates, and reducing microbial pollution and reaction medium viscosity.

MATERIALS AND METHODS

Sample Collection:

From July to December 2021, samples of oil-contaminated soil were collected from numerous Egyptian governorates. Giza, Dakahlia, Cairo, and Gharbia soil samples were obtained, including contaminated soil. All samples were placed in sterile screw-capped tubes, and care was made to ensure that the collection spots included a wide range of features such as soil color and topographical distribution.

Isolation of Bacterial Strains:

In 45 mL of sterile saline water (0.85% w/v aqueous NaCl solution) from each sample, 5 grammes of soil were suspended. Up to 10-7 serial dilutions were made. 0.1 mL of each dilution was applied to a culture plate containing selective media. The selective media consisted of the following ingredients (g/L): Na2HPO4 (12.0), KH2PO4 (3), NaCl (0.5), NH4Cl (1.0), MgSO4 (2.0), CaCl2 (0.1), olive oil (2.0% v/v), agar (20), and the pH were adjusted to 7.0. The inoculation plates were then kept at 37°C. (Janata *et al.,* 2018).

Screening of Bacterial Isolates for Lipase Enzyme:

Using a qualitative rapid plate assay, the ability of the acquired bacterial isolates to generate lipase enzyme was tested. The modified medium contained the following components in the specified concentrations: The manufacturing medium utilize in this study had the following composition: (%) Agar, 2.0; $CaCl_2$, 0.1; Olive Oil, 0.01; Phenol Red, 0.01. pH 7.0. At 37 °C, culture plates were incubated. (Ertugrul, *et al*. (2007)

Screening for Lipase Production:

The manufacturing medium utilised in this investigation has the following composition: (%w/v) Pepton, 0.2, $NH_4H_2PO_4$, 0.1, NaCl, 0.25, $CaCl_2.2H_2O$, 0.04, $MgSO_47H_2O$, 2.0 (v/v), pH 7.0, and 1-2 drops as an emulsifier, Tween 80. The inoculum for the pre-culture was overnight cultures suspended in 5 ml of sterile deionized water and used to provide an initial cell density to modify the turbidity of the 0.5 McFarland standard. A rotary shaker set to 150 rpm was used to incubate submerged microbial cultures in 500 ml Erlenmeyer flasks with 100 ml of liquid media. The incubation temperature was set at 37 °C. The culture was centrifuged at 10,000 rpm for 20 minutes at 4 °C after 24 hours of incubation, and the cell-free culture supernatant fluid was employed as the extracellular enzyme source. (Mobarak-Qamsari *et al.*, 2011).

Optimizing the Production Medium for Lipase:

The study aimed to optimize the media components for the highest lipase production by bacterial isolates. The researchers examined several factors, such as the incubation time (from 2 to 7 days), the pH level (from 3 to 9 adjusted with 1 N Hydrochloric acid or 1 N Sodium hydroxide), temperature (ranging from 25°C to 45°C), the olive oil concentration (from 0% to 2%), and

additional carbon sources. They also tested different nitrogen sources.

RESULTS

Isolation And Screening of Isolated Bacteria:

lipase-producing microbes from various samples obtained in several Egyptian governorate regions. Lipase-producing organism colonies (25 isolates) were detected by their yellow color, as shown in (Fig. 1). 25 different bacterial isolates in all were recovered at random from oil-contaminated soil, with Dakahlia (7 isolates) and Cairo (8 isolates) providing the most isolates, followed by Gharbia (6 isolates) and Giza (4 isolates). The presence of a yellow color around the colonies of eleven bacterial isolates (D2, D5, GH1, GH4, GH5, GH6, G4, S2, S4, S6, and S7) indicated lipase production. These isolates were then quantitatively tested for lipase production (Table 1).

Control **Positive result**

Fig 1. Screening plate assay technique for isolating isolates on the production of Lipase**.**

Table 1. Screening for production of lipase from bacterial isolated by qualitative plate assay technique

Sample	Result	Sample	Result	Sample	Result
D 1	**-**	**GH 4**	**+**	**S 4**	**+**
D 2	**+**	**GH 5**	**+**	**S 5**	**-**
D 3	**-**	**GH 6**	**+**	**S 6**	**+**
D 4	**-**	**G1**	**-**	**S 7**	**+**
D 5	**+**	**G 2**	**-**	**S 8**	**-**
D 6	**-**	**G 3**	**-**		
D 7	**-**	**G 4**	**+**		
GH 1	**+**	**S 1**	**-**		
GH 2	**-**	**S 2**	**+**		
GH 3	**-**	**S 3**	**-**		

Confirmation of the Screening for Lipase Production by Bacteria:

The assessment of specific activity, enzyme synthesis, and protein estimate for each isolation was assessed and revealed that isolate (S6) had the highest specific activity as indicated in (Table. 2). Isolates with typical lipase-producing capabilities were then submitted to additional identification, characterization, and Optimization techniques.

Table 2. Conducting a quantitative screening of bacterial isolates for Lipase formation.

Samples	Total activity (U)	Total protein (mg)	Specific activity (U/mg)
D2	5.3	1.5	3.53
D 5	4.9	1.6	3.06
GH 1	7.1	1.3	5.46
GH 4	6.6	1.8	3.66
GH 5	4.8	1.9	2.52
GH 6	5.9	1.5	3.93
G 4	6.2	1.2	5.16
S 2	7.0	1.8	3.88
S 4	3.9	1.6	2.43
S6	7.5	1.1	6.81
S 7	5.8	1.8	3.22

Factors Influencing Lipase Production:
Effect of Time:

The time of incubation influences the bacterial isolate's synthesis of lipase (S6). (Fig 2.) indicates that lipase synthesis gradually increased for up to 4 days, reaching a maximum of 7.85 U/mL of enzyme production. However, enzyme activity began to drop after this point.

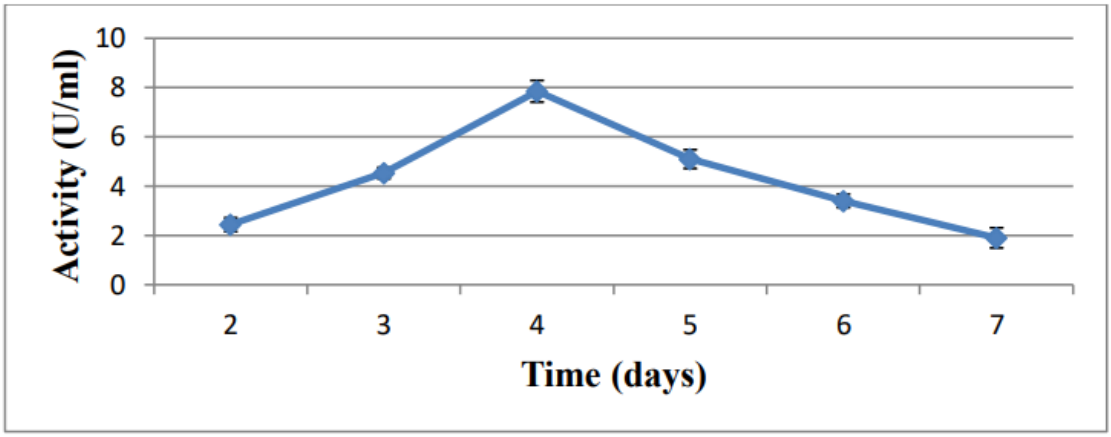

Fig 2. Influence of incubation time on lipase.

Effect of pH:

The synthesis of lipase by bacteria isolate (S6) was influenced by the pH of the medium. (Fig. 3**)** demonstrates an investigation of the influence of pH on enzymes. The results indicate that the maximal synthesis of the enzyme (8.1 U/ml) optimum result was achieved at a pH of 7.0. Deviating from this optimal pH value resulted in reduced enzyme synthesis, which can be attributed to either enzyme inhibition or a suboptimal pH condition in the medium.

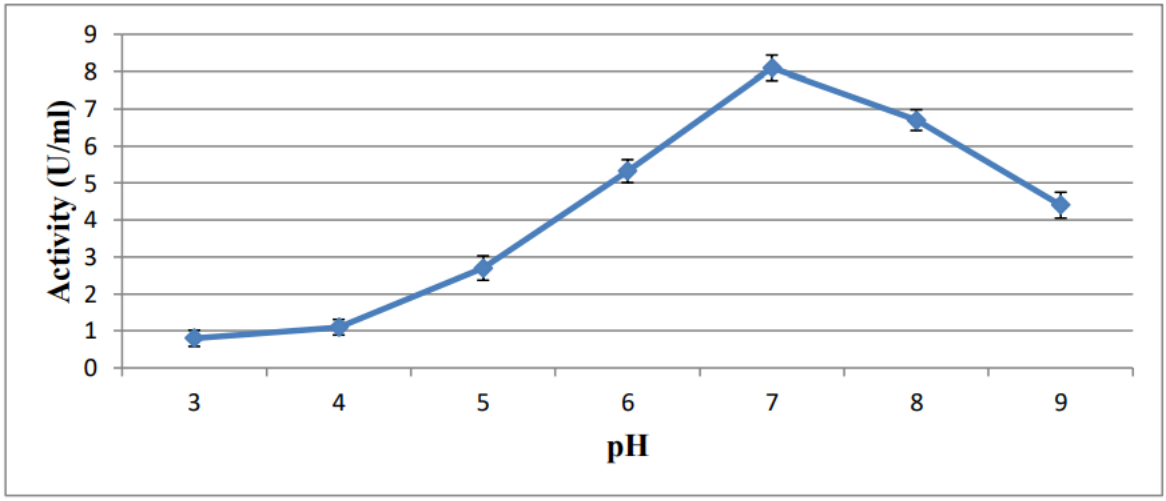

Fig. 3. Impact of pH on lipase synthesis.

Temperature Effect:

The medium's incubation temperature has an important impact on the expansion and synthesis of enzymes of microbial strains. In the case of lipase synthesis by isolated bacteria (S6), it was observed that the maximum enzyme production (8.9 U/ml) occurred at a temperature of 40°C. (Fig. 4) illustrates the relationship between temperature and bacterial lipase synthesis, showing that any deviation from the optimum temperature results in reduced enzyme production.

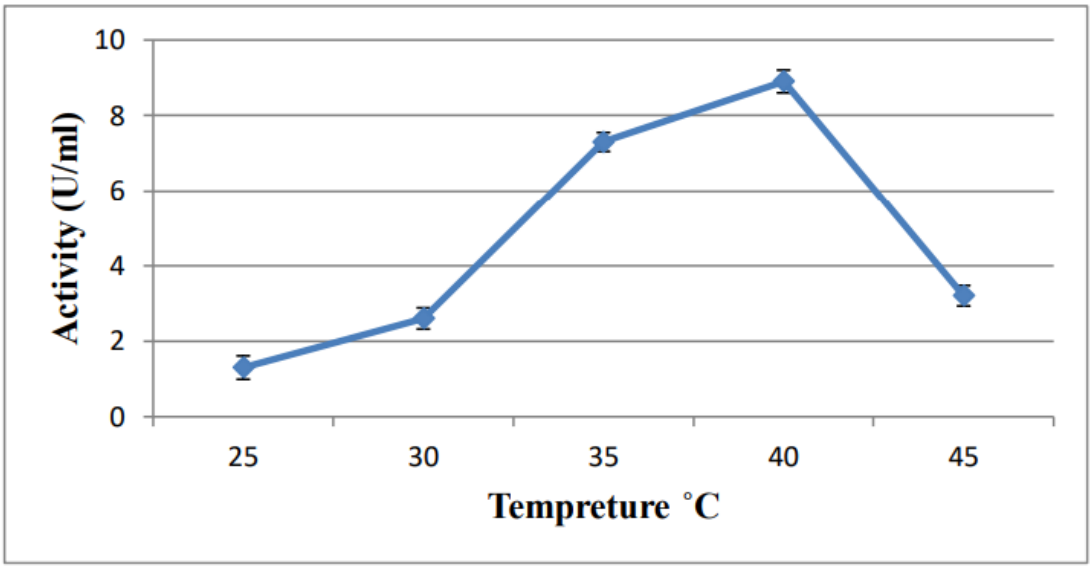

Fig. 4. Temperature impact on lipase synthesis.

Impact of Different Sources of Carbon:

The choice of carbon source in the growth medium can greatly impact the lipase production by the bacterial isolate numerous carbon sources were tested According to the data in (Fig. 5**)** It was found that olive oil gave the maximum production of enzyme (8.88 U/ml).

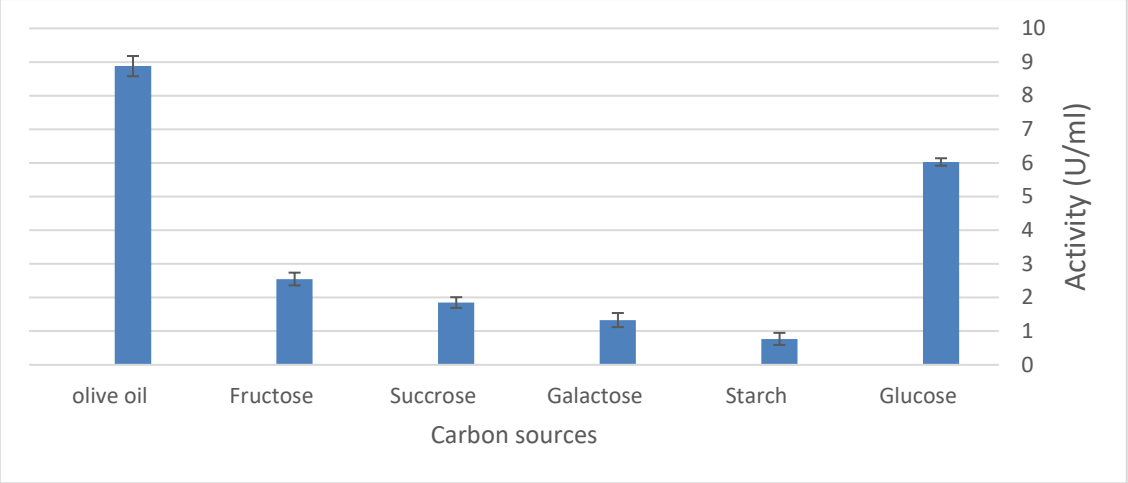

Fig. 5. Impact of different sources of carbon on lipase.

Effect of Different Nitrogen Sources:

The influence of additional organic and inorganic nitrogen sources on lipase synthesis was Investigated, and the results are as follows: Peptone exhibited the highest effectiveness as a nitrogen source, supporting the highest enzyme synthesis with a value of 9.1 U/ml. Different levels of enzyme production were also seen with other nitrogen sources. Sodium Nitrate showed the lowest-efficiency nitrogen source, with a lipase synthesis value of 1.2 U/ml, as depicted in (Fig. 6).

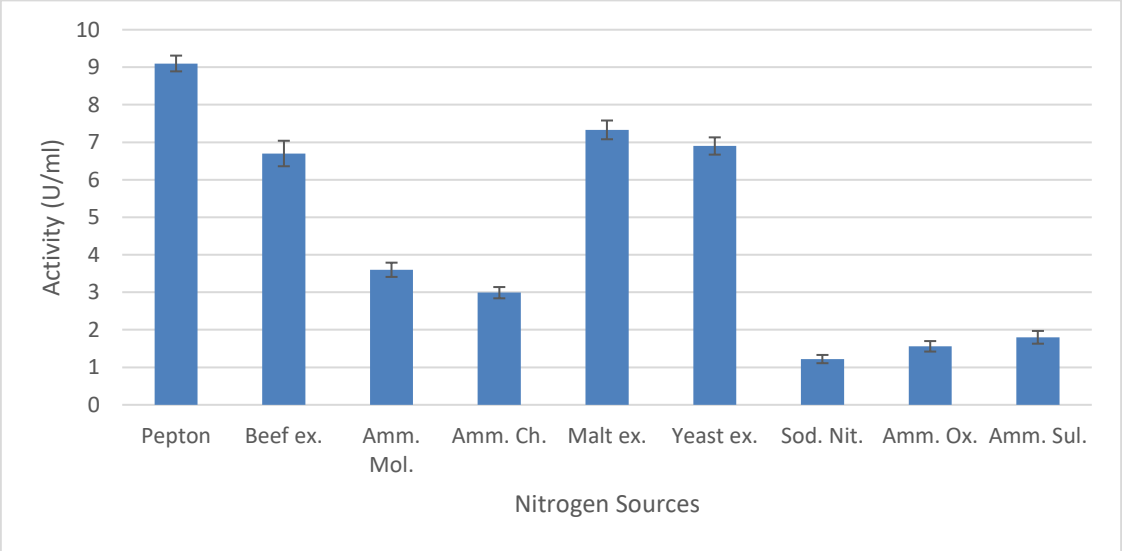

Fig. 6. Impact of different nitrogen sources on lipase enzyme synthesis.

DISCUSSION

Microorganisms capable of producing lipase can be discovered in diverse environmental conditions, industrial waste, vegetable-oil processing factories, dairies, oil-contaminated soil, oil seeds, decomposing food, compost piles, coal tips, and hot springs are all examples. (Mobarak-Qamsari *et al.*, 2011).

Microbial lipases have already demonstrated their broad utility in a variety of industries. (Bora L, Kalita MC, 2008). In recent decades, there has been an increasing interest in microbial lipase production due to their significant potential in various industrial applications. These applications include the use of lipases as food additives for flavour enhancement, the synthesis of esters for fine chemical production, the treatment of

wastewater to decompose and remove oil substances, the removal of lipids in cosmetic products, the oil and fat digestion in foods for pharmaceutical purposes, removing fats from animal skins in the leather industry, and the utilization of lipases in medicine for blood triglyceride assays (Nadia *et al.,* 2010; Sebdani *et al.,* 2011)

Only eleven bacterial isolates (D2, D5, GH1, GH4, GH5, GH6, G4, S2, S4, S6, and S7) out of 25 were capable of producing lipase. Following the identification and isolation of organisms with the greatest lipase enzyme activity, by employing one variable at a time approach for lipase synthesis optimization, multiple growth conditions that affect lipase synthesis were altered. Beginning with the incubation period, at 4 days, the greatest activity of lipase was measured, followed by a notable drop. The decrease could be attributed to a decrease in dietary element consumption. (Gupta *et al.,* 2004). Lipase has been identified as an inducible enzyme, and carbon has been identified as a significant factor in the expression of lipase activity. When different carbon sources were examined, olive oil demonstrated the highest lipase activity. Subsequently, the pH of the production medium was changed from 3 to 9 to determine the optimal pH. The pH range of 6–8 displayed high lipase activity, with the optimum activity observed at pH 7. Bacteria capable of producing lipase have been found to favour pH levels around 7. (Gupta *et al.,* 2004 and Oluwaseye I Ilesanmi *et al.,* 2020). All nitrogen sources have been designated as suppliers of nitrogen, Amino acids and growth factors that are essential for enzyme production. These nitrogen sources contribute to the overall nutrient composition required for the production of enzymes. (Thakur *et al.,* 2014) Out of the nine tested nitrogen sources, Peptone, Yeast Extract, and Malt Extract exhibited remarkably high lipase activity. These organic nitrogen sources were found to be particularly conducive to lipase production. On the other hand, Sodium Nitrate displayed the lowest lipase activity among the tested nitrogen sources.

According to our findings, the developed lipase activity was better at40 °C than it was at 37 °C, with a slight decrease at higher temperatures The source and type of lipase determine the ideal temperature for lipase production; Pseudomonas stutzeri MTCC 5618 lipase production was optimal at 50°C (Bharathi *et al.,* 2019) Other publications, however, recommend a range of temperatures for other bacterial or fungi strains to produce lipase.For instance, some studies claim that different bacterial isolates produce lipases best at 37°C (Gupta *et al.,* 2004) or 20°C Mazhar, Additionally, lipases from various Pseudomonas species, including P. fluorescens and P. fluorescens HU380, have been reported .(Kojima, Y., & Shimizu, S. 2003), P. fragi(Mencher, J. R., & Alford, J. A. 1967), and P. mendoncina (Makhzoum *et al.,* 1996), were discovered to be the most effective between 35 and 45°C. Compared to other lipases in this genus, P. aeruginosa lipases appear to be more thermostable. While certain fungal isolates produce lipase best at 36°C (Cesário *et al.,* 2021). As a result, depending on the source and type of your particular lipase, you might need to change the temperature.

Conclusion

This study describes the discovery of bacterial isolate was isolated from soil and contaminated soil from different locations in Egypt. Then, screen for the creation of lipase with a rapid assay method. highest lipase production was found with optimum pH 7, temperature 40 °C. and an incubation time of 4 days. Olive oil was the most effective carbon source, and peptone was the most effective nitrogen source.

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