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# Isolation and Identification of Lipase-producing *Actinobacteria* from the Lut Desert of Iran

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**ABSTRACT**

Lipases are the third category of widely used hydrolytic enzymes that catalyze the hydrolysis of triacylglycerols, glycerol, and free fatty acids. *Actinobacteria*, which are Gram-positive bacteria, have the largest genome size among bacteria and produce various secondary metabolites, including enzymes. This study aimed to isolate lipase-producing *Actinobacteria* strains from the Lut Desert in Iran. In this study, microbial samples isolated from the Lut Desert region of Iran in 2017-2018 were cultured on specific *Actinobacteria* growth media. These samples were activated and cultivated on Tween 80 agar plates for lipase production. After seven days of bacterial growth, the samples were assessed based on the transparent clear zone diameter around the colonies. The lipase activity was measured spectrophotometrically using para-nitrophenyl palmitate as the specific substrate, and the best lipase-producing strain was identified based on its 16S rRNA sequence. Among the six isolated *Actinobacteria* strains, three strains exhibited lipase production capability. Strain Ga7 had the largest clear zone with a diameter of 7 mm and lipase activity of $5.45\times10^{-7}$ µmol/min, making it the most promising potential lipase producer. Based on its 16S rRNA sequence, strain Ga7 belongs to the genus *Streptomyces sp*., with 99% similarity to *Streptomyces indoligenes*. This study highlights the significance of using bacteria as a microbial source for lipase production for future industrial and biotechnological applications. Based on the findings of this study, it was determined that the Ga7 strain, due to its bacterial genus and its isolation from the Gandom Beryan area of the Lut Desert as the hottest place on earth, can be a suitable candidate for lipase production in various industries. These findings emphasize the importance of studying microorganisms from extreme environments like the Lut Desert as rich sources for discovering and utilizing industrial enzymes.



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## Introduction

The Industrial Revolution of the 19th century strengthened activities that significantly contributed to the destruction of the environment (Ilesanmi *et al*., 2020; Narayanan, 2009). The current state of the environment and the need to save it has led manufacturing industries to reach decisions that include sustainable and green production processes, including enzyme catalysis (Ilesanmi *et al*., 2020; Sarmah *et al*., 2018). Enzymes are protein molecules that living organisms use to carry out biochemical reactions. They play essential roles in various processes, including digestion, metabolism, and intracellular thermoregulation. Unlike chemical catalysts used in chemistry, enzymes are specific

for the catalyzed reaction type and the substrate or set of substrates. Enzymatic catalytic activity occurs through specific regions known as active sites. The primary enzyme examples are amylase, lactase, diastase, sucrose, maltase, invertase, glucoamylase, alpha glycosidase, protease, peptidase, and lipase (Kirana *et al*., 2016; Robinson, 2015). In general, enzymes are classified into seven groups based on their function, including oxidoreductases (EC1), transferases (EC2), hydrolases (EC3), lyases (EC4), isomerases (EC5), ligases (EC6), and translocases (de Souza Vandenberghe *et al*., 2020). Hydrolases account for more than 75% of commercial enzymes, and lipases (triacylglycerol acyl hydrolase, E.C.3.1.1.3) are part of this family of hydrolases which catalyze the hydrolysis of triglycerides to fatty acids and glycerol (Naveed *et al*., 2021; Sharma *et al*., 2001). After proteases and amylases, lipases are the industry's third-most-used enzyme category (Borrelli and Trono, 2015).

Lipases are classically defined as enzymes, generally monomeric and water-soluble and the molecular weight of lipases is in the range of 19-60 kDa (Hari Krishna and Karanth, 2002; Tong et al., 2016). Natural oils such as olive oil, coconut oil, and vegetable oil are substrates that increase lipase production (Fatima *et al*., 2021).

Lipases can hydrolyze long-chain triacylglycerols into monoglyceride, diglyceride, fatty acids, and glycerol at the interface between water and substrate, produced by different microorganisms alone or with esterase. Lipases catalyze a wide range of chemical reactions, such as complete or incomplete hydrolysis of triglycerides and chemical reactions of esterification, interesterification, and transesterification in lipids (Abdelmoez *et al*., 2013; Colla *et al*., 2010; Fatima *et al*., 2021). The 3D structures of lipases from different microbial sources are not the same; they show high sequence diversity and have at least 170 structures resolved to date (rcsb.org). The position of the fatty acid in the glycerol backbone, the chain length of the fatty acid, and its degree of unsaturation are the factors, and the physical properties of lipases depend on it (Carpen *et al*., 2019). The "Active site" in lipases has a unique structure and amino acid composition, enabling these enzymes to interact with specific substrates (such as triglycerides). The catalytic structure of lipases typically includes a "catalytic triad" composed of three key amino acids: serine (Ser), histidine (His), and either aspartic acid (Asp) or glutamic acid (Glu). These three amino acids are arranged in a specific spatial pattern, forming a complex mechanism that facilitates the cleavage of ester bonds in lipid molecules (Schrag *et al*., 1991). Consequently, these enzymes are unique and specific to the type of biotransformation processes they catalyze and are relevant in a wide range of industrial processes (Ilesanmi *et al*., 2020). Lipase enzyme is naturally present in pancreatic juice and stomach, and lipases also maintain the correct functioning of the gallbladder. They also control the amount of body fat synthesized and burned by reducing adipose tissue (Kirana *et al*., 2016). In general, lipases are found in animals, plants, and microorganisms, but it is known that those originating from bacteria are more stable than others (Hou, 2002; Joshi & Kuila, 2018; Kirana *et al*., 2016). Bacterial lipases are more commercially important mainly due to their ease of cultivation and optimization to obtain higher yields, and the industrial demand for new sources of lipases with different catalytic properties has stimulated the isolation and selection of new strains (Patel *et al*., 2021). In particular, microbial lipases have a wide range of enzymatic properties and substrate specificities, making them very useful for industrial applications such as processing fats and oils, additives, detergents, cosmetics, paper production, and pharmaceuticals (Hasan-Beikdashti *et al*., 2012; Ray, 2012). On the other hand, among the medical uses of lipases, we can mention their role as digestive aids in the treatment of digestive disorders and indigestion (Hasan-Beikdashti *et al*., 2012; Hasan *et al*., 2006). In dairy industries, lipases increase cheese's taste and aroma, accelerate cheese's ripening, and break down the fat of milk or butter and cream to create a unique taste (Ray, 2012). In terms of diet, lipase can help digest fats, so they are widely used in the livestock and poultry industry. In agriculture, many chemicals, pesticides, and fungicides are produced by lipases (Chandra *et al*., 2020). In addition, lipase can be used for environmentally friendly

degreasing in the leather processing industry, and it has also been found that microbial lipases produce biodiesel by transesterification (Ray, 2012)

Many bacteria can produce lipase, including the genera *Bacillus* and *Pseudomonas*, *Staphylococcus* and genus *Burkholderia*, bacteria of the genera *Serratia* and *Actinobacteria*, such as various species of *Streptomyces*, one of the most well-known genera of actinobacteria. The reason for producing lipases resistant to environmental conditions has been studied. *Nocardia spp*, a genus of actinobacteria, can also produce lipases with industrial applications (Arpigny and Jager, 1999; Dharmsthiti and Kuhasuntisuk, 1998; Gupta *et al*., 2004; Hasan *et al*., 2006; Lee *et al*., 1999). Also, halophiles have received considerable attention because of their enzymes, which could overcome the deleterious effects of salt. Besides, they can remain active in the presence of many surfactants and organic solvents (Satari Faghihi *et al*., 2018).

Actinomycetes are prokaryotes that live freely, saprophyte, and symbiotic with plants. They can be found in all ecosystems, including soil, water, marine sediments, and warm waters, but their primary environment is soil and the population. They form a large part of its natural flora (Bredholt *et al*., 2008; Singh *et al*., 2006). *Actinomycetes* have the largest genome among bacteria, and their G+C percentage is very high. Thousands of transcription factors express this long genome, and depending on the specific need, they can produce a variety of secondary metabolites, such as lipases (Anderson & Wellington, 2001).

In general, actinobacteria lipases have many advantages over lipases produced by other microorganisms, with features such as high stability against temperature and pH, variety of enzyme activity, resistance to harsh environmental conditions, easy production on a large scale, and the ability to be used in biodegradable processes. These characteristics have made actinobacteria a valuable source for the production of industrial lipases, which are very practical and helpful in various applications, from the food and pharmaceutical industries to biological purification (Sharma *et al*., 2001). Considering the critical role of lipases in various industries and the importance of lipases produced by actinobacteria, in this study, we investigated the native lipase-producing *Actinobacteria* strains isolated from the Gandom Beryan region located in the Lut desert and the level of microbial lipase enzyme activity.

## Materials and Methods

### The bacteria and their activation

In this study, 11 samples of bacteria were used, which were isolated in 1396-1397 from the Gandom Beryan region in the Lut desert of Iran, at coordinates 53° 57' 39" longitude and 30° 35' 12.596" latitude and were stored in stocks containing glycerol 50 percent were stored at -80°C (Mohammadi *et al*., 2022). At first, to activate the strains, specific liquid media were used: Isp4 medium (containing $CaCO_3$ 2 g/L, $K_2HPO_4$ 1 g/L, $MgSO_4$ 1 g/L, NaCl 1 g/L, $(NH_4)_2SO_4$ 2 g/L, starch 10 g/L, trace elements 1000 µL, agar 20 g/L) and Gause medium (containing starch 20 g/L, $K_2HPO_4$ 0.5 g/L, $KNO_3$ 1 g/L, $MgSO_4$ 0.5 g/L, $FeSO_4$ 0.01 g/L, agar 20 g/L, and NaCl 0.5 g/L). Subsequently, the strains were incubated at 30℃ for seven days with shaking at 100 rpm (Mohammadi *et al*., 2022). After seven days passed and turbidity was observed in the culture medium, 100 µl of each sample was transferred to the solid culture medium to ensure their purity, and petri dishes were incubated at 30 °C for seven days. After the bacteria grew, the morphological characteristics of each strain were examined. Those with colony morphology and bacteria similar to actinobacteria (chalky or powdery colonies with irregular margins, different color spectrum, and gram-positive or variable) were selected, and their ability to produce lipase was investigated.

### Screening of lipase-producing strains

For screening lipase-producing strains, Tween-80 agar medium was used, which includes 10 g/L peptone, 0.5 g/L NaCl, 0.1 g/L $CaCl_2 \cdot H2O$, 15 g/L agar, 10 mL Tween-80, and 1000 mL water (Panyachanakul *et al*., 2020). Strains were cultured on Tween-80 agar plates and incubated at 30°C. After seven days, the clear zone around the colonies was visually assessed, and the clear zone diameter was measured with a ruler to detect lipase production.

### Examination of lipase activity

At first, the isolated lipase-producing strain that had the largest diameter of the lipase clear zone around the colony was cultured in tween 80 liquid medium at 30 °C; after seven days of bacterial growth and lipase production in the medium, the sample was centrifuged at 10,000 rpm for 10 minutes and the supernatant separated. Then, the lipase activity in the supernatant was investigated, and an enzymatic assay was performed using the spectrophotometric method. For this purpose, the amount of para nitrophenol (pNP) released from para nitrophenyl palmitate (pNPP) (Sigma) is measured. Therefore, the reaction mixture containing gum arabic, Tris Hcl-buffer, isopropanol, para nitrophenyl palmitate, and triton x-100 was prepared in appropriate concentration. Then, 1.35 ml of this prepared enzyme substrate mixture was added to 150 µl of the enzyme, and then absorption was measured at a wavelength of 410 nm (Kordel *et al*., 1991; Pencreac'h & Baratti, 1996).

For the optimum time of lipase activity, every 12 hours, 3 ml of the Tween-80 culture medium in which the novel strain was cultured (having the largest clear halo diameter in the initial lipase production screening) was taken over ten days. After centrifugation, the supernatant was separated, and its activity was measured according to the protocol to determine the optimal time for lipase activity. The process was repeated three times, and the average result was calculated. The results were then plotted using Microsoft Excel 2016 (Thomson *et al*., 1999).

### Growth curve of lipase-producing strain

The novel strain's growth was checked during 12-hour intervals to check the bacterial growth rate. For this purpose, the desired strain was cultured in 200 ml of Tween 80 medium and placed in an incubator at 30 °C and 100 rpm. Then, during specific times (every 12 hours), 2 ml of Tween 80 culture medium containing bacteria was taken. Its absorbance was measured at 630 nm wavelength, and the growth curve was drawn using Microsoft Excel 2016 software.

### DNA extraction and quality confirmation

Bacterial DNA was extracted using the phenol-chloroform method. Electrophoresis was performed with a 1% gel to check the success of DNA extraction. About 2µl of genomic DNA sample extracted with 2µl of loading dye were loaded into the well. After the loading time of electrophoresis with a current of 7V/CM for 30 minutes, the gel was transferred to a container containing ethidium bromide, placed in the gel dock device, and photographed (Irshad *et al*., 2023).

### Molecular identification

The *Actinobacteria* isolate was identified by examining the 16SrRNA sequence. After the DNA extraction process, the PCR reaction was initiated using the universal primers 27F and 1492R, along with the specific primers ACT235f and ACT878r of Actinobacteria (Table 1).

**Table 1.** Specific and universal primers*

| Primers | Oligomer sequence (5'→3') | PCR products (bp) |
|---|---|---|
| *ACT235f* | 5'CGCGGCCTATCAGCTTGTTG | 600 bp |
| *ACT878r* | 5'CCGTACTCCCCAGGCGGGG | |
| *27F* | 5'AGAGTTTGATCCTGGCTCAG | 1400 bp |
| *1492R* | 5'GGTTACCTTGTTACGACTT | |

*These primers reported by Mohammadi *et al*., 2022; bp= base pairs.

The amplified products were then subjected to electrophoresis on a 1% agarose gel and sequenced by the South Korean Macrogen company using the Sanger sequencing method. The resulting sequence was then analyzed using the widely used BLAST tool in the NCBI database, and a phylogeny tree was constructed using the MEGA-X software.

Molecular identification of Actinobacteria strains was performed by sequencing the 16S rRNA gene. Following DNA extraction, PCR amplification was carried out using universal primers 27F and 1492R and specific primers ACT235f and ACT878r for Actinobacteria (Table 1). The PCR products were separated on a 1% agarose gel electrophoresis and sequenced by Macrogen Inc., South Korea, using the Sanger sequencing method. The obtained sequences were analyzed using the BLAST tool in the NCBI database, and a phylogenetic tree was constructed using MEGA-X software (Irshad *et al*., 2023).

## Results

### The bacteria and their activation

*Actinobacteria's* morphology was microscopically examined after incubating the samples at 30 °C for 7 days to grow. Gram

staining and microscopic observation of bacterial morphology confirmed filamentous Gram-positive *Actinobacteria*. Additionally, these bacteria were analyzed using specific and universal primers, and a single band on agarose gel confirmed that the strains were *Actinobacteria*. The results indicated that the stored isolates were pure. Based on the colony morphology characteristics, out of the 11 cultured strains, only six colonies, Ga1, Ga2, Ga3, Ga5, Ga7, and Ga9, exhibited typical Actinobacteria colony characteristics (chalky or powdery colonies with irregular margins and varying color spectra). Each isolate's colony morphology and characteristics are shown in the table below (Table 2). For confirmation and final verification of the isolates belonging to the Actinobacteria class, specific primers ACT235f and ACT878r were used. The observation of a 600 bp band confirmed that the six colonies (Ga1, Ga2, Ga3, Ga5, Ga7, and Ga9) belong to the *Actinobacteria* class (Table 2) (data not shown). Also, the colony morphology characteristics of the selected isolates were investigated, which showed colored chalky or powdery colonies with irregular margins (Fig. 1).

**Table 2.** Bacterial strains were isolated in 2017-2018 from the Gandom Beryan area of the Lut desert, Iran.

| No. | Strain | Medium | Colony Morphology | Bacterial Morphology |
|---|---|---|---|---|
| 1 | Ga1 | ISP4 | pink, circular, chalk and powder | Gram-positive cocci with chain arrangement |
| 2 | Ga2 | ISP4 | white, round, fine, chalky and powdery | diplococci gram positive |
| 3 | Ga3 | ISP4 | white, round, fine, chalky, powdery and raised center | diplococci |
| 4 | Ga5 | Gause | white, round, chalky and powdery with tiny drops | Gram-positive cocci with chain arrangement |
| 5 | Ga7 | Gause | white, circle, chalky, powdery and produce droplets in center | Gram-positive cocci with chain arrangement |
| 6 | Ga8 | Gause | white, round, chalky, powdery with irregular border | coccus with chain arrangement |
| 7 | Ga9 | Gause | white, round, rough | Gram-positive bacilli |
| 8 | Ga11 | Gause | circle, plaster and powder | coccus with chain arrangement |
| 9 | Ga13 | Gause | circle, chalk, expanding border | coccus with chain arrangement |
| 10 | Ga17 | Gause | milky and mucoid | coccobacilli |
| 11 | Ga21 | Gause | dark and scattered mucoid | diplococci |

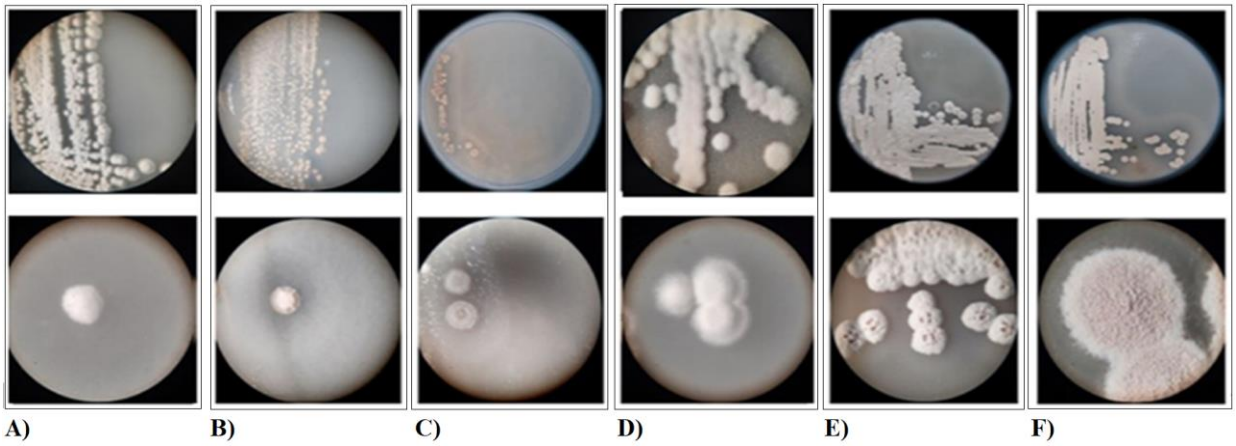


**Fig. 1.** Morphology of strain colonies: A) Ga1; B) Ga2; C) Ga3; D) Ga5; E) Ga7; F) Ga9 (with 2.5× and 40× magnification).

## Screening of lipase-producing actinobacteria

To check the lipase enzyme production ability of the six selected strains, they were cultured on the culture medium containing Tween 80 as a substrate, and the clear zone around the colonies was examined. The results indicated that among the 6 *Actinobacteria* strains isolated from the Gandom Beryan in the Lut desert, only three strains, Ga2, Ga3, and Ga7, had a clear zone around their colony (Fig. 2). The diameter of this clear zone in the Ga2 and Ga3 strains was 4 mm, and in the Ga7 strains, it was 7 mm. The presence of this zone indicated the production of lipase by these strains. On the other hand, the observation of deposition in the lipase zone indicated calcium deposition resulting from lipase activity. According to the results, the Ga7 strain was selected as the novel strain, and

further experiments were performed on this strain.

### Examination of lipase activity

After the enzyme extract was added to the specific substrate solution of para-nitrophenyl palmitate, a yellow color appeared in the environment. Its absorption was measured at 410 nm wavelength. Then the enzyme activity was checked, which was done at 12-hour intervals for 252 hours, i.e., about ten days. No lipase activity was observed until the second day (48 hours) after bacterial culture.

After 60 hours of bacterial growth, lipase activity was observed in the sample. Until the middle of the seventh day, 180 hours after bacterial culture, there was an increasing trend in lipase activity. It was found that the highest lipase activity of the Ga7 strain was observed in the sample after eight days, which was $5.4501\times10^{-9}$ µM/min. After the 8th day, a decreasing trend was observed in the lipase activity of the sample, which continued until the 10th day when the activity was measured (Fig. 3A).

### Growth curve of lipase-producing strain

To check the growth of bacteria and to determine which stage of bacterial growth produces the highest amount of lipase, bacterial growth was checked for 252 hours (10 days) at 12-hour intervals using a spectrophotometer at a wavelength of 630 nm. Bacteria did not grow until 48 hours (2 days) and in the lag phase. After two days, the bacteria entered its logarithmic phase, and its absorption increased. This increasing process continued until the sixth day (144 hours), and from the sixth to the eighth day, it was in its stationary phase.

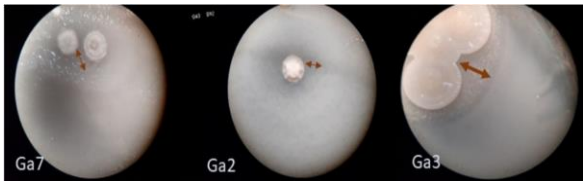


**Fig. 2.** Screening results of lipase-producing strains: The clear halo around the colonies in the Tween 80 culture medium indicates the ability to produce lipase in Ga2, Ga3, and Ga7 strains.

From the eighth day onwards, that is, 204 hours after the cultivation of the bacteria, until the last day, we saw a decrease in sample absorption, which indicated that the bacteria had entered its death phase. The highest absorbance in this study was 3.3542 nm, obtained 144 hours (6 days) after bacterial culture in Tween 80 medium and corresponds to the end of the stationary phase of the bacterial growth curve (Fig. 3B).

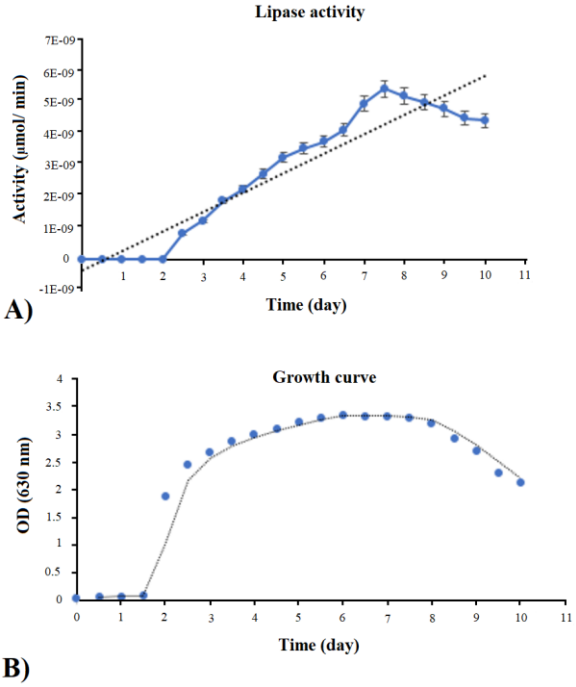


**Fig. 3.** Enzyme activity and Growth curve: A) Examining enzyme activity at 12-hour intervals during 252 hours (10 days); B) Growth curve of Ga7 strain at 12-hour time intervals during 252 hours (10 days).

### Molecular identification of novel strain

To identify the Ga7 strain, the DNA of the strain was first extracted, and then to ensure that the desired strain belonged to Actinobacteria, specific primers ACT235f and ACT878r were used. The PCR product was loaded on a 1% agarose gel, and the presence of bands with a weight of 600 bp confirmed that this strain belongs to the Actinobacteria group. 27F and 1492R general primers were used to identify the target strain. The PCR product with a size of 1400bp was sequenced, and the obtained sequence was placed in the NCBI database. Based on the sequencing results and to identify the Ga7 strain, its phylogenetic tree was

constructed using MEGA-X software (Fig. 4), which showed that the Ga7 strain belongs to *Streptomyces sp*. and is 99% similar to *Streptomyces indigenes* species.

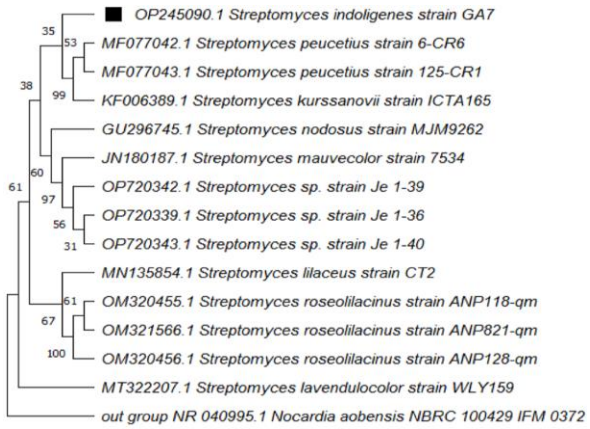


**Fig. 4.** Phylogenetic tree of isolate Ga7, based on the neighbor-joining method of 16S rRNA gene sequences. The tree topology was evaluated in 500 bootstrap iterations.

## Discussion

The industrial enzyme market has always seen steady growth in quantity and income. However, a steady increase has been observed in developing economies such as Africa, Eastern Europe, the Middle East, and Asia-Pacific. Among the necessary industrial enzymes, microbial lipases have shown great potential due to their ability to catalyze various reactions in aqueous and non-aqueous environments. The international lipase market is expected to exceed $797.7 million by 2025 (Farooq *et al*., 2022).

Soil habitats are rich sources of decomposing microorganisms, which has led to numerous studies focusing on isolating and identifying microorganisms from various soils around the world, including the desert soils of Iran. The Lut Desert is one of the main deserts of the Iranian plateau and the 25th largest desert in the world. Gandom Beryan, a naturally scorching and arid area in the Lut Desert, is one of the hottest places on earth and is located near Kerman in southeastern Iran. The temperature in Gandom Beryan, due to intense sunlight and the region's geological features, can soar above 70 °C on certain days, making it one of the hottest spots on the planet. Consequently, this area may host resilient microorganisms such as *Actinobacteria*, which are capable of producing various industrial enzymes, including stable and resistant lipases (Mohammadi *et al*., 2022).

In this research, among the 11 isolated strains from Lut desert, 6 were *Actinobacteria* strains, and three strains named Ga2, Ga3, and Ga7 were recognized as lipolytic strains by secreting lipase enzyme, which was observed by creating a clear halo around the colonies. Therefore, 27.2% of the strains could produce lipase and were compared by measuring the diameter of the lipase halo. The Ga7 strain with the largest lipase halo diameter equal to 7 mm was selected as the novel strain to evaluate the enzyme activity. Our assumption to prove the fact that the transparent aura and sediment around the colony indicates the presence of lipase enzyme is that lipase hydrolyzes Tween 80 into smaller units and fatty acid, then the calcium in the culture medium is released with fatty acid as a result of hydrolysis. It reacts, forming a calcium-fatty acid complex, which causes precipitation around the lipase-producing colonies.

A quantitative investigation of the enzyme activity of the Ga7 strain was done using the spectrophotometric method and the specific substrate para-nitrophenyl palmitate. Lipase creates ester cleavage between palmitic acid and para-nitrophenol; after this process, para-nitrophenol enters the environment and causes the yellow color, and the higher the amount of para-nitrophenol released, the more yellow color is produced, and spectrophotometric optical absorption at 410 nm goes up (Kordel *et al*., 1991; Pencreac'h and Baratti, 1996). The result of the lipase activity of the selected strain Ga7 in the optimal state was 5.45×10 -7 µmol/min. Optimal enzyme activity was obtained after 7.5 days of bacterial growth in the given medium. By examining the bacterial growth curve and measuring the absorbance of the liquid culture medium at a wavelength of 630 nm, it was found that the highest lipase activity occurs at the end of the bacterial resting phase. This indicates that bacteria secrete lipase to survive in nutrient-deficient conditions.

In 2020, Chanakul and colleagues investigated the lipase production capabilities of actinomycetes by cultivating them on a Tween-80 agar medium. Their results revealed that out of 63 actinomycete isolates, 23 exhibited lipase activity indicated by forming a clear zone in the

Tween-80 agar medium, as a sign of lipolytic activity. Among these, 23 isolates displayed clear zones with a diameter greater than 5 mm around the colonies, but only 11 isolates were capable of producing lipase with a high activity ranging from 60 to 108 U/mL. In our study, the presence of this clear lipase halo around the colony and subsequent lipase activity in the Ga7 strain was also confirmed, supporting the correlation between clear zone diameter and lipase activity. Larger clear zones typically indicate higher levels of lipase secretion, which can be attributed to the amount of enzyme produced and its efficiency in hydrolyzing the substrate. For this reason, we considered the strain Ga7 as the novel strain (Panyachanakul *et al*., 2020).

In a Dhavala Swarna and J. Joel Gnanadoss study, 25 *actinobacteria strains* were isolated from forest soil, and their enzymatic activities were evaluated. Meanwhile, five strains exhibited the highest lipase activity when cultured on an appropriate agar medium. These isolates were subsequently selected for quantitative analysis. The production of extracellular enzymes by the top-performing strains was measured at regular intervals over 12 days, with maximum enzyme production observed on the eighth day, showing lipase activity ranging from 30 to 60 U/mL. Similarly, our study observed optimal enzyme production by the novel strain in the middle of the seventh day (Swarna & Gnanadoss, 2020).

In 2023, Jassim and Jarallah isolated actinomycetes from various soils in the Hilla province of Iraq and analyzed their enzymatic activities. To assess the lipase activity of the strains, they used peptone-tween agar (PTA) plates, where the formation of turbid, milky precipitates around the colonies indicated lipase activity. Six strains were found to be capable of producing lipase. Similarly, in our study, three strains produced these precipitates around the colonies, and the novel strain, which exhibited the largest lipase clear zone, was further quantitatively analyzed for lipase production (Jassim & Jarallah, 2023). another study conducted by Mahadik et al. *Aspergillus niger strain* was reported with a lipase activity of $3.8\times10^{-7}$ µmol/min and a clear halo diameter of 6 mm. (Mahadik *et al*., 2002). In the work of Gupta *et al*., (2003) it is reported that the *Bacillus subtilis* strain had a lipase activity of 3.5 $\times10^{-7}$ µmol/min and a clear halo diameter of 6 mm. In comparison, our isolated Ga7 strain showed better performance in terms of lipase activity and clear halo diameter, which can be due to the specific environmental conditions of the Lut desert and the adaptation of microorganisms in this area to harsh environmental conditions (Gupta *et al*., 2003).

## Conclusion

Due to the biodiversity and special ecological conditions of the Lut desert, this area has great potential to discover microorganisms that produce industrial enzymes. Because of its high lipase activity, the Ga7 strain can be used as a potential source for industrial lipase production. Future research should focus on optimizing this strain's cultivation conditions and enzyme production and identifying more biodiversity of Lut desert microorganisms. The findings of this research highlight the importance of investigating extreme environments such as the Lut desert as valuable sources of industrial biocatalysts.

## Conflict of interests

The authors declare no conflict of interest.

## References

Abdelmoez, W., Mostafa, N., & Mustafa, A. (2013). Utilization of oleochemical industry residues as substrates for lipase production for enzymatic sunflower oil hydrolysis. *Journal of Cleaner Production*, 59, 290-29. https://doi.org/10.1016/j.jclepro.2013.06.032

Anderson, A. S., & Wellington, E. M. (2001). The taxonomy of *Streptomyces* and related genera. *International Journal of Systematic and Evolutionary Microbiology*, 51(3), 797-814. https://doi.org/10.1099/00207713-51-3-797

Arpigny, J. L., & Jaeger, K.E. (1999). Bacterial lipolytic enzymes: classification and properties. *Biochemical Journal*, 343(1), 177-183. https://portlandpress.com/biochemj

Borrelli, G. M., & Trono, D. (2015). Recombinant lipases and phospholipases and their use as biocatalysts for industrial applications. *International Journal of*

*Molecular Sciences*, 16(9), 20774-20840. https://doi.org/10.3390/ijms160920774

Bredholt, H., Fjærvik, E., Johnsen, G., & Zotchev, S. B. (2008). Actinomycetes from sediments in the Trondheim fjord, Norway: diversity and biological activity. *Marine Drugs*, 6(1), 12-24. https://www.mdpi.com/journal/marinedrugs

Carpen, A., Bonomi, F., Iametti, S., & Marengo, M. (2019). Effects of starch addition on the activity and specificity of food-grade lipases. *Biotechnology and Applied Biochemistry*, 66(4), 607-616. https://doi.org/10.1002/bab.1761

Chandra, P., Enespa, Singh, R., & Arora, P. K. (2020). Microbial lipases and their industrial applications: a comprehensive review. *Microbial Cell Factories*, 19, 1-42. https://doi.org/10.1186/s12934-020-01428-8

Colla, L. M., Rizzardi, J., Pinto, M. H., Reinehr, C. O., Bertolin, T. E., & Costa, J. A. V. (2010). Simultaneous production of lipases and biosurfactants by submerged and solid-state bioprocesses. *Bioresource Technology*, 101(21), 8308-8314. https://doi.org/10.1016/j.biortech.2010.05.086

de Souza Vandenberghe, L. P., Karp, S. G., Pagnoncelli, M. G. B., von Linsingen Tavares, M., Junior, N. L., Diestra, K. V., Viesser, J. A., & Soccol, C. R. (2020). Classification of enzymes and catalytic properties. In *Biomass, Biofuels, Biochemicals* (pp. 11-30). Elsevier . http://dx.doi.org/10.1016/B978-0-12-819820-9.00002-8

Dharmsthiti, S., & Kuhasuntisuk, B. (1998). Lipase from *Pseudomonas aeruginosa* LP602: biochemical properties and application for wastewater treatment. *Journal of Industrial Microbiology and Biotechnology*, 21(1-2), 75-80. https://doi.org/10.1038/sj.jim.2900563

Farooq, S., Ganai, S. A., Ganai, B. A., Mohan, S., Uqab, B., & Nazir, R. (2022). Molecular characterization of lipase from a psychrotrophic bacterium *Pseudomonas* sp. CRBC14. *Current Genetics*, 68(2), 243-251.. https://doi.org/10.1007/s00294-021-01224-w

Fatima, S ,.Faryad, A., Ataa, A., Joyia, F. A., & Parvaiz, A. (2021). Microbial lipase production: A deep insight into the recent advances of lipase production and purification techniques. *Biotechnology and Applied Biochemistry*, 68(3), 445-458. http://dx.doi.org/10.1002/bab.2019

Gupta, R., Gupta, N., & Rathi, P. J. A. M. (2004). Bacterial lipases: an overview of production, purification and biochemical properties. *Applied Microbiology and Biotechnology*, 64, 763-781. https://doi.org/10.1007/s00253-004-1568-8

Gupta, R., Rathi, P., Gupta, N., & Bradoo, S. (2003). Lipase assays for conventional and molecular screening: an overview. *Biotechnology and Applied Biochemistry*, 37(1), 63-71. https://doi.org/10.1042/ba20020059

Hari Krishna, S., & Karanth, N. G. (2002). Lipases and lipase-catalyzed esterification reactions in nonaqueous media. *Catalysis Reviews*, 44(4), 499-591. http://dx.doi.org/10.1081/CR-120015481

Hasan-Beikdashti, M., Forootanfar, H., Safiarian, M. S., Ameri, A., Ghahremani, M. H., Khoshayand, M. R., & Faramarzi, M. A. (2012). Optimization of culture conditions for production of lipase by a newly isolated bacterium *Stenotrophomonas maltophilia*. *Journal of the Taiwan Institute of Chemical Engineers*, 43(5), 670-677. https://doi.org/10.1016/j.jtice.2012.03.005

Hasan, F., Shah, A. A., & Hameed, A. (2006). Industrial applications of microbial lipases. *Enzyme and Microbial Technology*, *39*(2), 235-251. https://doi.org/10.1016/j.enzmictec.2005.10.016

Akoh, C. C., & Xu, X. (2002). Enzymatic production of Betapol and other specialty fats. *In Lipid biotechnology* (1st ed., pp. 18). CRC.

Ilesanmi, O. I., Adekunle, A. E., Omolaiye, J. A., Olorode, E. M., & Ogunkanmi, A. L. (2020). Isolation, optimization and molecular characterization of lipase producing bacteria from contaminated soil. *Scientific African*, 8, e00279. https://doi.org/10.1016/j.sciaf.2020.e00279

Irshad, G., Naz, F., Ghuffar, S., Khalid, A. R., Arif, S., Maqsood, A., Raja, M. U., & Din, R. U. (2023). Molecular studies of postharvest fungal peach fruit rots: *Fusarium sporotrichioides, Aspergillus niger, Aspergillus flavus, Penicillium chrysogenum*, and *Cladosporium pseudocladosporioides*. *Pakistan Journal of Phytopathology*, 35(2),

349-356. https://doi.org/10.33866/PHYTOPATHOL.035.02.0910

Jassim, Y. A., & Jarallah, E. M. (2023). Screening for antimicrobial activities and enzymatic activities production of some actinomycetes spp. isolated from different soil samples from Hilla Province. *British Journal of Multidisciplinary and Advanced Studies*, 4(4), 17-25. https://doi.org/10.37745/bjmas.2022.02533

Joshi, R., & Kuila, A. (2018). Lipase and their different industrial applications: A review. *Brazilian Journal of Biological Sciences*, 5(10), 237-247. http://dx.doi.org/10.21472/bobs.051004

Kirana, S., Arshada, Z., Nosheenb, S., Kamala, S., Gulzara, T., Majeeda, M. S., Jannata, M., & Rafiquec, M. A. (2016). Microbial lipases: production and applications: a review. *Journal of Biochemistry, Biotechnology and Biomaterials*, 1(2), 7-20.

Kordel, M., Hofmann, B., Schomburg, D., & Schmid, R. D. (1991). Extracellular lipase of *Pseudomonas* sp. strain ATCC 21808: purification, characterization, crystallization, and preliminary X-ray diffraction data. *Journal of Bacteriology*, 173(15), 4836-4841. https://doi.org/10.1128/jb.173.15.4836-4841.1991

Lee, D.-W., Koh, Y.-S., Kim, K.-J., Kim, B.-C., Choi, H.-J., Kim, D.-S., Suhartono, M. T., & Pyun, Y.-R. (1999). Isolation and characterization of a thermophilic lipase from *Bacillus thermoleovorans* ID-1. *FEMS Microbiology Letters*, 179(2), 393-400. https://doi.org/10.1111/j.1574-6968.1999.tb08754.x

Mahadik, N. D., Puntambekar, U. S., Bastawde, K. B., Khire, J. M., & Gokhale, D. V. (2002). Production of acidic lipase by *Aspergillus niger* in solid state fermentation. *Process Biochemistry*, 38(5), 715-721. https://doi.org/10.1016/S0032-9592(02)00194-2

Mohammadi, M., Khaleghi, M., Shakeri, S., Hesni, M. A., Samandari-Bahraseman, M. R., & Dalvand, A. (2022). Isolation of Actinobacteria strains from environmental samples and assessment of their bioactivity. *Avicenna Journal of Clinical Microbiology and Infection*, 9(1), 8-15. http://dx.doi.org/10.34172/ajcmi.2022.03

Narayanan, P. (2007). Environmental pollution: Principles, analysis and control. CBS Publishers & Distributors.

Naveed, M., Nadeem, F., Mehmood, T., Bilal, M., Anwar, Z & ,.Amjad, F. (2021). Protease-a versatile and eco-friendly biocatalyst with multi-industrial applications: an updated review. *Catalysis Letters*, 151, 307-323. https://link.springer.com/article/10.1007/s10562-020-03316-7

Panyachanakul, T., Lomthong, T., Lorliam, W., Prajanbarn, J., Tokuyama, S., Kitpreechavanich, V & ,.Krajangsang, S. (2020). New insight into thermo-solvent tolerant lipase produced by Streptomyces sp. A3301 for re-polymerization of poly (DL-lactic acid). *Polymer*, 204, 122812. http://dx.doi.org/10.1016/j.polymer.2020.122812

Patel, G. B., Shah, K. R., Shindhal, T., Rakholiya, P., & Varjani, S. (2021). Process parameter studies by central composite design of response surface methodology for lipase activity of newly obtained Actinomycete. *Environmental Technology and Innovation*, 23, 101724. https://doi.org/10.1016/j.eti.2021.101724

Pencreac'h, G., & Baratti, J. C. (1996). Hydrolysis of p-nitrophenyl palmitate in n-heptane by the *Pseudomonas cepacia* lipase: a simple test for the determination of lipase activity in organic media. *Enzyme and Microbial Technology*, 18(6), 417-422. https://doi.org/10.1016/0141-0229(95)00120-4

Ray, A. (2012). Application of lipase in industry. *Asian Journal of Pharmacy and Technology*, *2*(2), 33-37 . https://doi.org/10.5958/2231–5713

Robinson, P. K. (2015). Enzymes: principles and biotechnological applications. *Essays in Biochemistry*, 59, 1- 14. https://doi.org/10.1042/bse0590001

Sarmah, N., Revathi, D., Sheelu, G., Yamuna Rani, K., Sridhar, S., Mehtab, V., & Sumana, C. (2018). Recent advances on sources and industrial applications of lipases. *Biotechnology Progress*, 34(1), 5-28. https://doi.org/10.1002/btpr.2581

Satari Faghihi, L., Seyedalipour, B., Riazi, G., & Ahmady-Asbchin, S. (2018). Introduction of two halo-alkali-thermo-stable biocatalysts: Purification and characterization. *Catalysis Letters*, 148(3), 831-842. https://doi.org/10.1007/s10562-018-2295-6

Schrag, J. D., Li, Y., Wu, S., & Cygler, M. (1991). Ser-His-Glu triad forms the catalytic site of the lipase from *Geotrichum candidum*. *Nature*, 351(6329), 761-764. https://doi.org/10.1038/351761a0

Sharma, R., Chisti, Y., & Banerjee, U. C. (2001). Production, purification, characterization, and applications of lipases. *Biotechnology Advances*, 19(8), 627-662. https://doi.org/10.1016/s0734-9750(01)00086-6

Singh, L. S., Baruah, I., & Bora, T. C. (2006). Actinomycetes of Loktak habitat: isolation and screening for antimicrobial activities. *Biotechnology*, 5(2), 217-221. https://doi.org/10.3923/biotech.2006.217.221

Swarna, D., & Gnanadoss, J. J. (2020). Screening and molecular characterization of actinomycetes from mangrove soil producing industrially important enzymes. *Journal of Scientific Research*, 64(2), 87-95. https://doi.org/10.37398/JSR.2020.640211

Thomson, C. A., Delaquis, P. J., & Mazza, G. (1999). Detection and measurement of microbial lipase activity: a review. *Critical Reviews in Food Science and Nutrition*, 39(2), 165-187. https://doi.org/10.1080/10408399908500492

Tong, X., Busk, P. K., & Lange, L. (2016). Characterization of a new sn-1, 3-regioselective triacylglycerol lipase from *Malbranchea cinnamomea*. *Biotechnology and Applied Biochemistry*, 63(4), 471-478. https://doi.org/10.1002/bab.1394.