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Original research

Isolation and Genetic Identification of Lipase Producing Bacteria from Oil-Contaminated Sites

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ABSTRACT

Lipase-producing bacteria can be isolated from various environments, including industrial and agricultural waste, vegetable oils, dairy factories, and oil-contaminated soil. Lipase is the third most important enzyme in biotechnology due to its broad catalytic properties and ability to function in heterogeneous media. The current study aims to isolate, screen, and determine the prevalence of lipase-producing bacteria from various oil-contaminated soils in Basrah province, Iraq. Eleven soil samples and five water samples were collected from various oil-contaminated sites. Identification of lipase-producing bacteria was performed using the *16S rDNA* sequencing technique. A total of fifty-one lipase-producing bacterial isolates were identified. Seven isolates exhibited high efficiency in lipase enzyme production, including A5, A1, A3, J3, A4, A2, and G3, with lipase activity values of 49 U/mL, 28 U/mL, 24 U/mL, 23 U/mL, 23 U/mL, 20 U/mL, and 20 U/mL, respectively. The isolate A5 was the most promising, exhibiting the highest activity at 49 U/mL. Based on the sequencing of the *16S rDNA* gene, these seven isolates were identified as *Bacillus subtilis* strain QD517, *Bacillus velezensis* strain Bac104, *Bacillus subtilis* strain PK9, *Enterobacter cloacae* strain YY-2, *Bacillus cereus* strain RB1, *Lysinibacillus xylanilyticus* strain D, and *Brevibacillus borstelensis* strain LDH-b. Seven lipase-producing bacterial isolates were characterized as new bacterial strains, and their sequences were registered in the NCBI GenBank database. The production of large quantities of the lipase enzyme requires optimization of culture conditions using various factors to enable applications in multiple fields.

INTRODUCTION

Pollution of the environment due to pipeline leakage, spillage, and oil disruption is common and represents a major environmental issue for communities, environmentalists, and governments (Ikuesan et al. 2017, Almansoory et al. 2019).

The ecosystem suffers considerable damage and alterations as a result of oil spills. Hydrocarbon (HC) toxicity to organisms and soil is a significant global concern today. The effects of this pollution can lead to changes in soil physicochemical and microbiological properties, resulting in soil infertility or stunted and delayed plant growth, or both. Additionally, both surface and underground water can become contaminated, posing risks to human health (Abdul-Ameer Ali et al. 2019).

The natural environment can manage minor oil spills, but only over an extended period. Consequently, there are still negative impacts on various components of the environment before it can fully regain its integrity. This has prompted scientists and environmentalists to search for effective methods to restore oil-polluted sites. The most common technologies and strategies for oil containment and removal from water include floating devices, barriers, oil collection devices, collection vessels, absorbent materials, chemical dispersants, surfactants, physical degradation, biological breakdown, and onsite burning. Cleanup efforts typically employ mechanical or physical methods (sorbents, booms, and skimmers), chemical treatments, and biological techniques (Pete et al. 2021).

Biological techniques for oil spill remediation have generated interest in the microbiological study of oil pollution in the environment. This trend encompasses several areas, including the effects of oil spills on microbial populations, the microbial degradation and biodeterioration of crude oil and petroleum products, bioremediation of oil spills, and the production of useful products by crude oil-degrading microorganisms. Notable products include biosurfactants and enzymes (Auta et al. 2022). The demand for these enzymes is rising across various industries due to their numerous advantages, such as minimal environmental toxicity (Thabet et al. 2023).

Most microorganisms enzymatically attack pollutants, converting them into safe molecules. Biodegradation involves treating petroleum pollutants with aliphatic-degrading microorganisms that possess various enzymes, notably lipases (Aransiola et al. 2022). Lipases are among the most valuable biotechnological enzymes, produced by microorganisms (bacteria and fungi), plants, and animals. However, microbial lipases especially those from bacteria are more effective than their plant and animal counterparts (Thabet et al. 2023). These microorganisms are primary sources of lipolytic enzymes, making them relatively low-cost, viable, and efficient at breaking down pollutants in a short time (Abubakar et al. 2024). Additionally, microbial enzymes tend to be more stable than those from plants and animals, and their production is more convenient and safer, enhancing their importance for commercial applications. The oily environments of vegetable oil-processing factories, industrial waste sites, soil contaminated with oil, and diesel-fuel-polluted soil provide suitable habitats for lipase-producing bacteria (Alhebshi et al. 2023). Various bacterial strains isolated from these environments have shown the ability to produce lipase. The most notable genera known for their lipase production include *Acinetobacter, Bacillus, Burkholderia, Pseudomonas, Staphylococcus, Microbacterium, Lactobacillus, Serratia, Aeromonas, Arthrobacter, Stenotrophomonas*, and *Thermosyntropha* (Gururaj et al. 2016, Sethi et al. 2023).

Bacteria generally produce lipase enzymes in small quantities under natural environmental conditions. However, this production can be increased by altering nutritional sources, such as carbon and nitrogen, as well as cultivation conditions like temperature, pH, and inoculum size. These adjustments help identify the most suitable variables for enhancing bacterial growth and enzyme production. While bioremediation typically occurs in an aerobic environment, it can also take place under anaerobic conditions (Abbas et al. 2020, Alyousif et al. 2022). The study aims to investigate the diversity of lipase-producing bacteria in oil-contaminated environments in Basrah province, Iraq, and identifies species using molecular tools for potential biotechnological applications.

MATERIALS AND METHODS

Collection of Samples

Eleven soil and five water samples contaminated with hydrocarbons and oil waste were collected from several areas in Basrah province, Iraq (Table 1 and Fig. 1). The soil samples were collected at a depth of 5-10 cm below the surface. Approximately 100 g of soil was taken using a sterile laboratory spatula and placed in sterile, clean plastic containers. At the same time, 500 ml of water samples were collected in sterile, clean plastic containers. The samples were transported to the laboratory in the Department of Ecology, College of Science, University of Basrah, and were kept in a refrigerator at 4 °C until laboratory analysis could be conducted.

Table 1. The sites and types of collected samples.

Type of samples	No. of Samples	Site of Samples	Samples code	**Latitude (N)**	**Longitude (E)**
Soil	2	Basrah Center	A	30˚ 30' 0 "N	47˚ 47'0"E
			L	30˚ 30' 0 "N	47˚ 47'0"E
Soil	5	Khor Al-Zubair	B	30˚ 13' 0 "N	47˚ 50'0"E
	.		C	30˚ 16' 0 "N	47˚ 46'0"E
	.		F	30˚ 13' 0 "N	47˚ 41'0"E
	.		G	30˚ 15' 0 "N	47˚ 42'0"E
	.		M	30˚ 18' 0 "N	47˚ 42'0"E
Water	4	Khor Al-Zubair	N	30˚ 13' 0 "N	47˚ 46'0"E
	.		O	30˚ 15' 0 "N	47˚ 46'0"E
	.		P	30˚ 13' 0 "N	47˚ 46'0"E
	.		Q	30˚ 11' 0 "N	47˚ 50'0"E
Soil	1	Al-Najebia	D	30˚ 35' 0 "N	47˚ 47'0"E
Water	1		E	30˚ 13' 0 "N	47˚ 42'0"E
Soil	2	Khums mile	H	30˚ 29' 0 "N	47˚ 50'0"E
			J	30˚ 31' 0 "N	47˚ 47'0"E

Soil	1	AL-Rumila	K	30˚ 31' 0 "N	47˚ 18'0"E

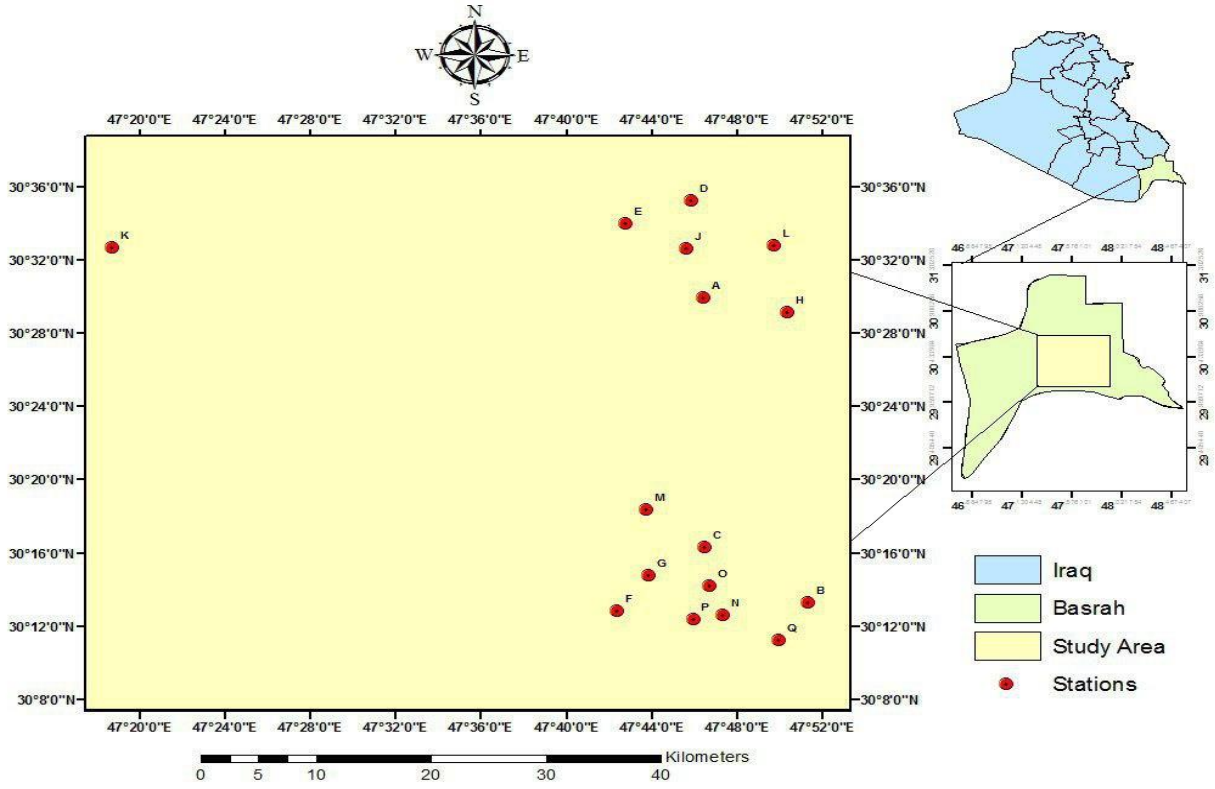


Figure 1. The map of study areas in Al-Basrah Province, Iraq.

Isolation of lipase-producing bacteria

One gram of oil-contaminated soil and 1 ml of oil-contaminated water were added to tubes containing 9 ml of distilled water separately and mixed well by vortex. A series of dilutions was performed, ranging from 10^{-1} to 10^{-6}. Next, 0.1 ml of each dilution was pipetted and spread onto a petri dish containing Tween 80 agar medium using an L-shaped spreader. The plates were then incubated at 37 °C for 24 hrs. Bacterial colonies that showed the establishment of a clear zone surrounding them were noted as positive (Muhsin et al. 2016).

Screening of lipase-producing bacteria

The positive isolates were purified on nutrient agar medium and activated in 5 mL of nutrient broth, for 24 h. at 37 °C. Then, 2.5 mL of nutrient broth was added to 100 mL of mineral salt medium (MSM) containing NaCl (0.25 g/L), peptone (0.2 g/L), $NH_4H_2PO_4$ (0.1 g/L), $MgSO_4 \cdot 7H_2O$ (0.04 g/L), $CaCl_2 \cdot 2H_2O$ (0.04 g/L), Tween (1-2 drops), and olive oil (2 mL). The mixture was then incubated for 3-5 days at 37 °C (Mobarak-Qamsari et al. 2011).

Lipase enzyme assay

Lipase activity was estimated using a mixture of 2 ml of phosphate buffer (pH 10), prepared by dissolving 1 g of phosphate buffer in 100 ml of distilled water, 4 ml of olive oil, 2.5 ml of distilled water, 0.5 ml of $CaCl_2$ (2.19 g of $CaCl_2$ in 100 ml of distilled water), and 1 mL of diluted lipase enzyme. The mixture was incubated in a shaker at 27 °C for 15 min. After incubation, 1 ml of 96% ethanol and 2-3 drops of phenolphthalein (0.5 g in 50 ml of ethanol) were added. The mixture was then titrated with NaOH (0.2 g in 100 ml of distilled water) from a burette, gradually adding the base until the color changed to light pink. A control sample was prepared without the diluted lipase enzyme, and the reaction was stopped by adding NaOH (Rasmey et al. 2017). The amounts of fatty acids liberated in each sample was calculated based on the volume of NaOH used to reach the titration endpoint by following equation:

μmol fatty acid/ml subsample (U) = [(ml NaOH for sample − ml NaOH

for blank) × N × 1000] / 5 ml

U = μmol of fatty acid released/ml

N = The normality of the NaOH titrant used

Identification of lipase-producing bacteria by 16S rDNA gene Sequencing

The isolated lipase-producing bacteria were identified via sequencing and analysis of the *16S rDNA* gene. Bacterial DNA was extracted using the Presto TM Mini gDNA bacteria kit (Geneaid, Taiwan). The *16S rDNA* gene was amplified with universal primers 27F and 1492R (Miyoshi et al. 2005). The PCR mixture was prepared in a total volume of 25 μL, which included 12.5 μL of master mix (Promega, USA), 2 μL of DNA, 1 μL each of forward and reverse primers, and 9.5 μL of nuclease-free water. The PCR program for amplifying the *16S rDNA* gene consisted of an initial denaturation at 96 °C for 3 min, followed by 27 cycles of 96 °C for 30 seconds, primer annealing at 56 °C for 25 seconds, extension at 72 °C for 15 seconds, and a final extension at 72 °C for 10 min.

Sequencing the PCR products of 16S rDNA gene

The PCR products of the *16S rDNA* gene were sent to Macrogen company in South Korea for purification and sequencing. The obtained *16S rDNA* gene sequences were proofread using Chromas and aligned with nucleotide sequence databases from NCBI using BLAST tools http://www.ncbi.nlm nih.gov to identify and assess the sequence homology of the lipase-producing bacterial isolates. The phylogenetic tree was generated using MEGA X, which aligned the sequences using the Clustal W program included in the software (Kumar et al. 2018).

Data analysis

The lipase assays were done in duplicate the average of duplicate determination was used to represent the result of lipase assay for all bacterial isolates.

RESULTS

Isolation of Lipase-Producing Bacteria

Sixteen samples collected from different sites were used in the present study to isolate lipase-producing bacteria. The results showed that 51 bacterial isolates were able to produce the lipase enzyme. Gram staining indicated that most of the isolated bacterial species were Gram-positive, 42 (82.35%), while 9 (17.65%) were Gram-negative, as shown in (Table 2).The occurrence and distribution of lipase-producing bacteria in each sample were reported as 7 bacterial isolates from sample A, 4 isolates from sample B, 8 isolates from sample C, 5 isolates from sample D, 4 isolates from sample E, 1 isolate from sample F, 5 isolates from sample G, 2 isolates from sample H, 3 isolates from sample J, 2 isolates from sample K, 1 isolate from sample L, 3 isolates from sample M, 3 isolates from sample N, 1 isolate from sample O, 2 isolates from sample P, and 1 isolate from sample Q, as listed in (Table 2).

Table 2. Gram's staining characteristics of lipase-producing bacterial isolates.

Samples	Isolates code	Gram's staining	Percentage of Gr+ve	Percentage of Gr-ve
A	A1	Gr+ve	100%	0%
	A2	Gr+ve		
	A3	Gr+ve		
	A4	Gr+ve		
	A5	Gr+ve		
	A6	Gr+ve		
	A7	Gr+ve		
B	B1	Gr+ve	100%	0%
	B2	Gr+ve		
	B3	Gr+ve		
	B4	Gr+ve		
C	C1	Gr-ve	50%	50%
	C2	Gr-ve		
	C3	Gr+ve		
	C4	Gr-ve		
	C5	Gr-ve		
	C6	Gr+ve		
	C7	Gr+ve		
	C8	Gr+ve		
D	D1	Gr+ve	100%	0%
	D2	Gr+ve		
	D3	Gr+ve		
	D4	Gr+ve		
E	E1	Gr+ve	100%	0%
	E2	Gr+ve		
	E3	Gr+ve		
	E4	Gr+ve		
F	F1	Gr+ve	100%	0%
G	G1	Gr+ve	100%	0%
	G2	Gr+ve		
	G3	Gr+ve		
	G4	Gr+ve		
	G5	Gr+ve		
H	H1	Gr+ve	100%	0%
	H2	Gr+ve		
J	J1	Gr-ve	0%	100%
	J2	Gr-ve		
	J3	Gr-ve		
K	K1	Gr+ve	100%	0%
	K2	Gr+ve		
L	L1	Gr+ve	100%	0%

M	M1	Gr+ve	66.66%	33.33%
	M2	Gr+ve		
	M3	Gr-ve		
N	N1	Gr+ve	100%	0%
	N2	Gr+ve		
	N3	Gr+ve		
O	O1	Gr+ve	100%	0%
P	P1	Gr-ve	50%	50%
	P2	Gr+ve		
Q	Q1	Gr+ve	100%	0%

Screening of Lipase-Producing Bacteria

Bacterial isolates displayed varying abilities to produce lipase enzymes. The isolates that yielded positive results in the preliminary screening underwent further evaluation to assess their lipase production using mineral salt medium (MSM), with olive oil as the sole carbon source and peptone as the sole nitrogen source (Fig. 2). These isolates demonstrated different lipase activity when tested with phosphate buffer, ranging from 49 U/mL to 3 U/mL. Seven isolates A5, A1, A3, J3, A4, A2, and G3 emerged as the most promising lipase enzyme producers, exhibiting the highest lipase activities of 49 U/mL, 28 U/mL, 24 U/mL, 23 U/mL, 23 U/mL, 20 U/mL, and 20 U/mL, respectively, out of the 51 bacterial isolates that tested positive for lipase activity. Among these, isolate A5 was identified as the most promising lipase producer with an activity of 49 U/mL, while isolate D3 displayed the lowest activity at 3 U/mL, as shown in (Table 3).

Table 3. Screening of bacterial isolates for the production of lipase enzyme.

Samples	Isolates code	Lipase assay (U/mL) *
A	A1	28
	A2	20
	A3	24
	A4	23
	A5	49
	A6	24
	A7	5
B	B1	3.5
	B2	4.5
	B3	6
	B4	4.5
C	C1	13
	C2	17
	C3	7
	C4	9
	C5	7
	C6	11
	C7	12
	C8	18
D	D1	8
	D2	10
	D3	3
	D4	16
	D5	18
E	E1	11
	E2	15
	E3	10
	E4	15

F	F1	17
G	G1 G2 G3 G4 G5	18 16 20 20 11
H	H1 H2	12 15
J	J1 J2 J3	12 12 23
K	K1 K2	16 14
L	L1	9
M	M1 M2	11 8
	M3	9
N	N1 N2 N3	14 12 10
O	O1	12
P	P1 P2	10 7
Q	Q1	10

* Mean ± SD, n=2

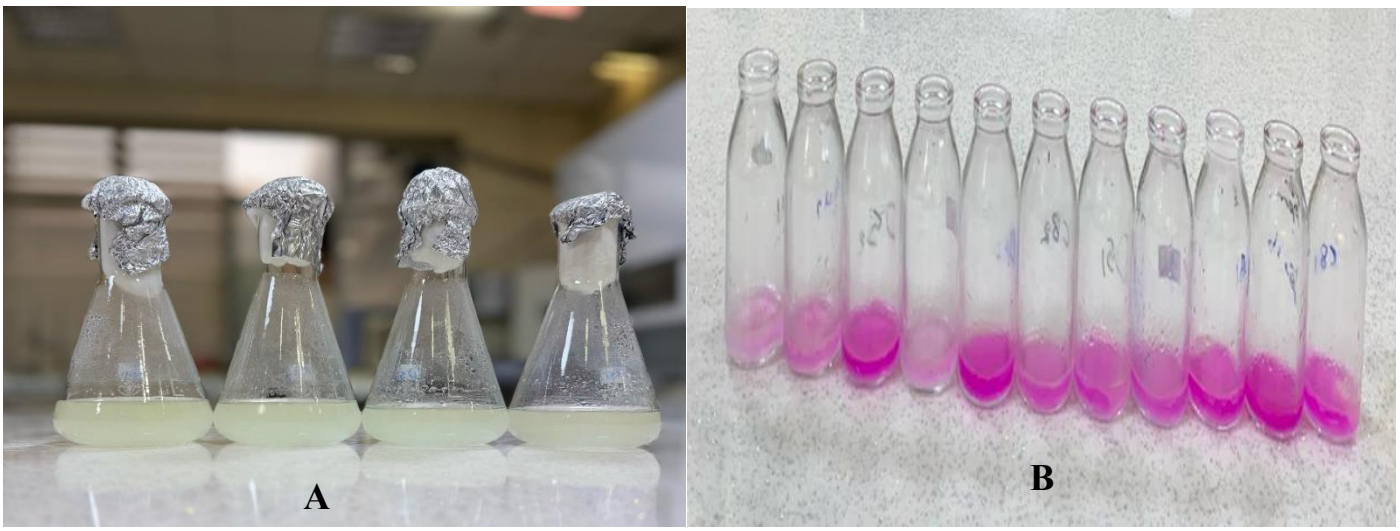
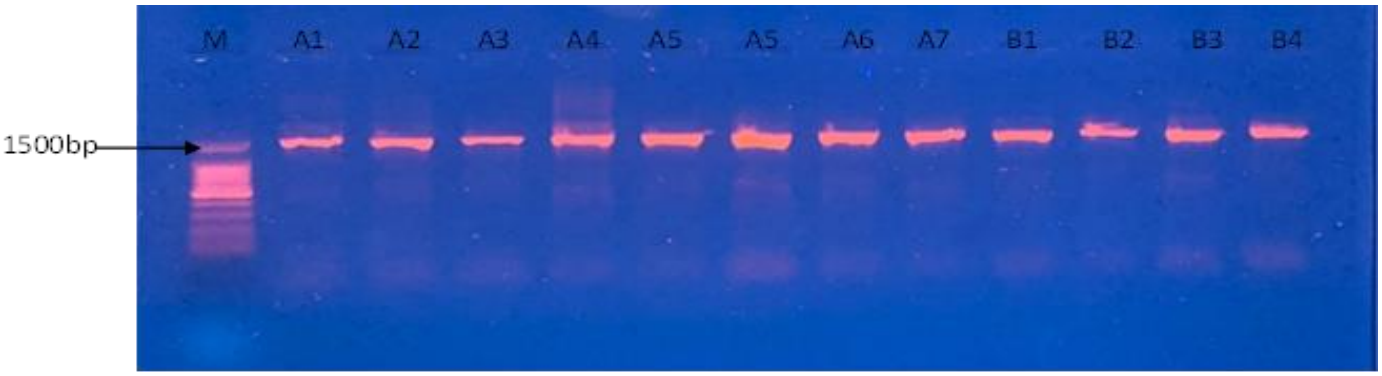


Figure 2. Screening of bacterial isolates for the production of lipase enzyme, A: flask containing MSM with bacterial isolate**, B:** Lipase enzyme assay by titration method**.**

Characterization of Lipase-Producing Bacteria by *16S rDNA* Gene Sequencing

The *16S rDNA* gene of all bacterial isolates was amplified using the PCR technique, and the results were visualized by electrophoresis under a UV transilluminator, showing a band size of nearly 1500 bp when compared to the DNA ladder, as illustrated in Fig. 3. The bacterial isolates were identified through *16S rDNA* gene sequencing and analysis. Molecular identification and sequencing of the *16S rDNA* genes were conducted using the NCBI BLAST tool to characterize the



lipase-producing bacterial isolates by comparing the obtained sequences with those in the NCBI database. The identification of the lipase-producing bacterial isolates was confirmed through sequencing and analysis of the *16S rDNA* gene.

Figure 3. Agarose gel electrophoresis for PCR products of *16S rDNA* gene (1500 bp) for lipase-producing bacteria. lane M: DNA ladder, lane A1-B4: Bacterial isolates.

Most lipase-producing bacterial isolates in the present study belong to spore-forming, Gram-positive bacilli. The results of the *16S rDNA* gene sequences of these isolates indicate that, at the genus level, they belong to *Bacillus* (34 isolates), *Brevibacillus* (3 isolates), and 2 isolates each of *Lysinibacillus, Achromobacter, Stutzerimonas, Enterobacter,* and *Exiguobacterium*. Additionally, 1 isolate belongs to each of *Staphylococcus, Cronobacter, Pigmentiphage,* and *Acinetobacter*. The most prevalent bacterial species among the samples were *Bacillus subtilis*, *Bacillus amyloliquefaciens*, and *Bacillus cereus* (Table 4, Fig. 4). The phylogenetic tree was constructed using partial *16S rDNA* sequences from lipase-producing bacterial isolates obtained in the current study to determine their relationships and evolutionary connections. The analysis of phylogenetic tree revealed bacterial lipase producing isolates were divided into two main groups (Fig. 5), one group represented Gram-positive bacteria, predominantly spore-forming bacteria, which included several subgroups Based on the degree of closeness between the isolates, regardless of their source, and the other group consisted of Gram-negative bacteria, which also appeared in subgroups that which show the extent of similarity between these isolates. These subgroups indicate the degree of similarity among bacterial isolates, regardless of their types or source of isolation.

Table 4. Characterization of lipase-producing bacteria by *16S rDNA* gene sequencing, isolates code and similarity percentage.

Isolates code	Closet species	Accession no.	identity %
A1	*Bacillus velezensis* strain Bac104	PQ813838.1	100
A2	*Lysinibacillus xylanilyticus strain D*	KF923754.1	100
A3	*Bacillus subtilis strain PK9*	PQ289147.1	100
A4	*Bacillus cereus strain RB1*	MK418365.1	100
A5	*Bacillus subtilis strain QD517*	EF472261.1	100
A6	*Bacillus amyloliquefaciens strain IRHB18*	OP364585.1	100
A7	*Bacillus subtilis strain QD517*	EF472261.1	100
B1	*Bacillus licheniformis strain HNXS-W29*	PP092139.1	100
B2	*Staphylococcus warneri strain HBUM06952*	MF662224.1	100
B3	*Bacillus amyloliquefaciens*	KU143924.1	100
B4	*Bacillus nakamurai strain PGPR corkoak AJ34*	MZ700084.1	99.92
C1	*Cronobacter sakazakii strain KYU46*	GU227669.1	100
C2	*Achromobacter spanius strain 22STR569*	OR831916.1	100

C3	*Bacillus cereus strain LT419*	PQ782563.1	100
C4	*Achromobacter marplatensis strain KLK37*	MT634501.1	100
C5	*Pigmentiphaga daeguensis strain PT-16*	HQ848120.1	98.30
C6	*Lysinibacillus macroides strain CRDT-EB-7.2*	MN192407.1	100
C7	*Bacillus subtilis strain Bs31*	OQ423166.1	100
C8	*Bacillus subtilis strain SPA N1*	JQ308575.1	100
D1	*Bacillus subtilis strain MA139*	DQ415893.3	100
D2	*Bacillus badius strain M9-20*	KT382256.1	100
D3	*Bacillus amyloliquefaciens subsp. plantarum strain Hk8-20*	JF899260.1	99.18
D4	*Bacillus velezensis strain FJ23*	OR551414.1	99.93
E1	*Bacillus amyloliquefaciens strain MX66*	OP753626.1	100
E2	*Bacillus amyloliquefaciens strain Ba09*	MT250917.1	100
E3	*Bacillus amyloliquefaciens strain MX66*	OP753626.1	100
E4	*Bacillus encimensis strain SGD-V-25*	KF413433.2	100
F1	*Bacillus amyloliquefaciens strain MX66*	OP753626.1	100
G1	*Bacillus subtilis strain HY-34*	MZ895403.1	100
G2	*Brevibacillus borstelensis strain UTM105*	KF952566.1	100
G3	*Brevibacillus borstelensis strain LDH-b*	MW686864.1	100
G4	*Brevibacillus borstelensis strain UTM105*	KF952566.1	100
G5	*Bacillus subtilis strain LE24*	MG980567.1	100
H1	*Bacillus cereus strain H23B00114*	PQ093572.1	100
H2	*Bacillus subtilis strain B4*	MW776610.1	100
J1	*Stutzerimonas balearica strain ROD040*	OK135687.1	99.93
J2	*Enterobacter cloacae strain HG-1*	MN582993.1	100
J3	*Enterobacter cloacae strain YY-2*	OR378485.1	99.89
K1	*Bacillus oceanisediminis strain 4S3*	KM374766.1	100
K2	*Bacillus subtilis strain L4*	KU179323.1	100
L1	*Bacillus subtilis strain EPP2 2*	JQ308548.1	100
M1	*Exiguobacterium aestuarii*	MW192903.1	100
M2	*Exiguobacterium profundum strain B09*	JX112643.1	100
M3	*Acinetobacter junii strain BASN28*	OQ780942.1	100
N1	*Bacillus tropicus strain SS9*	MK467544.1	100
N2	*Bacillus subtilis strain ME2*	JQ900623.1	100
N3	*Bacillus cereus strain ABC12*	ON631071.1	100
O1	*Bacillus oceanisediminis strain 4S3*	KM374766.1	100
P1	*Stutzerimonas stutzeri strain XX1*	MW820278.1	100
P2	*Bacillus cereus strain WX2*	KF624695.1	100
Q1	*Bacillus albus strain FA135*	MK993477.1	99.81

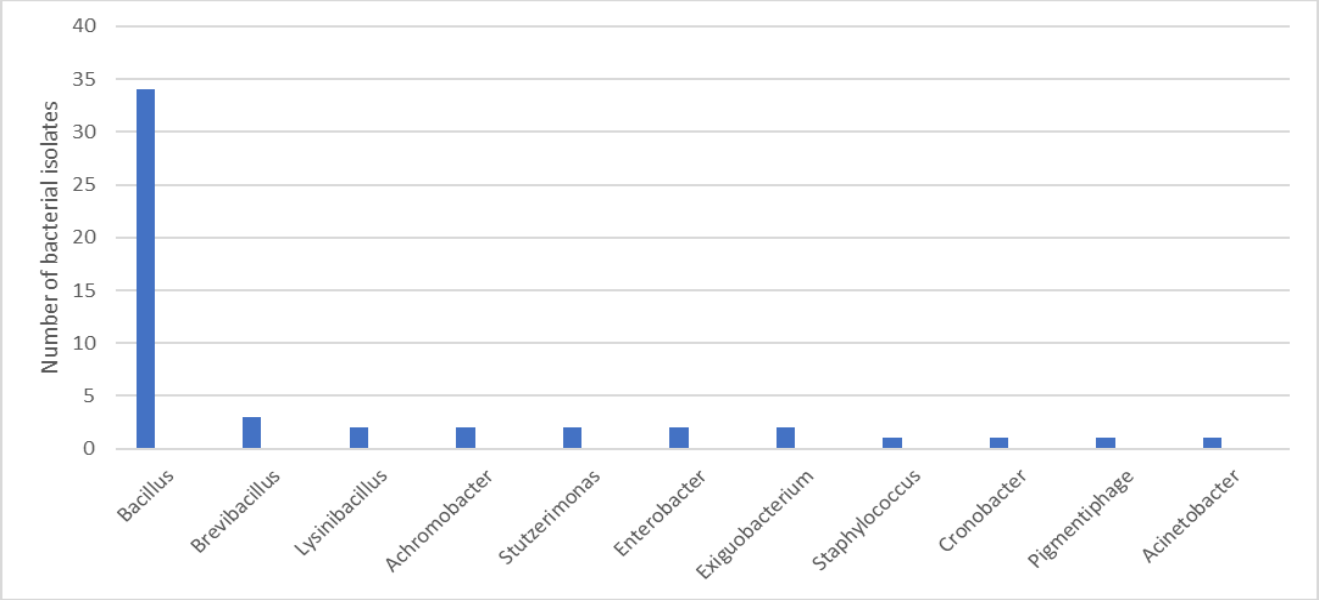


Figure 4. The number of total lipase-producing bacteria of the respective genera (n=51).

Recording of New Lipase-Producing Bacterial Strains

The lipase-producing bacterial isolates in the present study were 100% similar based on *16S rDNA* sequencing, except for seven bacterial isolates, namely B4, C5, D3, D4, J1, J3 and Q were characterized as a new bacterial strain. The sequences of these new bacterial strains have been recorded at the National Center for Biotechnology Information (NCBI) under the accession numbers listed in (Table 5). The new lipase-producing bacterial strains that have been recorded include Gram-negative and Gram-positive bacteria.

Table 5. The lipase-producing bacterial isolates were recorded as new bacterial strains.

Samples	Isolates code	New bacterial strains	Sequence identity (%)	Accession no. of new strain
B	B4	*Bacillus nakamurai* strain ZAINABBSRB4	99.92	PV050379.1
C	C5	*Pigmentiphaga daeguensis* strain ZAINABBSRC5	98.30	PV050380.1
D	D3	*Bacillus amyloliquefaciens* strain ZAINABBSRD3	99.18	PV050381.1
	D4	*Bacillus velezensis* strain ZAINABBSRD4	99.93	PV050382.1
J	J1	*Stutzerimonas balearica* strain ZAINABBSRJ1	99.93	PV050383.1
	J3	*Enterobacter cloacae* strain ZAINABBSRJ3	99.89	PV050384.1
Q	Q	*Bacillus albus* strain ZAINABBSRQ	99.81	PV050385.1

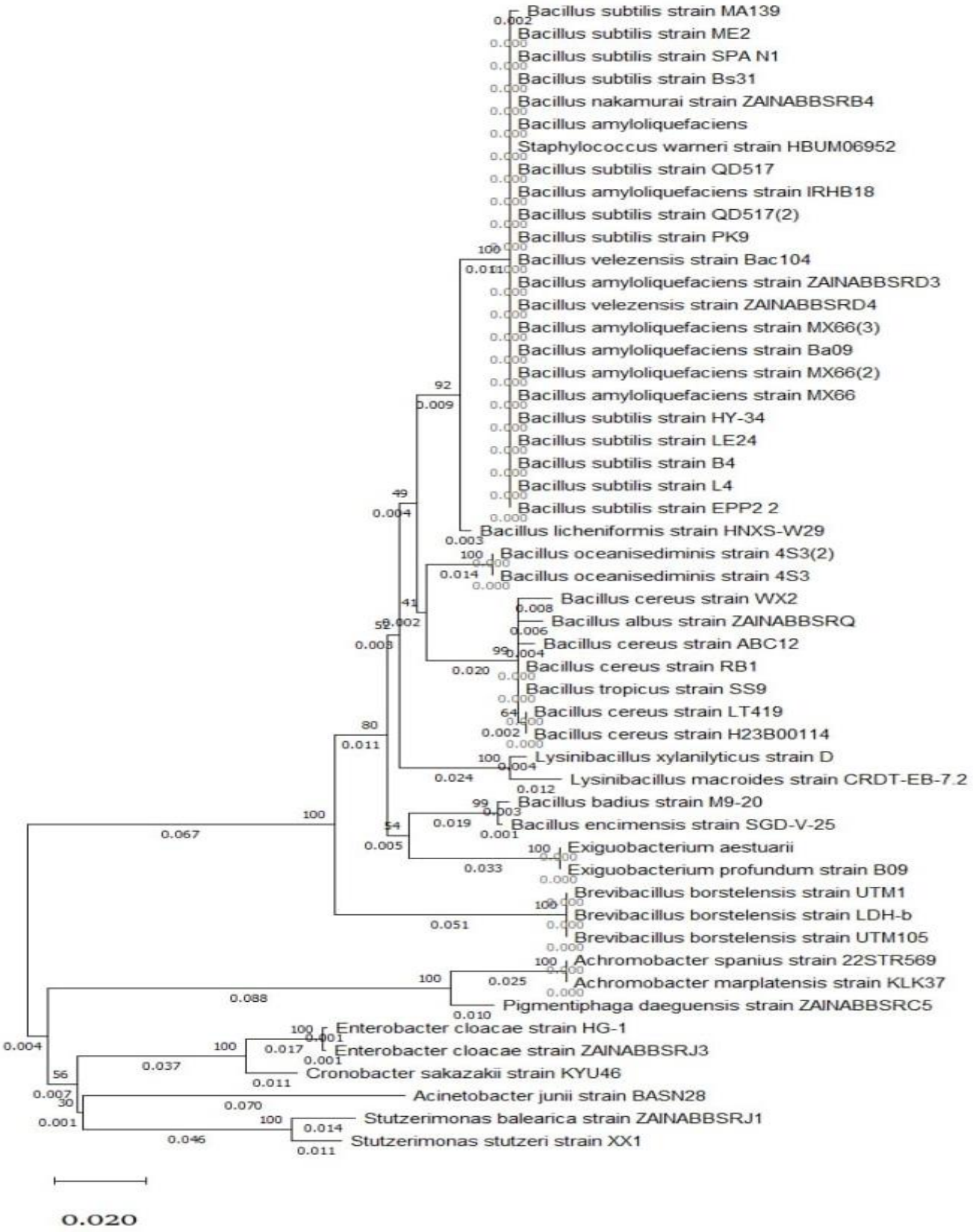


Figure 5. Neighbor-joining phylogenetic tree illustrating the evolutionary relationships among lipase-producing bacteria.

DISCUSSION

Lipase is the third most widely used enzyme in biotechnology, following protease and amylase, due to its broad catalytic properties and ability to function in heterogeneous media (Yao et al. 2021). This enzyme is utilized across various industries, including biodiesel, food and beverages, leather, textiles, detergents, pharmaceuticals, tea processing, and cosmetics, among others. Lipase is characterized by its high selectivity, catalytic efficiency, and stability (Remonatto et al. 2022). It exhibits significant temperature resistance and pH stability, and it remains highly active in the presence of various industrial organic solvents and metal ions. Additionally, lipase demonstrates strong tolerance to commonly used enzyme inhibitors and commercial detergents (Zhao et al. 2021). While lipases can be isolated and purified from bacterial, fungal, plant, and animal sources, bacterial lipases have shown to be more efficient and stable (Snellman et al. 2002).

Lipase-producing bacteria can be isolated from various environments, such as industrial and agricultural waste, vegetable oils, dairy factories, and soil contaminated with oils (Bashir et al. 2024). The ability of these bacteria to produce lipase enzymes enables them to thrive in areas contaminated with oil waste (Lee et al. 2015).

Most bacterial species isolated in the current study belonging to the genera *Bacillus, Brevibacillus, Lysinibacillus, Achromobacter, Stutzerimonas, Enterobacter,* and *Exiguobacterium* have been isolated and identified in previous studies, which isolated them from different environmental samples in Basrah province, including soil, water, sewage, and food samples (Alyousif 2022, Mohammad & Alyousif 2022, AL Khafaji et al. 2023, AL-Amery & Alyousif 2024). Species belonging to the genus *Bacillus* are prevalent at the sampling sites, appearing in 14 out of 16 locations due to their ability to withstand harsh environments, such as dry and hot conditions and areas with high solar radiation. These *Bacillus* sp can form thick spores and are resistant to endospores (Alyousif 2022). Bacterial species associated with *Bacillus* are an important group of microorganisms in many soils. Many of these species are significant for practical applications because they produce enzymes and other industrially useful products, and they play essential roles in nutrient recycling, nitrogen stabilization, and soil enrichment with minerals (Yahya et al. 2021). According to lipase activity assays, species belonging to the genus *Bacillus* have the potential to produce lipase enzymes in substantial amounts, which aligns with previous studies (Al Mohaini et al. 2022; Oni et al. 2022; Saravanakumar et al. 2024).

Bacillus encimensis, Bacillus badius, Bacillus nakamurai, Bacillus oceanisediminis, and *Pigmentiphaga daeguensis* have not been previously reported for their production of lipase enzymes, nor has their ability to secrete lipase and treat oil pollution been studied. These isolates are being reported for the first time in Iraq. *B. encimensis* was isolated from a marine sediment sample in India (Dastager et al. 2015), *B. nakamurai* from soil (Dunlap et al. 2016), *B. oceanisediminis* from the South Sea in China (Zhang et al. 2010), and *P. daeguensis* from wastewater collected from a dye works in Korea (Yoon et al. 2007).

The current results indicate that several Gram-positive and Gram-negative bacteria, which do not form spores, are effective at secreting lipase enzymes in varying amounts depending on the bacterial species. Examples include *Enterobacter cloacae*, *Staphylococcus warneri*, *Achromobacter* spp., and *Stutzerimonas balearica*. These species have been shown to secrete lipase enzymes that were isolated from various sources, including environmental and food samples (Ali et al. 2015, Yele & Desai 2015, AL-Lami & AL-Kazaz 2016, Ugras & Uzmez 2016, Iboyo et al. 2017, Issa et al. 2023).

Mubarak-Qamsari et al. (2011) studied several lipase-producing bacteria from the wastewater of an oil processing plant and found that maximum lipolytic activity occurred at 45°C and a pH of 7-10. Sagar et al. (2013) isolated lipase-producing bacteria from a waste-contaminated site. Among 18 bacterial isolates, two demonstrated significant lipolytic activity; their optimal conditions were 45°C, pH 8, and incubation times of 18 hrs at 37°C and 24 hrs, respectively. Habibolahi & Salehzadeh (2018) isolated and identified lipase-producing bacteria from the soil of oil extraction plants, identifying them as *Pseudomonas* spp. They determined that the optimal conditions for lipase production by this strain included 48 hrs of incubation at 37°C, a pH of 7, an agitation speed of 150 rpm, peptone as the nitrogen source, and olive oil as the carbon source.

In the current study, we recorded seven new bacterial strains through the sequencing of the *16S rDNA* gene, which have been registered in the NCBI GenBank database under specific accession numbers. The DNA sequences of these bacterial isolates may change due to exposure to mutagens such as chemicals, radiation, and other factors that can cause DNA damage and affect inheritance (Mohammad & Alyousif 2022).

Soil and water contaminated with oil and its derivatives are significant sources of bacterial isolates that produce lipase enzymes. Lipase-producing bacteria hold biotechnological and ecological importance, attracting researchers seeking new and more efficient isolates (Yao et al. 2021). The potential lipase-producing bacteria identified in this study are excellent candidates for large-scale applications in the future. Industrial applications of lipase enzymes span various sectors, including oil processing, detergents, food processing, and chemicals and pharmaceuticals. Environmental applications involve treating petroleum-contaminated soil and water, providing an economical and eco-friendly alternative. To achieve a high lipase yield from these candidate isolates, subsequent optimization of culture conditions is necessary to enhance lipase production, which has been further addressed. Therefore, additional experiments in specific applications should be conducted on both lab and large scales as the next steps following the screening experiments in this study.

CONCLUSIONS

In conclusion, 51 lipase-producing bacterial isolates were obtained from oil contamination sites, all capable of producing lipase enzymes with varying activity levels. Seven isolates demonstrated high efficiency in lipase production, among these, isolate A5 was the most promising, exhibiting the highest activity at 49 U/mL. Based on the sequencing of the *16S rDNA* gene, these seven isolates were identified as *Bacillus subtilis* strain QD517, *Bacillus velezensis* strain Bac104, *Bacillus subtilis* strain PK9, *Enterobacter cloacae* strain YY-2, *Bacillus cereus* strain RB1, *Lysinibacillus xylanilyticus* strain D, and *Brevibacillus borstelensis* strain LDH-b. The production of large amounts of lipase enzymes requires the optimization of culture conditions, considering various factors for application in diverse fields.

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