Detection and molecular characterization of lipase-producing bacteria

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Background
Lipase is a type of hydrolytic enzyme that has several applications and industrial efforts. Lipases are used as biological catalysts to manufacture products such as food ingredients and applied in making fine chemicals. The type of lipase produced from microbes, mainly from bacteria and fungi, represents the most widely used class of enzymes in biotechnological applications and organic chemistry. Microbial enzymes are also more stable than their corresponding plant and animal enzymes, and their production is more convenient and safer, which makes them more important in commercial uses. The oily environment of vegetable oil-processing factories, industrial wastes, soil contaminated with oil, and diesel fuel-polluted soil provides a suitable habitat for lipase-producing microorganisms.
Objective
This study aims to detect new strains of lipase-producing bacteria from diverse sources and different areas in Jeddah, Saudi Arabia. Furthermore, the detected bacterial strains have been identified based on morphological, biochemical, and molecular characterization. The plasmid profile of some isolated bacterial strains has been detected.
Materials and methods
A total of 36 soil samples contaminated with fuel and engine oil were collected from different areas in Jeddah, Saudi Arabia. Tween 20 medium was used to detect the lipolytic activity of the bacterial strains. The isolated bacteria in this study were identified by morphological and biochemical tests and 16SrRNA.
Results and discussion
Results showed that 53 isolates were positive and able to produce lipase, and 15 isolates have been selected as strong lipase-producing bacteria. The sequences were submitted to the NCBI GenBank under accession numbers, accession numbers, ON360988.1 for *Acinetobacter sp.* **(FS5)**, ON360990.1 for *Alcaligenes faecalis* **(FS8)**, ON360991.1 for *Acinetobacter baumannii* **(FS9)**, ON360992.1 for *Bacillus tropicus* (FS10), ON360993.1 for *A. baumannii* **(FS11)**, ON360994.1 for *Sphingomonas aeria* **(FS15)**, and ON360996.1 for *A. baumannii* **(FS17)**. Plasmids were isolated from selected strains that showed lipase production using a plasmid-isolation miniprep. Results indicated that isolates FS6 and FS15 have no plasmids, whereas FS8 has one plasmid (≈1295.5 bp). Furthermore, isolates FS10 and FS11 have two plasmids (≈1539.3 and 1295.5 bp). In addition, isolate **FS9** has three plasmids (≈1539.3, 1295.5, and 417.7 bp). The isolates showed strong lipase activity and could be good sources for the production of lipase.

Keywords:
16SrRNA, bacterial strains, lipase; plasmid

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Introduction

Lipases, known as triacylglycerol acyl hydrolases (EC 3.1.1.3), participate in the breakdown of fats and oils to produce glycerol and free fatty acids [1]. Therefore, lipase is a type of hydrolytic enzyme that has several application industrially [2].

Furthermore, they are involved in conversion reactions, like esterification, interesterification, transesterification, alcoholic lysis, acidolysis, and aminolysis [3]. Lipases are used as biological catalysts to manufacture products such as food ingredients and are applied in making fine chemicals. Lipids are important in almost all parts of our lives, in which they have a role in nutrition, flavors, cosmetics, and stains [2,4]. Lipase can be found naturally in plants, animals, and microorganisms, but there is a more stable type and easy to get with a high amount, which originates from bacteria, which makes

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it more important in commercial uses [5–7]. *Candida rugosa* lipase is another scientifically significant lipase from the yeast 'generally recognized as safe' and used in the food industry [8,9].

Bacterial lipases can hydrolyze milk fat and enrich the flavor and lipolysis of butter fat and cream, so it is widely used in the dairy and food industry [10]. Moreover, lipases are used in the detergent and textile industry and for the synthesis of decomposable compounds [11–13]. Tetrahydrolipstatin (also known as orlistat, Xenical, and Alli) is the only FDA-approved antiobesity medication for long-term use. It contains lipostatin, the controller of pancreatic lipase digestion activity. Lipostatin was first isolated from *Streptomyces toxytricini* [9,14]. Furthermore, lipases are also used for the curing of hair loss and skin scalp disease. It is also used for diagnostic purposes of tuberculosis (Tributyrin Agar), a lung disease caused by *Mycobacterium tuberculosis* [15].

The oily environment of vegetable oil-processing factories, industrial wastes, soil contaminated with oil, and diesel fuel polluted soil provides a good habitat for lipase-producing microorganisms [16,17]. Bacterial lipases are seriously influenced by many factors, such as temperature, pH, nitrogen and carbon sources, inorganic salts, and dissolved oxygen concentration [18]. The lipase from the genus Pseudomonas has attracted the most attention in many research studies and gets the interest of biotechnologists owing to its versatile metabolic machinery and its ability to adapt to stable environmental conditions [19,20]. However, Verma *et al.* [21] mentioned that *Bacillus* species are more potential candidate for industrial purposes owing to their remarkable properties because it is nonpathological, and thus can be easily produced on a large industrial scale as they have the potential to be used in food and chemistry industries [22,23]. Lipases from *Bacillus* sp. are considered to be lidless and small (actually the smallest lipases known). Other strains of lipase-producing bacteria were reported by many authors such as *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Bacillus coagulans*, *Bacillus cereus*, *Staphylococcus aureus*, and *Staphylococcus hyicus* [24]. Moreover, Bharathi *et al.* [4] isolated lipase-producing bacterial strains of *Pseudomonas* sp., *Klebsiella* sp., *Corynebacterium* sp., *Streptococcus* sp., *Escherichia* sp., *Proteus* sp., *Bacillus* sp., and *Staphylococcus* sp., and by using rRNA sequencing for bacteria, the species, genus, and high taxon levels can be differentiated [25].

B. cereus was isolated by Ghaima *et al.* [17] from diesel fuel-polluted soil samples, whereas strains from the genera of *Corynebacterium* sp., *Streptococcus* sp., *Escherichia* sp., *Proteus* sp., *Bacillus* sp., *Staphylococcus* sp., *Pseudomonas* sp., and *Klebsiella* sp. were isolated by [4] from petrol-spilled soil. Moreover, the *P. aeruginosa* strain was isolated by Mobarak-Qamsari *et al.* [19] from wastewater of an oil-processing plant. In Saudi Arabia, water samples were collected from thermal hot springs located in the Gazan area conducted to screen and characterize the thermophilic bacteria (lipase, cellulase, and amylase producers) [26]. Looking at the huge predictive value and applications, Singh *et al.* [27] isolated lipase-producing microorganisms from soil of several natural sources, and then the better enzyme-producing microbial isolate was screened and investigated for lipase production and activity in a plan for measuring bioprospecting of lipolytic microorganisms. Some lipase-producing halophilic bacteria (*Bacillus* and *Staphylococcus*) were isolated by Ghasemi *et al.* [28] from the Maharla, a hypersaline lake in south of Iran, and identified using 16SrRNA as a molecular marker. Moreover, the same study indicated the presence of lipases in halophilic bacteria, which could be applied in industrial processes. Earlier study of plant growth regulators showed that production of hydrolytic enzymes such as protease, lipase, pectinase, and amylase is one of the activities of rhizosphere bacteria [29]. In general, lipase production occurs on lipidic carbon, such as oils, fatty acids, glycerol, or tweens in the presence of an organic nitrogen source [18].

The present study aimed to detect new strains of lipase-producing bacteria from different areas in Jeddah, Saudi Arabia. Furthermore, the detected bacterial strains have been identified based on morphological, biochemical, and molecular characterization. Plasmid profile of some isolated bacterial strains have been detected.

Materials and methods

Collection of samples

A total of 36 soil samples contaminated with fuel and engine oil were collected from different areas in Jeddah, Saudi Arabia, using sterilized containers and stored at 4°C until examination.

Isolation of bacteria

One gram of each soil sample was suspended in 9 ml of sterilized distilled water to generate serial dilution of up to 10^{-5}–10^{-7}, and 100 µl of suspensions of these dilutions was spread onto nutrient agar plates and incubated at 30°C for 24 h.

Purification and preservation of isolated bacteria

The obtained bacteria were purified by streaking and subculturing on nutrient agar plate and incubated at 30°C until pure cultures were obtained. Then, they were transferred to slants of the same medium and preserved at 4°C. For long preservation, strains were kept in 20% glycerol and stored at −80°C.

Screening of lipase-producing bacteria using the agar plate method

Selected bacterial strains were inoculated into 10 ml of nutrient broth and incubated in a shaking incubator for 24–36 h at 30°C. Then, 15 µl of enriched media was inoculated into Tween 20 agar medium containing 1% Tween 20 as a substrate for lipase activity, and then incubated at 30°C for 3 days according to Rajan *et al.* [30]. Zone formation indicates lipolytic activity.

Diameter of lipolytic zones for lipase-producing bacteria

The index was determined after measuring the diameter of the clear zone (halo) and the colony using the following formula (index=colony diameter +halo zone diameter/colony diameter) according to Edi-Premono *et al.* [31].

Identification of the isolated bacteria

The isolated bacteria in this study were identified by morphological and biochemical tests and 16SrRNA.

Morphological and biochemical characteristics

The Gram staining procedure modified by Rueckert and Morgan [32] was used in the microscopic identification of bacteria. A clean microscope slide was labeled with the code for the unknown organism using a marking pencil. One loopful of unknown bacteria culture was applied on the slide using an inoculating loop. The shape of the bacteria and the staining effects were recorded [33].

Molecular identification of lipase-producing bacteria.

Isolation of genomic DNA

Lipase-producing bacteria was characterized molecularly using the 16SrRNA sequencing. The genomic DNA was extracted using a GeneJET Genomic DNA Purification kit according to the manufacturer's instructions.

PCR amplification

The DNA of the isolates was subjected to PCR amplification by using the forward and reverse primers of 16 S rRNA.

Forward primers	5′-AGA GTT TGA TCM TGG CTC AG-3′
Reverse primers	5′-GGG TTG CGC TCG TTG-3′

PCR amplifications were usually performed on a model A 200 gradient thermal cycler (long gene) in a final volume of 50 µl of reaction mixture, containing 50 U/ml Taq polymerase, 10 mM of an equimolar dNTPs mix, 10× PCR buffer supplemented with 3 mM $MgCl_2$, 19 µl of ultrapure PCR water, 0.5 µM of each primer, and 2 µl of genomic DNA, as template, with a concentration between 0.1 and 10 ng. The standard protocols followed for amplification consisted of an initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation for one minute at 95°C, annealing at temperature 58°C for 1 min, first elongation at 72°C for 2 min, and final elongation at 72°C for 5 min. The amplification process was confirmed by ethidium bromide fluorescence in 1% agarose gel.

Gel electrophoresis

Overall, 1% agarose gel was dissolved in 1× TAE buffer, melted, and poured into an electrophoresis chamber. A volume of 10% (v/v) loading buffer was added to the DNA samples. Samples were loaded onto the gel, and the electrophoresis was performed at 50 V/cm in 1× TAE buffer. DNA was visualized by soaking in a dilute solution of ethidium bromide. DNA fragments were separated, and their sizes examined and determined under UV light and used Gel Documentation System model 286-760 DigiDoc-ItTM for the imaging and documentation of agarose gels.

Sequencing of amplified fragments of isolate 16SrRNA genes

For further studies (sequencing) of amplified samples, 16SrRNA gene was sent to Macrogen Korea. Blast search was used for analysis of sequences, and the nearest match was found in GenBank. Finally, the phylogenetic analysis was performed for the blast results.

Plasmid profiling

Plasmid isolation

Plasmid was isolated using the miniprep method according to Rodriguez and Tait [34]. One milliliter from bacterial culture was transferred to Falcon tubes, and the bacterial cells were collected by centrifugation at 4500 rpm for 10 min using a Sigma centrifuge. The pellet was washed by 1 ml of SET buffer and recentrifuged as above. Then, 750 µl of SET buffer and 1750 µl of lysing solution were added to the pellet, vortexed briefly, and placed on an ice bath for 15 min.,

followed by adding 1250 µl of 3 M sodium acetate. The tube was inverted gently several times and incubated on the ice bath for 30 min. The mixture was then centrifuged at 10 000 rpm at 40°C for 20 min, and the supernatant was transferred to a new Falcon tube, which was then filled with 3750 µl of isopropanol. Tubes were inverted several times, and plasmid DNA was collected by centrifugation at 4500 rpm at room temperature for 10 min. Isopropanol was removed. DNA pellet was washed two times with 3 ml of 70% ethanol and centrifuged for 10 min. The DNA pellet was then air-dried and suspended in 50 µl of sterilized distilled water.

Gel electrophoresis

Plasmid was characterized by agarose gel electrophoresis according to the standard procedure of [35]. Agarose gel electrophoresis was done through a horizontal slab gel of 1% agarose submerged in 100 ml 1× TAE supplemented with 2 µl of ethidium bromide running buffer at 120 V for 1 h., in the proportion of 7 µl of sample with 1 µl of loading buffer dye. DNA bands were visualized on UV. Molecular weight of the plasmids was determined using Lambda DNA Hind III Digest.

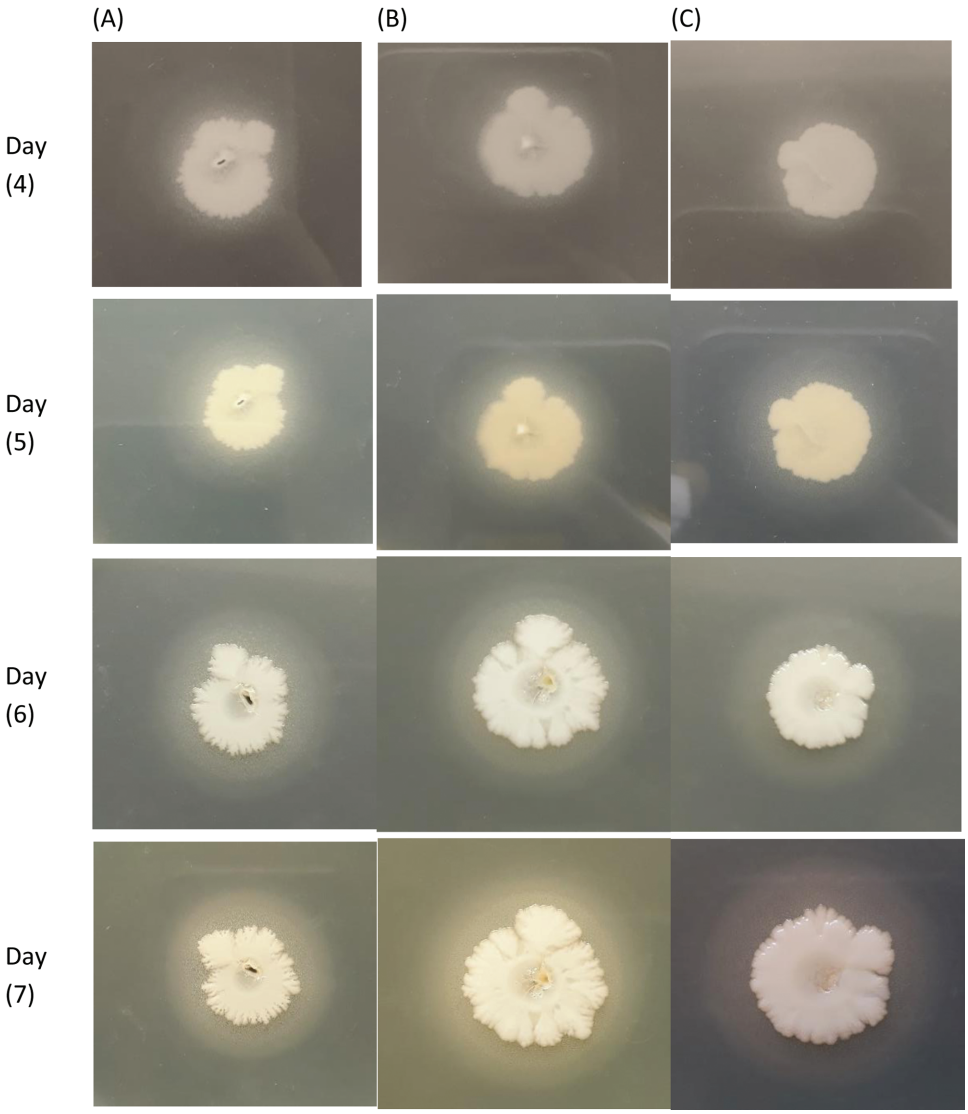
Antibiotic resistance test

Antibiotic sensitivity and resistance of bacterial isolates have been tested on NA medium. The test was

Table 1 List of bacterial isolates and area sources

Bacterial isolates	Sources
FS3	Mechanical shop at western area
FS5, FS6, FS7, and FS8	Gas stations at western area
FS9, FS10, FS11, FS13, and FS14	Mechanical shops at eastern area
FS15, FS16, FS17, and FS19	Mechanical shops at southern area

Figure 1



Zone of precipitation on Tween 20 agar plates for some isolates after incubation at 30°C for 4–7 days (a: FS5, b: FS13, and c: FS19).

performed by the disc diffusion method as described by Bauer [36], using seven types of commercially available antibiotics disc listed below: amikacin (30 µg), ceftazidime (30 µg), aztreonam (30 µg), piperacillin (100 µg), imipenem (10 µg), ciprofloxacin (5 µg), and teicoplanin (30 µg).

The inhibition zone has been recorded after 24 h of incubation at 30°C. After incubation, the antibiotic inhibition zone diameters were measured in mm. The strains were classified as being resistant, intermediate resistant, or susceptible to a particular antibiotic, where (–) sensitive (S) more than or equal to 21 mm; intermediate (I), 16–20 mm; and resistant (R), less than or equal to 15 mm [37].

Results

Sample collection and bacterial isolation

Bacterial samples were collected from the soil samples obtained from different locations in Saudi Arabia (Jeddah) (Table 1). Bacterial isolates were purified, serial dilutions were done, and isolates were separately plated with nutrient agar medium, and then incubated at 30°C for 24 h. Bacterial isolates were stored at 4°C until test their ability for lipase production.

Lipase-producing bacteria screening using Tween 20 plate

Screening of lipase production bacteria was tested on Tween 20 agar plate after incubation at 30°C for 4–7 days. Results showed that 53 isolates were positive and able to produce lipase. The index for precipitation zone of the isolates was assayed using rulers, which varied from 2.8 to 4 cm on day 4 and 3.3–5.9 cm on day 7 (Fig. 1), and 15 isolates have been selected as strong lipase-producing bacteria, as shown in Table 2. Index was determined by measuring the diameter of the precipitation zone (halo) and the colony using the following equation formula as reported by Edi-Premono *et al.* [31]:

Morphological and biochemical characteristics

The morphological characteristics of the bacterial isolates were tested on the nutrient agar through a direct examination using a light microscope based on morphology, aggregation, and Gram staining.

Molecular identifications of the isolated bacteria

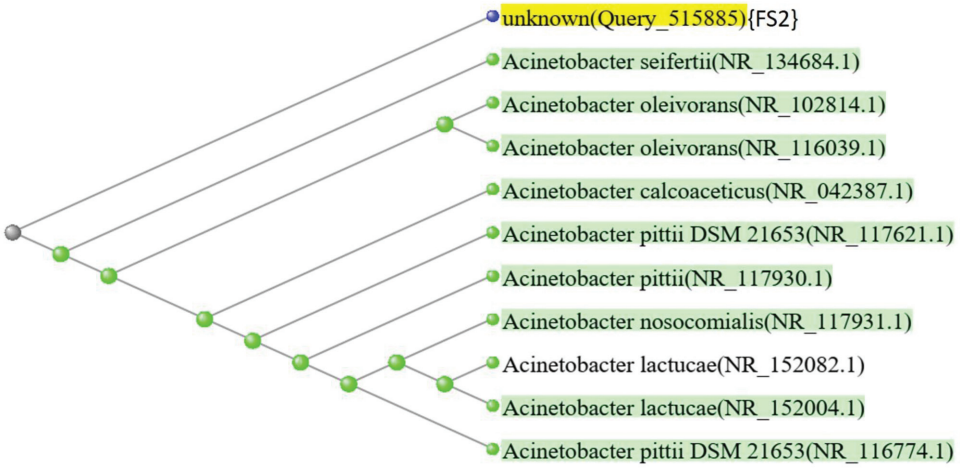
Identification of the 15 selected bacterial isolates was confirmed using 16SrRNA gene sequencing. The sequences were submitted to the NCBI GenBank (www.ncbi.nlm.nih.gov) under accession numbers, ON360988.1 for *Acinetobacter* sp. **(FS5)**, ON360990.1 for *Alcaligenes faecalis* **(FS8)**, ON360991.1 for *Acinetobacter baumannii* **(FS9)**, ON360992.1 for *Bacillus tropicus* (**FS10**), ON360993.1 for *A. baumannii* **(FS11)**, ON360994.1 for *Sphingomonas aeria* (**FS15)**, and ON360996.1 for *A. baumannii* (**FS17**) as in Table 3.

Finally, the phylogenetic analysis was performed for the blast results as in (Figs 2–8)

Isolation of plasmid

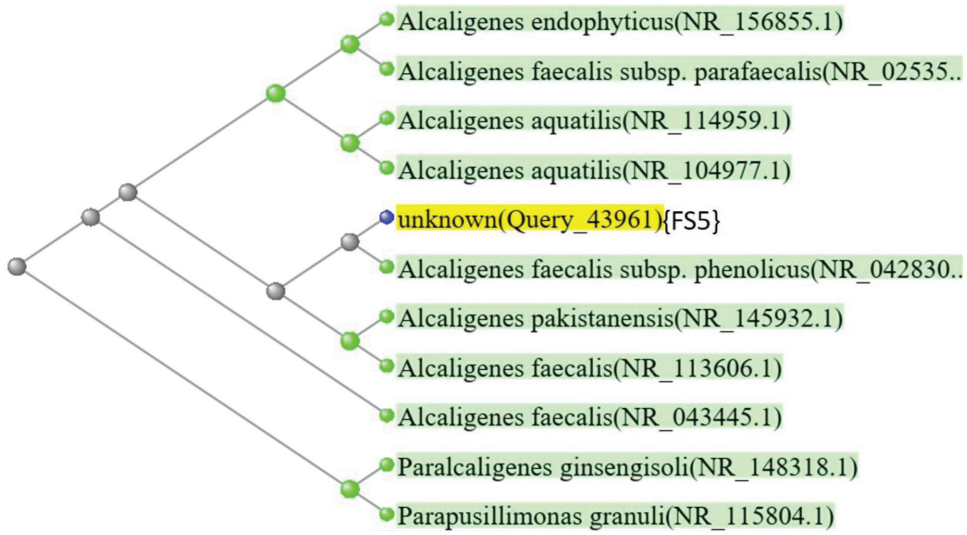
Plasmids were isolated from selected strains that showed lipase production using plasmid isolation miniprep (Promega Plasmid Kit). Results of electrophoresis indicated that isolate FS6 and FS15 have no plasmids, whereas FS8 has one plasmid

Figure 2



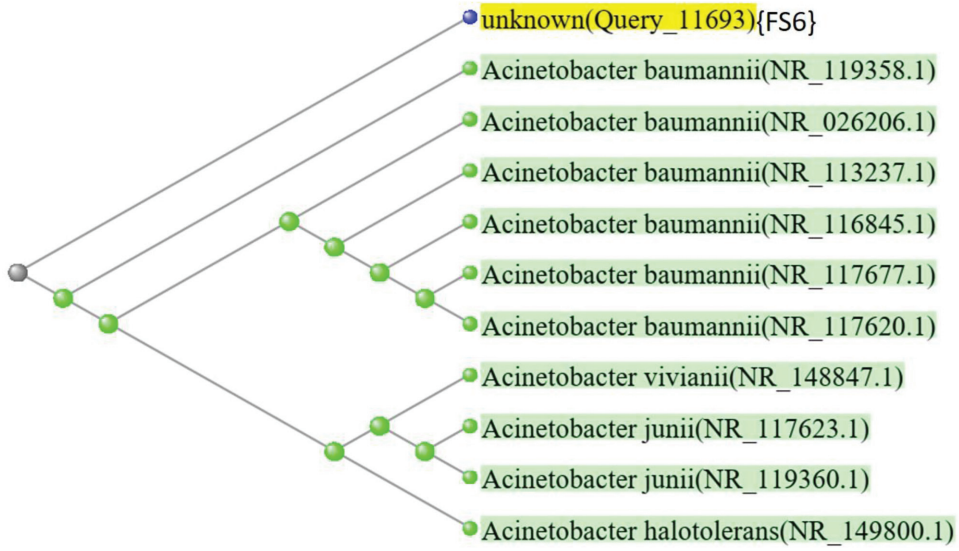
A phylogenetic tree of the taxonomic position of the isolated bacterial strain (FS2) isolated from soil, created using the Neighbor-Joining method, based on the 16S-DNA sequences and other closely related species in database.

Figure 3



A phylogenetic tree of the taxonomic position of the isolated bacterial strain (FS5) isolated from soil, created using the Neighbor-Joining method, based on the 16S-DNA sequences and other closely related species in database.

Figure 4



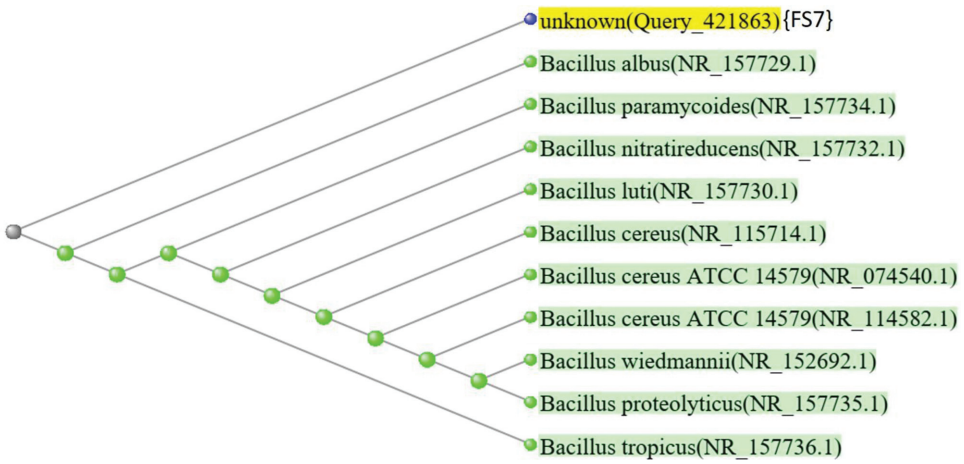
A phylogenetic tree of the taxonomic position of the isolated bacterial strain (FS6) isolated from soil, created using the Neighbor-Joining method, based on the 16S-DNA sequences and other closely related species in database.

(≈1295.5 bp). Furthermore, isolates FS10 and FS11 have two plasmids of ≈1539.3 and 1295.5 bp. In addition, isolate FS9 has three plasmids (≈1539.3, 1295.5, and 417.7 bp) (Fig. 9).

Antibiotic resistances

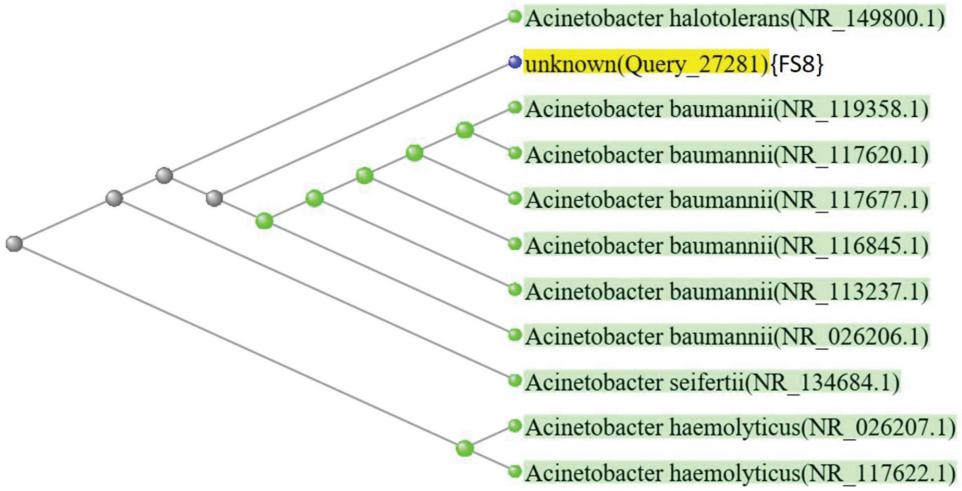
Resistance to antibiotics was determined on NA plates. The disc method results are shown in Table 4, which showed the effect of seven antibiotics on the 15 bacterial strains as follows: FS1, FS13, and FS23 were highly sensitive to imipenem and ciprofloxacin with inhibition zones of 30 and 40 mm, respectively, whereas the antibiotic piperacillin had a low effect with an inhibition zone of 20 mm, and there was no obvious effect for amikacin, ceftazidime, aztreonam, and teicoplanin. *Acinetobacter calcoaceticus* (FS5) was highly sensitive to imipenem, with an inhibition zone of 30 mm, but the antibiotic ciprofloxacin had low effect with an inhibition zone of 20 mm, and there was no clear effect of amikacin, ceftazidime, aztreonam, piperacillin, and teicoplanin. *A. baumannii* (FS6 and FS17) was sensitive to imipenem and ciprofloxacin with an inhibition zone of 22 mm, and there was no obvious effect for

Figure 5



A phylogenetic tree of the taxonomic position of the isolated bacterial strain (FS7) isolated from soil, created using the Neighbor-Joining method, based on the 16S-DNA sequences and other closely related species in database.

Figure 6



A phylogenetic tree of the taxonomic position of the isolated bacterial strain (FS8) isolated from soil, created using the Neighbor-Joining method, based on the 16S-DNA sequences and other closely related species in database.

piperacillin, amikacin, ceftazidime, aztreonam, and teicoplanin.

B. cereus (FS7) was sensitive to imipenem, amikacin, and ciprofloxacin with inhibition zones of 23, 40, and 24 mm, respectively, but the antibiotics piperacillin and teicoplanin had low effects, with inhibition zone of 18–20 mm, and there was no clear effect of ceftazidime and aztreonam.

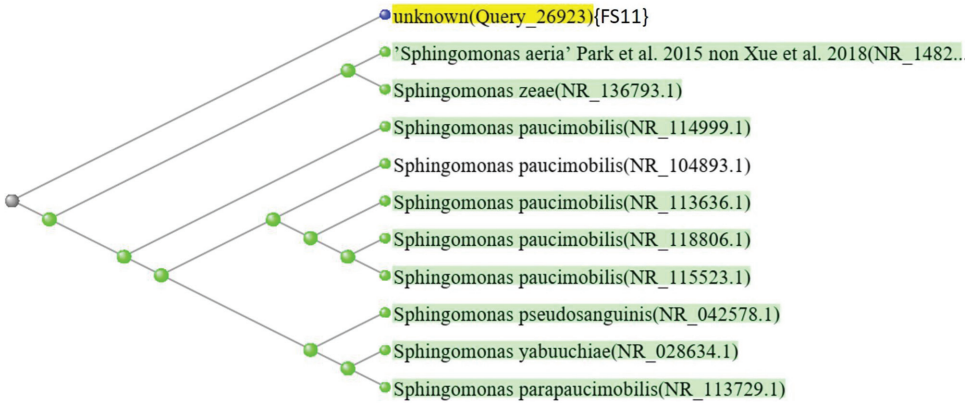
A. faecalis (FS8) was highly sensitive to imipenem, with an inhibition zone of 33 mm, but there was no clear effect of amikacin, ceftazidime, aztreonam, piperacillin, ciprofloxacin, and teicoplanin.

A. baumannii (FS9) was sensitive to imipenem and ciprofloxacin with inhibition zones of 25 and 23 mm, respectively, and there was no obvious effect for amikacin, ceftazidime, aztreonam, piperacillin, and teicoplanin.

Bacillus sp. (FS10) was sensitive to amikacin, imipenem, and ciprofloxacin, with inhibition zones of 23, 40, and 24 mm, respectively, but the antibiotic teicoplanin had a low effect with an inhibition zone of 20 mm, and there was no clear effect of ceftazidime, aztreonam and piperacillin.

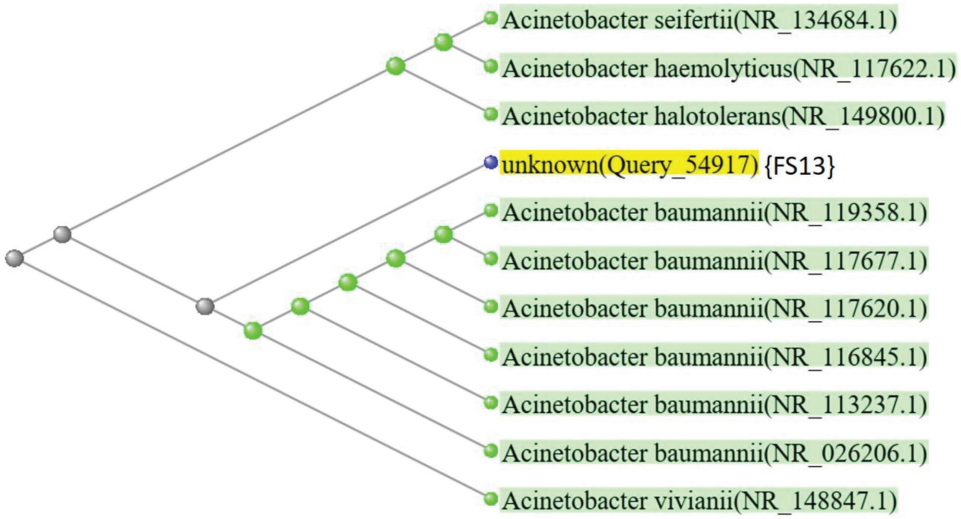
A. baumannii (FS11) was sensitive to imipenem with an inhibition zone of 24 mm, but the antibiotics

Figure 7



A phylogenetic tree of the taxonomic position of the isolated bacterial strain (FS11) isolated from soil, created using the Neighbor-Joining method, based on the 16S-DNA sequences and other closely related species in database.

Figure 8



A phylogenetic tree of the taxonomic position of the isolated bacterial strain (FS13) isolated from soil, created using the Neighbor-Joining method, based on the 16S-DNA sequences and other closely related species in database.

ciprofloxacin and teicoplanin had a low effect with an inhibition zone of 20 mm, and there was no clear effect of amikacin, ceftazidime, aztreonam, and piperacillin.

Bacillus thuringiensis (FS14) was sensitive to amikacin, imipenem, and ciprofloxacin, with inhibition zones of 25, 40, and 27 mm, respectively, but the antibiotics piperacillin and teicoplanin had a low effect with an inhibition zone of 20–18 mm, respectively, and there was no clear effect of ceftazidime and aztreonam.

S. aeria (FS15) was sensitive to imipenem and ciprofloxacin with inhibition zones of 38 and 23 mm, respectively, but the antibiotic amikacin had a low effect with an inhibition zone of 18 mm, and there was no a clear effect of ceftazidime, aztreonam, piperacillin, and teicoplanin.

Klebsiella sp. (FS16) was sensitive to amikacin, imipenem, and ciprofloxacin with inhibition zones of 23–26 mm, but the antibiotics piperacillin and teicoplanin had low effect, with an inhibition zone of 20 mm, and there was no clear effect of ceftazidime and aztreonam.

Finally, *Bacillus subtilis* (FS19) was sensitive to imipenem, with an inhibition zone of 22 mm, but the antibiotics aztreonam and ciprofloxacin had low effect with an inhibition zone of 20 mm, and there was no clear effect of amikacin, ceftazidime, piperacillin,

Table 2 List of bacterial isolates with positive result on Tween 20 agar plate

	Colony diameter (cm)				Zone diameter (cm)				Index			
Bacterial isolates	Day (4)	Day (5)	Day (6)	Day (7)	Day (4)	Day (5)	Day (6)	Day (7)	Day (4)	Day (5)	Day (6)	Day (7)
FS3	1.3	1.3	1.8	1.8	2.8	2.8	3	3	3.2	3.2	3.5	3.5
FS5	1.4	1.5	1.7	1.8	2.7	3	3	4	3.3	3.5	3.5	4.0
FS6	1	1	1.4	1.7	3	3	3.2	3.8	4.0	4.0	3.7	3.9
FS7	1.2	1.9	1.9	2.1	3	3.2	3.2	3.2	3.7	3.6	3.6	3.6
FS8	1	1	1	1.1	3	4	4.8	5.3	4.0	5.0	5.8	5.9
FS9	1.7	1.9	2	2	2.9	3	3.2	3.5	3.4	3.5	3.6	3.8
FS10	1.8	2	2	2	3	3.1	3.3	4	3.5	3.6	3.7	4.0
FS11	1.8	1.9	2	2.2	3	3	3.2	3.5	3.5	3.5	3.6	3.8
FS13	1.6	1.7	1.8	2	3	3	3.4	3.8	3.5	3.5	3.7	3.9
FS14	2	2.1	2.3	2.5	3.3	3.7	4	4.7	3.7	3.9	4.0	4.4
FS15	1.2	1.3	1.3	1.3	2.8	2.6	3	2.8	3.5	3.3	3.6	3.5
FS16	1.5	1.5	1.6	1.7	2.7	2.7	3	3.1	3.3	3.3	3.5	3.5
FS17	0.9	1	1.2	1.2	1.7	1.7	1.7	2.5	2.8	2.7	2.6	3.3
FS19	1.5	1.6	1.7	1.8	3	3	3.2	3.7	3.5	3.5	3.6	3.9
FS23	3	3	3.5	3.8	3.1	3.1	3.6	4.2	4.0	4.0	4.5	4.9

Table 3 Accession number, closest phylogenetic relative, and identity presents of seven acterial isolates (FS2–FS13) obtained from soil

Isolate code on NCBI	Name	Accession number	Closest phylogenetic relative and accession number	Identity %
FS2	*Acinetobacter* sp. (FS5)	ON360988.1	*Acinetobacter* NR_117930.1	95.32
FS5	*Alcaligenes faecalis* (FS8)	ON360990.1	*Alcaligenes faecalis* NR_113606.1	98.41
FS6	*Acinetobacter baumannii* (FS9)	ON360991.1	*Acinetobacter baumannii* NR_117620.1	97.38
FS7	*Bacillus tropicus* (FS10)	ON360992.1	*Bacillus tropicus* NR_157736.1	98.78
FS8	*Acinetobacter baumannii* (FS11)	ON360993.1	*Acinetobacter baumannii* NR_117620.1	99.42
FS11	*Sphingomonas aeria* (FS15)	ON360994.1	*Sphingomonas aeria* NR_148286.1	97.82
FS13	*Acinetobacter baumannii* (FS17)	ON360996.1	*Acinetobacter baumannii* NR_117620.1	98.55

and teicoplanin. In conclusion, all strains were sensitive to imipenem but resistant to ceftazidime (Table 4).

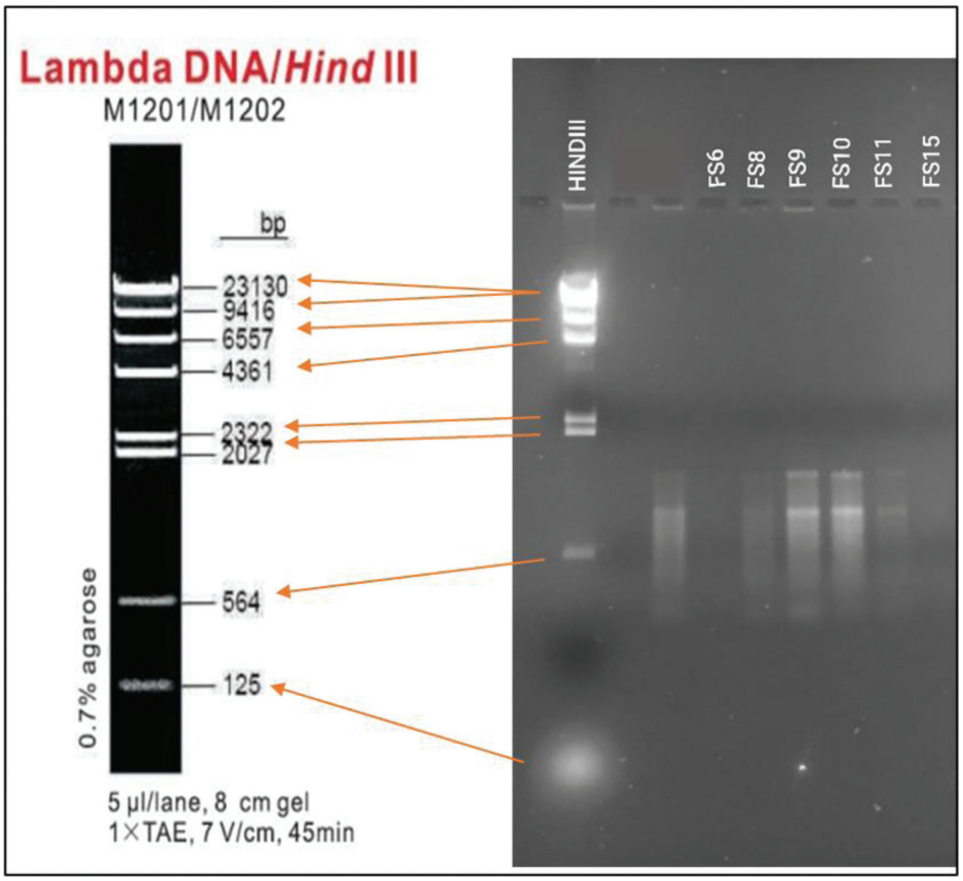
Discussion

Oily soil provides a good and rich media for lipase-producing bacteria. So, it attracts a lot of interest among scientists, such as in India, samples were collected from oil and fat contaminated soils of diary and oil refinery by Sirisha *et al.* [38]; soil samples were collected from a mechanic's workshop in Jericho G.R. A, Ibadan, by Ilesanmi *et al.* [39]; and eight strains were isolated by Bharathi *et al.* [4] from a petrol-spilled soil sample. In the present study, 36 soil samples contaminated with fuel and engine oil were collected from different areas in Jeddah, Saudi Arabia. Bacterial isolates were collected, purified, and screened as lipase-producing bacteria. A total of 53 isolates were positive and able to produce lipase through the precipitation zone (halo) that was observed around the colonies of lipase-producing bacteria. These results were achieved by culturing the different tested bacterial isolates on Tween 20 agar medium containing 1% Tween 20 as a substrate for lipase activity. A total of 15 isolates have been selected as strong lipase-producing bacteria where the lipase index of the isolates varied from 2.8 to 4 cm at day 4 and 3.3–5.9 cm at day 7.

The preliminary identification of these isolates was based on the morphological characteristics of the culture, biochemical, and molecular identification through 16SrRNA gene sequencing, identifying the following isolates: *B. cereus strain* **(FS3**, **FS13**, and **FS23)**, *A. calcoaceticus* **(FS5)**, *A. baumannii* **(FS6** and **FS17)**, *B. cereus* **(FS7)**, *A. faecalis* **(FS8)**, *A. baumannii* **(FS9)**, *Bacillus* sp. **(FS10)**, *A. baumannii* **(FS11)**, *B. thuringiensis* **(FS14)**, *S. aeria* **(FS15)**, *Klebsiella* sp. **(FS16)**, and *B. subtilis* **(FS19)**.

B. cereus strains (FS3, FS13, and FS23) in the current study have been isolated from mechanical shops, whereas FS7 from a gas station. Many reports have indicated different sources for *B. cereus* strain such as from diesel fuel-polluted soil samples in Baghdad that were revealed by Ghaima *et al.* [17], and Dutta and Ray [40] found the same strains from spoiled coconut, which was capable of producing alkaline-stable

Figure 9



Plasmid bands isolated from strains FS6, FS8, FS9, FS10, FS11, and FS15.

Table 4 Results of antibiotic sensitivity test of bacterial strains

	Diameter of the inhibition zone (mm)						
Bacterial isolates	Amikacin	Ceftazidime	Aztreonam	Piperacillin	Imipenem	Ciprofloxacin	Teicoplanin
Bacillus cereus **(FS3)**	15 (R)	0 (R)	0 (R)	20 (I)	30 (S)	40 (S)	0 (R)
Acinetobacter sp. **(FS5)**	15 (R)	0 (R)	0 (R)	0 (R)	30 (S)	20 (I)	8 (R)
Acinetobacter baumannii **(FS6)**	15 (R)	0 (R)	0 (R)	0 (R)	22 (S)	22 (S)	12 (R)
Bacillus cereus **(FS7)**	23 (S)	0 (R)	0 (R)	18 (I)	40 (S)	24 (S)	20 (I)
Alcaligenes faecalis **(FS8)**	15 (R)	0 (R)	0 (R)	0 (R)	33 (S)	10 (R)	0 (R)
Acinetobacter baumannii **(FS9**	13 (R)	0 (R)	0 (R)	0 (R)	25 (S)	23 (S)	8 (R)
Bacillus sp. **(FS10)**	23 (S)	0 (R)	0 (R)	15 (R)	40 (S)	24 (S)	20 (I)
Acinetobacter baumannii **(FS11)**	12 (R)	0 (R)	0 (R)	0 (R)	24 (S)	20 (I)	20 (I)
Bacillus cereus **(FS13)**	13 (R)	0 (R)	0 (R)	10 (R)	25 (S)	22 (S)	15 (R)
Bacillus *thuringiensis* **(FS14)**	25 (S)	0 (R)	0 (R)	20 (I)	40 (S)	27 (S)	18 (I)
Sphingomonas aeria **(FS15)**	18 (I)	0 (R)	0 (R)	0 (R)	38 (S)	23 (S)	0 (R)
Klebsiella sp. **(FS16)**	22 (S)	0 (R)	0 (R)	20 (I)	26 (S)	26 (S)	20 (I)
Acinetobacter baumannii **(FS17)**	15 (R)	0 (R)	0 (R)	0 (R)	25 (S)	25 (S)	18 (I)
Bacillus subtilis **(FS19)**	15 (R)	0 (R)	20 (I)	0 (R)	22 (S)	20 (I)	0 (R)
Bacillus cereus **(FS23)**	20 (I)	0 (R)	0 (R)	12 (R)	20 (I)	18 (I)	18 (I)

(S) Sensitive (≥21 mm). (I) Intermediate (16–20 mm). (R) Resistant (≤15).

extracellular lipase. Moreover, *Bacillus* sp strains were isolated from agricultural soils of Turkey by Demirkan *et al.* [41].

A novel thermophilic-organic solvent stable lipase from Acinetobacter strain was isolated and characterized by Uttatree *et al.* [42]. In the current

study, five different isolates FS5, FS6, FS9, FS11, and FS17 have been identified as Acinetobacter genera with different lipase zone diameters varying from 2.5 to 4 cm at day 7 with index of 3.3 to 4 cm. Snellman and Colwell [43] reviewed different sources of Acinetobacter genera including skin of human, food, and dairy products in addition to various soils and aqua places, which were described as strictly aerobic, gram-negative coccobacillus and lipolytic strains, which is consistent with this study, and their lipases have numerous biochemical characteristics that can be used for biotechnological applications.

Other isolated *Bacillus* strains in this study (FS10, FS14, and FS19) were also revealed by other authors [44–46].

Furthermore, Farinde *et al.* [47] described *Bacillus* sp. OS1 strain in matured dried lima bean seed samples in Nigeria as positive gram and catalase strain, which is consistent with the current study, but no earlier studies about its lipase production have been founded.

B. thuringiensis strain was obtained from the Linow Lake volcanic site in Indonesia [48]. The same strain was isolated from biotopes situated in Sfax (Tunisia) and was lipase positive [49].

The isolated strain FS8 was identified in this study as *A. faecalis,* and the result showed its ability in lipase production, which is consistent with the finding that described *A. faecalis* as lipase-producing organism, and its lipase was used as bio-detergent which has good stability against exposure to commercial detergents [50]. Furthermore, commercial capacity of lipase production was examined [51]. *S. aeria* was isolated from air at the foot of Xiangshan mountain and was negative to Tween 20 and starch hydrolysis, whereas was positive in the current study. Furthermore, it was oxidase negative and catalase positive [52]. No studies have yet shown lipase production from the genus *S. aeria*, which were isolated from soil samples contaminated with fuel and engine. The present study is the first report on the identification of *S. aeria* with high levels of lipase enzyme production.

Klebsiella sp. (FS16) was described and isolated from benthic soil of an aquaculture farm in East Kolkata Wetlands, India [53].

In the present study, seven isolates belonging to Bacillus species were identified as lipase producers. This is consistent with various studies, which mentioned that bacterial Bacillus species are the prominent source of lipases enzyme as [46], *B. subtilis* [54], *Bacillus pumilus* [55], and *Bacillus licheniformis* [56,57].

Lipase production by Bacillus species in the current study showed a significant lipase zone diameter that varied from 3 to 4.7 cm at day 7 with an index of 3.5–4.9 cm. Bacillus strains have been studied on Tributyrin Agar by Demirkan *et al.* [41], where a large hydrolytic zone was found with index=2 formed by *B. cereus* strain. Other *Bacillus* spp. [58] have been studied, where lipase secretion of *Bacillus* spp. was assessed on solid media containing Tween 20 and olive oil and found the diameter of the zone of clearance varied from 2 to 3 cm.

The plasmid profile in the present study indicated that strains *Bacillus sp.* (FS10) and *A. baumannii* (FS11) harbored two plasmids, one plasmid of greater than 1200 bp and one plasmid of greater than 1500 bp. *A. faecalis* (FS8) harbored one plasmid of greater than 1200 bp. However, *A. baumannii* (FS6) and *S. aeria* (FS15) harbored no plasmid. Four plasmids ranging in size from 4.7 to 44.7 kb were found in the extensively antibiotic resistant *A. baumannii* [59], whereas two plasmids isolated by Mindlin *et al.* [60].

Conclusion

Lipase-producing bacteria have a biotechnological and ecological importance that attract lots of scientists to find new and more efficient strains. Oil and fuel contaminated soil is a good source for the isolation of lipase-producing bacteria, as 15 bacterial isolates were screened as lipase producers. The lipase-producing isolates belonged to *B. cereus*, *A. calcoaceticus*, *A. baumannii*, *B. cereus*, *A. faecalis*, *A. baumannii*, *Bacillus* sp., *A. baumannii*, *B. thuringiensis*, *S. aeria*, *Klebsiella* sp., and *B. subtilis*. No studies have yet shown lipase production from aeria species of the genus Sphingomonas, Therefore, the present study may be the first to detect *S. aeria* as a lipase producer, where the isolates showed strong lipase activity and could be a good source to produce lipase enzyme.

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Conflicts of interest

There are no conflicts of interest.

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