International Journal of Environment and Pollution

Volume 1, Issue 1

Research Article

Date of Submission: 28 November 2025
Date of Acceptance: 23 December 2025
Date of Publication: 30 December 2025

Isolation and Screening of Indigenous Bacteria from Hydrocarbon-Contaminated Soil for Petroleum Biodegradation

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Citation: Hazaimeh, M., Alsowayeh, N., Kanaan, B. (2025). Isolation and Screening of Indigenous Bacteria from Hydrocarbon-Contaminated Soil for Petroleum Biodegradation. *Int J Environ Pollut*. 1(1), 01-16.

Abstract
Due to the risks posed by the petroleum spill to ecological systems, it has become a primary environmental concern. Nowadays, the world claims to have developed efficient methods for removing petroleum pollution. Bioremediation using bacterial cells is a cost-effective and environmentally friendly technique. It depends on removing or converting petroleum pollution into a harmless form. The present research aims to isolate single bacterial strains capable of degrading petroleum contamination. The isolated strains were assessed for their ability to degrade petroleum and then tested for their capacity to produce biosurfactants. Then, it was identified using morphology, biochemical tests, and 16S rRNA. The results of 16S rRNA revealed that different Nocardia and Rhodococcus spp were isolated from the contaminated soil. The two strains of Rhodococcus sp. KSA1 strain (ON526877) and Rhodococcus sp. KSA4 strain (ON526878) degraded 49.35% and 32.78% of petroleum, respectively, and the two strains of Nocardia spp were Nocardia sp. KSA6 strain (ON421650) and Nocardia KSA10 strain (ON421651) degraded 33.78% and 19.17% of petroleum, respectively, within five days. Nocardia isolates are closely related to Nocardia species (KM233637) isolated from the USA, and Rhodococcus isolates are closely related to Rhodococcus isolates from Malaysia (MN566071 & MN566083) and Thailand (MT484133), as determined by sequencing and phylogenetic analyses of the 16S rRNA gene. Furthermore, these isolates produced a stable E24 emulsion for 72 hours. Finally, these isolates can be used in environmental conditions to remediate petroleum contamination in harsh conditions in Saudi Arabia.

Keywords: Bacterial Hydrocarbon Degrader, 16S rRNA, Bioremediation, Emulsification Test, Drop Collapse

Introduction

Crude oil consists mainly of poly-aromatic hydrocarbons (PAHs), paraffins, naphthene, and aromatic compounds such as sulfur compounds, organic nitrogen, and phenols [1]. Millions of barrels of petroleum, a key component of crude oil, have been consumed by developed countries as their primary source of energy. The exploration, refining, and transportation of petroleum and its products often result in spills of petroleum into the environment [2]. Petroleum contamination leads to environmental degradation and significant damage to the ecosystem, which can persist for a long time if left untreated [3]. The contamination can be detected after many years due to the persistence of these compounds in the environment [4,5].

Petroleum compounds are the primary sources of soil contamination, which can have a significant impact on human health and the ecosystem. Plant roots absorb it, get transferred to food chains, and accumulate in living organisms' bodies. It also changes biological and physicochemical properties and affects microbial communities' diversity and enzymatic activities [6]. Petroleum contamination changes soil properties, such as changing total organic carbon, nitrogen, and phosphorus contents, conductivity, and soil moisture [7,8]. PAH compounds are characterized by high hydrophobicity, which results in a strong affinity for soil. This phenomenon replaces water content in soil with PAH and reduces soil moisture [9].

Petroleum pollution is a primary pollutant due to its mutagenic, immunotoxic, carcinogenic, and neurotoxic properties [10]. Humans and animals living near petroleum contamination sources are exposed to high doses of petroleum contamination, which may weaken their immune systems and increase the likelihood of infections, anaemia, genotoxicity, changes in white blood cell numbers, and cancer [11]. Plant cells can also absorb low-molecular-weight hydrocarbons that can destroy entire plants. Touching plants such as leaves, stems, and roots has phytotoxic effects that generate plant stress, including anaerobic conditions, oxidative stress, and water and nutrient deficiency [12,13]. Humans who consume these plants may be affected by cancer, eye irritation, and a depressed nervous system, as shown by medical diagnosis [13]. Gradual contamination of groundwater converts the agricultural soil into a desert by eliminating biodiversity [14]. Many physicochemical techniques are used to treat petroleum contamination in environments, including sorbent materials, booms, washing, burning, and skimmers. However, these techniques have several drawbacks, including increased waste, high costs, and low efficiency in reducing pollution [10,15].

Bioremediation is a treatment approach for removing different types of contaminants from soil, including petroleum contamination [1]. Several bioremediation methods are employed to remove petroleum contamination from the environment, as outlined in Table 1. Bioremediation using microbial species has been established as an effective technique to restore environments with low ecological and economic impact [3]. It is aimed at removing or detoxifying petroleum contamination and converting it to harmless products such as CH_4, H_2O, and CO_2 [16]. Various microorganisms, particularly bacterial cells, are highly effective in biodegradation and can break down all classes of hydrocarbons. Bacterial cells have many advantages, such as being able to adapt to different environments, having diverse genes and metabolic processes, and fast reproduction [10]. In addition, bacterial cells are more active in biosurfactant production, which is the easiest way for hydrocarbon degradation [14].

Table 1: Bioremediation Methods Used to Remove Petroleum Pollution from the Environment

Method	Contaminant	References
Bio piles	Diesel	Jabbar et al. 2019
Immobilized microorganism consortium	Oil spills	Faria et al. 2021
Bioaugmentation using microorganisms	Petroleum	Nafal & Abdulhay 2020
Biostimulation using fertilizer	Petroleum Crude oil	Udume et al. 2023 Agbor et al. 2023 (2)
Biostimulation using composting	Crude oil	Camacho et al. 2024
Biostimulating using surfactants	Petroleum compounds	Zahed et al., 2022
Microbial genetic engineering	Hydrocarbon compounds	Maqsood et al., 2024
Phytoremediation & rhizoremediation	Petroleum	Hussain et al. 2018
Electrobioremediation	Organic compounds	Cameselle & Reddy, 2022

The industrial area in Majmaah City, Riyadh, Kingdom of Saudi Arabia (KSA), has numerous workshops specializing in car repair and oil changes for trucks. Leaking oil from cars and lorries contaminates the soil for long periods. The aged hydrocarbon in soil may contribute to the abundance and diversity of bacterial hydrocarbon degraders. The present work aimed to assess the petroleum degradation capacity of indigenous bacteria thriving in the soil of the Majmaah industrial area. In this study, we report bacterial isolates that can efficiently degrade petroleum hydrocarbon mixtures.

Materials and Methods

Sample collection

The industrial area of Al-Majmaah in Saudi Arabia has been affected by contaminated sandy soil spots containing petroleum derivatives, such as diesel and oil spills. These spots were formed due to the industrial area, which contains many mechanical workshops. The samples were obtained using a small shovel from a depth of approximately 10 cm and transferred to labeled, sterile glass. Then, the samples (30g from each site) were placed in a container and moved to the Research Laboratory at Majmaah University. Samples were then preserved at 4 °C for use within 48 hours of collection.

Isolation of hydrocarbons degrading bacteria

A test tube containing 10 mL of normal saline (0.85% NaCl) was filled with 1 gram of contaminated soil, and the tube was agitated vigorously using a shaker. Then, the suspension was left to stand for one minute. Autoclaved Erlenmeyer flasks containing 98 mL of autoclaved minimal salt media (MSM) were inoculated with one mL of the suspension. The culture media were prepared as described by [17]. In briefly, 1g K_2HPO_4, 1g KH_2PO_4, 1g NH_4NO_3, 0.02g $CaCl_2$. 0.2 g $MgSO_4$ and 0.05 g $FeCl_3$ were dissolved in 1 liter of double-distilled water. One mL of diesel oil was added as a carbon source. The samples

were incubated for 5 days at 30 °C and 150 rpm on a rotary shaker. The control was prepared with the same procedure and under the same conditions, but without inoculum or suspension. Bacterial growth was detected by comparing the turbidity of the culture medium with that of the control. To ensure the growth of the bacterial hydrocarbon degrader only in the medium, 1 mL of culture medium was transferred to another flask containing fresh MSM with 1 mL of diesel and incubated for 5 days under identical conditions. The process was performed five times [18].

MSM agar media was prepared by adding 15g agar to one liter of MSM media and autoclaving. The bacterial hydrocarbon degraders were qualitatively tested using MSM agar medium. The autoclaved media were poured into a petri dish plate and divided into two groups: one group was layered with 1 mL of diesel oil as a carbon source, and the other was without diesel oil as a control. Then, 100 µL of growth media solution was applied to the top layer and maintained at 30 °C for 5 days. All samples with or without a diesel layer were conducted in triplicate. Using brain heart infusion (BHI) agar media, single colonies of bacteria cultivated on MSM medium were separated and cultured successively to ensure purity. The putative bacterial hydrocarbon degrader isolates were partially characterized using colony morphology, cell morphology, Gram staining, biochemical analysis, and 16S rRNA sequencing.

Bacterial cell morphology and biochemical characteristics

Biochemical characteristics and cell morphology were used as preliminary indicators for identifying the obtained isolates. Colony characteristics, including colony growth, colour, elevation, surface shape, and the microscopic cellular structure, were documented. The characteristics of the isolates were studied using several standard biochemical tests, including Gram staining, indole test, citrate utilization, urease production, Methyl Red Test, hydrogen sulfide production, Voges-Proskauer reaction, catalase reaction, and oxidase production [19]. Methyl red is used to differentiate bacterial cells that produce acids, such as succinic, acetic, and lactic acid, from those that ferment glucose. The Voges-Proskauer test is used to differentiate bacterial cells that produce acetone during glucose fermentation [20].

Molecular Identification

DNA extraction and 16S rRNA PCR

DNA was extracted from the isolated colonies by QIAamp DNA Mini kits (QIAGEN), following the manufacturer's protocol. The eluted DNA was stored at -20 °C for later use. PCR was performed on the extracted DNA by amplifying the 16S rRNA gene utilizing forward primer (27F, 5'AGAGTTTGATC(A/C)TGGCTCAG-3') and reverse primer (1492R, 5'-TACGG(C/T)TACCTTGTTACGACTT-3') [21]. The PCR product (1300 bp) was amplified in a 20 µl total reaction volume, including 0.2 µl Koma-Taq (2.5 µl/µl), 2 µl of 10X Tag PCR buffer,1.6 µl 2.5 mM dNTP buffer mixture, one µl of each primer (10 pmol/µl), 2 µl extracted DNA template (20 ng/µl), and PCR-grade water in a total volume of 20 µl. The deoxynucleotide triphosphate (dNTP) buffer mixture contains each dNTP (dATP, dCTP, dGTP, and dTTP) at a concentration of 2.5 mM. It offers convenience, minimizes pipetting, and can be added directly to amplification reactions. Thermal cycling for amplification began with an initial denaturation step of 5 minutes at 95 °C, followed by 30 cycles of 95 °C for 0.5 minutes, 55 °C for 2 minutes, and 72 °C for 1.5 minutes, and a final extension cycle of 10 minutes at 72 °C. Positive and negative (no DNA template) controls were included. The amplified PCR product was separated via electrophoresis on a 1.5% agarose gel, subsequently stained with EtBr (0.5 µg/µL), and visualized under ultraviolet illumination.

Sequencing and phylogenetic analysis

Four 16S rRNA PCR amplicons were selected based on section 2.2. The bacteria were cultured several times in broth MSM media and then grown on MSM agar media layered with diesel hydrocarbon. Then, they were isolated utilizing the Montage PCR Clean-up Kit (Millipore). The purified amplicons were sequenced utilizing 785F 5'-GGATTAGATACCCTGGTA-3' and 907R 5'-CCGTCAATTCMTTTRAGTTT-3' (Macrogen). The sequencing was performed using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA). Macrogen, Inc., based in Seoul, Korea, resolved the sequencing data with an Applied Biosystems 3730XL automated DNA sequencing device. The sequences were read utilizing DNA BaserV3 software and compared using the Basic Local Alignment Search Tool (BLAST) (http://www.ncbi.nlm.nih.gov/BLAST). The 16S rRNA sequences from four isolates were aligned using ClustalW. Additionally, a Neighbor-Joining tree was constructed using MEGA X. The evolutionary distances are represented in terms of base substitutions per site. It was computed using the Maximum Composite Likelihood technique. In the process of building the trees, all gaps and missing locations were eliminated. There were 1000 replications in the bootstrap analysis. The four sequences from the study were banked in GenBank and assigned the accession numbers ON421650 and ON421651 (Nocardia species), ON526877, and ON526878 (Rhodococcus species).

Petroleum biodegradation

Following the isolation of a bacterial hydrocarbon-degrading colony, each colony was transferred separately into BHI broth and cultivated for 24 hours. The cells in the culture media were pelletized by centrifugation at 3000 rpm for 15 minutes. Next, the pellet was resuspended in normal saline and diluted to an optical density (OD) of 0.5 at 600 nm using a spectrophotometer. This concentration was used for petroleum biodegradation tests. The efficiency of these isolates in degrading petroleum was assessed by transferring 1 mL of bacterial cells (OD 0.5) into 98 mL of fresh MSM media and 1 mL of petroleum hydrocarbon and then pouring the mixture into a sterile Erlenmeyer flask under aseptic conditions. The flasks were placed in a shaker at 150 rpm and 30 °C for one week. Each sample was conducted in triplicate.

A petroleum refinery in Riyadh provided a petroleum mixture in our study. In parallel, flasks prepared under the same conditions but without inoculum serve as a control for studying the effects of abiotic conditions. The percentage of petroleum biodegradation was measured using the method of Dussauze et al [22]. The contents of the Erlenmeyer flask were poured into a separation funnel, and 30 mL of hexane was added to separate the residual petroleum from the aqueous phase. The mixture was then shaken vigorously by hand and left to stand for 10 minutes. The residual petroleum contents were collected and evaporated using evaporated flasks. Then, the spectrophotometer was used to measure the residual petroleum at a wavelength of 390 nm, expressed in mg/L as described by Fusey and Oudot [23].

Screening of Biosurfactants Production

Biosurfactant synthesis by bacterial strains is determined using screening tests such as the emulsification index and the drop collapse test ([24]. The culture media was centrifuged at 3000 rpm for 15 minutes, and the pellet was removed. To measure the emulsification index (E24), 3 ml of supernatant was poured into a test tube, followed by 3 ml of petroleum. The two phases were blended by vortexing. The test tube was then coated with paraffin and left for 24 hours. The (E24) was determined according to the Bader et al [25] equation:

$$E24 = \frac{\text{Height of emulsion}}{\text{Total height}}$$

The drop collapse assay is performed by adding 20 µL of culture supernatants to a glass slide coated with petroleum hydrocarbons. If the drop collapses and spreads, the bacterial cell is a biosurfactant producer. The control is designed by adding distilled water or culture media from the control to slides covered with a petroleum layer [26].

Statistical analysis

Statistical analysis was performed utilizing a one-way analysis of variance (ANOVA) test with SPSS v20.0. Software.

Results and Discussion

Bacterial isolates identification

When soil samples were added to the MSM broth, turbidity in the culture media began to appear after one day of incubation, unlike the control. This turbidity indicates successful bacterial growth, as it correlates with increasing bacterial cell numbers. The rise in turbidity suggests that the bacteria are using hydrocarbons as a carbon source and degrading them effectively [27,28]. The bacterial growth on the MSM agar media exhibited varied colony colors and morphologies. This diversity in appearance indicates the presence of different bacterial strains that have utilized diesel oil as a source of energy [29]. We isolated four different colonies based on the colony morphology and color. These pure colonies were then transferred and regrown onto BHI agar plates and designated as SA1, SA4, SA6, and SA10. The growth characteristics as well as the morphological features are summarized in Table 2. The results showed that all bacterial strains exhibited rapid growth, with well-developed, smooth, and white colonies.

Table 2: Morphological Characteristics of the Isolated Bacterial Strains

Isolates	Slow/Fast Growth	Colony Growth	Elevation	Surface	Color of Colony
SA1	Fast	Well Grown	Raised	Smooth	Pinkish white
SA4	Fast	Well Grown	Raised	Smooth	Pinkish white
SA6	Fast	Well Grown	Flat	Smooth	Bright white
SA 10	Fast	Well Grown	Flat	Smooth	Bright white

The data presented in Table 3 show the results of the biochemical assays. The results show that the four targeted isolates exhibit different biochemical characteristics. All strains were found to be gram-positive and tested negative for oxidase but positive for catalase in biochemical assays. Positive catalase indicates the isolates are decomposing H_2O_2 to overcome oxidative stress, and the cells are most probably aerobic [30]. SA1 and SA4 exhibited negative citrate utilization, while SA6 and SA10 showed positive results. The results demonstrated the positive citrate utilization ability of SA 6 and SA 10 isolates, which degrade and utilize citrates as a carbon source. The negative results of the urea test indicate that all the strains are most likely not H. pylori [31] .

Table 3: Biochemical Characterization of the Isolated Strains

Strains Name	Gram Staining	Oxidase Test	Catalase Test	Indole Test	Urea	Methyl Red	Voges-Proskauer	Citrate Utilization	H_2S Production
SA 1	+	-	+	-	-	+	-	-	-
SA 4	+	-	+	-	-	+	-	-	-
SA 6	+	-	+	-	-	-	-	+ (Growth & No Color change)	-
SA 10	+	-	+	-	-	-	-	+ (Color change)	-

Sequencing analysis of the 16S rRNA gene, spanning 659 bp, from two isolates taxonomically classified as Nocardia sp., revealed that the two bacterial isolates belong to the genus Nocardia and were deposited in the GenBank database as Nocardia sp. KSA6 strain (ON421650) and Nocardia KSA10 strain (ON421651). The pairwise alignment showed that the two strains are 100% identical and have ≥99.85 nucleotide identity with other Nocardia species sequences from North America (USA), Asia (Iran, China, Japan, Iraq, Turkmenistan, and Turkey), and Europe (Germany), and are closely related to Nocardia sp. X1654 strain isolated from the USA (Figure 1).

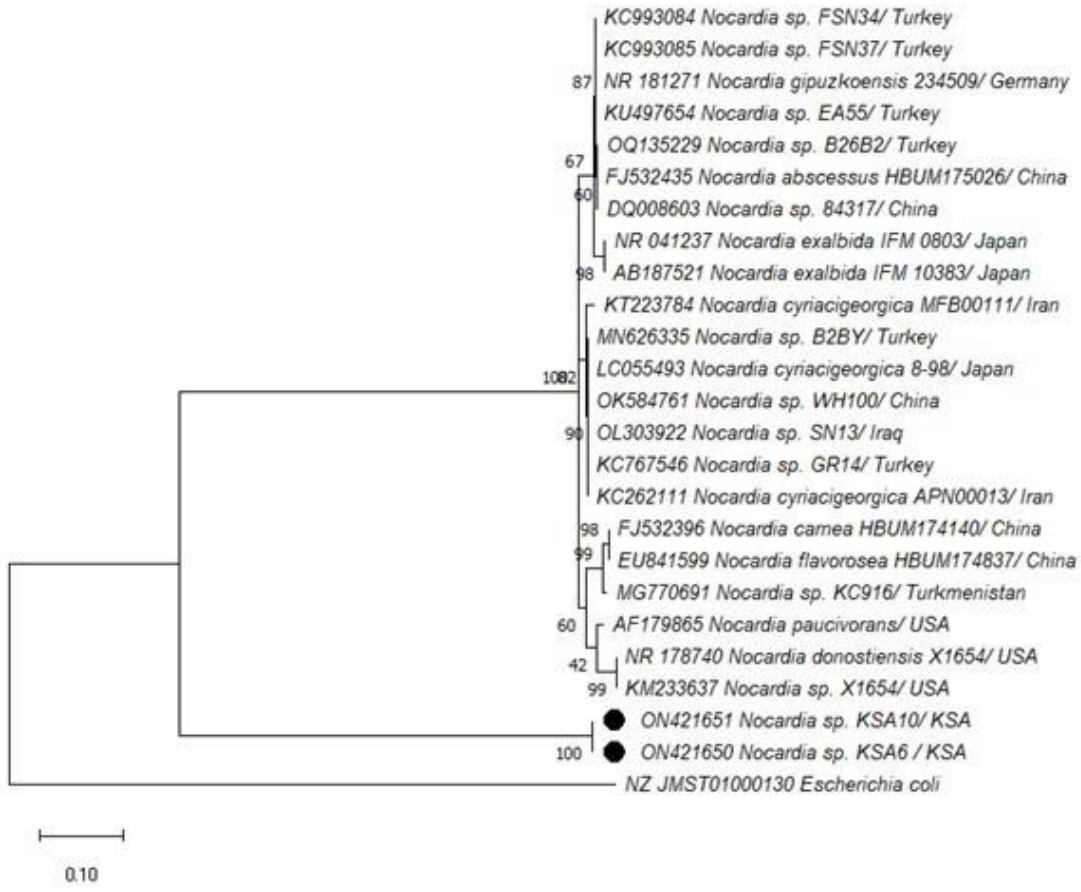

Figure 1: Phylogenetic Tree of Nocardia sp. (GenBank Accession No. ON421650-1) with Other

Nocardia sequences from GenBank are based on the 16S rRNA gene. The bootstrap test, the maximum Likelihood method, and the Jukes-Cantor model. In MEGAX (1000 replicates), the tree. Nocardia sp. isolates in this study are marked with black circles.

Additionally, sequencing analysis of the 16S rRNA gene, spanning over 680 bp, from two isolates taxonomically classified as Rhodococcus sp., revealed that the two bacterial isolates belong to the genus Rhodococcus and were deposited in the GenBank database as Rhodococcus sp. KSA1 strain (ON526877) and Rhodococcus sp. KSA4 strain (ON526878). The pairwise alignment showed that the two Rhodococcus strains have ≥99.6% identity with 0.002 evolutional divergence (3 nucleotide substitutions) and have >99.5 nucleotide identity with other Rhodococcus sequences from North America (USA and Costa Rica), South America (Argentina), Asia (Thailand, China, India, and Turkey, Europe (UK, Germany and Spain) and are closely related to Rhodococcus sequences from Malaysia (R. biphenylivorans, MN566071. MN566083) and Thailand (R. rhodochrous, MT484133) (Figure 2). Diversity within the same species may result from mutations, and gene flow introduces genetic variability into identical lineages of the new generation of daughter cells [32].

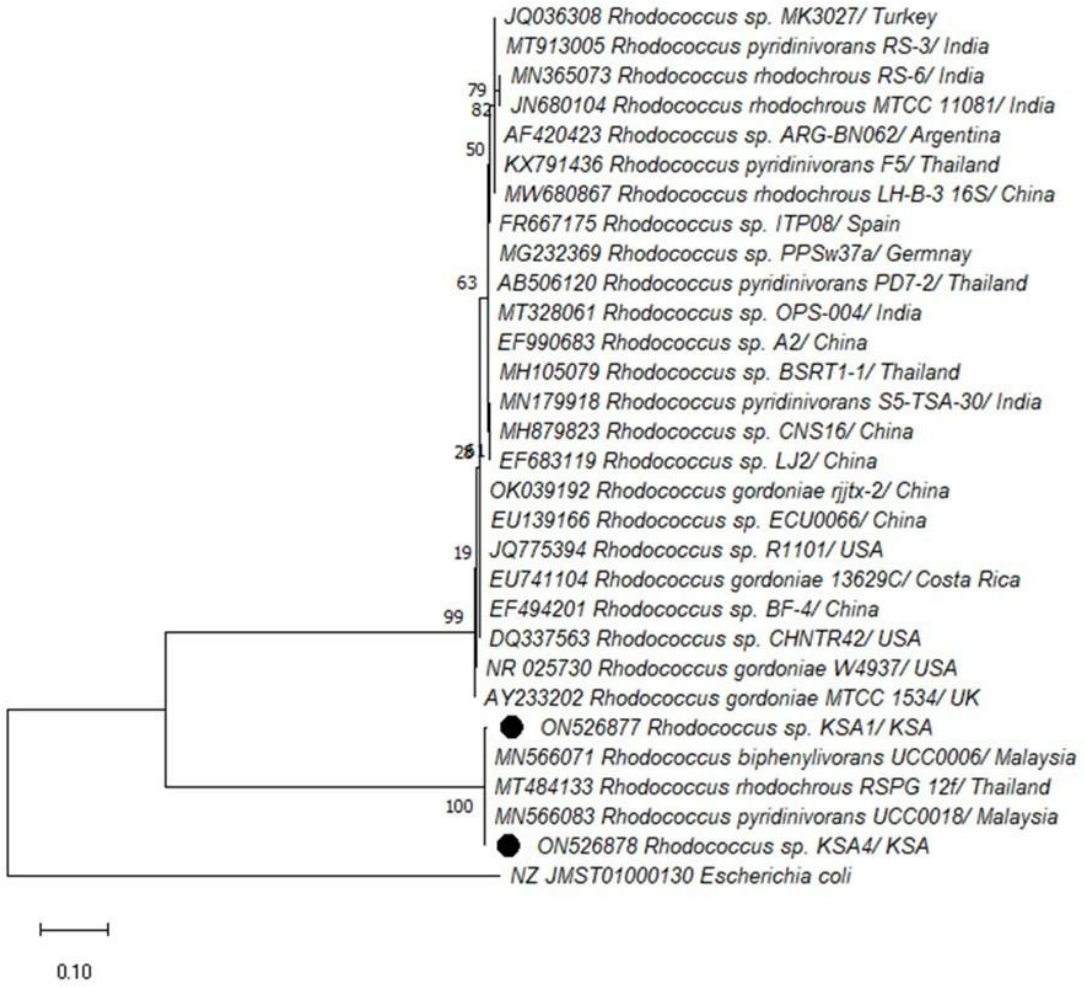

Figure 2: The phylogenetic tree of Rhodococcus sp. (GenBank Accession No. ODN526877-8) was constructed using sequences from GenBank, based on the 16S rRNA gene. The tree was drawn using

the Maximum Likelihood method and the Jukes-Cantor model, implemented in MEGAX with 1000 bootstrap replicates. Rhodococcus sp. isolates from this study are marked with black circles.

Petroleum biodegradation

Figure 3 shows the biodegradation results of petroleum hydrocarbons after five days of incubation. The results revealed different capacities for degrading petroleum hydrocarbons among the isolated strains. All isolated strains showed significantly degraded petroleum compared to the control ($p < 0.05$). The strains Nocardia sp. labeled SA 10 and SA 6 degraded 19.17% and 33.78% of petroleum, respectively. In contrast, the strains Rhodococcus sp. were labeled as SA4 and SA1, which degraded 32.46% and 49.46% of the substrate, respectively. Thus, strain SA 1 had the highest biodegradation percentage. R. erythropolis was reported to degrade 36.02% of petroleum within 66 days [33]. Alaidaroos described the ability of Rhodococcus sp. to enhance petroleum breakdown by altering its membrane composition, which resulted in increased cell surface hydrophobicity [34]. In addition, Rhodococcus spp. Enhances petroleum component degradation using different enzymes, such as alkane 1-monooxygenase [35]. Thi Mo et al. [36] reported the ability of Rhodococcus to adapt to the hydrocarbon-contaminated medium by forming a specialized cellular structure consisting of intracellular multi-membranous assemblies and surface vesicles connected using fibers. This architecture increases the surface contact area between the enzymes bound to the cell surface and the hydrocarbon compounds, enhancing the degradation process.

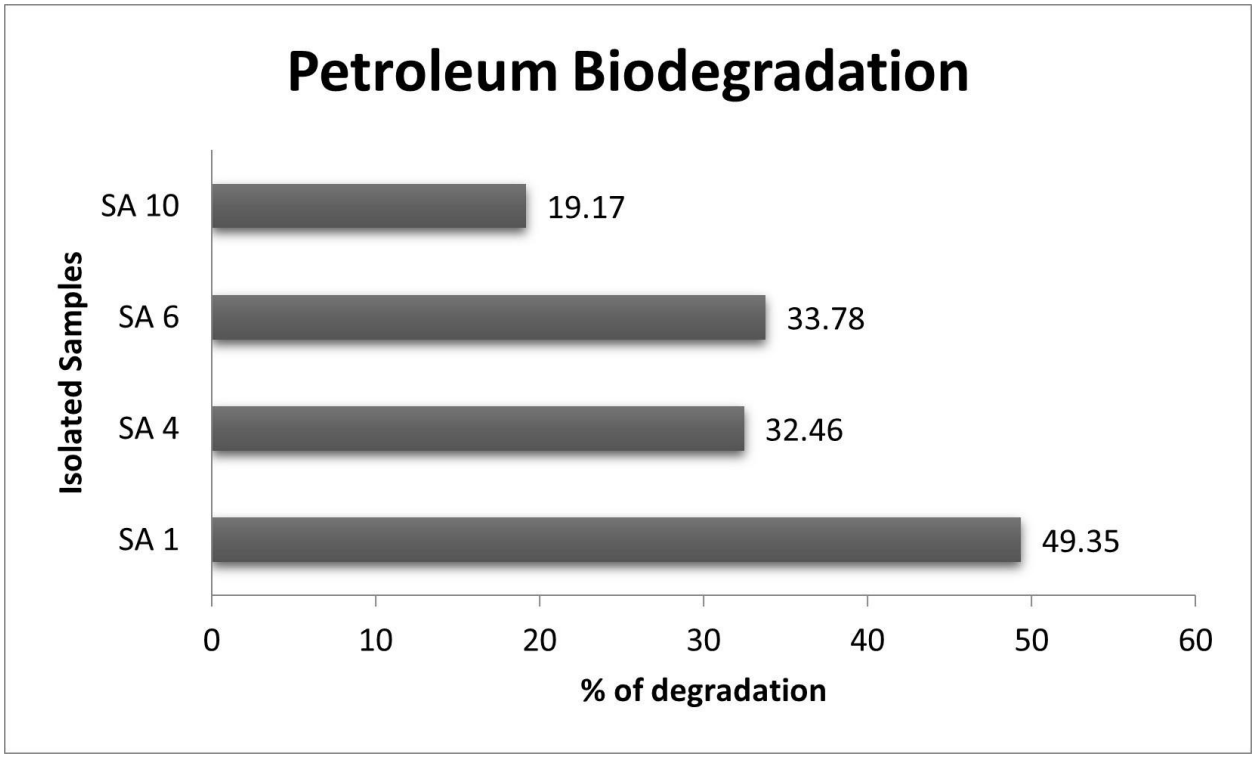

Figure 3: Petroleum Bioremediation Using an Isolated Bacterial Hydrocarbon Degrader.

Nocardia strains have been reported to degrade different hydrocarbon compounds such as paraffin wax, crude oil, phenol, rubber, and monocyclic aromatic hydrocarbons, and polyethylene [34,37]. It could be active in the degradation of polyaromatic hydrocarbons such as BTEX using the

monooxygenase and dioxygenase pathways [38]. Several species of Nocardia have been found to degrade various petroleum hydrocarbon compounds and produce new bioactive substances. Alotaibi et al. demonstrated that only a few species of Nocardia are isolated and capable of biodegrading hydrocarbon compounds [39]. They also reported that the Nocardia sp. strain utilizes various enzymes, including cytochrome P450 hydroxylase, Alkane 1-monooxygenase, and naphthalene dioxygenase, to biodegrade aerobically the petroleum hydrocarbon compounds present in soil.

Biosurfactants activity

The four isolates utilized petroleum oil in MSM media within 5 days of incubation. The emulsification test (E24%) and drop collapse tests demonstrated the capability of these isolates to generate biosurfactants. The E24% test showed emulsification activity for Rhodococcus sp. and Nocardia sp., as outlined in Table 4. The ability of an emulsion to resist the changes in its physicochemical properties is called emulsion stability [40]. Both Rhodococcus sp. strains (ON526877) and (ON526878) showed stable emulsion columns for 72 hours (E72% = 28±0.87% and 27±0.7%), respectively. Also, Nocardia sp. (ON421651) strain and (ON421650) strain both showed stability (E72% = 24±0.87% and 23±0.7%), respectively. This result indicates the stability of the produced emulsion. The emulsion is unstable by nature, and the two phases will separate if they stand for a while. The instability of emulsions may result in a decrease in the efficiency of biosurfactants and a shortening of their shelf life [41]. Thus, long-term stability of the emulsion may support petroleum biodegradation.

Table 4: Emulsification Activities and Drop Collapse for Isolated Strains

Bacterial strain	E24%	Drop collapse
Rhodococcussp. strain (ON526877)	29±1%	+
Rhodococcussp. strain (ON526878)	29±0.9%	+
Nocardiasp. strain (ON421651)	27±0.75%	+
Nocardiasp. strain (ON421650)	26±.83%	+

The significance of emulsifiers lies in their ability to reduce the interfacial tension between oil and water significantly. This reduction enhances the sweeping and displacement efficiency of petroleum droplets, increasing the availability of petroleum to be degraded by bacteria [42]. Additionally, the drop collapse test revealed that the free bacterial culture media drops from all culture media were flat droplets, unlike the control and distilled water. This result indicated positive results for biosurfactant production. The drop collapse test is susceptible to the presence of biosurfactants, providing results even with minimal quantities of biosurfactants [43].

Thi Mo et al. reported the ability of Rhodococcus to produce trehalolipid biosurfactants freely in culture media or bound to cell walls [36]. The biosurfactant types are ionic or nonionic trehalolipid. Rhodococcus can use biosurfactants to replace the phospholipid of the membrane. This property enables the cell membrane to regulate by attaching or detaching to hydrocarbon compounds. Juárez et al. reported the ability of Rhodococcus species to degrade long-chain alkanes and aromatic compounds under aerobic conditions [44]. Some Rhodococcus species possess unique genes related to the degradation of alkanes and aromatic compounds, such as benA, benB, benC, catA, catB, catC, and five alkB genes.

Javadi et al. reported that Nocardia sp. produced glycolipid biosurfactants, which have high potential to emulsify petroleum hydrocarbons and create stable emulsions [45]. Biosurfactant production enhances bacterial growth in culture media amended with petroleum and increases the bioavailability of petroleum compounds for degradation [19,46]. Some microbial cells produce biosurfactants to enhance the bioavailability of immiscible and unmixed hydrophobic compounds for biodegradation. Increasing the bioavailability of unmixed compounds for biodegradation provides a better chance for bacterial cells to survive under harsh conditions. Biosurfactant formation involves hydrophobic molecules as a carbon source in the growth medium. This alternative approach could be cost-effective, utilizing waste materials as feedstock for bacterial cells [25]. Alotaibi et al. have isolated Nocardia sp from soil contaminated with petroleum compounds [39]. They found that Nocardia sp. thrives at the plant roots in contaminated soil and plays a role in promoting plant growth in such environments. Although Nocardia sp. has demonstrated the ability to degrade petroleum compounds, the species has received limited attention in the literature, with few studies examining its capabilities to degrade petroleum and its derivatives.

Microbial cells can persist in hostile environments and remediate the pollutants in soil, water, and sediments [47]. When the bioremediation process is coupled with biosurfactant and bio-stimulant production, it offers shorter remediation periods and promises large-scale application in the future [48]. Bioremediation harnesses the capability of microorganisms and/or their enzymes to transform harmful organic pollutants to harmless compounds that no longer pose risks to organisms [49].

Conclusion

The current investigation showed that soil contaminated with hydrocarbon spills is rich in indigenous microbial hydrocarbon degraders. The morphological and biochemical tests for the isolated strains showed that all the isolates were Gram-positive. However, it differs in biochemical activities such as oxidase and catalase. The molecular identification approach, which involved constructing a neighbor-joining tree, identified the strains as Rhodococcus and Nocardia. This article further investigated the potential of Rhodococcus and Nocardia spp to break down crude oil petroleum in polluted milieus. These bacterial strains degraded between 19.17 and 49.35% of petroleum within a short period, which is an impressive rate. This proficiency in petroleum contaminants degradation leads to their progress in the practical environmental bioremediation of petroleum contamination. The emulsification test and drop collapse technique prove the ability of these isolates to produce biosurfactants. It can efficiently emulsify petroleum compounds and impact the biodegradation process. The isolates were able to produce stable emulsions for up to 72 hours. The results of this study suggest the potential for producing future commercial biodegradation products for petroleum contamination. Furthermore, immobilizing the isolated bacterial cells in natural materials may enhance petroleum bioremediation under environmental conditions.

Funding: No funding received
Ethical approval: Not applicable.

Author's contribution: Conceptualization: MH, BK; Methodology: NA, MH, BK, MME; Investigation: NA, MH, BK, MME; Software: MH, BK; Validation: NA, MH, BK, MME; Formal analysis: NA, MH, BK, MME; Resources: MH, BK; Data Curation; NA, MH; Writing - Original Draft: NA, MH, BK; Writing -

Review & Editing: MH, MME; Visualization: MH, NA; Supervision: MH, MME; Project administration: MME, MFK; Funding acquisition: None.

Declaration of competing interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability: No data was used for the research described in the article.

Acknowledgments: The author extends appreciation to the Deanship of Postgraduate Studies and Scientific Research at Majmaah University for funding this research work.

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