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16S Rrna-Based Molecular Identification of Indigenous Hydrocarbon-Associated Bacteria from Crude Oil-Contaminated Soil in Kirkuk, Iraq

Mustafa Abbas Askar

Department of Environmental Engineering, Faculty of Engineering, Bursa Uludağ University, 16059 Nilüfer, Bursa, Türkiye.

ABSTRACT: Petroleum hydrocarbon contamination can alter soil physicochemical conditions and select for microbial populations with potential relevance to natural attenuation and bioremediation. This study aimed to isolate and molecularly identify culturable indigenous bacteria from crude oil-contaminated soil collected near the North Oil Company facilities in Kirkuk, northern Iraq. The contaminated soil was characterized by visible petroleum contamination, alkaline pH, high organic carbon, and high total petroleum hydrocarbon concentration. Bacterial isolates were cultured, genomic DNA was extracted using a commercial DNA extraction kit, and the 16S rRNA gene was amplified by PCR. PCR products were verified by agarose gel electrophoresis and sequenced by Sanger sequencing. BLASTn analysis identified five bacterial isolates affiliated with *Bacillus subtilis*, *Stutzerimonas balearica*, *Ochrobactrum* sp., *Alcaligenes faecalis*, and a putative *Ralstonia* sp. The closest BLAST matches showed sequence identities ranging from 94.70% to 100.00%. Phylogenetic analysis supported the placement of the isolates within their respective bacterial lineages. The occurrence of these culturable taxa in highly hydrocarbon-contaminated soil suggests the presence of indigenous hydrocarbon-associated bacteria that may be relevant for future bioremediation studies. However, because hydrocarbon degradation activity was not directly tested, the isolates should be considered hydrocarbon-associated rather than confirmed hydrocarbon-degrading bacteria.

KEYWORDS: crude oil-contaminated soil; 16S rRNA; bacterial isolation; hydrocarbon-associated bacteria; BLASTn; phylogenetic analysis; Kirkuk; Iraq; bioremediation

1. INTRODUCTION

Petroleum hydrocarbon contamination is a major environmental problem in oil-producing and oil-processing regions because crude oil and petroleum-derived wastes can enter soil during extraction, refining, storage, transportation, leakage, and accidental spills [1], [2]. Petroleum hydrocarbons are chemically complex mixtures that include aliphatic hydrocarbons, aromatic hydrocarbons, resins, asphaltenes, and polycyclic aromatic hydrocarbons (PAHs). Their behavior in soil depends on volatility, solubility, sorption, biodegradability, and weathering stage [1], [3]. Once introduced into soil, petroleum hydrocarbons can alter soil structure, reduce aeration and water movement, coat soil particles, and negatively affect microbial and plant activity [1], [2]. Heavy petroleum fractions and high-molecular-weight PAHs are often more persistent because they are less soluble and more strongly sorbed to soil organic matter and fine particles [3], [4].

Microorganisms play a central role in natural attenuation and bioremediation of petroleum-contaminated soils because many bacteria and fungi can transform or degrade hydrocarbon compounds under suitable environmental conditions [1], [2], [5]. Microbial degradation is influenced by contaminant composition, oxygen availability, nutrient balance, moisture, pH, temperature, and bioavailability [1], [2], [5]. Indigenous microorganisms are especially important because long-term exposure to petroleum contamination may select microbial populations that tolerate hydrocarbons or participate in their transformation. However, detection of a bacterial taxon in contaminated soil does not by itself prove hydrocarbon-degradation activity; functional confirmation requires degradation assays, hydrocarbon-loss measurements, functional-gene analysis, enzyme assays, or related activity-based tests [2], [5].

Culture-based isolation remains useful in petroleum microbiology because it allows viable bacterial strains to be recovered, preserved, identified, and later tested for hydrocarbon-degradation potential [1], [2]. Although culture-dependent methods represent only the culturable fraction of the microbial community, they provide candidate isolates for future biodegradation, bioaugmentation, or mechanistic studies. Molecular identification using the 16S rRNA gene is widely applied for bacterial identification because the gene contains conserved and variable regions that allow comparison among bacteria and reference sequences [6]. The 16S rRNA gene is especially useful for genus-level bacterial identification, although species-level assignment may be less reliable for some bacterial groups and should be interpreted carefully when sequence identity or query coverage is low [6], [7].

The contaminated soil used in the present study was collected near the North Oil Company hot-processing complex in Kirkuk, northern Iraq, from the 0-10 cm soil layer. The same soil was previously described as dark, petroleum-odorous, and highly contaminated with total petroleum hydrocarbons of approximately 38,751 mg kg-1 and measurable PAHs. The soil also showed alkaline pH, high organic carbon, and nutrient conditions that may influence microbial activity in hydrocarbon-contaminated environments. These characteristics make the soil a relevant source for isolating and identifying culturable hydrocarbon-associated indigenous bacteria.

Therefore, the objective of this study was to isolate and molecularly identify culturable indigenous bacteria from crude oil-contaminated soil collected in Kirkuk, Iraq, using 16S rRNA gene amplification, Sanger sequencing, BLASTn analysis, and phylogenetic tree construction. The study aimed to determine the taxonomic identity and phylogenetic placement of recovered bacterial isolates and to discuss their possible relevance to petroleum-contaminated soil environments. Because direct hydrocarbon-degradation assays were not performed, the isolates are interpreted as hydrocarbon-associated bacteria rather than confirmed hydrocarbon-degrading strains.

2. MATERIALS AND METHODS

2.1. Soil sampling site and contamination background

The contaminated soil used for bacterial isolation was collected from an oil-impacted area near the North Oil Company hot-processing complex in Kirkuk, northern Iraq, located at approximately 35.406998° N and 44.214748° E. Soil was collected from the upper 0-10 cm layer at several points within the contaminated area and pooled to obtain a representative composite sample. The soil was visibly dark, had a strong petroleum odor, and showed weak structure, indicating substantial petroleum hydrocarbon contamination. Large stones and debris were removed, and the composite soil was manually homogenized before subsequent microbiological and physicochemical analyses. The location of the study site is shown in Figure 1.

Initial characterization showed high total petroleum hydrocarbon (TPH) contamination, with a concentration of 38,751.520 ± 846.204 mg kg-1. The soil also contained measurable PAHs, with total PAHs of 12.818 ± 0.754 mg kg-1. Baseline physicochemical characterization showed alkaline pH (8.17 ± 0.40), high total organic carbon (14.07 ± 0.44%), high organic matter content (24.32 ± 0.74%), total nitrogen of 0.107 ± 0.005%, and total phosphorus of 0.107 ± 0.008%, corresponding to a TOC-based C:N:P ratio of 100:0.76:0.76. These characteristics indicate a highly petroleum-contaminated soil matrix with elevated carbon loading and relatively limited nitrogen and phosphorus availability.

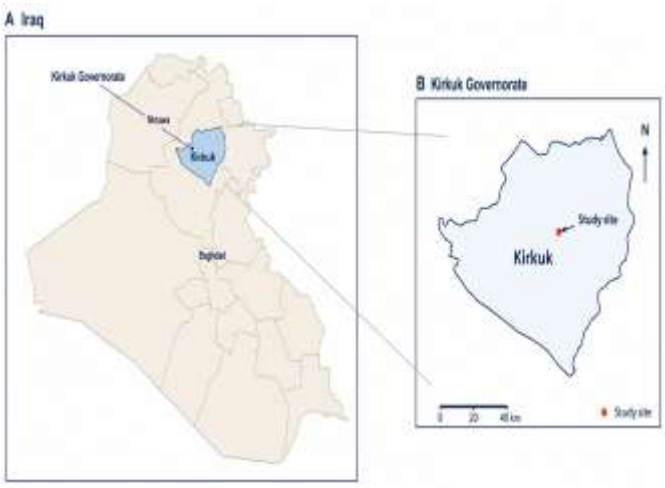

Figure 1. Location of the soil sampling site in Kirkuk Governorate, Iraq: (A) location of Kirkuk Governorate within Iraq; and (B) location of the study site within Kirkuk Governorate.

2.2. Bacterial isolation and culture

Culturable bacteria were isolated from the hydrocarbon-contaminated soil using a culture-dependent approach. Briefly, a soil suspension was prepared by mixing contaminated soil with sterile distilled water or sterile physiological saline, followed by serial dilution under aseptic conditions. Aliquots from appropriate dilutions were spread or streaked onto agar plates and incubated until visible bacterial colonies developed. Colonies showing distinct morphological characteristics were selected and purified by repeated streaking on fresh agar plates to obtain pure bacterial isolates.

Pure bacterial isolates were transferred into nutrient broth and incubated for 24 h at 30 °C to obtain sufficient bacterial biomass for genomic DNA extraction. After incubation, 1-2 mL of each bacterial culture was transferred into sterile 2 mL microcentrifuge tubes and centrifuged at 13,000 rpm for 1 min to pellet the bacterial cells. The supernatant was discarded, and the bacterial pellet was used for subsequent DNA extraction.

2.3. Genomic DNA extraction

Genomic DNA was extracted from pure bacterial cultures using the G-spin™ Total DNA Extraction Kit according to the manufacturer's protocol with minor procedural adjustments. For cell lysis, 200 µL of Buffer CL, 20 µL of Proteinase K, and 5 µL of RNase A solution were added to each bacterial pellet and mixed thoroughly by vortexing. The lysate was incubated at 56 °C for 10-30 min. After lysis, 200 µL of Buffer BL was added, mixed thoroughly, and incubated at 70 °C for 5 min. The lysate was centrifuged at 13,000 rpm for 5 min to remove unlysed cell debris, and approximately 350-400 µL of supernatant was transferred into a new 1.5 mL microcentrifuge tube.

An equal binding step was performed by adding 200 µL of absolute ethanol to the lysate, followed by pulse vortexing and brief centrifugation. The mixture was applied to a spin

column and centrifuged at 13,000 rpm for 1 min. The column was washed sequentially with 700 µL of Buffer WA and 700 µL of Buffer WB, each followed by centrifugation at 13,000 rpm for 1 min. Finally, DNA was eluted by adding 30-100 µL of Buffer CE directly onto the membrane, incubating for 1 min at room temperature, and centrifuging at 13,000 rpm for 1 min.

2.4. 16S rRNA gene PCR amplification

The extracted genomic DNA was used as a template for amplification of the bacterial 16S rRNA gene by polymerase chain reaction (PCR). Each PCR reaction was prepared in a final volume of 25 µL, containing 12.5 µL of master mix, 1 µL of forward primer, 1 µL of reverse primer, 5 µL of DNA template, and 5.5 µL of distilled water.

The 16S rRNA gene was amplified using the primer pair GTGTAGCGGTGAAATGCG (forward) and ACGGGCGGTGTGTACAA (reverse). PCR amplification was performed under the following thermal cycling conditions: initial denaturation at 95 °C for 5 min; 35 cycles of denaturation at 95 °C for 40 s, annealing at 58 °C for 1 min, and extension at 72 °C for 40 s; followed by final extension at 72 °C for 7 min. PCR products were checked by agarose gel electrophoresis before sequencing.

2.5. Agarose gel electrophoresis

The amplified 16S rRNA PCR products were examined by agarose gel electrophoresis to confirm successful amplification before sequencing. PCR products were separated on a 1.5% agarose gel containing ethidium bromide and visualized under ultraviolet (UV) light. The presence of clear amplification bands was used to verify the PCR products prior to sequence analysis.

2.6. Sanger sequencing and BLASTn identification

The amplified 16S rRNA PCR products were submitted to Macrogen Inc. (Seoul, South Korea) for Sanger sequencing. Sequence chromatogram files were obtained in ABI format (.ab1) and checked for sequence quality before taxonomic identification. Low-quality terminal regions were trimmed where necessary, and the resulting nucleotide sequences were converted into FASTA format for downstream analysis.

The cleaned 16S rRNA sequences were compared with reference sequences available in the NCBI nucleotide database using BLASTn [7]. The closest bacterial matches were evaluated based on percentage identity, query coverage, E-value, alignment score, and taxonomic consistency. Because 16S rRNA gene sequencing is generally more reliable for genus-level identification than for species-level assignment in some bacterial groups, species names were assigned conservatively when sequence similarity and taxonomic placement supported the result [6].

2.7. Phylogenetic analysis

Phylogenetic analysis was performed using MEGA software. The cleaned 16S rRNA sequences obtained from the bacterial isolates were combined with closely related reference sequences downloaded from GenBank in FASTA format. Reference sequences were selected based on the closest BLASTn matches, taxonomic relevance, query coverage, and percentage identity.

The sequences were aligned using MUSCLE or ClustalW in MEGA. Phylogenetic trees were constructed using the Neighbor-Joining method with the Kimura 2-parameter model. Tree topology reliability was evaluated using 1000 bootstrap replications. Gaps and missing data were treated using the partial deletion option, and bootstrap values above 50% were displayed at branch nodes. The phylogenetic placement of each isolate was evaluated by comparing its clustering pattern with closely related reference sequences.

3. RESULTS

3.1. Soil contamination background

The soil used for bacterial isolation showed clear evidence of petroleum hydrocarbon contamination. The sample was dark in color, had a strong petroleum odor, and was collected from an oil-impacted area near the North Oil Company hot-processing complex in Kirkuk, northern Iraq. The initial TPH concentration was 38,751.520 ± 846.204 mg kg-1, indicating a highly contaminated soil matrix.

The soil also contained measurable PAHs, with an initial total PAH concentration of 12.818 ± 0.754 mg kg-1. Baseline physicochemical characterization showed slightly alkaline pH (8.17 ± 0.40), TOC of 14.07 ± 0.44%, organic matter of 24.32 ± 0.74%, total nitrogen of 0.107 ± 0.005%, and total phosphorus of 0.107 ± 0.008%, corresponding to a TOC-based C:N:P ratio of 100:0.76:0.76. These characteristics indicate a petroleum-contaminated soil environment with high carbon loading and relatively limited nutrient availability.

3.2. PCR amplification and sequencing

The extracted genomic DNA from purified bacterial isolates produced successful 16S rRNA gene amplification. Clear PCR bands were observed on the 1.5% agarose gel, indicating that the target 16S rRNA gene fragments were successfully amplified and were suitable for sequencing. Sanger sequencing generated readable chromatogram files for the bacterial isolates. After quality checking and trimming, the cleaned sequences were used for BLASTn-based taxonomic identification and phylogenetic analysis.

3.3. BLASTn identification and phylogenetic placement of bacterial isolates

BLASTn analysis of the cleaned 16S rRNA gene sequences allowed taxonomic identification of five bacterial isolates recovered from the hydrocarbon-contaminated soil (Table 1). The isolates were affiliated with the genera *Bacillus*, *Stutzerimonas*, *Ochrobactrum*, *Alcaligenes*, and *Ralstonia*. The closest BLASTn matches showed sequence identities ranging from 94.70% to 100.00%, supporting reliable genus-level identification and, for most isolates, species-level assignment.

Isolate I1 showed 100.00% identity and 100% query coverage with *Bacillus subtilis* strain AZ, supporting its identification as *Bacillus subtilis*. Isolate I2 showed 100.00% identity and 100% query coverage with *Stutzerimonas balearica*, supporting its identification as *Stutzerimonas balearica*. Isolate I3 showed high similarity to *Ochrobactrum* sp., with 99.52% identity and 98% query coverage to *Ochrobactrum* sp. strain 7002-009; therefore, it was conservatively identified as *Ochrobactrum* sp. Isolate I4 showed 98.98% identity with *Alcaligenes faecalis*, supporting its identification as *Alcaligenes faecalis*. Isolate I5 showed the closest match to *Ralstonia pickettii* strain C2, but the sequence identity was relatively low (94.70%) despite 96% query coverage. Therefore, isolate I5 was conservatively reported as a putative *Ralstonia* sp. rather than a confirmed *Ralstonia pickettii* isolate.

Phylogenetic analysis supported the BLASTn-based identification of the isolates (Figure 2). Isolate I1 clustered within the *Bacillus subtilis* lineage (Figure 2a), while isolate I2 was placed within the *Stutzerimonas balearica* lineage (Figure 2b). Isolate I5 was positioned within the *Ralstonia* lineage (Figure 2c), isolate I4 clustered with *Alcaligenes faecalis* reference sequences (Figure 2d), and isolate I3 grouped with closely related *Ochrobactrum* sp. sequences (Figure 2e).

Table 1. BLASTn-based identification of bacterial isolates recovered from crude oil-contaminated soil.

Isolate code	Closest BLASTn match	GenBank accession no.	Query cover (%)	Identity (%)	Suggested identification
I1	*Bacillus subtilis* strain AZ	OR362822.1	100	100.00	*Bacillus subtilis*
I2	*Stutzerimonas balearica*	OR362823.1	100	100.00	*Stutzerimonas balearica*
I3	*Ochrobactrum* sp. strain 7002-009	KY770438.1	98	99.52	*Ochrobactrum* sp.
I4	*Alcaligenes faecalis*	OQ834459.1	96	98.98	*Alcaligenes faecalis*
I5	*Ralstonia pickettii* strain C2	JX010987.1	96	94.70	Putative *Ralstonia* sp.

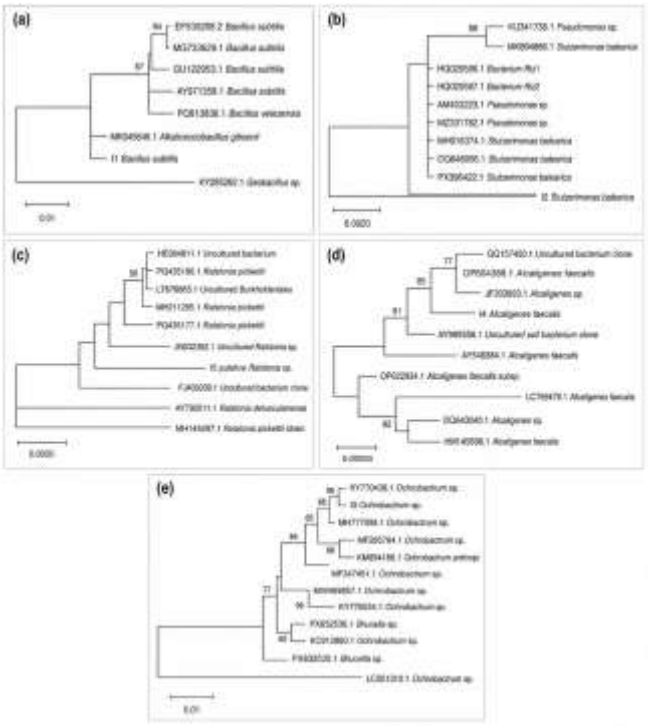

Figure 2. Phylogenetic trees based on partial 16S rRNA gene sequences showing the relationships between bacterial isolates recovered from crude oil-contaminated soil and closely related reference sequences retrieved from GenBank: (a) isolate I1, *Bacillus subtilis*; (b) isolate I2, *Stutzerimonas balearica*; (c) isolate I5, putative *Ralstonia* sp.; (d) isolate I4, *Alcaligenes faecalis*; and (e) isolate I3, *Ochrobactrum* sp. Trees were constructed using the Neighbor-Joining method with the Kimura 2-parameter model and 1000 bootstrap replications. Bootstrap values greater than 50% are shown at branch nodes.

4. DISCUSSION

4.1. Overview of identified hydrocarbon-associated bacteria

The culture-dependent and 16S rRNA-based approach used in this study identified five culturable bacterial isolates from crude oil-contaminated soil. The isolates were assigned to *Bacillus subtilis*, *Stutzerimonas balearica*, *Ochrobactrum* sp., *Alcaligenes faecalis*, and a putative *Ralstonia* sp. based on BLASTn similarity and phylogenetic placement. The recovery of these taxa from petroleum-contaminated soil suggests the presence of culturable indigenous bacteria that may tolerate hydrocarbon-associated stress. However, because direct hydrocarbon-degradation assays were not performed, the isolates should be interpreted as hydrocarbon-associated bacteria rather than confirmed hydrocarbon-degrading strains.

The identification of *Bacillus subtilis* is consistent with previous studies reporting *Bacillus* strains from petroleum-polluted environments. Wang et al. [10] reported *Bacillus subtilis* BL-27 isolated from petroleum-polluted soil and showed that the strain could degrade petroleum hydrocarbons

under experimental conditions. Wu et al. [11] investigated a defined co-culture of *Bacillus subtilis* and *Pseudomonas aeruginosa* and reported enhanced crude-oil degradation compared with individual cultures. Biosurfactant-producing *Bacillus subtilis* has also been reported in crude-oil-related bioremediation studies, where biosurfactant production supported hydrocarbon emulsification and removal [12]. These reports support the environmental relevance of detecting *B. subtilis* in crude oil-contaminated soil, although the degradation ability of the present isolate still requires direct testing.

The detection of *Stutzerimonas balearica* is also relevant to petroleum-contaminated environments. Salvà-Serra et al. [13] reported that *S. balearica* occurs in diverse environments, including polluted habitats, and described genomic and phenotypic features related to aromatic compound degradation. The isolate identified as *Ochrobactrum* sp. also fits the context of hydrocarbon-contaminated soil. Dike et al. [14] evaluated *Ochrobactrum* sp. immobilized on biochar for remediation of petroleum hydrocarbon-contaminated soil and described it as an autochthonous hydrocarbonoclastic bacterium. Similarly, *Alcaligenes faecalis* has been linked to crude oil-contaminated soil and aromatic hydrocarbon degradation; Singha et al. [15] reported the draft genome sequence of *A. faecalis* BDB4, a PAH-degrading bacterium isolated from crude oil-contaminated soil.

In contrast, the isolate related to *Ralstonia pickettii* was interpreted cautiously because the closest BLASTn match showed only 94.70% identity. This identity value is not sufficient for confident species-level assignment, so the isolate was retained as a putative *Ralstonia* sp. rather than being reported definitively as *Ralstonia pickettii*. Overall, the identified isolates represent a taxonomically diverse culturable bacterial group from highly petroleum-contaminated soil and provide a useful basis for future degradation assays, biosurfactant screening, crude-oil growth tests, and functional-gene analysis.

4.2. Interpretation of hydrocarbon association and functional limitations

The bacterial isolates recovered in this study were obtained from a highly hydrocarbon-contaminated soil; therefore, their occurrence suggests possible adaptation or tolerance to petroleum-associated stress. However, taxonomic identification based on 16S rRNA gene sequencing does not by itself confirm that an isolate can actively degrade crude oil, TPH fractions, or PAHs. The terms hydrocarbon-associated or potentially relevant to bioremediation are therefore more appropriate than confirmed hydrocarbon-degrading bacteria for the present isolates.

This distinction is important because 16S rRNA sequencing mainly provides taxonomic information, while hydrocarbon degradation is a functional trait. Although 16S rRNA sequencing is widely used for bacterial identification, it may not always provide reliable species-level resolution, and additional methods may be needed when identification is uncertain or when functional capacity is being claimed [6], [9]. Functional confirmation of hydrocarbon-degrading ability usually requires growth or degradation experiments in which hydrocarbons are provided as the main or sole carbon source, followed by chemical measurement of hydrocarbon loss.

For the present isolates, future confirmation could include growth on mineral salt medium containing crude oil, diesel, n-alkanes, or selected PAHs as the sole or main carbon source; measurement of TPH loss by GC-FID; PAH measurement by GC-MS; biosurfactant screening by emulsification index, oil-spreading assay, drop-collapse test, or surface-tension reduction; and detection of functional genes related to alkane or aromatic hydrocarbon degradation [17], [18]. These tests would help determine whether the identified isolates are active hydrocarbon degraders or mainly hydrocarbon-tolerant bacteria present in the contaminated soil.

4.3. Implications for future bioremediation studies

The identified isolates provide a useful starting point for future bioremediation-oriented investigations. Since they were recovered from highly crude oil-contaminated soil, they may possess tolerance to petroleum-associated stress and may include candidates with hydrocarbon-degradation potential. However, before they can be proposed for bioaugmentation or applied remediation, their functional abilities must be tested under controlled laboratory conditions.

A logical next step would be to screen each isolate individually and in mixed consortia. Mixed bacterial consortia may perform better than single isolates because different taxa can complement one another by degrading different hydrocarbon fractions or by producing metabolites that support cooperative degradation. For example, one isolate may contribute biosurfactant production, while another may be more efficient in degrading specific aliphatic or aromatic fractions [19].

The isolates may also be evaluated together with organic amendments such as date-palm waste. Since palm-waste biostimulation has shown strong improvement in TPH and PAH removal in the same contaminated soil system, future studies could compare natural attenuation, biostimulation with palm waste, bioaugmentation with selected isolates, and combined biostimulation-bioaugmentation. This would help determine whether the isolated bacteria can further improve remediation performance beyond amendment-based stimulation alone.

4.4. Study limitations

This study provides an initial culture-dependent molecular identification of indigenous bacteria isolated from crude oil-contaminated soil; however, several limitations should be considered. First, the study was based on culturable bacterial isolates only. Culture-dependent methods are useful because they provide viable strains for future testing, but they do not

represent the complete microbial community present in soil. Many environmental bacteria may remain uncultured under standard laboratory conditions; therefore, the five isolates identified here should be considered a partial representation of the culturable bacterial fraction rather than a complete description of the soil microbiome [8].

Second, bacterial identification was based mainly on partial 16S rRNA gene sequencing, BLASTn comparison, and phylogenetic placement. This approach may have limited species-level resolution for some bacterial groups, especially when sequence identity is low or when closely related taxa have highly similar 16S rRNA sequences [6], [9]. This limitation is particularly relevant for isolate I5, which showed only 94.70% identity to the closest *Ralstonia pickettii* reference sequence.

Third, this study did not include direct hydrocarbon-degradation assays or functional-gene analysis. Therefore, the identified isolates cannot be described as confirmed hydrocarbon-degrading bacteria. Their recovery from crude oil-contaminated soil and their taxonomic similarity to bacteria previously reported in contaminated environments indicate possible hydrocarbon association, but functional confirmation requires additional tests. Finally, the study was conducted using isolates obtained from one contaminated soil source; therefore, the findings should be interpreted as site-specific.

5. CONCLUSIONS

This study identified culturable indigenous bacteria isolated from crude oil-contaminated soil collected from an oil-impacted area in Kirkuk, northern Iraq. Based on 16S rRNA gene sequencing, BLASTn analysis, and phylogenetic placement, five bacterial isolates were assigned to *Bacillus subtilis*, *Stutzerimonas balearica*, *Ochrobactrum* sp., *Alcaligenes faecalis*, and a putative *Ralstonia* sp. BLASTn results showed high sequence similarity for most isolates, with identities ranging from 98.98% to 100.00% for four isolates, while the putative *Ralstonia* sp. isolate was interpreted cautiously because of its lower identity value of 94.70%.

The recovery of these bacteria from highly petroleum-contaminated soil suggests the presence of culturable hydrocarbon-associated indigenous bacteria that may be relevant for future bioremediation studies. Several of the identified taxa have previously been reported in petroleum-contaminated environments or hydrocarbon biodegradation-related studies, supporting their environmental relevance. However, because direct hydrocarbon-degradation assays were not performed, the isolates should not be described as confirmed hydrocarbon-degrading bacteria at this stage.

Overall, the study provides a useful taxonomic foundation for future applied work. The identified isolates can be prioritized for further screening using crude oil or selected hydrocarbons as carbon sources, TPH and PAH degradation assays, biosurfactant tests, functional-gene analysis, and replicated soil microcosm or pilot-scale experiments. These additional tests would help determine whether the isolated bacteria can contribute actively to hydrocarbon removal and whether they have practical potential for bioaugmentation or combined biostimulation-bioaugmentation strategies in petroleum-contaminated soils.

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