

Article

Isolation and Characterization of Antibiotic-Resistant and Biosurfactant-Producing Hydrocarbon-Degrading Bacteria from Industrial Contaminated Soils

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Abstract

In the current study, soil samples were collected from regions contaminated with industrial waste containing petroleum and its derivatives, including areas around a tire factory in Kırşehir, a chrome factory in Mersin and Kazanlı, and the Karaduvar refinery regions in Mersin, Türkiye. A total of 40 bacteria were isolated from the soil samples. The isolates were identified using molecular methods as well as morphological, physiological, and biochemical tests. Based on the results of 16S rRNA sequence analysis, it was revealed that the isolates belonged to the genera *Bacillus*, *Diaphorobacter*, *Cupriavidus*, *Acinetobacter*, *Massilia*, *Staphylococcus*, and *Azospirillum*. The antibiotic and heavy metal resistance of the strains were determined. Furthermore, the Drop Collapse method was employed to evaluate the biosurfactant production abilities of the strains, confirming that certain strains possess biosurfactant-producing capabilities. Consequently, distinct bacterial species capable of degrading polyaromatic hydrocarbons, resisting antibiotics and heavy metals, and producing biosurfactants were successfully isolated from contaminated areas in the current study. It is thought that the utilization of species with these properties in bioremediation studies will contribute to the restoration of ecosystem balance.

Keywords: bioremediation; polycyclic aromatic hydrocarbons (PAHs); biosurfactant production; heavy metal resistance; industrial waste



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1. Introduction

The rapid increase in industrial development, urbanization, and the intensive use of agrochemicals and anthropogenic activities has introduced numerous pollutants into the environment, such as pesticides, herbicides, heavy metals, and petroleum-based products [1]. Among these hazardous industrial wastes, petroleum and its derivatives cause critical damage to aquatic and terrestrial ecosystems due to high global demand. Today, a significant portion of the energy resources required for industrial and domestic needs is met by oil and petroleum products [2,3]. It is well established that petroleum and its derivatives contain various detrimental components, including n-alkanes, cycloalkanes, and PAHs [4].

Hydrocarbons derived from oil, such as diesel, kerosene, and petrol, frequently contaminate soil and surrounding ecosystems during oil drilling, fuel waste disposal, and accidental spills from tanker ships [5,6].

The interaction of petroleum residues with nature alters their original properties due to the combined effects of numerous physical and biological factors. The long-term presence of these hydrocarbon-containing derivatives leads to environmental pollution, resulting not only in the death of the affected flora and fauna but also in various genetic mutations [1,7]. Moreover, chronic exposure to excessive petroleum can lead to internal organ diseases, bone marrow damage, and a high risk of cancer due to the presence of mutagenic substances [7,8]. Consequently, there is an increasing demand for remediation methods that alleviate these problems while minimizing harm to the ecosystem. Various approaches, including chemical, physical, thermal, and biological strategies, have been developed [9,10]. However, biological applications, such as microbial bioremediation, offer more eco-friendly and cost-effective approaches that are gaining wider attention [11,12].

To date, numerous hydrocarbon-degrading bacteria have been isolated and molecularly identified from industrial wastes, as well as from soil and water exposed to oil products [5,13,14]. Molecular identifications have revealed that genera such as *Acinetobacter*, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Corynebacterium*, *Micrococcus*, *Pseudomonas*, *Methylobacterium*, and *Rhodococcus* are among the most common hydrocarbon degraders. Many of these bacteria also exhibit resistance to heavy metals and antibiotics, alongside biosurfactant-producing properties [15,16]. Furthermore, PAH contamination in industrial areas is often coupled with the presence of heavy metals. Heavy metal contamination can negatively affect the bioremediation of hydrocarbons due to the synergistic cytotoxic effects of these dual contaminants. Therefore, searching for bacterial strains that can degrade PAHs while exhibiting high resistance to heavy metals in the soil has become a critical research priority [12]. Additionally, the biological degradation capacity of microorganisms is often restricted by the hydrophobic nature of petroleum. While chemical surfactants have been developed to enhance degradation, microbial-derived biosurfactants are more effective for decontaminating oil spills due to their superior biodegradability [17].

The present study aimed to isolate and characterize native bacterial species from industrial waste-contaminated areas and petroleum and its derivatives that are capable of degrading polyaromatic hydrocarbons, resisting antibiotics and heavy metals, and producing biosurfactant, predicting the ability of strains in various environmental conditions.

2. Materials and Methods

2.1. Collection of Soil Samples

Soil samples were collected from industrial areas contaminated with petroleum and its derivatives in Türkiye. The sampling sites included a tire factory in Kırşehir, a chromium factory in Mersin, and refinery regions in the Kazanlı and Karaduvar districts of Mersin. Samples were collected aseptically, stored in sterile plastic zip-lock bags, and transported to the Microbiology Laboratory at Kırşehir Ahi Evran University. All samples were maintained at 4 °C until further analysis.

2.2. Crude Oil and Reference Strain

The crude oil used in this study was obtained from the Aliğa Tüpraş Petroleum Refinery (İzmir, Türkiye). *Pseudomonas aeruginosa* ATCC 27853 (Boston-USA) was utilized as the reference strain (it was used because it belongs to the genus (*Pseudomonas*) and species that degrade hydrocarbons).

2.3. Isolation and Screening of Hydrocarbon Degrading Bacteria

For the isolation process, a mineral salt medium (MSM) was prepared containing 1.0 g KNO₃, 0.2 g MgSO₄, 0.1 g NaCl, 0.1 g CaCl₂, and 1.0 g K₂HPO₄ per liter. The chemicals were purchased from Merck (Darmstadt, Germany). To this, 1% crude oil/Triton-X-100 (1:1) was added as the carbon source. Ten grams of soil sample were inoculated into 100 mL of this medium and incubated in a shaker incubator (MAXQ 4450) at 28 °C and 180 rpm for three days. Following this, 10 mL of the culture was transferred into fresh medium for a second round of enrichment; this process was repeated a total of three times. After the final incubation, 0.1 mL of the culture was spread onto Nutrient Agar (NA) plates. All experiments were repeated three times. Morphologically distinct colonies were selected using a binocular microscope (Novex P-20, Euromex Microscopen bv, Arnhem, The Netherlands) and isolated for further characterization [18,19].

2.4. Morphological and Biochemical Characterization

The isolated hydrocarbon-degrading strains were subcultured on NA medium. Morphological characteristics, including colony shape and color, were recorded. Then, Gram staining, 3% KOH, motility, catalase, and oxidase tests were exploited for assessment of morphological, physiological, and biochemical characterizations of bacterial strains by utilizing conventional methods according to the Harley and Prescott (2002) method [20].

2.5. Determination of Antibiotic Resistance

Antibiotic susceptibility testing was conducted on Mueller–Hinton Agar (MHA) using the disc diffusion method. The following ten antibiotics were utilized: Ampicillin (AM, 10 µg), Nalidixic acid (NA, 30 µg), Chloramphenicol (C, 30 µg), Tetracycline (TE, 30 µg), Nitrofurantoin (F/M, 300 µg), Gentamicin (GM, 10 µg), Imipenem (IPM, 10 µg), Ciprofloxacin (CIP, 5 µg), Cefotaxime (CTX, 30 µg), and Erythromycin (E, 15 µg). *P. aeruginosa* ATCC 27853 served as the reference strain to ensure antibacterial efficacy [21]. Isolates were activated in nutrient broth, adjusted to a 0.5 McFarland turbidity standard, and inoculated onto MHA plates using the spread-plating technique. Antibiotic discs were placed at specific intervals. Following incubation at 28 °C for 24 h, the diameters of the inhibition zones were measured and recorded in millimeters [22].

2.6. Determination of Heavy Metal Resistance Levels

Metals such as cobalt (Co), zinc (Zn), manganese (Mn) and mercury (Hg) are among the most well-known heavy metals for bioremediation studies [23]. In this regard, heavy metal resistance was evaluated using the well-diffusion method [23]. Based on preliminary experiments, concentrations were set at 5, 10, 15, and 20 mM to identify the most resistant strains. Aqueous solutions of CoCl₂·6H₂O, ZnSO₄·7H₂O, MnCl₂·2H₂O, and HgCl₂ were sterilized using 0.2 µm PTFE filters before being added to the media. *P. aeruginosa* ATCC 27853 was used as the control. Strains exhibiting growth at higher metal concentrations than the control were considered resistant.

2.7. Cultivation for Biosurfactant Production

To induce biosurfactant production, stock cultures were first activated in Tryptic Soy Broth (TSB). The activated cultures were then inoculated into Mineral Salt Medium (MSM) at a 1/20 (*v/v*) ratio under sterile conditions. Incubation was performed at 35 °C for 10 days in a shaking incubator (Thermo MAXQ 4450, Thermo Fisher Scientific, Marietta, OH, USA) at 150 rpm [24].

2.8. Detection of Biosurfactant Activity

Biosurfactant production was detected using the “Drop Collapse” method. Cultures were centrifuged at 10,000 rpm for 20 min (Micro CL 17), and the supernatants were filtered through 0.2 µm PTFE membranes. For the assay, 96-well microwell plates were coated with 7 µL of mineral oil and equilibrated at room temperature for 24 h. Subsequently, 25 µL of the filtrate was dropped onto the oil surface at a 45° angle. Sterile water and uninoculated medium served as negative controls. Biosurfactant activity was confirmed by observing the collapse or spreading of the droplets compared to the stable droplets of the controls [24].

2.9. Molecular Identification via 16S rRNA

Molecular identification of selected bacterial strains was realized by using 16S rRNA gene sequencing. Genomic DNA extraction was performed according to the following method, and the 16S rDNA gene region was amplified via Polymerase Chain Reaction (PCR) using the universal primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'). The 30 µL reaction mixture contained 10× PCR buffer, 10 mM dNTPs, 1.2 µL DMSO, 50 µM MgCl₂, 0.3 U/mL Taq DNA polymerase, and 100 ng/µL template DNA. PCR amplification was performed with an initial denaturation at 95 °C for 2 min, followed by 36 cycles of 94 °C for 1 min, 54 °C for 1 min, and 72 °C for 2 min, with a final extension at 72 °C for 5 min. The resulting PCR products were analyzed using 1% agarose gel electrophoresis, stained with ethidium bromide, and visualized under a gel documentation system (DNR Bio-Imaging Systems).

3. Results

3.1. Morphological and Biochemical Characterization

In the present study, a total of 40 bacterial strains were isolated from the specified contaminated sites. Morphological analysis revealed that 25 isolates (62.5%) exhibited smooth (S-type) colonies, 12 (30%) showed rough (R-type) colonies, 2 (5%) were mucoid (M-type), and 1 (2.5%) was characterized as L-type. Gram staining results indicated that 17 isolates were G (+), while 23 were G (−). These results were further confirmed by the 3% KOH test, which yielded 23 positive and 17 negative results, consistent with the Gram reactions. Regarding motility, 24 isolates were found to be motile, while 16 were non-motile.

Biochemical testing showed that 38 isolates were catalase-positive, forming characteristic gas bubbles upon the addition of hydrogen peroxide, whereas only 2 isolates were catalase-negative. In the oxidase test, 25 isolates (62.5%) tested positive by oxidizing the p-aminodimethylaniline reagent to a purple-blue color, while 15 isolates were oxidase-negative. The morphological and biochemical properties of the strains with the highest hydrocarbon-degrading potential are summarized in Table 1. (Note: *P. aeruginosa* ATCC 27853 was excluded from these specific counts.)

Table 1. Morphological, physiological and biochemical characterization results for bacterial isolates.

Bacterial Code	Cell Morphology	Gram Property	Motility	KOH (%3)	Catalase Activity	Oxidase Activity
YH 9-2	Bacilli	+	+	—	+	—
YH 9-4	Bacilli	—	—	+	+	+
YH 9-5	Bacilli	—	—	+	+	+
YH 9-6	Bacilli	—	—	+	+	+
YH 9-8	Coccobacillus	—	—	+	+	—
YH 9-12	Bacilli	+	+	—	+	+

Table 1. Cont.

Bacterial Code	Cell Morphology	Gram Property	Motility	KOH (%3)	Catalase Activity	Oxidase Activity
YH 10-11	Bacilli	+	+	—	+	+
YH 10-12	Coccobacillus	—	—	+	+	—
YH 11-1	Bacilli	—	+	+	+	+
YH 13-1	Bacilli	—	+	+	+	+
YH 13-2	Bacilli	—	+	+	+	+
YH 13-3	Bacilli	+	—	—	+	—
YH 13-4	Bacilli	+	—	—	+	—
YH 13-5	Bacilli	+	+	—	+	—
YH 15-2	Bacilli	+	—	—	+	—
YH 15-3	Bacilli	—	—	+	+	+
YH 16-1	Bacilli	—	—	+	+	+
YH 16-4	Bacilli	—	—	+	—	—
YH 16-6	Bacilli	—	+	+	+	+
YH 16-7	Bacilli	—	+	+	+	+

“+” as positive, “—” as negative.

3.2. Antibiotic Resistance Profiles

The resistance rates among the 20 selected isolates were as follows: Tetracycline (75%), Nitrofurantoin (65%), Ampicillin (60%), Nalidixic Acid (60%), Ciprofloxacin (55%), Erythromycin (50%), Chloramphenicol (45%), Cefotaxime (40%), Gentamicin (25%), and Imipenem (15%).

Tetracycline was identified as the antibiotic with the highest resistance rate (75%), whereas Imipenem proved to be the most effective, with only 15% resistance. Notably, isolate YH 13-1 exhibited multidrug resistance against all tested antibiotics. Conversely, isolates YH 13-4 and YH 16-1 were found to be susceptible to all antibiotics (Table 2 and Figure 1).

Table 2. Antibiotic resistance of bacterial strains.

Bacterial Code	Nalidixic Acid (NA, 30 µg)	Imipenem (IPM, 10 µg)	Cefotaxime (CTX, 30 µg)	Gentamicin (GM, 10 µg)	Erythromycin (E, 15 µg)
YH 9-2	R	S	S	S	S
YH 9-4	S	S	S	S	R
YH 9-5	S	S	R	R	R
YH 9-6	S	R	S	R	R
YH 9-8	R	S	R	S	R
YH 9-12	R	S	S	S	S
YH 10-11	R	S	S	S	S
YH 10-12	R	S	R	R	R
YH 11-1	R	S	S	R	S
YH 13-1	R	R	R	R	R

Table 2. Cont.

YH 13-2	S	S	S	S	S
YH 13-3	R	S	R	S	S
YH 13-4	S	S	S	S	S
YH 13-5	R	S	S	S	S
YH 15-2	R	S	S	S	R
YH 15-3	R	S	R	S	S
YH 16-1	S	S	S	S	S
YH 16-4	S	S	S	S	R
YH 16-6	S	S	S	S	R
YH 16-7	R	S	R	S	R
<i>P. aeruginosa</i>					
ATCC 27853	R	R	R	S	R
Bacterial Code	Tetracycline (TE, 30 µg)	Ciprofloxacin (CIP, 5 µg)	Chloramphenicol (C, 30 µg)	Ampicillin (AM, 10 µg)	Nitrofurantoin (F/M, 300 µg)
YH 9-2	R	S	R	R	R
YH 9-4	R	S	S	R	R
YH 9-5	R	S	S	R	R
YH 9-6	R	R	S	R	R
YH 9-8	R	R	R	R	R
YH 9-12	R	S	R	S	R
YH 10-11	R	R	S	R	R
YH 10-12	R	R	R	R	R
YH 11-1	R	R	S	S	R
YH 13-1	R	R	R	R	R
YH 13-2	R	S	S	S	S
YH 13-3	R	R	R	R	R
YH 13-4	S	S	S	S	S
YH 13-5	S	R	S	S	S
YH 15-2	R	R	S	R	R
YH 15-3	R	R	S	R	R
YH 16-1	S	S	S	S	S
YH 16-4	S	S	S	R	S
YH 16-6	R	S	R	S	S
YH 16-7	S	S	R	R	S
<i>P. aeruginosa</i>					
ATCC 27853	R	R	R	S	R

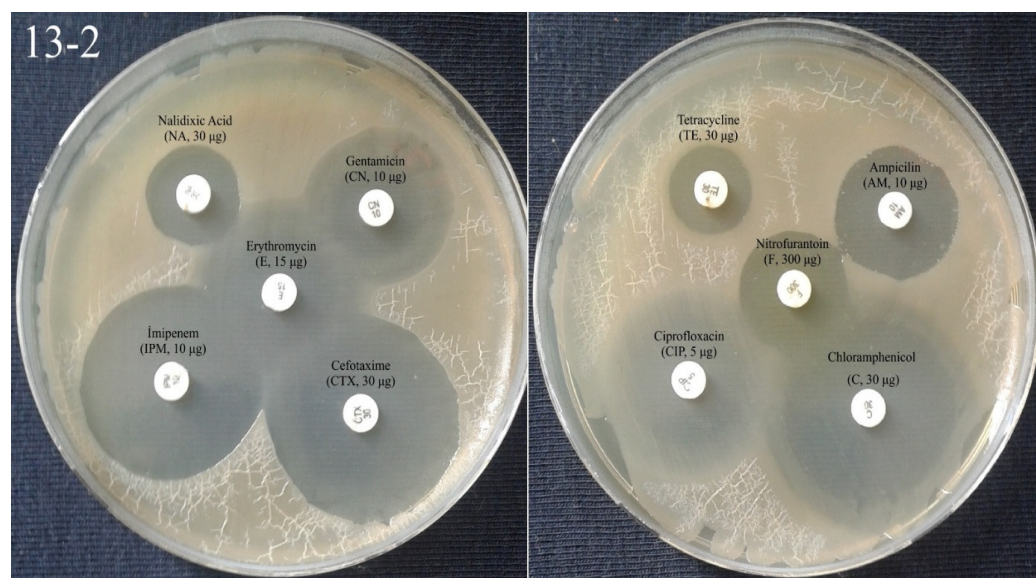


Figure 1. Antibiotics resistance results of YH13-2.

3.3. Determining Heavy Metal Resistance Levels of Bacterial Strains

Heavy metal resistance was evaluated using *P. aeruginosa* ATCC 27853 as a control strain (Table 3). For cobalt, 25% of the isolates were resistant to 5 mM, 5% to 10 mM, and 10% to 20 mM, while 60% were sensitive. Regarding zinc, 10% were resistant to 5 mM and 10 mM, and 15% to 20 mM; however, 65% of the total isolates showed resistance to cobalt at various levels. Manganese resistance was significantly higher, with 40% of the isolates tolerating concentrations up to 20 mM. In contrast, resistance to mercury was minimal; only isolate YH 10-12 exhibited resistance at 5 mM, while all other isolates were sensitive across all tested concentrations (Figure 2). Isolate YH 13-1 was found to be resistant to all antibiotics, as well as heavy metals except HgCl_2 . Also, similar results were found in the other bacterial strains (Tables 2 and 3).

Table 3. Heavy metal resistance of bacterial strains.

Bacterial Isolates	Concentration (mM)	$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	$\text{MnCl}_2 \cdot 2\text{H}_2\text{O}$	HgCl_2
YH 9-2	5	S	S	R	S
	10	S	S	R	S
	15	S	S	R	S
	20	S	S	R	S
YH 9-4	5	R	S	R	S
	10	S	S	R	S
	15	S	S	S	S
	20	S	S	S	S
YH 9-5	5	R	S	R	S
	10	S	S	S	S
	15	S	S	R	S
	20	S	S	S	S
YH 9-6	5	S	R	R	S
	10	S	R	R	S

Table 3. Cont.

Bacterial Isolates	Concentration (mM)	CoCl ₂ 6H ₂ O	ZnSO ₄ 7H ₂ O	MnCl ₂ 2H ₂ O	HgCl ₂
YH 9-6	15	S	R	R	S
	20	S	R	R	S
YH 9-8	5	S	R	R	S
	10	S	S	R	S
	15	S	S	S	S
	20	S	S	S	S
YH 9-12	5	S	S	R	S
	10	S	S	R	S
	15	S	S	R	S
	20	S	S	R	S
YH 10-11	5	R	S	S	S
	10	S	S	S	S
	15	S	S	S	S
	20	S	S	S	S
YH 10-12	5	S	R	R	R
	10	S	R	R	S
	15	S	R	S	S
	20	S	R	S	S
YH 11-1	5	S	R	R	S
	10	S	S	R	S
	15	S	S	R	S
	20	S	S	R	S
YH 13-1	5	R	R	R	S
	10	R	R	R	S
	15	R	R	R	S
	20	R	R	R	S
YH 13-2	5	R	R	S	S
	10	R	R	S	S
	15	S	S	S	S
	20	S	S	S	S
YH 13-3	5	R	S	R	S
	10	S	S	R	S
	15	S	S	R	S
	20	S	S	R	S
YH 13-4	5	R	S	S	S
	10	R	S	S	S
	15	R	S	S	S
	20	R	S	S	S

Table 3. Cont.

Bacterial Isolates	Concentration (mM)	CoCl ₂ 6H ₂ O	ZnSO ₄ 7H ₂ O	MnCl ₂ 2H ₂ O	HgCl ₂
YH 13-5	5	R	S	R	S
	10	S	S	R	S
	15	S	S	S	S
	20	S	S	S	S
YH 15-2	5	S	S	R	S
	10	S	S	S	S
	15	S	S	S	S
	20	S	S	S	S
YH 15-3	5	S	S	R	S
	10	S	S	R	S
	15	S	S	R	S
	20	S	S	S	S
YH 16-1	5	S	R	R	S
	10	S	R	S	S
	15	S	S	S	S
	20	S	S	S	S
YH 16-4	5	S	S	R	S
	10	S	S	R	S
	15	S	S	R	S
	20	S	S	R	S
YH 16-6	5	S	S	R	S
	10	S	S	R	S
	15	S	S	R	S
	20	S	S	R	S
YH 16-7	5	S	S	R	S
	10	S	S	R	S
	15	S	S	R	S
	20	S	S	R	S

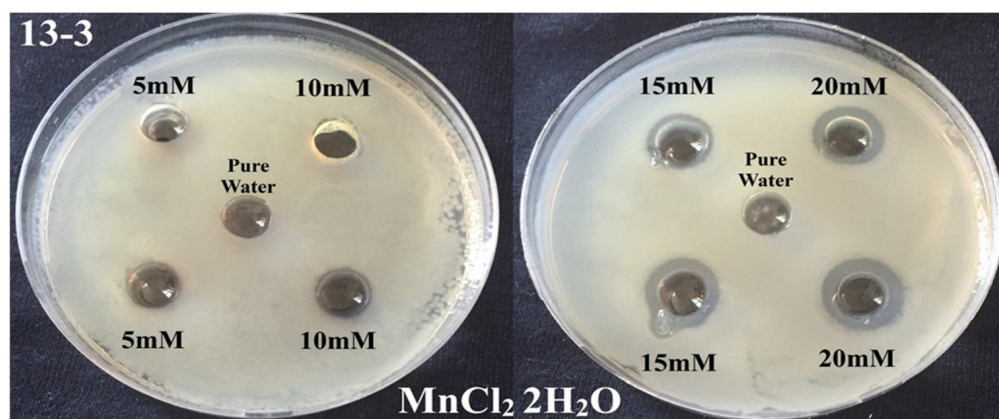


Figure 2. Cont.

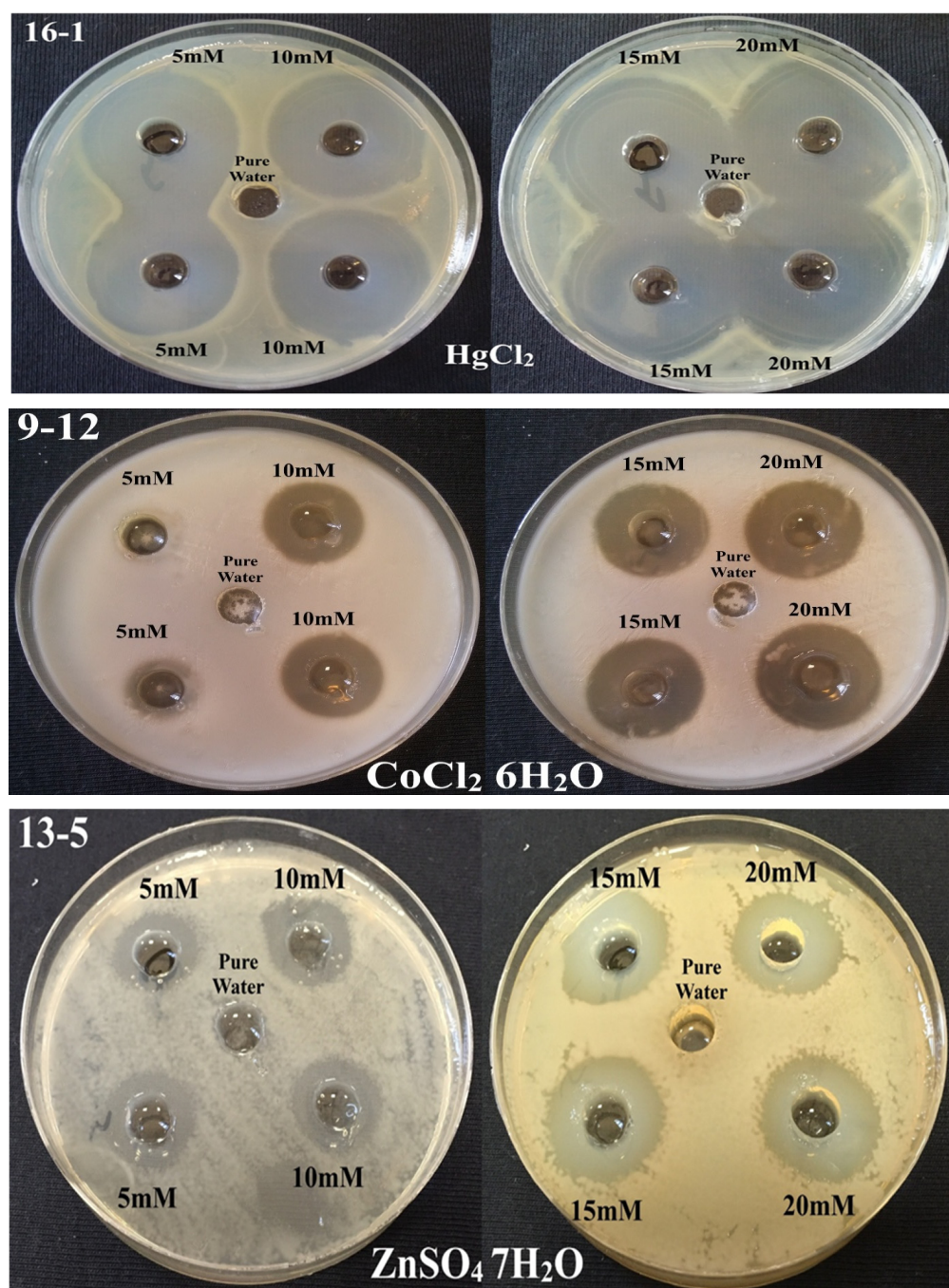


Figure 2. Heavy metal resistance results of some isolates.

3.4. Biosurfactant Production

Based on the results, 15 out of 20 isolates (75%) demonstrated biosurfactant production, while 5 isolates (YH 9-4, YH 9-6, YH 13-3, YH 13-4, and YH 15-2) did not exhibit this ability. The YH 13-1 isolate, which tested positive for biosurfactant production, was found to be resistant to both antibiotics and heavy metals (except HgCl_2). Detailed results are presented in Table 4.

Table 4. Biosurfactant production results of isolates.

Bacterial Code	Biosurfactant Production
YH 9-2	+
YH 9-4	—

Table 4. Cont.

Bacterial Code	Biosurfactant Production
YH 9-5	+
YH 9-6	—
YH 9-8	+
YH 9-12	+
YH 10-11	+
YH 10-12	+
YH 11-1	+
YH 13-1	+
YH 13-2	+
YH 13-3	—
YH 13-4	—
YH 13-5	+
YH 15-2	—
YH 15-3	+
YH 16-1	+
YH 16-4	+
YH 16-6	+
YH 16-7	+
<i>Pseudomonas aeruginosa</i> ATCC 27853	+

“+” as positive, “—” as negative.

3.5. Molecular Identification

The 16S rDNA sequences of 20 isolates were determined and compared with the GenBank database using BLAST analysis. Identification results, including base pair numbers and similarity percentages, are provided in Table 5. A neighbor-joining phylogenetic tree was constructed using MEGA 12 software [21] to illustrate the evolutionary relationships of the strains (Figure 3).

Table 5. GenBank accession numbers and information of the bacterial strains.

Bacterial Code	Base Number	Name	Simillarity (%)	Genbank Number
YH 9-2	1418	<i>Bacillus megaterium</i>	100	KY010267
YH 9-4	1344	<i>Diaphorobacter</i> sp.	100	KY010268
YH 9-5	1346	<i>Diaphorobacter</i> sp.	100	KY010269
YH 9-6	1392	<i>Cupriavidus</i> sp.	99	KY010270
YH 9-8	1396	<i>Acinetobacter calcoaceticus</i>	99	KY010271
YH 9-12	1415	<i>Bacillus megaterium</i>	100	KY010272
YH 10-11	1429	<i>Bacillus subtilis</i>	100	KY010273
YH 10-12	1379	<i>Acinetobacter calcoaceticus</i>	100	KY010274
YH 11-1	1373	<i>Cupriavidus</i> sp.	99	KY010275
YH 13-1	1380	<i>Achromobacter xylosoxidans</i>	100	KY010276
YH 13-2	1382	<i>Massilia alkalitolerans</i>	99	KY010277

Table 5. Cont.

Bacterial Code	Base Number	Name	Simillarity (%)	Genbank Number
YH 13-3	1399	<i>Bacillus simplex</i>	100	KY010278
YH 13-4	1364	<i>Massilia alkalitolerans</i>	99	KY010279
YH 13-5	1421	<i>Bacillus simplex</i>	99	KY010280
YH 15-2	1422	<i>Staphylococcus</i> sp.	100	KY010281
YH 15-3	1303	<i>Azospirillum brasilense</i>	99	KY010282
YH 16-1	1352	<i>Massilia alkalitolerans</i>	99	KY010283
YH 16-4	1015	<i>Azospirillum brasilense</i>	100	KY010284
YH 16-6	1017	<i>Azospirillum brasilense</i>	99	KY010285
YH 16-7	1329	<i>Azospirillum brasilense</i>	98	KY010286

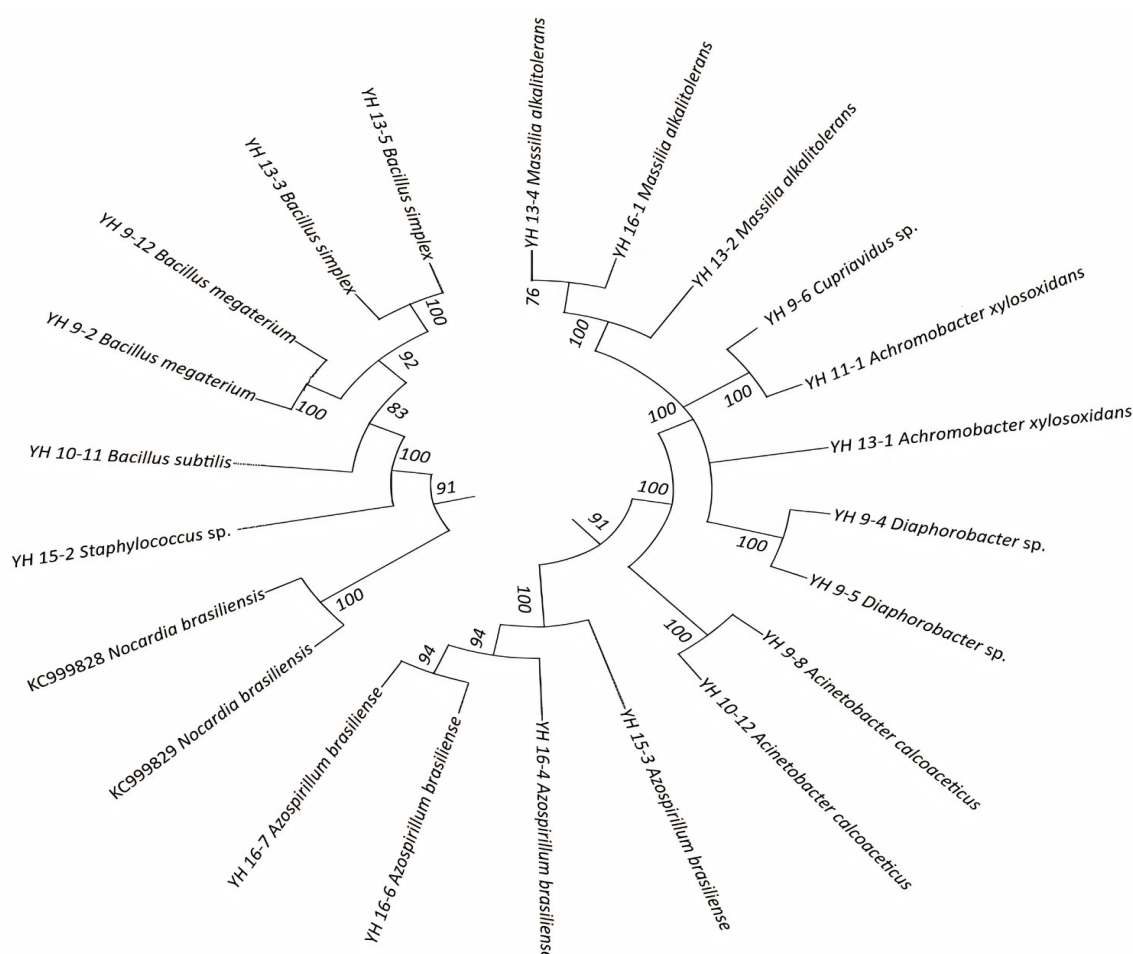


Figure 3. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences of the strains.

4. Discussion

Since the 19th century, industrially extracted crude oil often referred to as “black gold” has become the world’s most essential energy source, underpinning the economies of many nations [8,25]. However, the environmental cost of this dependency is significant. Anthropogenic activities release approximately 8 million tons of crude oil into the environment annually, leading to severe soil and water contamination [25]. Conventional remediation methods, such as adsorption and ozone catalytic oxidation, provide rapid solutions but are often limited by high costs and the risk of secondary contamination [10].

Consequently, biotechnological approaches like bioremediation have become a cornerstone for the sustainable elimination of petroleum hydrocarbons, utilizing native microorganisms to convert toxins into non-toxic metabolites [12].

In the current study, 40 bacterial strains were isolated from industrial sites in Türkiye (Kırşehir and Mersin), with 20 isolates identified as high-potential petroleum degraders. These isolates belong to the genera *Bacillus*, *Diaphorobacter*, *Cupriavidus*, *Acinetobacter*, *Achromobacter*, *Massilia*, *Staphylococcus*, and *Azospirillum*. Our findings are strongly supported by previous literature that identifies these genera as key players in hydrocarbon degradation and environmental resilience.

For instance, Abbaszade et al. (2020) utilized *Cupriavidus campinensis* as a model organism to demonstrate simultaneous heavy metal and antibiotic resistance alongside aromatic compound degradation [26]. Our isolation of *Cupriavidus* sp. from refinery areas mirrors these results, confirming the genus's robust biotechnological potential. Similarly, Al Disi et al. (2022) isolated *Bacillus* and *Pseudomonas* strains from oily soils in Qatar, which achieved 70–80% heavy metal removal and 73% diesel degradation. This aligns with our observation of *Bacillus megaterium* and *Bacillus subtilis* exhibiting high survival rates in multi-polluted soils [12].

The identification of *Diaphorobacter* sp. in our study is consistent with reports by Wen et al. (2022), who found that *Diaphorobacter* sp. strain MNS-0 could impressively degrade phenanthrene while maintaining resistance to a wide spectrum of antibiotics, including ampicillin and chloramphenicol [27]. This parallels our results where our *Diaphorobacter* isolates showed multidrug resistance. It is well known that heavy metal and antibiotic resistance genes often co-localize on plasmids, facilitating horizontal gene transfer and activating survival pathways in multi-pollutant environments. This co-resistance provides dual adaptive advantages that sustain bacterial viability in contaminated sites. Consequently, these plasmids drive efficient bioremediation through the simultaneous detoxification and catabolism of organic hydrocarbons and inorganic co-contaminants [28].

Furthermore, the isolation of *Achromobacter xylosoxidans* in our study supports the work of Kopella et al. (2023), who identified this species as a potent biosurfactant producer capable of emulsifying lubricant oil-contaminated soils [29].

Our findings regarding *Massilia alkalitolerans* and *Azospirillum brasilense* also find support in the recent literature. Li et al. (2021) successfully utilized *Massilia* sp. for phenanthrene degradation [13], while Nanekar and Kokitkar (2023) identified *Azospirillum* sp. as a key degrader in petroleum-contaminated regions in India [9]. Moreover, the biosurfactant production capabilities of our *B. megaterium* and *Acinetobacter calcoaceticus* isolates are corroborated by Shokranian et al. (2025) and Hoa Kieu et al. (2025), who highlighted these species as promising strains for the biodegradation of complex hydrocarbons [16,17].

The diversity of genera found in our study extending to *Staphylococcus* mirrors the findings of Omrani et al. (2025), who identified similar hydrocarbon-utilizing strains in diverse environments in Tunisia. Collectively, these comparisons validate that our native isolates possess the necessary metabolic machinery for effective bioremediation. As the global shift toward green technology intensifies, the use of these multi-functional bacterial strains offers a reliable, permanent, and economically viable technique for cleaning polluted territories [30]. Especially, *P. aeruginosa* ATCC 27853 strains possess the ability to degrade and process petroleum-based compounds. They produce specific enzymes that pseudo-solubilize hydrocarbons, making it easier for them to detach from soil particles. This ability gives these microorganisms great potential for the bioremediation of a crude oil-polluted environment [31].

Overall, while limited studies have focused on functional bacterial communities and their microbial diversity during the bioremediation process, our findings confirm our

hypothesis that native functional bacterial populations play pivotal roles in the bioremediation of PAH-contaminated soil. Hence, this study successfully identified diverse bacterial strains with high potential to degrade PAHs in contaminated regions, offering a more extensive perspective on the bioremediation of soils industrially contaminated with PAHs and their derivatives. Therefore, our study is unique in that it demonstrates in detail the specific bacterial strains that can be used in remediating areas contaminated with industrial wastes containing petroleum and petroleum derivatives from the chromium factory and the Kazanlı and Karaduvar refinery regions in Mersin, Türkiye.

5. Conclusions

The present study highlights microbial bioremediation as an environmentally friendly and cost-effective strategy to mitigate the impact of petroleum-derived pollutants on human life and the environment. Our results demonstrate that the isolated bacterial strains, characterized by their high hydrocarbon degradation potential, biosurfactant production, and resistance to both heavy metals and antibiotics, offer a viable solution for the remediation of industrial waste-contaminated sites. Among these strains, YH 13-1 was one of the best hydrocarbon-degrading bacteria and found to be resistant to all antibiotics, as well as heavy metals except HgCl_2 . Also, similar results were found in the other bacterial strains. These native isolates represent promising candidates for restoring ecosystem balance in regions heavily impacted by petroleum pollution.

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Abbreviations

The following abbreviations are used in this manuscript:

PAHs Polycyclic Aromatic Hydrocarbons

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