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Screening and Identification of Diesel Biodegrading Bacteria Isolated From Petroleum Hydrocarbon Contaminated Garage Soils of Kericho County, Kenya

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ABSTRACT

Despite the benefits of petroleum hydrocarbon as essential raw energy sources in many industries, they cause major global environmental pollution. Petroleum hydrocarbons pollutants are highly toxic and recalcitrant, making them dangerous and persistent over long periods in an ecosystem. However, oil contaminated soil is enriched with microorganisms that can utilize petroleum products and hydrocarbons for their growth, nutrition, and metabolic activities. This study aimed to isolate and characterize hydrocarbons-degrading bacteria capable of degrading hydrocarbons in soil samples obtained from oil-polluted garage sites in Kericho County, Kenya. One hundred and ten (110) bacterial isolates were isolated after enrichment, with 67 of the isolates (60.9%) having visible petroleum diesel-degrading capability. The bacteria were characterized based on phenotypic characteristics and 16S rRNA gene sequence analyses. Forty-nine of the isolates were Gram negative rods, and majority (56) of the isolates reacted positively for catalase and negatively for oxidase (38), methyl red (59), and Voges Proskauer (65); 50.9% of the isolates tested positive for citrate utilization. More than half of the isolated bacteria (69.7%) demonstrated strong evidence of diesel degradation. Bacteria with moderate diesel degradation demonstration accounted for 18.2% of the isolates, while isolates with substantial diesel residues contributed 12.1%. Following 16S rRNA gene sequence analysis, the bacterial strains were identified as belonging to the genera *Acinetobacter* (8), *Pseudarthrobacter* (4), *Corynebacterium* (2), *Gordonia* (2), *Athrobacter* (2), *Microbacterium* (2), *Acidivorax* (1), *Pseudoxanthomonas* (1), *Priestia* (1), *Cellulosimicrobium* (1), *Cupriavidus* (1), *Paenarthrobacter* (1), *Exiguobacterium* (1), *Shewanella* (1), *Stutzerimonas* (1), and *Pseudomonas* (1). This study has demonstrated that garage soils with petroleum hydrocarbon contamination in Kericho County harbor a rich and diverse indigenous population of microbes with the ability to biodegrade diesel. The findings suggest potential application of these bacterial strains to facilitate the biodegradation of petroleum hydrocarbons.

1 | Introduction

Many economic activities depend on fossil fuels to meet their energy demands, leading to the growth of the petrochemical industry [1, 2]. Fossil fuels (crude oil) are naturally formed hydrocarbons derived from the remains of dead plants and animals in the Earth's crust [3]. Crude oil is a dark, viscous, and flammable liquid mixture containing (83%–87%) hydrocarbons, along with varying amounts of hydrogen (10%–14%), oxygen (0.05%–1.5%), sulfur (0.005%–6.0%), nitrogen (0.1%–0.2%), and metals such as nickel, iron, and copper [2]. Despite the availability of alternative energy sources, fossil fuels remain the most affordable option, which contributes to the continued growth of the petrochemical industry [4]. The production of crude oil, its transportation, chemical processing, and distribution are considered the main sources of anthropogenic hydrocarbons pollution [5]. Crude oil are toxic residual pollutant substances that negatively impact the environment [6]. This is especially true in developing countries where environmental regulations on toxic waste disposal are not adhered to, especially by small enterprises [7]. This is particularly evident in garages and motor vehicle repair sites, where soils become major recipients of waste petroleum products spilled during maintenance activities. However, concerns about environmental pollution due to crude oil remain prevalent, as research indicate that accumulating it can severely damage living organisms, leading to death or mutations in plants and animals [1].

Petroleum hydrocarbons are among the major and most commonly occurring environmental pollutants [8]. Hydrocarbons can be classified based on their chemical structure, namely aliphatic hydrocarbons or saturated hydrocarbons, aromatic hydrocarbons (monocyclic aromatic and polycyclic aromatic hydrocarbons [PAHs]), and heteroatomic compounds (saturated and aromatic ones), including resins and asphaltenes [9]. Petroleum diesel is primarily composed of hydrocarbons with carbon chain lengths ranging from C11 to C25, principally consisting of normal alkanes. Branched alkanes and PAHs are also among key components of petroleum hydrocarbons [1].

Hydrocarbons interact with both the biotic and abiotic components of the ecosystem in various ways that can be either natural or anthropogenic. Because of their complex characteristics, the lightest and most volatile hydrocarbons fractions are released into the atmosphere, the amphipathic and hydrophilic fractions dissolve in water, and the lipophilic bind to soil/sediment particles and organic matter [3]. PAHs can potentially affect the nervous, immune, and excretory systems and cause tumors and mutations. The toxicity of hydrocarbons affects humans, plants, animals, and microorganisms, compromising ecosystem biodiversity and functioning [10]. In addition, environmental contamination by hydrocarbons and their derivatives has been associated with the extinction of many plant and animal species [3]. Arising from these challenges, there is a growing global interest to research on environmental pollution associated with hydrocarbons to identify microorganisms that thrive in contaminated sites [11]. Such microbes can be used in managing petroleum hydrocarbons contamination through biodegradation and bioremediation [12, 13].

Hydrocarbons in the environment are biodegraded primarily by bacteria, yeast, and fungi. Bacteria are the most active agents in petroleum degradation that work as primary degraders of spilled oil. Multiple studies have demonstrated that bacterial communities exposed to hydrocarbons quickly transition to species capable of degrading and utilizing hydrocarbons compounds as carbon sources [3]. This shift is also essential for alleviating the physiological stress induced by the presence of petroleum hydrocarbon [2, 14]. Hydrocarbons-degrading bacteria have evolved adaptive mechanisms such as the ability to emulsify and metabolize the hydrocarbons using specialized enzymes like oxygenases [14]. These traits can be readily transferred to other bacteria within the same environment, facilitated by plasmids and evolutionary forces that favor specific traits. This enables bacteria to develop the ability to break down persistent waste, such as petroleum hydrocarbons [15]. Because of this ability, the use of bacteria for environmental biodegradation has become the preferred approach.

Over time, various crude oil products have been discharged into the environment, highlighting the need to identify hydrocarbons-degrading microorganisms that can aid in cleaning up contaminated sites in Kenya. This study focused on characterizing bacteria from soils polluted with petroleum hydrocarbons across three sub-counties in Kericho County, Kenya.

2 | Materials and Methods

2.1 | Description of the Study Area

Soil samples were obtained from motor vehicle garages within the three most populous sub-Counties of Kericho County (latitude 0° 22′ 1.2″ [0.367°] South, longitude 35° 18′ 10.63″ [35.3°] E, altitude 2094 m and annual rainfall 1735 mm), Kenya. The County has a population of 901,777 [16] occupying an area of 2111 km^2. The County is divided into six subcounties (Figure 1; Table 1).

2.2 | Collection of Soil Samples

The study was undertaken between October 2020 and July 2021. A total of 63 motor vehicle garages in operation for more than 5 years in Ainamoi, Belgut and Bureti subcounties, Kericho County were selected and numbered. Oil contamination of the soil was assessed by visual examination in the field as described by Kostka et al. [18], where 0 = *clean or no visual contamination*, 1 = *low*, and 2 = *high levels of contamination*. In each garage, three soil samples were collected from a depth of 10 cm using a soil auger and homogenized, then a composite representative sample of 50 g were collected into clean brown 1 kg sugar paper. The soil samples were coded based on the collection order and sites, stored in a cool box and immediately transported to the Kenya Agricultural and Livestock Research Organization-Tea Research Institute (KALRO-TRI) plant molecular laboratory. Thereafter, the soil samples were sieved using a 2-mm sieve to remove stones and plant debris, and stored at 4°C.

2.3 | Preparation of Culture Media

The diesel-degrading bacteria were isolated on Bushnell Hass (BH) broth (Himedia, India). The medium was supplemented with 1.25% v/v of a 0.22-μm pore size filter (Sigmaaldrich, United States), sterilized petro-diesel. Subculturing was done on soya casein digest agar medium (SCDA) (Himedia, India) to obtain

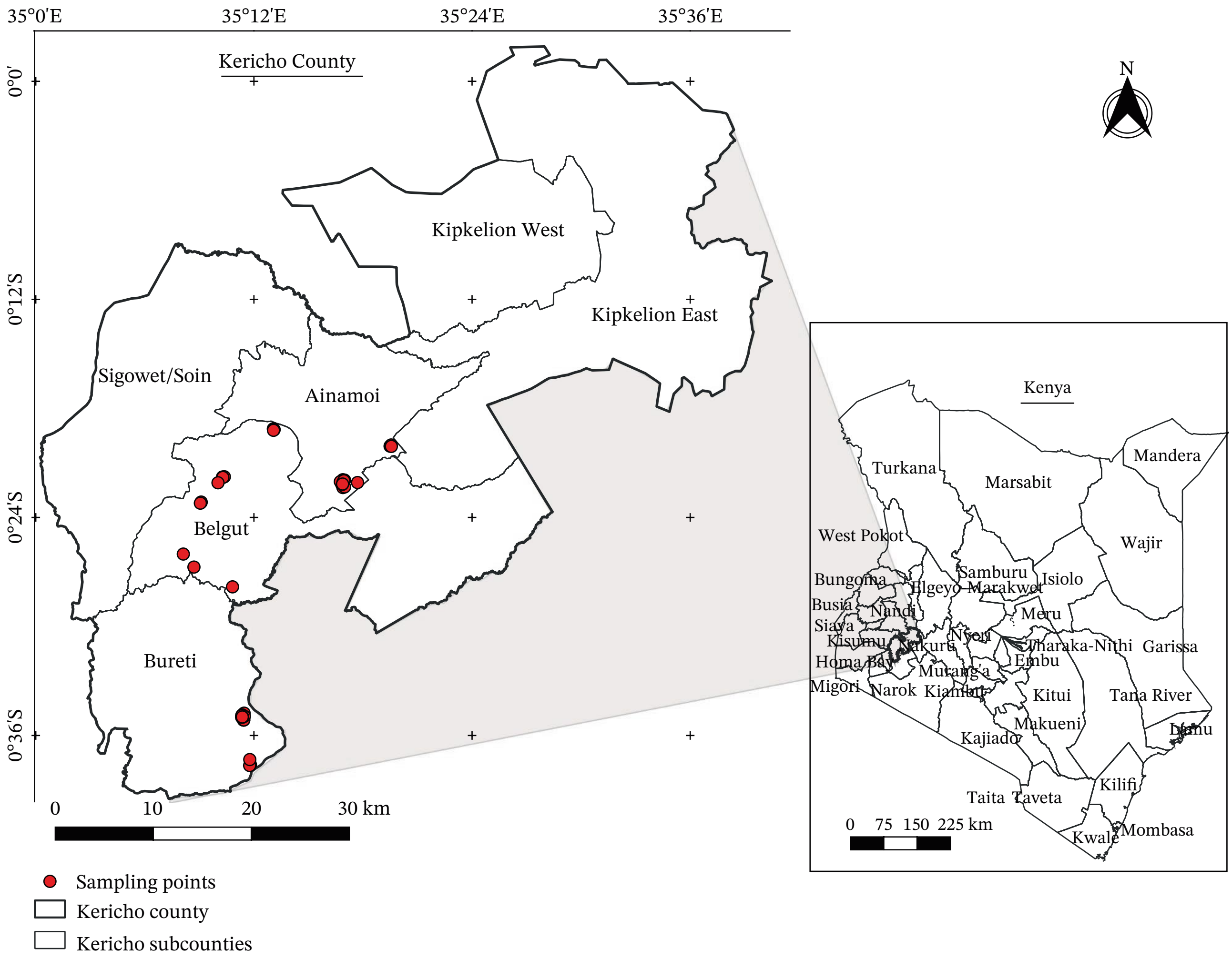

FIGURE 1 | Map showing the distribution of sampling points in three (Ainamoi, Belgut and Bureti) selected subcounties of Kericho County, Kenya. The country, county, and subcounty boundaries were obtained from Global Administrative Areas [17] Version 4.1, accessed June 23, 2023. Geographical coordinates of the sampling points were obtained using a handheld Global Positioning System (GPS) with Position Dilution of Precision (PDOP) of less than 25–100 M during the fieldwork period between October 2020 and July 2021.

pure colony cultures. Nutrient broth (NB) (Himedia, India) was used to grow cultures for DNA extraction.

2.4 | Isolation of Petroleum Diesel-Degrading Bacteria

The soil sample was pulverized with a spatula on a large clean polythene paper. Ten grams of soil was suspended in 100 mL of sterile physiological saline (0.85% NaCl, Lab prepared) and stirred for 30 min at approximately 200 ×g with a Teflon-coated magnetic bar (Sigma-Aldrich, United States) to suspend the bacteria [19]. The soil suspension was serially diluted by adding 1 mL of the soil suspension to a test tube with 9 mL physiological saline (Lab prepared), vortexed (Stuttgart, Germany) at approximately 150 ×g for 1 min, then a 1-mL aliquot was rapidly transferred to a new test tube with 9 mL saline. Subsequently, other dilutions were serially made from 10^{-1} to 10^{-5}. Of the 10^{-5} soil dilution, 10 mL was introduced into the 40 mL BH media with 1.25% v/v diesel in loosely capped flat bottom flasks and incubated for 7 days in an incubator shaker (SNS INNOVA 42 Labnet International, United States) at 30°C and 100 ×g [18]. Non-inoculated flasks containing BH broth with 1.25% v/v diesel served as controls. After 7 days of incubation, 500 μL of the BH enrichment media bacteria culture was inoculated on SCDA (Himedia, India and incubated for 48 h at 30°C. The cultures were re-coded and pure colonies obtained by serial plating on SCDA for 24 h until a pure culture was obtained [20].

2.5 | Morphological and Biochemical Characterization of Isolates

The bacterial isolates were characterized for cell shape, size, pigmentation, elevation, margin, form, and mucoid consistency using both dissecting and compound microscopy [19, 20]. Gram staining was performed to determine the shape and size of the isolate cells, with observations made using oil immersion microscopy. The results were further confirmed through the 3% (w/v) potassium hydroxide (KOH) string test. Biochemical tests on diesel-degrading bacteria included citrate utilization, catalase, oxidase activity, and carbohydrate use [20].

TABLE 1 | Coordinates of the sample collection garage.

S/N	Sample site	Latitude	Longitude
1	Taplotin Ronda spares	0.38633	35.15148
2	Taplotin Nyota spares	0.38593	35.15159
3	Taplotin Elshaddai spares	0.38696	35.15084
4	Sosiot booster spares	0.36275	35.17284
5	Sosiot power saw	0.36314	35.17124
6	Sosiot Weston spares	0.36296	35.17119
7	Sosiot power saw market	0.36336	35.17149
8	Sosiot Joykem spares	0.36825	35.16701
9	Chepnyogaa junction	0.43363	35.13521
10	Kabianga centre 1	0.44558	35.14496
11	Premier puncture workshop	0.46372	35.18034
12	Kapsoit Tripoli	0.31972	35.21821
13	Kapsoit Ramogi	0.31873	35.21777
14	Kapsoit Josee	0.31958	35.21835
15	Kapsoit Dan	0.31942	35.21845
16	Kapsoit tractor garage	0.32014	35.21791
17	Litein Onyancha	0.57957	35.19093
18	Kapkatet precious	0.62677	35.19672
19	Litein bush mouth	0.58595	35.19059
20	Kapkatet opp. Police station	0.62758	35.19605
21	Litein transformer	0.58585	35.19029
22	Litein mabwai	0.58497	35. 18985
23	Litein Josam	0.58447	35.18987
24	Litein mama Neno tractor	0.58581	35.19068
25	Litein Davis Tvs service	0.58111	35.18918
26	Litein boxer spare services	0.58242	35.19119
27	Litein power saw	0.57996	35 18981
28	Litein Jakarabok tractor	0.58331	35.18864
29	Litein Malel general	0.58335	35.18927
30	Kapkatet Nick's services	0.62211	35.19616
31	Litein modern spares	0.58288	35.18959
32	Litein Vinny Tvs service	0.58197	35.18868
33	Litein sebuleni parking	0.58306	35.18932
34	Kericho town four bob	0.36827	35.28053
35	Town Bovaline KCC	0.3672	35.28177
36	Town Guru Nanak	0.36968	35.28045
37	Town Mutua	0.36797	35.28125
38	Town Auma KCC	0.36722	35.28137
39	Town KCC mosque	0.36726	35.28188
40	Town Kericho industrial enterprices	0.36592	35.283
41	Town Lomet	0.36712	35.28189
42	Town Amani	0.36771	35.2807
43	Town Kiptebes	0.36796	35.28044
44	Town nyota garage lower	0.36546	35.28146
45	Brook Halane energy	0.33467	35.32479
46	Brook Mutai Spares kapsaos	0.33438	35.3251
47	Town Jua Kali Magret	0.36752	35.28085
48	Town Jadeja garage	0.36795	35.2805
49	Town Jumbo garage	0.37263	35.28154
50	Town next to stage garage	0.3642	35.28451
51	Town KCC kwa Ken garage	0.36416	35.28306
52	Town Kiprono Tegecha	0.37044	35.28144
53	Town jua kali	0.36747	35.28158
54	Town fountain garage	0.36721	35.27919
55	Brook tea view garage	0.33357	35.32578
56	Town Sinendet	0.37204	35.2831
57	Town nyota upper	0.36812	35.28149
58	Town Tengecha Mutua	0.36969	35.28109
59	Total Kipsigis town	0.36803	35.29497
60	Brook next to Brook inn	0.33487	35.32596
61	Town blue triangle	0.36579	35.28294
62	Town Kaplong garage	0.36641	35.28238
63	Town new highway garage	0.36939	35.28125

2.6 | Molecular Identification of the Diesel-Degrading Bacteria

2.6.1 | Genomic DNA Extraction

Genomic DNA was extracted from overnight cell cultures grown on Nutrient broth (NB) at 30°C as described by [21]. The cell pellets were suspended in 200 μL of solution 1 [50 mM Tris (pH 8.5; Sigma-Aldrich, United States), 50 mM EDTA (pH 8.0; Sigma-Aldrich, United States), and 25% sucrose solution (Fluka United States)], 5 μL of lysozyme (20 mg/mL; Glentham, United Kingdom), and 5 μL of RNase A (20 mg/mL; Sigma-Aldrich, United States), mixed gently and incubated at 37°C for 1 h. Thereafter, 600 μL of solution 2 [10 mM Tris (pH 8.7), 5 mM EDTA (pH 8.0), and 1% sodium dodecyl sulfate (Avantor, United States)], and 10 μL proteinase K (20 mg/mL; Glentham, United Kingdom) were added, mixed gently, and the mixture incubated at 55°C for 30 min. Phenol-chloroform (Sigma-Aldrich, United States) extraction was performed, followed by chloroform alcohol (Lab prepared) washing. The DNA was precipitated with ice-cold absolute ethanol (Sigma-Aldrich, United States) and sodium chloride (Sigma-Aldrich, United States), left overnight at −20°C, and centrifuged. The pellet was washed with 70% ethanol, air-dried, and re-suspended in TE buffer (Lab prepared).

2.6.2 | Amplification of Bacterial 16S rRNA Gene

The isolated bacteria were identified using the 16S rRNA gene sequencing, and gene amplification was performed using the bacterial primer set, 27-F (5′-TAGAGTTTGATCCTGGCTCAG-3′) and 1392-R (5′-GACGGGCGGTGTGTACA-3′) [22]. Polymerase chain reaction (PCR) reactions were performed in 25 μL volume using 12.5 μL one Taq Quick-Load 2× Master Mix with a standard PCR buffer (New England Biolabs, United Kingdom), 10 m*M* primer, 50 ng template DNA, and the reaction mixtures were topped up to 25 μL with nuclease-free water (ElgaBiotech, Germany). The negative control reaction contained all the PCR components except the DNA template. Amplifications were performed in a Veriti thermocycler (Bio-Rad, Singapore), the reaction mixtures were subjected to the following thermal cycling profiles: 1 cycle of initial denaturation at 94°C for 5 min, followed by 32 cycles of denaturation at 94°C for 1 min, primer annealing at 49°C for 1 min, extension at 72°C for 2 min, and a final extension at 72°C for 10 min [23].

The amplified PCR product (7 μL) was resolved on a 1% agarose gel (Sigma-Aldrich, United States) stained with ethidium bromide (Fisher Scientific, United States) in 1× TBE buffer, electrophoresis (Clever Scientific, United Kingdom) carried out at 70 V for 60 min and the PCR product was visualized using a Gel-Doc XR+ Imaging System (Bio-Rad, Germany). The molecular weight of the PCR product was estimated using a Gene Ruler 1 kb Plus DNA marker (Thermo Fisher Scientific, United States), a blank control was run alongside the ladder and samples for quality check. The PCR product was purified using Zymoclean Gel DNA Recovery Kit (Zymo Research, United States) according to the manufacturer's instructions. The purified amplicon was Sanger sequenced at Inqaba Biotechnical Industries, Pretoria, South Africa.

2.6.3 | Phylogenetic Analysis

The 16S rRNA gene sequence of the bacterial isolates were preprocessed using ChromasPro 2.1.8 software package (http://technelysium.com.au/wp/, accessed March 19, 2024). Annotation of high-quality transcripts was performed by searching against available nucleotide sequences deposited in the National Center for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov/accessed March 19, 2024) using Basic Local Alignment Search Tool (BLAST) e-value threshold of zero. The 16S rRNA gene sequence with high similarity to those determined in the study was retrieved, added to the database, and aligned with MEGA (Molecular Evolutionary Genetic Analysis) 7.0 software package [24]. The trees' topologies were evaluated using the bootstrap resampling method [25] based on 1000 replicates. The sequence of *Trichoderma* (HQ630962.1) was used as an out group.

2.7 | Data Analysis

Numerical data were presented as percentiles, while the data on cultural, morphological, and biochemical characteristics were presented as tables and graphs using Microsoft Excel 2021. Correlation was computed using R (version 4.5.1, accessed July 13, 2025). The same data was scored as binary data for presence (1) or absence (0) and used to generate distance matrices based on Jaccard's similarity coefficient. These similarity matrices were analyzed using cluster analysis via the unweighted pair group method with arithmetic mean (UPGMA), and a dendrogram was constructed using FigTree software (Version 1.4.2; accessed on April 30, 2024). The similarity coefficient data from the 67 bacterial isolates were then used to reduce the sample size based on relatedness before molecular characterization. 16S rRNA gene sequences of the bacterial isolates were viewed and edited using Chromas Pro 5 software package. They were aligned using CLUSTAL W 1.6 to provide a full sequence of about 1500 nucleotide bases, and compared with sequences in the public databases with the BLAST search program on the National Center for Biotechnology Information (NCBI) website to find closely related bacterial 16S rRNA gene sequence, parameters such as percentage similarities and E-values were used. The 16S rRNA gene sequence of the isolates and those of the closely related bacteria were then aligned and processed to produce phylogenetic trees using MEGA 5 software package.

3 | Results

3.1 | Soil Bacteria Communities in Garage Sites

A total of 110 bacterial isolates were obtained from the 63 composite motor vehicle garage soil samples from the three subcounties in Kericho County. Of these, 75 (68%), 15 (11.8%), and 20 (16.4%) were from Ainamoi, Belgut, and Bureti subcounties, respectively. Mixed bacterial communities were obtained from 24 (72.7%), 5 (15.2%), and 4 (12.1%) soil samples collected from Ainamoi, Belgut, and Bureti, respectively (Table 2).

The 110 bacterial isolates exhibited three distinct diesel degradation capabilities (Table 3). Most of the isolates (67, representing 61%), degraded the added diesel with no traces of diesel droplets, while the remaining isolates had either low (23, representing 21%) or large (20, representing 18%) traces of diesel droplets, the negative controls run along the experiment did not show any microbial growth (Table 3).

Pearson correlation analysis was conducted to assess the relationship between diesel residue levels and the number of bacterial isolates recovered per the sampling sites. At 5% level of significance, analysis showed that there was a weak negative relationship between diesel residue levels and the number of bacterial isolates recovered per the sampling sites [Calculated Pearsons correlation coefficient $\rho = -0.344$, with p value $= 0.004$].

3.2 | Morpho-Cultural Characteristics of Bacteria

Most colonies grew within 48 h of incubation at 30°C. The isolates had different cultural characteristics on SCDA medium (Figure 2). The colonies presented different colors: white, whitish, pink, cream, orange, brownish, luminous green, and cream white. They were either nonmucoid, sticky on media, or mucoid. The margins were entire, filamentous or irregular with either round, filamentous, or irregular colonies. The colony margins appeared to be raised, umbonate, with spreading edges or growing into the media. The morphology of the bacterial cells was either cocci or rods (Table 4).

TABLE 2 | Number of bacteria isolates from selected garage sites in three sub-counties.

Subcounty	Sampling sites	Number of isolates
Ainamoi	35	75
Belgut	11	15
Bureti	17	20
Total	63	110

3.3 | Morphological Characteristics of Degrading Bacteria

Morphological characteristics of the bacterial isolates were categorized based on color, consistency, elevation, shape, margin, and the whole colony. Eight different colony colors were observed, isolate consistency was mainly nonmucoid (65.7%) and mucoid (34.3%). More than half of the isolates had convex elevation (71.6%) compared with umbonate (17.9%), and others (10.5%). In addition, slightly over half of the isolated bacteria shapes were rods (53.7%), with slightly less than half exhibiting a cocci shapes (46.3%). A significant number of the isolates (91.0%) had entire margins as compared with filamentous (6.0%), and irregular margins (3.0%). Based on colony characteristics, almost all of the isolates (89.6%) had round margins.

TABLE 3 | Diesel degradation properties by bacteria isolated from soil sampled from garages in Kericho County.

Biodegradation capability	Number of isolates	Bacteria associations[a]	Group/diesel residue
No traces of diesel	67	23	1/Low
Low traces of diesel	23	6	2/Moderate
Large traces of diesel	20	4	3/High
Negative control (media add treatment only)	0	0	
Total	110	33	

[a] Number of samples, which yielded more than one pure bacteria isolate after enrichment culture.

Isolate 8a, *Pseudomonas* spp. Isolate 42a, *Priestia* spp. Isolate 37b, *Pseudoxanthomona* spp.

Isolate 60b, *Acinetobacter* spp. Isolate 37b, *Acinetobacter* spp. Isolate 61a, *Acinetobacter* spp.

FIGURE 2 | Cultures of different diesel-degrading bacteria isolated from soils sampled.

3.4 | Biochemical Characteristics of Diesel-Degrading Bacteria

The diesel-degrading bacterial isolates had diverse biochemical characteristics, over half (60%) of high diesel residue isolates demonstrated the capability to utilize citrate as its sole carbon and energy source, unlike group one (49.3%), and group two (47.8%). The capacity to produce the catalase enzyme was significant among the isolates in low diesel residue group (83.6%) followed by the high diesel residue group (80%). There was significant Gram negative isolates in the moderate diesel degrading group (82.6%) and in the low diesel residue group (70.1%), as compared with the high residue group (55%). A significant number of bacterial groups exhibited unstable acid production during glucose fermentation, as demonstrated by the Methyl Red (MR) test, which showed similar results to the Voges–Proskauer (VP). In the VP test, over half of the isolates across all three degradation groups tested negative for the production of acetylmethyl carbinol from glucose fermentation. There was variation in the outcome of oxidase test among the groups, with almost equal positive (43.3%) and negative (56.7%) observations in low residue group, in contrast, moderate residue group (60.9%) of isolates showed positive reactions and (80%) of high residue group isolates tested negative.

3.5 | Clustering of Bacterial Isolates

The cultural, morphological, and biochemical diversity, and relationships among the bacteria isolates were determined by Jaccard's similarity coefficient using the UPGMA method. The dendrogram divided the bacteria into seven major clusters (Figure 3). The similarity coefficient among the 67 isolates ranged from 0.177 to 0.977 with an average of 0.577. The seven clusters comprised 1 to 26 bacterial isolates with majority placed in cluster six (26 isolates) and cluster seven (22 isolates). Cluster 6 had multiple isolates that were highly similar, while cluster seven had two highly similar isolates according to the cultural, morphological, and biochemical characteristics.

3.6 | Molecular Identification of Diesel-Degrading Bacteria

The partial 6S rRNA gene sequence of diesel-degrading bacterial isolates was compared with nucleotide sequence in the GenBank to determine the degree of similarity between them, and closely related strains. BLASTn results revealed percentage identities between diesel-degrading bacterial isolates with closely related bacteria in the GenBank (Table 5). The accession numbers listed in Table 5 represent reference 16S rRNA gene sequences from GenBank that were used for comparative analysis with the sequences obtained in this study Table 5.

Phylogenetic analysis of the 16S rRNA gene sequence identified the diesels degrading bacteria isolated from the petroleum contaminated soils in Kericho County to belong to the genera *Acinetobacter*, *Arthrobacter*, *Pseudoxanthomonas*, *Corynebacterium*, *Gordonia*, *Pseudarthrobacter*, *Paenarthrobacteria*, *Acidovorax*, *Cellulosimicrobacter*, *Microbacterium*, *Priestia*, *Cupriavidus*, *Microbacterium*, *Exiguobacterium*, *Shewanella*, *Stutzerimonas*, and *Pseudomonas*.

The phylogenetic tree (Figure 4) illustrates the evolutionary connections among diesel-degrading bacterial isolates, marked with red diamonds and designated as Z isolates, alongside closely related reference strains. These relationships are inferred through an analysis of 16S rRNA gene sequence. The tree reveals two main clades with nine distinct sub-clades, highlighting genetic similarities and divergences among the isolates and reference species.

The first sub-clade consisted of sequences from seven isolates, which showed a high degree of sequence similarity with *Arthrobacter* spp. (MT373551.1, and MW033810.1), *Pseudarthrobacter* spp. (MN006554.1, OR964107.1, KU647201.1, and MN826535.1), respectively, forming a closely clustered sub-clade. They were assigned to the genera *Pseudarthrobacter*, *Arthrobacter*, *Paenarthrobacter*, and *Priestia*. The second sub-clade consisted of a sequence from three isolates, with *Cellulosimicrobium* spp. (MT533986.1), *Microbacterium* (OP847077.1, and MN314492.1). Z14, Z20, and Z39 were assigned to the genera *Cellulosimicrobium*, and *Microbacterium*.

The third sub-clade consists of two isolates, Z2 and Z55, both of which showed a sequence similarity of 99.89% with Corynebacterium variable (MT573863.1). Z2 and Z55 were assigned to the genus *Corynebacterium*. The fourth sub-clade comprises two isolates, Z6 and Z17, with a high similarity to *Gordonia* (MT549097.1, and MT5333952.1) both were assigned to the genus *Gordonia*. The fifth sub-clade had one isolate Z47, with a similarity of 99.89% with *Exiguobacterium* spp. (MT35579.1), assigned to the genus *Exiguobacterium*. The sixth sub-clade includes one isolate Z5, with a similarity to *Pseudoxanthomonas* spp. (LC133669.1), it was assigned to the genus *Pseudoxanthomonas*.

The seventh sub-clade contains three isolates Z11, Z38, and Z49, with a similarity to *Acidovorax* spp. (MT255158.1), *Cupriavidus alkaliphilus* (MN810330.1), and *Shewanella putrefacience* (KU163441.1). A bootstrap value of 37% was between Z11, Z38, and Z49, indicating a weak support for the relationship between these genera. A bootstrap value of 87% between Z11 and Z38 represents a high level of confidence in their close phylogenetic relationship based on 16S rRNA gene sequence analysis. Z11, Z38, and Z49 were assigned to the genera *Acidovorax*, *Cupriavidus*, and *Shewanella*, respectively.

The eighth sub-clade contains two isolates Z53 and Z62, with a similarity to *Stutzerimonas* spp. (OR742106.1) and *Pseudomonas* spp. (MN256239.1) based on 16S rRNA gene sequence analysis Z53 could be assigned to the genus *Stutzerimonas*, while Z62 to the genus *Pseudomonas*.

The ninth sub-clade consisted of sequence from eight isolates Z8, Z25, Z26, Z27, Z43, Z56, Z60, and Z61, which showed a high degree of sequence similarity of between 98.56% and 100% with *Acinetobacter* (MT613873.1, and GU566343.1), forming a closely clustered sub-clade. They were therefore assigned to the genus *Acinetobacter*.

TABLE 4 | Cultural, morphological, and biochemical characteristics of the 67 diesel-degrading bacteria.

Isolate code	Colony color	Colony size	Whole colony	Colony consistency	Colony margin	Colony elevation	Cell shape	CIT	CAT	Gram status	MR	VP	OXI
z1	Orange	Small	Round	Nonmucoid	Entire	Convex	Rods	+	+	+	+	—	—
z2	Cream white	Medium	Round	Nonmucoid	Entire	Raised	Rods	+	+	—	—	—	—
z3	White	Small	Filamentous	Nonmucoid	Filamentous	Umbonate	Cocci	—	—	—	—	—	—
z4	Brownish	Small	Round	Nonmucoid	Entire	Convex	Cocci	—	—	—	—	—	—
z5	White	Small	Filamentous	Nonmucoid	Filamentous	Umbonate	Cocci	—	+	+	+	+	—
z6	Whitish	Small	Round	Nonmucoid	Entire	Convex	Cocci	—	+	—	—	—	—
z7	White	Small	Round	Nonmucoid	Entire	Convex	Cocci	+	+	+	+	—	—
z8	Brownish	Large	Round	Mucoid	Entire	Convex	Cocci	+	+	—	—	—	—
z9	Orange	Small	Round	Nonmucoid	Entire	Convex	Rods	—	+	—	—	—	—
z10	Orange	Large	Round	Mucoid	Entire	Convex	Rods	+	+	+	+	—	—
z11	Orange	Small	Round	Nonmucoid	Entire	Convex	Cocci	+	—	—	—	—	—
z12	Brownish	Small	Round	Nonmucoid	Entire	Convex	Cocci	+	—	—	—	—	—
z13	Luminous green	Medium	Round	Nonmucoid	Entire	Convex	Cocci	—	+	—	—	+	+
z14	Luminous green	Medium	Round	Nonmucoid	Entire	Convex	Cocci	—	+	—	—	+	—
z15	Cream white	Large	Round	Nonmucoid	Entire	Convex	Rods	+	+	+	+	—	—
z16	Luminous green	Small	Round	Mucoid	Entire	Convex	Cocci	—	—	—	—	+	—
z17	Orange	Small	Round	Nonmucoid	Entire	Convex	Rods	—	+	—	—	—	—
z18	Cream white	Medium	Round	Nonmucoid	Entire	Convex	Cocci	+	+	—	—	—	—
z19	Luminous green	Small	Round	Mucoid	Entire	Convex	Cocci	—	+	—	—	—	—
z20	Orange	Medium	Round	Mucoid	Entire	Convex	Cocci	+	+	—	—	—	—
z21	Orange	Large	Round	Nonmucoid	Entire	Convex	Cocci	+	+	+	+	—	—

(Continues)

TABLE 4 | (CONTINUED)

Isolate code	Colony color	Colony size	Whole colony	Colony consistency	Colony margin	Colony elevation	Cell shape	CIT	CAT	Gram status	MR	VP	OXI
z22	Orange	Medium	Round	Mucoid	Entire	Convex	Cocci	—	+	+	+	+	—
z23	White	Large	Round	Mucoid	Entire	Umbonate	Rods	+	+	+	+	—	—
z24	Orange	Large	Round	Mucoid	Entire	Convex	Rods	+	+	+	+	—	—
z25	White	Large	Round	Mucoid	Entire	Umbonate	Cocci	+	+	—	—	—	—
z26	White	Large	Round	Mucoid	Entire	Convex	Cocci	+	+	—	—	—	—
z27	White	Small	Round	Nonmucoid	Entire	Convex	Cocci	—	+	+	+	—	—
z28	White	Small	Round	Mucoid	Entire	Convex	Cocci	+	+	—	—	—	—
z29	Orange	Small	Round	Nonmucoid	Entire	Convex	Rods	+	+	—	—	—	—
z30	Brownish	Large	Round	Mucoid	Entire	Umbonate	Cocci	+	+	—	—	+	—
z31	Cream	Small	Round	Mucoid	Entire	Convex	Rods	+	+	—	—	—	—
z32	Brownish	Medium	Round	Mucoid	Entire	Convex	Cocci	—	+	—	—	—	—
z33	Orange	Large	Round	Nonmucoid	Entire	Umbonate	Rods	+	+	—	—	—	—
z34	Orange	Medium	Round	Nonmucoid	Entire	Convex	Rods	—	—	—	—	—	—
z35	Luminous green	Small	Round	Nonmucoid	Entire	Convex	Cocci	—	+	+	+	—	—
z36	Orange	Small	Round	Nonmucoid	Entire	Convex	Rods	—	+	—	—	—	—
z37	Orange	Medium	Round	Mucoid	Entire	Convex	Rods	—	+	—	—	—	—
z38	White	Large	Round	Nonmucoid	Entire	Convex	Rods	+	+	+	+	—	—
z39	Orange	Medium	Round	Nonmucoid	Entire	Convex	Cocci	+	+	+	+	—	—
z40	Luminous green	Small	Round	Mucoid	Entire	Convex	Rods	—	—	—	—	+	—
z41	White	Large	Round	Mucoid	Entire	Convex	Cocci	+	+	+	+	—	—
z42	Brownish	Medium	Round	Nonmucoid	Entire	Convex	Cocci	—	+	—	—	—	—
z43	Brownish	Small	Round	Mucoid	Entire	Convex	Cocci	—	+	—	—	—	—
z44	White	Medium	Round	Nonmucoid	Entire	Convex	Cocci	—	+	+	+	—	—
z45	Brownish	Medium	Round	Nonmucoid	Entire	Convex	Cocci	+	+	—	—	—	—

(Continues)

TABLE 4 | (CONTINUED)

Isolate code	Colony color	Colony size	Whole colony	Colony consistency	Colony margin	Colony elevation	Cell shape	CIT	CAT	Gram status	MR	VP	OXI
z46	White	Medium	Round	Mucoid	Entire	Umbonate	Rods	+	+	+	+	—	—
z47	White	Medium	Round	Nonmucoid	Entire	Convex	Cocci	—	+	+	+	—	—
z48	Orange	Medium	Round	Nonmucoid	Entire	Convex	Rods	+	+	—	—	—	—
z49	Yellow	Large	Round	Nonmucoid	Entire	Umbonate	Rods	—	+	+	+	+	+
z50	White	Small	Round	Nonmucoid	Entire	Convex	Rods	—	+	+	+	—	—
z51	Orange	Large	Round	Nonmucoid	Entire	Umbonate	Rods	—	—	—	—	—	—
z52	Luminous green	Large	Filamentous	Nonmucoid	Irregular	Umbonate	Rods	+	—	—	—	—	—
z53	Orange	Large	Round	Nonmucoid	Entire	Umbonate	Rods	—	—	—	—	—	—
z54	Orange	Large	Irregular	Nonmucoid	Irregular	Umbonate	Rods	+	+	—	—	—	—
z55	Orange	Large	Filamentous	Mucoid	Filamentous	Umbonate	Rods	+	—	—	—	—	—
z56	Orange	Medium	Filamentous	Nonmucoid	Filamentous	Umbonate	Rods	+	+	—	—	—	—
z57	White	Medium	Round	Nonmucoid	Entire	Convex	Rods	—	+	—	—	—	—
z58	Orange	Large	Round	Mucoid	Entire	Convex	Rods	—	+	—	—	—	—
z59	Brownish	Small	Round	Nonmucoid	Entire	Convex	Rods	+	+	—	—	—	—
z60	Pink	Small	Filamentous	Nonmucoid	Entire	Flat	Rods	—	+	+	+	—	—
z61	Orange	Small	Round	Nonmucoid	Entire	Convex	Rods	—	+	—	—	—	—
z62	Brownish	Medium	Round	Nonmucoid	Entire	Umbonate	Cocci	+	+	—	—	—	—
z63	Orange	Large	Round	Nonmucoid	Entire	Umbonate	Rods	+	—	—	—	—	—
z64	Cream	Large	Round	Mucoid	Entire	Convex	Rods	—	+	—	—	—	—
z65	Orange	Large	Round	Mucoid	Entire	Umbonate	Rods	+	—	—	—	—	—
z66	Cream	Medium	Round	Nonmucoid	Entire	Convex	Rods	+	+	—	—	—	—
z67	Cream	Medium	Round	Nonmucoid	Entire	Convex	Rods	+	+	—	—	—	—

Note: NB: – Negative, + Positive, A, B, C, D, E – data codes representing different isolates from a single soil sample. Based on the cultural, morphological and biochemical characteristics, the probable bacteria genera that were isolated are *Acinetobacter*, *Arthobacter*, *Pseudomonads*, and *Corynebacteria*.

Abbreviations: CAT, catalase, CIT, citrate, MR, methyl red, OXI, oxidase, VP, Voges–Proskauer.

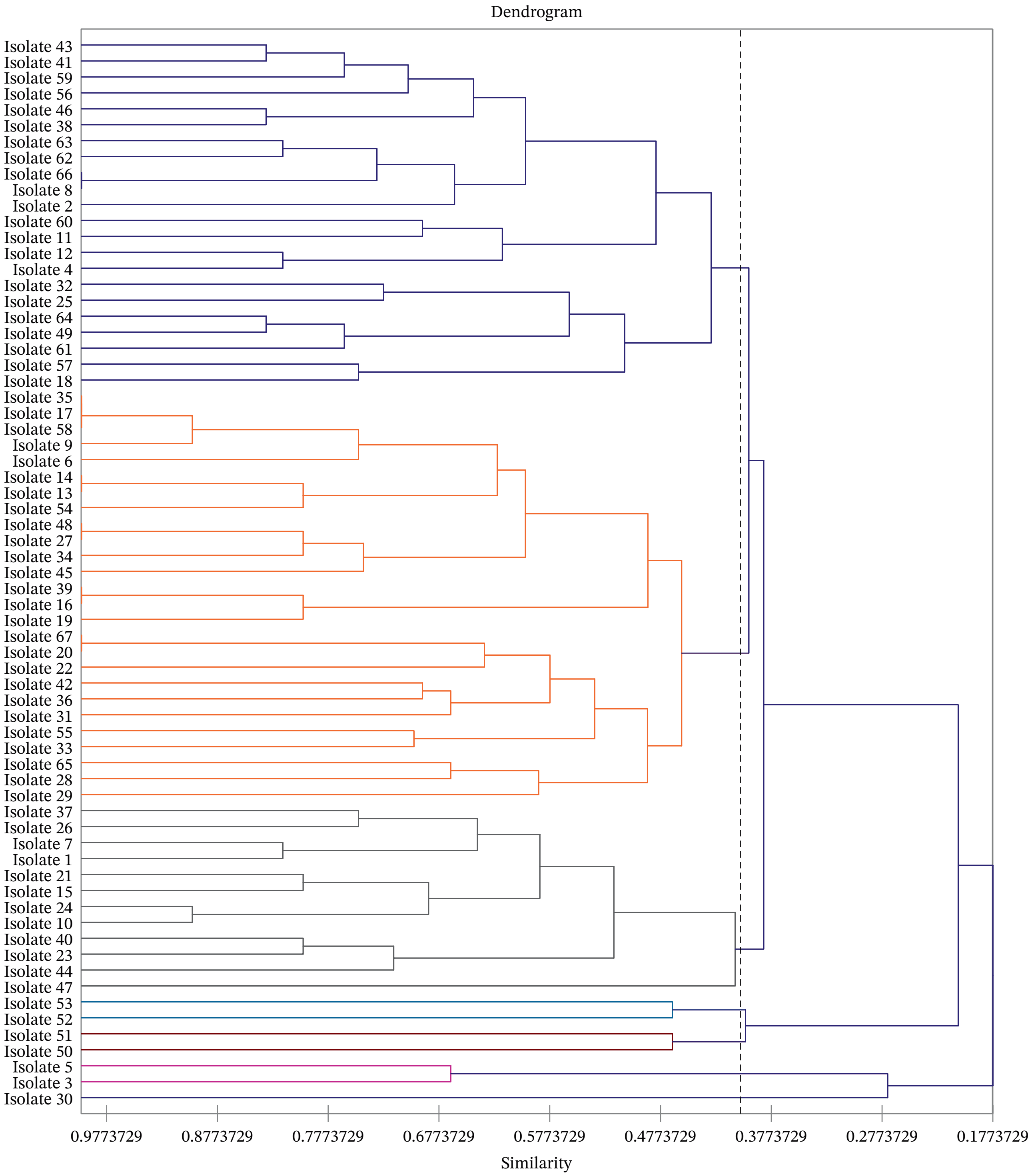

FIGURE 3 | Dendrogram based on UPGMA showing the relationships among 67 bacteria isolates—isolated from garage soils—based on their cultural, morphological, and biochemical properties on Jaccard's similarity index. The seven major bacterial clusters are presented by different colors, wherein isolates with similar colors are presumed close relatives.

4 | Discussion

The present study found that the prolonged exposure to petroleum hydrocarbons in garage soils appeared to promote microbial diversity. In previous studies, the scarcity of microorganisms capable of degrading hydrocarbons has been highlighted as a key factor limiting the biodegradability of oil contaminants [2].

A total of 110 bacterial isolates were obtained from a microbial community across 63 soil sampling sites. The high number of isolates may be attributed to the fact that the soil samples were collected from garages that had been operational for over 5 years. This observation is further supported by findings from four garages where surface re-carpeting with freshly excavated murram that may have disrupted microbial communities

TABLE 5 | Closest GenBank matches for the 16S rRNA gene sequences of the 29 diesel-degrading bacterial isolates based on BLASTn analysis.

S. no	Genus	Species	Query coverages (%)	E value	Percentage identity (%)	16S rRNA gene NCBI accession no.
z2_27F_D09	*Corynebacterium*	*Variabile*	100	0	99.89	MT573863.1
z5_27F_E09	*Pseudoxanthomonas* spp.		100	0	99.65	LC133669.1
z6_27F_F09	*Gordonia*	*Alkanivorans*	100	0	99.76	MT549097.1
z7_27F_G09	*Arthrobacter* spp.		100	0	99.89	MT373551.1
	Pseudarthrobacter	*Siccitolerans*	100	0	99.89	MN006554.1
z8_27F_H09	*Acinetobacter*	*Junii*	100	0	100.00	MT613873.1
z10_27F_A10	*Paenarthrobacter*	*Ureafaciens*	100	0	99.64	MT409549.1
z11_27F_B10	*Acidovorax* spp.		100	0	99.36	MT255158.1
z14_27F_D10	*Cellulosimicrobium*	*Cellulans*	100	0	99.39	MT533986.1
z15_27F_E10	*Pseudarthrobacter*	*Phenanthrenivorans*	100	0	100.00	OR964107.1
z17_27F_F10	*Gordonia*	*Amicalis*	100	0	99.51	MT533952.1
z20_27F_G10	*Microbacterium*	*Album*	100	0	98.61	OP847077.1
z23_27F_H10	*Pseudarthrobacter*	*Chlorophenolicus*	100	0	99.70	KU647201.1
z25_27F_A11	*Acinetobacter*	*Junii*	99	0	99.89	MT613873.1
z26_27F_B11	*Acinetobacter*	*Junii*	100	0	99.69	MT613873.1
z27_27F_C11	*Acinetobacter*	*Junii*	100	0	99.78	MT613873.1
z30_27F_D11	*Priestia*	*Megaterium*	100	0	99.55	MK318797.1
z38_27F_E11	*Cupriavidus*	*Alkaliphilus*	100	0	99.58	MN810330.1
z39_27F_F11	*Microbacterium*	*Saccharophilum*	100	0	99.37	MN314492.1
z43_27F_G11	*Acinetobacter* spp.		100	0	99.09	GU566343.1
z44_27F_H11	*Arthrobacter* spp.		100	0	99.38	MW033810.1
z45_27F_A12	*Pseudarthrobacter*	*Oxydans*	100	0	99.54	MN826535.1
z47_27F_B12	*Exiguobacterium* spp.		100	0	99.89	MT355759.1
z49_27F_C12	*Shewanella*	*Putrefaciens*	100	0	99.33	KU163441.1
z53_27F_D12	*Stutzerimonas*	*Frequens*	100	0	97.85	OR742106.1
z55_27F_E12	*Corynebacterium*	*Variabile*	100	0	99.89	MT573863.1
z56_27F_F12	*Acinetobacter*	*Junii*	100	0	98.56	MT613873.1
z60_27F_G12	*Acinetobacter*	*Junii*	100	0	99.47	MT613873.1
z61_27F_H12	*Acinetobacter*	*Junii*	100	0	99.90	MT613873.1
z62_27F_A01	*Pseudomonas* spp.		100	0	99.46	MT256239.1

yielded nothing during enrichment, despite continued pollution from auto repair activities. Indicating that biodegradability of petroleum oil contaminants is significantly restricted by the limited availability of colonizing hydrocarbon-degrading microorganisms in that environment.

The grouping of isolates into high, moderate, and low residue categories was based on the amount of diesel remaining after 7 days of incubation, with low residue indicating high degradation efficiency and high residue representing low degradation capacity. The study found that 67 out of 110 bacterial isolates (60.9%) exhibited a strong ability to degrade diesel. These isolates also showed a higher number of bacterial community associations, totaling 23 (69.7%) [1], stated that the ability of a soil's microbial community to degrade hydrocarbons depends on both their abundance and catabolic activity. Similarly, [26] reported that, mixed microbial

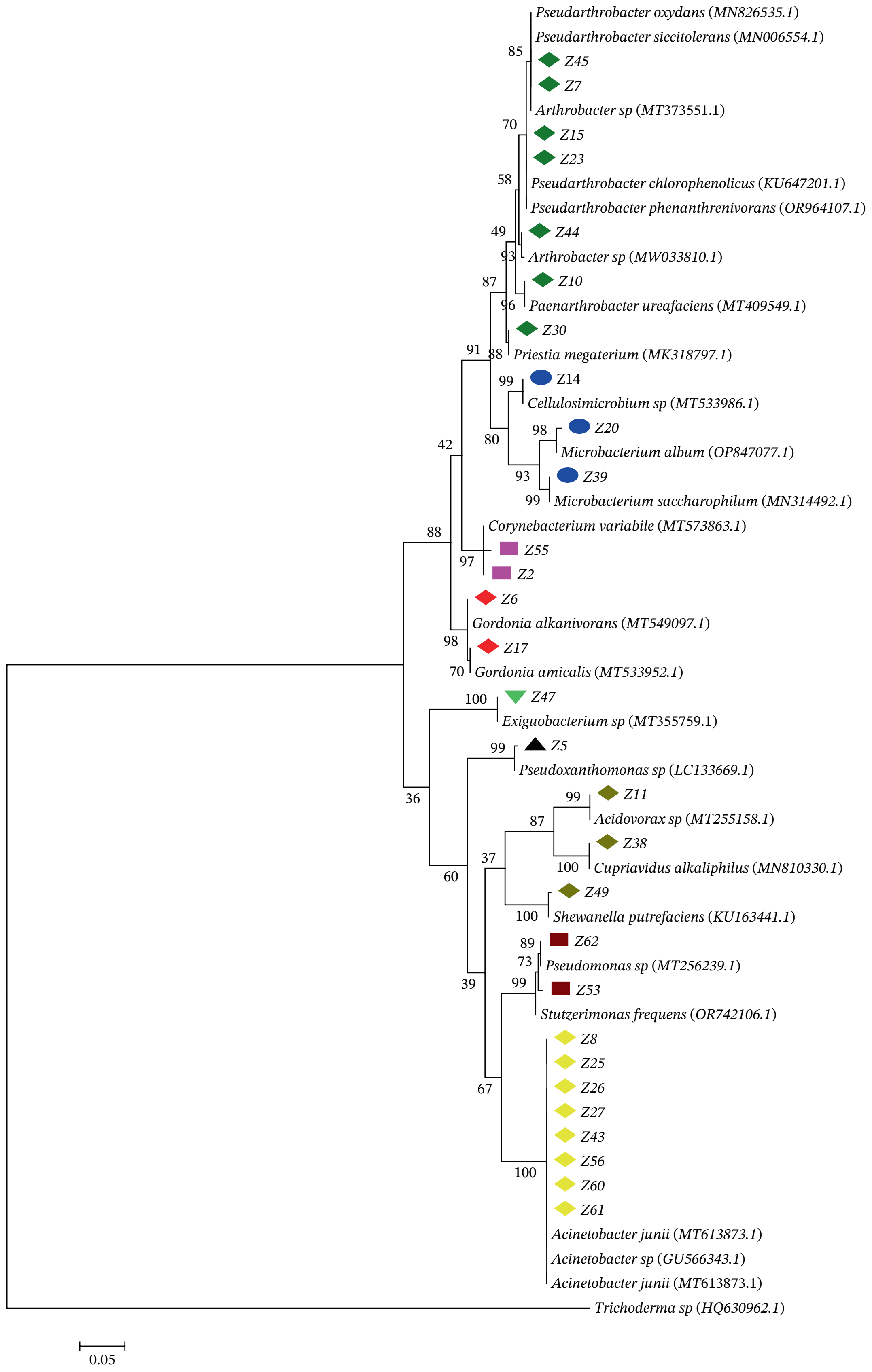

FIGURE 4 | A phylogenetic tree based on 16S rRNA gene sequence showing the relationship among the diesel degrading bacterial isolates and representatives of other related taxa. The tree is drawn to scale with branch lengths measured in the number of substitutions per site. The scale bar indicates 0.05 substitution per nucleotide position. The red diamonds represent the diesel degrading bacteria isolates. The number beside the node is the bootstrap value. In brackets are the GenBank accession numbers as accessed on March 16, 2024. The gene sequence of *Trichoderma* (HQ630962.1) was used as an out-group.

populations with broad enzymatic capacities are essential for breaking down complex hydrocarbon mixtures such as crude oil in soil, freshwater, and marine environments as they expand the range of degradable substrates and facilitate commensalism and co-metabolism. Consistent with the findings of the current study, Pearson correlation analysis indicated a weak negative association, $\text{rho} = -0.344$, with $p\,\text{value} = 0.0058$, between diesel residue concentrations and the number of culturable bacterial isolates. This relationship highlights the ecological significance of hydrocarbon-degrading bacterial communities and supports their critical role in the natural attenuation and biodegradation of diesel and other petroleum-based contaminants in affected environments. Moreover, the rich bacterial diversity in garage soils capable of thriving in petroleum-contaminated environments, may be attributed to the availability of diverse nutrient sources within this ecological niche [27, 28].

Morphological and biochemical profiling of bacterial isolates was performed to assess functional traits associated with diesel hydrocarbon degradation. Dissimilarities were observed in the morphological and biochemical characteristics of the isolated bacteria, the Gram-stain distribution across degradation groups showed that most isolates were Gram-negative. Gram-negative bacteria, especially genera like *Pseudomonas* and *Acinetobacter*, are widely recognized for their hydrocarbon-degrading ability due to biosurfactant production, outer membrane adaptations, and efficient substrate uptake systems [29], aligning with the findings of [30]. A similar trend was noted among group two microorganisms, which were classified as having moderate biodegradation capabilities. These findings, however, contrast with those of [31], who observed a higher prevalence of Gram-positive bacteria than Gram-negative ones. This discrepancy may stem from the experimental conditions in their study where contaminated soil was bioaugmented with vermicompost, biostimulants, and, in some cases, an additional nutrient consortium, which likely influenced the abundance of Gram-positive bacteria. A similar pattern was observed in group three isolates, which left noticeable diesel residues in the enrichment culture. This group also showed a higher proportion of Gram-positive bacteria compared with the first two groups.

A substantial proportion of highly efficient degraders (low residue group) exhibited catalase and oxidase positivity. Catalase decomposes hydrogen peroxide into water and oxygen, mitigating oxidative stress induced by reactive oxygen species during petroleum hydrocarbon oxidation [9, 32]. Oxidase activity reflects the presence of cytochrome oxidase, an enzyme essential in electron transport during aerobic respiration, which is the predominant mechanism for petroleum hydrocarbon degradation in many bacteria [28]. The MR and VP tests showed low positivity across all categories, these tests assess mixed acid fermentative pathways. However, slightly higher MR positivity among low-efficiency degraders may indicate accumulation of organic acids due to incomplete degradation or alternative metabolic stress responses by the bacterial isolates.

Citrate utilization was also common among moderate- and high-efficiency diesel-degrading isolates, suggesting enhanced metabolic versatility. Citrate-positive bacteria can exploit a broader range of organic acids and metabolic intermediates, many of which are by-products of diesel degradation. As a central intermediate of the tricarboxylic acid (TCA) cycle, citrate is endogenously produced by most aerobic bacteria; however, the capacity to utilize exogenous citrate depends on the presence of a functional citrate-permease system, which facilitates its transport into the cell, followed by enzymatic conversion to pyruvate and CO2 [20, 32]. Though these biochemical tests were primarily employed to assess the physiological and morphological relatedness of the isolates in this study, their outcomes also provide valuable insights into functional traits linked to hydrocarbon degradation. This metabolic adaptability likely supports bacterial survival and functional activity in petroleum hydrocarbon-contaminated environments, where carbon sources are chemically complex and often limited. Although this study provides preliminary evidence linking citrate utilization to diesel degradation potential, further targeted investigations are warranted to expound the pathways and regulatory controls governing citrate assimilation in petroleum hydrocarbon degrading bacteria. Notably, this study reinforces these trends, the clustering of positive biochemical traits including catalase activity, oxidase production, and citrate utilization within the low-residue (high-efficiency) group highlights their potential as key metabolic indicators of diesel degradation capacity. In contrast, the greater absence of these traits among high-residue (low-efficiency) isolates is consistent with their diminished biodegradation performance, though these tests were mainly used to for testing physiological and morphological relatedness of the isolates in this study.

Molecular analysis revealed a diverse community of biodegrading bacteria, including the genera *Corynebacterium*, *Pseudoxanthomonas*, *Gordonia*, *Pseudarthrobacter*, *Acinetobacter*, *Arthrobacter*, *Acidivorax*, *Cellulosimicrobium*, *Microbacterium*, *Cupriavidus*, Exiguobacterium, *Shewanella*, *Priestia*, *Paenarthrobacter*, *Stutzerimonas*, and *Pseudomonas*. These findings are consistent with previous studies which reported microbiomes dominated by bacterial species of the same genera [15, 28, 31].

In the current study, *Acinetobacter* was the most predominant microorganism isolated, aligning with the findings of [1, 28, 30]. Genera such as *Arthrobacter*, *Pseudomonas*, *Corynebacterium*, *Gordonia*, and *Microbacterium* were also isolated more than once. Other genera, including *Acidivorax*, *Cytobacillus*, *Cellulosimicrobium*, *Cupriavidus*, *Exiguobacterium*, *Shewanella*, and *Pseudoxanthomonas*, were represented by a single isolate each. All bacteria identified in this study are soil associated microorganisms and have been previously documented for their biodegradation capabilities [1, 26, 28, 31, 33]. They are known to degrade various compounds, including petroleum hydrocarbons and diesel [28, 33, 34].

Exiguobacteria is a Gram-positive, rod-shaped bacterium with activity on catalase and oxidase. *Exiguobacteria* is alkalophilic, halophilic, thermophilic, psychrophilic, and tolerant to heavy metals which enhances its biodegradation potential as well as thrive in extreme conditions. This genus has been shown to play a significant role in the biodegradation of various pollutants, including petroleum hydrocarbons [12, 13, 35]. Similarly, *Cytobacillus firmus* has been associated with the degradation of several environmental pollutants. It is a facultative anaerobe and thermophile, capable of surviving in diverse environments [28]. However, its specific role in petroleum hydrocarbon biodegradation is unknown. Notably, *Microbacterium* found in petroleum-contaminated environments has been shown to express alkane hydroxylases, a key enzyme required for bacterial degradation of petroleum hydrocarbons [36].

Pseudoxanthomonas species have been widely recognized for their involvement in breaking down various environmental contaminants [37]. Additionally, Hao et al. and Révész et al. [37, 38] found *Acidovorax* to be the predominant genus. Hao et al. and Yang et al. [37, 39] highlighted *Microbacterium* as an important microorganism in petroleum biodegradation. While *Arthrobacter* [28, 39] has been identified as a key contributor to the degradation of environmental pollutants. According to Li et al. [28], the major genera involved in petroleum hydrocarbons biodegradation are *Pseudoxanthomonas*, *Arthrobacter*, *Microbacterium*, *Acinetobacter*, and *Pseudomonas*.

Different bacterial genera exhibit varying capacities for petroleum hydrocarbons degradation. Many members of *Acinetobacter*, possess genes encoding hydrocarbon-degrading enzymes, such as *n*-alkane dioxygenase and *n*-alkane hydroxylase. These primarily act on C10–C30 petroleum products under aerobic conditions [1, 26, 28, 30]. Together with *Pseudomonas* [26, 28, 30] have biodegradation capabilities. *Acinetobacter* and *Pseudomonas* are most abundant in the environment particularly the soil ecosystem [26]. Additionally, the two genera are known to produce bio-surfactants that enhance the breakdown of petroleum hydrocarbons, leading to increased bacterial petroleum hydrocarbon uptake and improved biodegradation efficiency in the polluted soils [26, 30, 40]. However, Wang et al. [26] suggested that the effects of surfactants may involve complex modifications at the cellular and omics levels; they noted that while surfactants can enhance degradation, they may also have negative effects on microbial communities, necessitating further research to fully understand their impact on petroleum-degrading bacteria.

4.1 | Conclusion

A diverse and complex prokaryotic community resides in motor vehicle garage soils contaminated with petroleum hydrocarbons in Kericho County. The biochemical properties of some isolates, like oxidase, catalase, and citrate reactions, which most biodegrading microorganisms tested positive for could contribute to their ability to biodegrade petroleum hydrocarbons. This study has demonstrated that microorganisms with petroleum hydrocarbons-degrading potential exist in garage soils, with members of genera *Acinetobacter*, *Arthrobacter*, *Pseudomonas*, *Corynebacterium*, *Gordonia*, and *Microbacterium* are dominant.

The findings from this study can be used by environmentalists and institutions in selecting and improving the isolated bacteria for commercial exploitation in environmental cleaning, in the production of bacteria and in developing recombinant cells with enhanced capabilities in environmental petroleum hydrocarbon clean-up.

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Ethics Statement

The authors have nothing to report.

Consent

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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