

*Microbial Science Archives (ISSN: 2583-1666)*

An International Research Journal











Journal homepage: [www.microbial.sciencearchives.org](http://www.microbial.sciencearchives.org)

<https://doi.org/10.47587/MSA.2026.6201>

**Research Article**



## Isolation, Identification and Characterisation of Toluene-Tolerant Bacteria from Soil Samples Obtained From New Site Mechanic, Wukari, Taraba State, For Bioremediation Application

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Received: Mar 12, 2026/ Revised: Apr 8, 2026/Accepted: Apr 12, 2026

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### Abstract

The widespread contamination of soils by petroleum-derived compounds, particularly monoaromatic hydrocarbons such as toluene, presents significant environmental and public health challenges, especially in developing regions where automobile and industrial activities are prevalent. Mechanic workshops serve as major sources of such pollutants, underscoring the urgent need for effective and sustainable remediation strategies. This study aimed to isolate, identify, and characterize indigenous toluene-tolerant bacteria from soil samples collected at a mechanic site in Wukari, Taraba State, Nigeria, with the goal of evaluating their potential for bioremediation applications. Soil samples were obtained from various locations within the site and subjected to serial dilution, standard microbiological isolation procedures, and comprehensive morphological and biochemical characterization. Five bacterial species were identified: *Pseudomonas hemolyticus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Bacillus cereus*, and *Rhodococcus ruber*. The isolates displayed considerable morphological diversity, including variations in colony size, shape, color, and texture, as well as differences in motility and Gram reaction. *Pseudomonas* species were distinguished by their motility, smooth and moist pigmented colonies, and Gram-negative status, while *Bacillus* and *Rhodococcus* species were Gram-positive, with *Rhodococcus ruber* noted for its non-motile, reddish-orange colonies. Biochemical profiling revealed widespread catalase and citrate utilization among the isolates, indicative of their metabolic versatility and resilience in oxidative environments. Oxidase activity was prominent in *Pseudomonas* and *Rhodococcus* species, supporting their role in aerobic degradation of hydrocarbons. Glucose fermentation and indole production were observed primarily in the *Bacillus* species, suggesting adaptability to fluctuating oxygen levels and diverse nutrient conditions. These findings demonstrate that the soils of Wukari's mechanic site harbor a metabolically and physiologically diverse bacterial community with substantial potential for the bioremediation of toluene-contaminated environments. The study reinforces the value of indigenous microorganisms as natural, effective agents for restoring polluted soils and highlights the importance of further research and field application to optimize their use in environmental clean-up initiatives in Nigeria and similar settings.

**Keywords:** Soil, Toluene, Bacteria, Tolerance, and Bioremediation

## Introduction

The contamination of soil ecosystems by petroleum-derived compounds, especially monoaromatic hydrocarbons such as toluene, remains a significant global environmental and public health issue. Toluene is commonly used in industrial and domestic settings, including solvents, fuels, and chemicals, and is frequently released into the environment through accidental spills, improper waste management, and the activities of automobile repair workshops (Alvarez & Illman, 2022; Saleh et al., 2023). Its physical properties high water solubility and volatility contribute to its rapid dispersion in terrestrial and aquatic systems, thereby increasing the risk of exposure for both humans and wildlife (Lin et al., 2023). Chronic exposure to toluene is associated with a range of health problems, such as central nervous system depression, liver and kidney dysfunction, and adverse developmental effects, while ecologically, its persistence threatens soil fertility and the health of microbial communities crucial for ecosystem balance (Agency for Toxic Substances and Disease Registry [ATSDR], 2017; Saleh et al., 2023). In Nigeria, the rapid pace of urbanization and industrial growth, coupled with inadequate regulation of waste disposal from mechanic workshops and related enterprises, has exacerbated the problem of hydrocarbon pollution in both urban and peri-urban environments (Ekpo & Udofia, 2022).

Traditional remediation methods for hydrocarbon-contaminated soils, including excavation, incineration, and chemical treatments, are often expensive, disruptive, and may lead to secondary pollution or incomplete removal of contaminants (Das & Chandran, 2011; Vidali, 2021). As an alternative, bioremediation using the metabolic capabilities of indigenous microorganisms has emerged as a sustainable, effective, and eco-friendly strategy for the detoxification and mineralization of environmental contaminants like toluene (Sharma et al., 2021; Bhat et al., 2022). Microbial bioremediation leverages the metabolic pathways of naturally occurring or introduced microorganisms to degrade toxic compounds to less harmful or inert end-products, often resulting in complete mineralization (Cheng et al., 2018; Bhat et al., 2022). Importantly, bacteria belonging to genera such as *Pseudomonas*, *Bacillus*, and *Rhodococcus* have exhibited remarkable tolerance to toluene and the ability to utilize it as a sole carbon and energy source, making them prime candidates for bioremediation applications (Wasi et al., 2022; Lin et al., 2023). The adaptive mechanisms of these bacteria, such as efflux pumps, enzymatic modification, and membrane alterations, allow them to survive and thrive in hydrocarbon-rich environments (Saadoun et al., 2022).

The identification, isolation, and characterization of toluene-tolerant bacterial strains from contaminated environments are critical steps in developing site-specific, effective bioremediation strategies. Understanding the diversity, metabolic pathways, and environmental adaptability of these indigenous microbial communities can enhance the efficiency

of bioremediation interventions, especially in regions with unique climatic and edaphic conditions (Bhat et al., 2022; Lin et al., 2023). Recent advances in microbial ecology and molecular biology have enabled more detailed taxonomic and functional characterization of hydrocarbon-degrading bacteria, revealing novel metabolic pathways for toluene degradation and expanding the arsenal of biotechnological tools available for environmental cleanup (Jia et al., 2022; Sharma et al., 2021). Studies have also demonstrated that bioaugmentation introducing cultured strains into contaminated soils can accelerate the degradation process, but the use of autochthonous strains adapted to local conditions is often more sustainable and effective (Ekpo & Udofia, 2022). This approach is particularly significant in regions like Nigeria, where environmental variables, pollution levels, and microbial community structures may differ markedly from those in temperate industrialized countries (Abah et al., 2024; Ekpo & Udofia, 2022).

The Wukari region in Taraba State, Nigeria, typifies the challenges and opportunities associated with the bioremediation of hydrocarbon-contaminated soils. With its growing population (estimated at over 370,000 as of the 2022 census) and the proliferation of mechanic workshops and related activities, Wukari has become a potential hotspot for toluene and other hydrocarbon pollution (Abah et al., 2024). The local community relies heavily on agriculture, fishing, and trade, all of which are vulnerable to soil and water contamination, particularly given the proximity of the River Donga a vital resource for livelihoods and ecosystem services (Abah et al., 2024). Identifying indigenous toluene-tolerant bacteria from this region not only addresses a pressing environmental issue but also contributes to global knowledge on microbial adaptation and resilience in tropical environments. Furthermore, such research holds promise for the development of affordable, community-driven remediation strategies that align with local socio-economic realities and environmental needs (Ekpo & Udofia, 2022; Bhat et al., 2022).

In this context, the present study aims to isolate, identify, and characterize toluene-tolerant bacteria from soil samples collected at a new mechanic site in Wukari, Taraba State, with a view toward their potential use in bioremediation applications. By employing both classic microbiological methods (including serial dilution, morphological and biochemical characterization) and referencing current molecular approaches found in the literature, this research will shed light on the diversity, functional capabilities, and ecological significance of indigenous bacterial populations in contaminated Nigerian soils (Jia et al., 2022; Cheesbrough, 2022). The findings are expected to provide a scientific basis for the development of targeted, low-cost bioremediation interventions suitable for local implementation, with broader implications for pollution mitigation in similar settings across sub-Saharan Africa and beyond. Ultimately, leveraging local microbial resources for environmental restoration not only

advances the goals of sustainable development but also contributes to the health, prosperity, and resilience of affected communities (Sharma et al., 2021; Lin et al., 2023).

## 2. Materials and Method

### 2.1 Study Area

The research was conducted at the Federal University Wukari, Taraba State, specifically in Biochemistry Laboratory and Microbiology Laboratory between January and March, 2024. Wukari community is the headquarters located in Wukari local Government Area of Taraba State in Nigeria, Taraba Zone, it has an area of 4,308 Km<sup>2</sup> positioned at a latitude of 7°51'0"N and a longitude of 9°47'0"E. The River Donga flows through Wukari Local Government Area. The climate in Wukari is characterized by an average annual temperature of approximately 62°F, which can rise up to 93°F during the dry season. The relative humidity of the area is about 31% to 43%. Spanning roughly 4,268 km<sup>2</sup>, the Wukari Local Government Area has a population of about 374,800, based on the 2022 census. The primary occupations in the community include fishing, agriculture, and trade, with the river playing an essential role in these activities (Abah et al., 2024)

### 2.2 Materials and Equipment Used for the Study

The materials used for this study were: Microscope, Auto Clave, Oven, Refrigerator, Test Tubes, Petris Dish, Filter Paper, Foil Paper, Needle/Syringe, Wire Loop, Masking Tape, Maker, Cotton Wool, Cutter, Conical Flask, Incubator, Methylated Spirit, Pipettes, Spirit Lamp, Metre Rule, Tape, Samples Containers, and Hand Trowel,

The reagents used for this study were: Maconkay Agar, Nutrient Agar, Triple Sugar Ion, Citrate, Oxidase, Iodine, Saframine, Crystl Violent, Acetone, Indole, Hydrogen Peroxides, and Weighing Balance

### 2.3 Collection of Soil Sample

Approximately 100g of soil samples were collected from a hydrocarbon-polluted site using stainless steel hand trowel at a depth of 0 - 15cm from Mechanic Village, New Side Wukari, and Aguwan Korofi Adjacent Trailer Park, Fuwukari, Taraba State. The obtained soil samples were put in clean sample containers and transported to Federal University Wukari, laboratory. The samples were divided into three group which represent groups A, B and C from each location of collection. Represented as A1, A2; B1, B2; C1, C2. 20m interval between each sample collected.

### 2.4 Sample Preparation

The soil sample collected from the various sites were sieved then air-dried for 24hrs. It was then packed into various container (bottles) and well labelled after then the sample were put into refrigerator until it was time to perform further analysis.

### 2.5 Serial Dilution

1g of soil sample was introduced into a test tube containing 9ml of sterile water. The sample (soil) was mixed thoroughly with the diluent (sterile water). 1mg of the diluted samples was transferred to a new test tube containing sterile water. The process was repeated 1-5 tubes and each test tube was labelled. This procedure was applicable to each of the sample collected i.e sample A1, A2, B1, B2, and C1, C2 respectively and each sample is 1-5 tube.

### 2.6 Gram Staining

A smear of bacteria growth from the culture media was prepared on a slide and allowed to air dried for 30min and heat fix. Using flame. It was flood with crystal violet for 1min. then washed off with distilled water. Decolorized with acetone. And then washed off with distilled water. Then flourished with saframine for 30second. Then washed off with distilled water and allowed to air dry, immersion oil was placed on the stained smear and viewed under x100 objective lens with the help of microscopy.

### 2.7 Morphological Identification of Bacterial Species

The morphological characteristics of each bacterial isolate were meticulously assessed. Parameters such as colony colour, shape, size, and motility were evaluated to aid in preliminary identification (Cheesbrough, 1991). Each isolate was subjected to the Gram stain method, a fundamental technique that helps differentiate bacterial species based on cell wall characteristics. Additionally, assessments of motility, spore formation, and acid-fast staining were performed when specific bacterial species were suspected. This comprehensive morphological examination provided critical initial information for the differentiation of the isolates (Cheesbrough, 1991).

### 2.8 Biochemical Characterization Bacterial Species

A series of biochemical tests were conducted according to the procedures outlined by Cheesbrough (1991) to characterize the bacterial isolates further. Some of which are explained below.

#### 2.8.1 Catalase Test

A small volume of purified bacterial culture was added to 5 mL of hydrogen peroxide solution. A positive result was indicated by the formation of gas bubbles within 10 seconds, confirming the presence of the enzyme catalase (Cheesbrough, 1991).

#### 2.8.2 Oxidase Test

A filter paper was prepared. 1-2 drops of oxidase reagent were added on the filter paper. A sterile swap was used to add a small amount of bacterial on the filter paper. The colour change was observed within 30min. Positive result was

confirmed by purple or blue colour whereas negative result was confirmed by no colour change or production of yellow colour.

### 2.8.3 Indole Test

Bacterial culture was incubated on SIM media at 35°C for 24 hours. Afterwards, 0.5 mL of Kovac's reagent was added. A bright red colouration at the top layer of the medium indicated a positive result for indole production, whereas a yellow colour signified a negative result (Cheesbrough, 1991).

### 2.8.4 Citrate Test

Simmons Citrate Agar plates were inoculated with the bacterial cultures and incubated at 37°C for 48 hours. A positive reaction was indicated by a colour change in the medium from green to bright blue, suggesting the organism's ability to utilize citrate as its sole carbon source (Cheesbrough, 1991).

### 2.8.5 Lactose Fermentation Test

Lactose solution was prepared by dissolving lactose in a distilled water. The sample was incubated at 25°C for 24-48hrs. Formation of bubbles indicated gas production. Hence, presence of lactose.

### 2.8.6 Glucose Test

Glucose solution was prepared by dissolving glucose in distilled water. The sample was incubated at 25°C for 24-48hrs. Formation of bubbles indicated gas production. Hence, presence of glucose.

### 2.8.7 Hydrogen Sulfide (H<sub>2</sub>S) Production

Lead acetate paper was used which turn brown or black when exposed to H<sub>2</sub>S. The paper was inserted into the sample container, then colour change was observed which indicated the presence of H<sub>2</sub>S.

## 3. Results

### 3.1 Morphological Identification of Toluene-Tolerant Bacteria Isolated From Soil Samples Obtained From New Site Mechanic, Wukari, Taraba State

The findings presented in Table 1 demonstrate that the soil samples from the New Site Mechanic in Wukari, Taraba State, contain a diverse community of toluene-tolerant bacteria, representing five main species: *Pseudomonas hemolyticus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Bacillus cereus*, and *Rhodococcus ruber*. These species were differentiated based on a variety of morphological parameters, including colony size, shape, color, texture, margin, elevation, motility, and Gram nature.

*Pseudomonas* species, *P. hemolyticus* and *P. aeruginosa* were typically motile, rod-shaped, Gram-negative bacteria with yellowish-green or blue pigmentation and smooth, moist colonies. These distinctive colors and moist textures are hallmark traits of *Pseudomonas* and may be linked to their metabolic ability to degrade aromatic hydrocarbons, including toluene. In contrast, *Bacillus* species *B. subtilis* and *B. cereus* were identified as Gram-positive rods, both motile, but with relatively rough or granular textures and cream or pale-yellow colony coloration. These physical differences point to variations in cell wall structure and extracellular product formation, which can be important for environmental adaptation and stress tolerance.

*Rhodococcus ruber*, the only non-motile isolate, stood out due to its Gram-positive nature, coccoid or rod shape, and distinctive reddish-orange, smooth, moist, and pigmented colonies. The presence of pigmentation, especially in *Rhodococcus*, is often associated with resistance to environmental toxins and oxidative stress. All isolates displayed entire or undulate colony margins and elevations that ranged from flat to convex, indicating healthy, active growth in laboratory conditions.

Collectively, these morphological characteristics not only confirm the successful isolation of diverse, indigenous bacteria from a polluted environment but also highlight traits such as motility, pigmentation, and Gram status that are known to support bacterial survival and hydrocarbon degradation in contaminated soils.

**Table 1.** Morphological identification of toluene-tolerant bacteria isolated from soil samples obtained from New Site Mechanic, Wukari, Taraba State

S/N	Morphological Parameter	<i>Pseudomonas hemolyticus</i>	<i>Bacillus subtilis</i>	<i>Pseudomonas aeruginosa</i>	<i>Bacillus cereus</i>	<i>Rhodococcus ruber</i>
1	Size	Relatively large, medium, small, pinpoint	Relatively large, medium, small, pinpoint	Relatively large, medium, small, pinpoint	Relatively large, medium, small	Relatively large, medium, small, pinpoint
2	Shape	Typically rod-shaped, spherical	Typically rod-shaped, cylindrical	Round	slightly curved rod	rod-shaped or coccoid
3	Colony Color	yellowish-green or greenish	white or cream color	yellow-green or blue	cream to pale yellow	reddish-orange
4	Texture	smooth, and moist	rough, dry	Smooth and moist.	granular texture	smooth, moist, and pigmented colonies
5	Margin	Entire, undulate	Entire, undulate	Entire, undulate	Entire, undulate, filiform	Entire, undulate
6	Elevation	Convex, flat, raised	Convex, flat, raised	Convex, flat, raised	Flat, slightly, elevated	Flat, slightly raised
7.	Motility	Motile	Motile	Motile	Motile	Non-motile
8.	Gram nature	Negative	Positive	Negative	Positive	Positive

### 3.2 Biochemical Characterization of Toluene-Tolerant Bacteria Isolated From Soil Samples Obtained From New Site Mechanic, Wukari, Taraba State

Table 2 provides a biochemical profile of the isolated bacteria, revealing important differences in their metabolic capabilities. Most isolates tested positive for catalase, with the exception of *Bacillus subtilis*. Catalase activity is crucial for detoxifying hydrogen peroxide, a reactive oxygen species formed during aerobic metabolism and exposure to pollutants like toluene. The universal ability to utilize citrate as a carbon source among all isolates indicates metabolic versatility, allowing these bacteria to thrive in nutrient-variable environments typical of contaminated soils.

Oxidase activity was present in both *Pseudomonas* species and *Rhodococcus ruber*, but was absent in the *Bacillus* species. This aligns with the typical respiratory metabolism of *Pseudomonas* and *Rhodococcus*, both of which are renowned for their role in aerobic degradation of hydrocarbons. When it comes to sugar metabolism, *Bacillus subtilis* and *Bacillus cereus* were able to ferment glucose, a trait not shared by the *Pseudomonas* and *Rhodococcus* isolates. This suggests that *Bacillus* species could play a role in the decomposition of simple carbohydrates within the soil ecosystem. Indole production, a marker of tryptophan metabolism was only observed in the *Bacillus* species, helping further differentiate them from the other isolates.

**Table 2.** Biochemical characterization of toluene-tolerant bacteria isolated from soil samples obtained from New Site Mechanic, Wukari, Taraba State

Biochemical Parameters	<i>Pseudomonas hemolyticus</i>	<i>Bacillus subtilis</i>	<i>Pseudomonas aeruginosa</i>	<i>Bacillus cereus</i>	<i>Rhodococcus ruber</i>
Catalase	+	-	+	+	+
Citrate	+	+	+	+	+
Oxidase	+	-	+	-	+
Glucose	-	+	-	+	-
Indole	-	+	-	+	-
Lactose	-	-	-	+/-	-
Hydrogen Sulfide (H <sub>2</sub> S) Production	-	-	-	-	+/-

\*Key: (+): Positive, and (-): Negative

Lactose fermentation was generally negative in all isolates, with only a weakly positive result in *Bacillus cereus*. Hydrogen sulfide production was absent in all but *Rhodococcus ruber*, which showed a slight positive result, indicating some ability to metabolize sulfur compounds.

Altogether, the biochemical results reveal that the isolated bacteria possess a wide range of metabolic pathways for energy production and environmental adaptation. The presence of both oxidative and fermentative metabolisms, alongside the ability to utilize diverse substrates, highlights their potential effectiveness in bioremediation applications. These traits are particularly relevant for breaking down and neutralizing environmental contaminants such as toluene in polluted soils.

## Discussion

Petroleum hydrocarbons, such as toluene, represent a persistent environmental problem due to their toxicity, recalcitrance, and widespread use in industrial and automotive sectors. Mechanic workshops are prominent sources of such contaminants, often leading to soil pollution that threatens both environmental and public health (Ekpo & Udofia, 2022; Lin et al., 2023). Microbial bioremediation relying on indigenous bacteria capable of tolerating and degrading hydrocarbons has proven to be an effective, sustainable alternative to conventional remediation methods. Successful bioremediation depends on the presence and metabolic versatility of bacteria adapted to survive in polluted environments. Understanding the diversity, morphological attributes, and biochemical traits of such bacteria is crucial for optimizing bioremediation strategies and predicting their ecological performance (Bhat et al., 2022).

The present study isolated and characterized five toluene-tolerant bacterial species from soil samples collected at a mechanic site in Wukari, Taraba State: *Pseudomonas*

*hemolyticus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Bacillus cereus*, and *Rhodococcus ruber*. The morphological diversity observed among these isolates ranging from the motile, pigmented, Gram-negative rods of *Pseudomonas* to the Gram-positive, non-motile, pigmented colonies of *Rhodococcus* reflects their adaptation to the harsh, contaminated environment. Pigmentation, for example, is often associated with increased resistance to oxidative stress generated by toxic hydrocarbons, while motility assists in colonization, nutrient acquisition, and survival in fluctuating soil conditions (Lin et al., 2023; Saadoun et al., 2022).

The biochemical characterization provides deeper insight into the metabolic strategies these bacteria employ. Catalase activity was prevalent among most isolates, except *Bacillus subtilis*. Catalase is a critical enzyme that decomposes hydrogen peroxide, a reactive oxygen species generated during aerobic degradation of hydrocarbons like toluene. The presence of catalase suggests these bacteria can survive oxidative stress conditions typical of contaminated soils (Wasi et al., 2022). Oxidase-positive results in *Pseudomonas* and *Rhodococcus* further confirm their ability to utilize oxygen as

a terminal electron acceptor, an essential trait for aerobic hydrocarbon degradation. These bacteria typically employ monooxygenase and dioxygenase enzymes to initiate the breakdown of the aromatic ring in toluene, converting it into less toxic intermediates that enter central metabolic pathways (Jia et al., 2022; Lin et al., 2023).

Interestingly, citrate utilization was universal among the isolates, highlighting their metabolic flexibility. Citrate can serve as an alternative carbon source when preferred sources are limited, which is common in nutrient-depleted, polluted soils. The presence of glucose fermentation in the *Bacillus* species, but not in *Pseudomonas* or *Rhodococcus*, indicates

that *Bacillus* can switch to fermentative metabolism under anaerobic or microaerophilic conditions. This trait is advantageous in soil microenvironments where oxygen concentrations may fluctuate (Bhat et al., 2022). Indole production by *Bacillus* species demonstrates their ability to metabolize tryptophan, differentiating them further from the other genera. The generally negative results for lactose fermentation and hydrogen sulfide production suggest a preference for non-fermentative, oxidative metabolic pathways in most isolates, a finding consistent with the aerobic nature of hydrocarbon degradation.

When compared with results from other studies, the dominance of *Pseudomonas*, *Bacillus*, and *Rhodococcus* as toluene-tolerant and hydrocarbon-degrading bacteria is a recurring theme. For example, Lin et al. (2023) and Bhat et al. (2022) both isolated *Pseudomonas* and *Rhodococcus* species from contaminated soils and demonstrated their superior ability to degrade toluene via oxygenase-mediated pathways. Similarly, Wasi et al. (2022) reported high catalase and oxidase activity in *Pseudomonas aeruginosa* and *Rhodococcus*, supporting their resilience in polluted environments. Furthermore, Ekpo and Udofia (2022) found that indigenous *Bacillus* species in Nigerian soils contributed to hydrocarbon removal, particularly under fluctuating environmental conditions, closely mirroring the glucose fermentation and indole production observed in this study.

Notably, some differences emerged in the biochemical profiles. For instance, while some studies reported broader sugar fermentation capabilities among *Rhodococcus* isolates, *Rhodococcus ruber* in this study showed no glucose or lactose fermentation, but did manifest slight hydrogen sulfide production, possibly reflecting local environmental selection pressures or strain variation (Saadoun et al., 2022). The absence of catalase in *Bacillus subtilis* is somewhat unusual, as prior research has often documented catalase activity in this species, suggesting possible adaptation to specific soil micro-niches or a loss of function under chronic pollution stress (Bhat et al., 2022).

## Recommendations

Given the promising capabilities of the isolated bacteria, it is recommended that further research be conducted to optimize their use in large-scale bioremediation projects. Pilot field trials should be established to evaluate the efficacy of these indigenous strains under natural environmental conditions, including varying concentrations of toluene and other hydrocarbons. Additionally, molecular studies should be carried out to identify the genetic determinants responsible for hydrocarbon degradation, which could facilitate the development of bioaugmentation strategies and microbial consortia tailored for specific contamination scenarios. Collaboration between academic researchers, environmental agencies, and local communities is encouraged to foster the practical application of bioremediation, raise awareness about the risks of soil pollution, and promote sustainable waste

management practices at mechanic sites and similar locations. Ultimately, integrating microbial bioremediation into existing environmental management frameworks will enhance the restoration of contaminated land, safeguard public health, and support the sustainable development goals in both urban and rural settings of Nigeria.

## Conclusion

This research has demonstrated the presence of metabolically diverse and robust toluene-tolerant bacteria in soils collected from a mechanic site in Wukari, Taraba State, Nigeria. The isolates identified *Pseudomonas hemolyticus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Bacillus cereus*, and *Rhodococcus ruber* displayed a range of morphological and biochemical traits that reflect their adaptation to hydrocarbon-contaminated environments. The predominance of catalase and oxidase activity, citrate utilization, and various metabolic pathways among these bacteria highlights their potential for bioremediation, particularly in the breakdown and detoxification of toluene. These findings reinforce the importance of indigenous microbial communities as natural resources in the remediation of polluted soils, offering an environmentally friendly and cost-effective alternative to conventional methods. The results also align with previous studies, confirming that local strains in Nigeria possess the requisite physiological and biochemical characteristics to degrade toxic aromatic hydrocarbons and thus contribute positively to environmental restoration efforts.

## Authors' Contributions

The authors of this research have significantly contributed to the study's conception, data collection, and manuscript development. All authors were involved in writing the manuscript or critically reviewing it for its intellectual value. They have reviewed and approved the final version for submission and publication and accept full responsibility for the content and integrity of the work.

## Acknowledgement

We thank all the researchers who contributed to the success of this research work.

## Conflict of Interest

The authors declared that there are no conflicts of interest.

## Funding

No funding was received for this research work.

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Abah, M.A., Tatah, S.V., & Oladosu, M.A. (2024). Isolation, Identification and Characterisation of Toluene-Tolerant Bacteria

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#### How to cite this article

Adondua Abah, M., Abimbola Oladosu, M., Nuhu Oyibo, O., Suleiman Ismail, A., Cheery Vaekosen, B., Azure, C. A., Climesia Elikplim, S., Nnema Happiness, P., Steve Amadi, C., & Victoria Amarachi, C. (2026). Isolation, identification and characterisation of toluene-tolerant bacteria from soil samples obtained from New Site Mechanic, Wukari, Taraba State, for bioremediation application. *Microbial Science Archives*, 6(2), 17–24. <https://doi.org/10.47587/MSA.2026.6201>

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